Systematics and evolution of the Australian Dacini (Diptera: Tephritidae)

Melissa Starkie

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School of Biology and Environmental Science

Science and Engineering Faculty

Queensland University of Technology

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Abstract

The Dacini fruit flies (Diptera: Tephritidae) are a widely distributed clade that occupy tropical and subtropical forests across Africa, South-east Asia, Australia, and the Pacific. While there are existing systematic studies on this group, the Australian and Pacific fauna have been under-sampled and deeper evolutionary questions neglected. This study produces a molecular phylogenetic reconstruction based on targeted sampling of the Australian and Pacific Dacini in order to investigate biogeographic, systematic and evolutionary questions about the tribe.

The overarching aim of this thesis was to produce a Dacini phylogenetic tree and apply this tree to evolutionary, taxonomic and biogeographical questions concerning the group. The main aims of each chapter were to: (i) employ a targeted sampling method to expand the taxonomic and geographic collections available for analysis (Chapter 2); (ii) produce a multi-locus molecular phylogenetic tree (Chapter 3) and then, (iii) use this tree to investigate phylogenetic signal of the traits: male lure response and host breadth (Chapter 3); (iv) evaluate the ability of morphological character traits to resolve phylogenetic relationships (Chapter 4); (v) investigate the influence of biogeography in the Australian and Pacific region on divergence of the regional Dacini (Chapter 5); (vi) investigate basal lineages and inform a taxonomic review of the *Bactrocera aglaiae* species group (Chapter 6); and (vii) reconcile new genetic data with previous taxonomic relationships (based on morphology) in a taxonomic review of the *Bactrocera tryoni* species group (Chapter 7).

In Chapter 2, species were sampled along the east coast of Australia, using a combination of male lures, protein baits and fruit rearing in order to expand existing collections. Over 8600 specimens were collected during this study. New geographic distributions are recorded for five species, new lure responses are recorded for three species, a new species is described based on morphology.

Chapter 3 utilised 144 described species from Australia, the Pacific, and South-east Asia for a phylogenetic reconstruction of the tribe Dacini. The *Bactrocera aglaiae* species group was resolved as the oldest *Bactrocera* clade sampled in this study, distributed in northern Australia and Papua New Guinea. Consistent with other molecular phylogenies of the Dacini, there was poor agreement between systematic placement of species in the phylogeny and their morphologically based taxonomic

placement at the subgeneric and species complex levels. Divergence time estimates provided dates that were younger than the only previously dated phylogeny of this group, with the tribe estimated as diverging from its most recent common ancestor 43 million years ago. Ancestral trait reconstruction and tests for phylogenetic signal revealed that male lure response exhibits strong phylogenetic signal across the tree. Host diet breadth also exhibited phylogenetic signal, but was not as strong.

My phylogeny, like others before it, found poor alignment between Dacini systematic placements based on molecular data versus morphological data. Chapter 4 evaluated colour patterns and structural characters that are typically used in descriptions and diagnosis of Dacini species for their utility in phylogenetic reconstruction. When compared against datasets that contained only molecular data, the AU test found there was no significant improvement to the resolution of the tree when morphological characters were added to a molecular dataset. When morphological characters were used to reconstruct a phylogeny alone, species were not able to be resolved at the generic or species levels in a way congruent with current systematic understanding of the group.

Chapter 5 utilised the dated phylogeny from Chapter 3 to investigate divergence pathways. The analysis found that regional Dacini species moved eastward into the Pacific from Papua New Guinea and Australia, and that there was no westward movement of species back into those regions. There was evidence of multiple incursions via the Torres Strait land bridge into and out of Australia and Papua New Guinea, both in deeper and more recent evolutionary time. Within Australia, species have moved westward into the Northern Territory and southward out of north Queensland. There is no evidence, given the present-day distributions of fruit flies, that biogeographical land barriers have played a significant role in fruit fly speciation within Australia.

In Chapter 6 a taxonomic review of the *Bactrocera aglaiae* species group is provided. This included resolution of discrepancies between the descriptions of the holotype, and previous descriptions based on paratypes. Within the review, new species descriptions and identification of variation was provided. In addition, likelihood mapping tests confirmed the clade as the oldest *Bactrocera* clade.

The systematics of the *Bactrocera tryoni* species complex, which contains several of Australia's most important fruit fly pest species, was investigated in detail in Chapter 7 of the thesis based on paraphyly of species in the phylogeny produced in Chapter 3. Utilising a reduced genome source of SNP data, sequence data and morphological observations, it was found that the traditional concept of the complex as containing four species (*B. tryoni, B. neohumeralis, B. aquilonis* and *B. melas*) needed to be enlarged to include *B. ustulata, B. erubescentis, B. mutabilis* and *B. curvipennis.* Further *B. humilis* (a taxa morphologically very similar to *B. tryoni*) and *B. melas* showed no genetic evidence consistent with them being true species. To accommodate the extra species, the *B. tryoni* complex was taxonomically redefined as a species group, *B. tryoni* was redescribed, and *B. humilis* and *B. melas* were synonymised with *B. tryoni*. The potential for unrecognised cryptic species, morphologically similar to *B. tryoni* and *B. neohumeralis,* existing within the group is discussed.

Chapter 8 presents my final thoughts for the future of Dacini taxonomy and systematics. I recommend subgeneric groups be removed from use in Dacini taxonomy due to their lack of utility. In addition, using the term 'species group' instead of 'species complex' is also recommended based on the confusion this has caused other taxonomists. A case study using the *B. frauenfeldi* species complex is provided as an example of how results from each chapter can be used to investigate difficult species groups. Finally, I conclude by acknowledging this thesis has developed a comprehensive dataset which is a good starting point for any investigation of key species groups, trait analysis and large-scale biogeographic analyses.

Table of Contents

List of Figures

List of Tables

Statement of authorship

The work contained in this joint thesis undertaken between QUT and the Queensland Department of Agriculture and Fisheries has not been previously submitted to meet requirements for an award at these or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: QUT Verified Signature

Date: 19/01/2021

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Chapter 1: Introduction

1.1. General Introduction

Systematics is the practice of identifying biological units and reconstructing hypotheses of the evolutionary patterns of those units (Smith, 1994). This can be done by utilising numerous sources of information which can include morphological, molecular (Harvey et al., 2008), and ecological data (Kruckeberg, 1969). Systematics rarely operates as a single discipline, as the application of systematics can extend far beyond phylogenetic reconstruction. Crossover is most commonly observed (and utilised in this thesis) with taxonomic, biogeographic and ecological disciplines, with systematics capable of aiding in determining drivers of speciation, trait evolution, and providing evidence towards taxonomic revisions (McGuire et al., 2007, Dunnum and Salazar-Bravo, 2010, Smith et al., 2014, Manguilla et al., 2015).

The tribe Dacini of the family Tephritidae (Insecta: Diptera) is a very speciose clade, of which the evolutionary history of the group remains largely unknown. Additionally, there are a number of issues in the tribe that stem from a disconnect between taxonomy and phylogenetics, which for the former has been informed by morphological characters and the latter by predominantly molecular data. There have been many attempts to reconcile taxonomy and phylogenetics within the tribe, however the disconnect remains a problem for many systematists working on this group (Krosch et al., 2012, Doorenweerd et al., 2018, San Jose et al., 2018).

While numerous molecular phylogenetic studies have been published on the Dacini in the last 20 years (comprehensively reviewed in Chapter 3), taxon coverage has limited downstream applications of these phylogenies. Taxon sampling for previous Dacini phylogenies have often been pest species biased (Leblanc et al., 2015), focussed on supporting quarantine diagnostics (Boykin et al., 2013), or geographically scattered (San Jose et al., 2018), all of which has perpetuated taxon gaps with has limited further applications of these phylogenies to broader questions such as biogeographic history and trait evolution. Additionally, most previous Dacini phylogenetic studies have had the sole aim of producing the phylogeny and, while this is a worthwhile outcome in its own right, appropriately targeted sampling and the integration of biological and distributional data can allow for additional

evolutionary questions to be addressed, as evidenced in Virgilio et al. (2009) and Krosch et al. (2012).

In order to address these systematic and taxonomic issues within the tribe, the aim of this thesis is to develop a regionally robust phylogeny of the Dacini, focussed on the Australian fauna, a group consisting largely of non-pests which are frequently under-sampled in current literature. This phylogeny will then be used to address other evolutionary questions (trait evolution, biogeography) with a focus on this region.

The remainder of this chapter will illustrate the value of developing a comprehensively sampled phylogeny of the Australian and Pacific Dacini and the important ecological, biogeographic and taxonomic questions which can be answered by using such a phylogeny. Unlike most traditional monographic theses, this thesis does not focus the review of the literature to this chapter alone, but rather provides targeted, comprehensive literature reviews with each of the research chapters. Subsequently, this chapter is briefer and has the simplified role of introducing the thesis as a whole. It consists of four main sections: a general overview of the Dacini, including a background on their geographic distribution and general biology with a specific focus on biological traits that are investigated in later chapters (male lure response and host diet breadth); an overview of current taxonomy and systematics of the Dacini; specific systematic/taxonomic issues that need addressing within the tribe; and finally, an overview of biogeography and speciation of the Dacini.

1.2. The Dacini

With more than 4900 species in over 500 genera the Tephritidae, or 'true fruit flies', is one of the largest and most diverse families within the Insecta (Norrbom, 2004). The tribe Dacini (Tephritidae: Dacinae) is the most speciose tephritid clade with 932 species in four genera: 461 *Bactrocera* Macquart*,* 273 *Dacus* Fabricius*,* 196 *Zeugodacus* Hendel and 2 *Monacrostichus* Bezzi (Doorenweerd et al., 2018). The focus of this thesis is on the first three genera, of which there are 113 described Dacini species found in Australia (Hancock, 2013, Virgilio et al., 2015, Hancock and Drew, 2017c). This encompasses species inhabiting narrow distributional and host-plant ranges, to those that lay their eggs into multiple plant families and inhabit many natural and urban environments (Hancock et al., 2000). The two *Monachrostichus*

species have restricted distributions in South-east Asia where they infest *Citrus* species (Clarke, 2019), and are not further dealt with in this thesis because of their rarity and distribution outside of my area of geographic focus.

1.2.1. Distribution

The Dacini are found in temperate, tropical and subtropical regions of the world (Christenson and Foote, 1960), with the most extensive speciation in the tropics (Drew, 2004). The three primary genera, *Bactrocera, Dacus* and *Zeugodacus* have very different distributions; *Bactrocera* and *Zeugodacus* are found predominantly in tropical and subtropical South-east Asia, whereas *Dacus* dominates in savanna and dry tropical forests of Africa (Fig. 1.1) (Clarke, 2019). The widespread distribution of these three genera within the Dacini, combined with their adaptations to different habitats, raises questions surrounding their origin and evolutionary relationships.

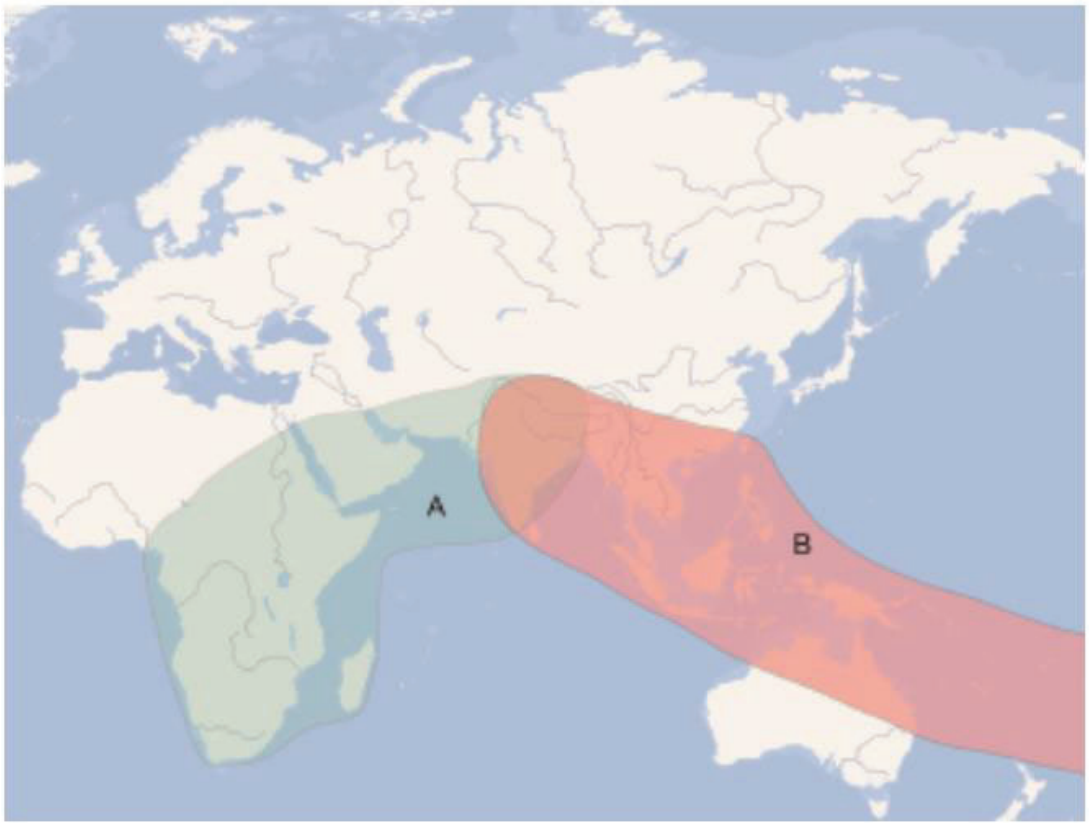


Figure 1.1: Broad distributions of the three Dacini genera sampled in this thesis. A: *Dacus;* and B: *Bactrocera* and *Zeugodacus.* Taken from Drew (2004).

1.2.2. Biology

The typical Dacini life cycle (Figure 1.2) begins after mating, when the female deposits fertilised eggs within ripening fruit (Christenson and Foote, 1960). The eggs develop into larvae which feed upon the fruit, before leaving the fruit as fully developed larvae to pupate within the soil, from which they subsequently emerge as adults (Dhillon et al., 2005).

Despite being referred to as 'fruit flies', most tephritids are non-frugivorous, instead laying eggs in diverse parts of the plant such as flower heads and shoots, with some species mining the crown and stems of a plant to form galls (Duan and Messing, 1997, Alberctsen, 2000, Kovac, 2015, Frias, 2008). The very small subfamily Tachiniscinae are not even herbivorous, but are endoparasitoids of saturnid butterflies (Clarke, 2019). The fruit feeding habit of a small number of frugivorous tephritids that attack commercial fruits has led to a common belief that all fruit flies are unwanted pests however, there are many species that are beneficial and have been observed performing key pollination duties (Dobson, 2006), sharing unique mutualistic behaviours with plants (Tan et al., 2002), and are used as weed biological control agents (Zwolfer, 1982, Foote et al., 1994), or simply have no pest status because they breed in non-commercial hosts (Hancock et al., 2000). For the purposes of this thesis the term 'fruit fly' will be used generically to refer to frugivorous species, unless otherwise noted.

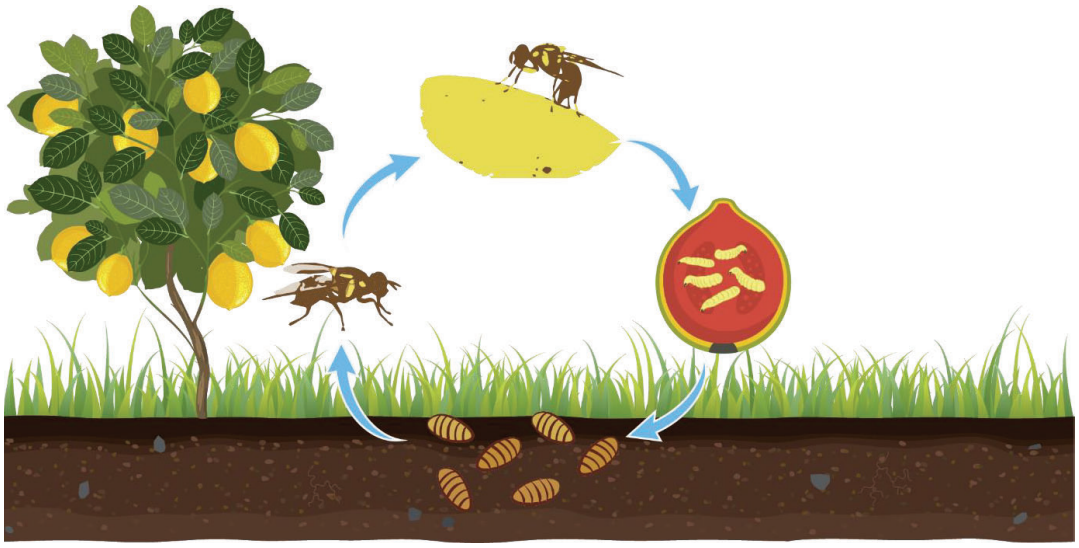


Figure 1.2: The typical fruit fly life cycle; egg laying, larval life stage, soil pupation and adult emergence. Illustration by J. Newman.

1.2.2.1. Host diet breadth

Some Dacini species are monophagous, and lay their eggs in a single plant species, whereas others utilise species from across multiple plant families (Hancock et al., 2000). While many insects are specialist host users, Clarke (2017) identified an extremely high rate of polyphagy within the genus *Bactrocera* (when compared to other insects) and hypothesized that, in contrast to the general pattern seen in most herbivorous insects (Bernays and Minkenberg, 1997, Loxdale et al., 2011), fruit flies may be shifting from specialist host use behaviours to polyphagous host use behaviours. The evolutionary patterns for polyphagous herbivore clades arising from specialist herbivores has been under explored in the literature (but see Hardy and Otto (2014) and Day et al. (2016)), and warrants further investigation. Very little is known of how generalism and specialism have evolved within the Dacini.

1.2.2.2. Male lure response

Male Dacini fruit flies can be trapped via species-specific responses to a small number of plant-derived phenylpropanoids and phenylbutanoids (Tan and Nishida, 2000) commercially formulated as lures. Roughly 80% of Australian Dacini species are responsive to the two most commonly used lures; methyl eugenol and cue-lure (Hancock et al., 2000, Royer, 2015, Royer et al., 2014). In the last decade, a large number of new analogues and chemical cues have been developed, which has renewed interest in testing lures both in new areas and for their attractiveness to previously unresponsive species (Fay, 2010, Royer et al., 2014, Royer, 2015, Royer et al., 2018, Royer et al., 2019). Additionally, flies were once thought to respond to one lure type, however it is now widely accepted that species can respond to more than one lure, tending to show a preference for one lure over another (Royer, 2015, Royer et al., 2018).

<u>Gaps in knowledge to be addressed</u>

i. Herbivory theory states that the normal evolutionary pattern for change in diet breadth is to progress from generalism to specialism (Loxdale et al., 2011), but the large number of polyphagous *Bactrocera* suggests that this

might not be the case in the Dacini (Clarke, 2017). The evolution of the character trait 'diet breadth' in the Dacini is a knowledge gap that will be addressed in this thesis.

ii. As more chemicals are identified which are attractive to male Dacini, the evolutionary patterns of lure response across the tribe may give insights into the origins of this trait (Raghu, 2004) and help identify the likely lure response of species for which an attractive lure has not yet been identified.

1.2.3. Current taxonomy and phylogenetic understanding of the Dacini tribe

The Australian Dacini taxa is classified into three genera, four subgeneric groups and 16 subgenera (Table 1.1). *Bactrocera* (*Bactrocera*) (83 species) and *Dacus* (*Neodacus*) (10 species) are the two largest subgenera: all others have three species or fewer. *Zeugodacus* has been elevated to genus level, from a subgenus within *Bactrocera,* only in the last few years (Virgilio et al., 2015). While this is generally accepted within the Dacini community (Doorenweerd et al., 2018, Clarke, 2019) this is not universally the case (Hancock and Drew, 2016). Confusion surrounds not only relationships at the genus level within the Dacini (e.g. the generic status of *Zeugodacus* (Virgilio et al., 2015, Hancock and Drew, 2016)), but also relationships among subgenera (Smith et al., 2003, Hancock, 2015), within species complexes (Liu et al., 2017, Schutze et al., 2015a) and at the species level (Leblanc et al., 2015), these issues are considered further below.

Non-traditional 'levels' of taxonomic rank, some of which, such as species complexes, are referred to as open nomenclature qualifiers (Sigovini et al., 2016). Open nomenclature qualifiers do not hold taxonomic status under the International Code of Zoological Nomenclature (Sigovini et al., 2016), but are used to name or group species that require further taxonomic definition. Open nomenclature qualifiers, along with other groupings, have been a routine part of Dacini taxonomy for over 100 years, when initially used to help better place species within existing genera (Bezzi, 1915, Bezzi, 1916). For the Dacini, subgeneric groups, subgenera, and species complexes (Drew, 1989, Drew and Romig, 2013, Hancock and Drew, 2018b) are all classifications that do not hold any taxonomic rank. Early Dacini taxonomists

also made extensive use of the sub-species (May, 1962) and 'varieties' (Hardy, 1951) (also considered to be open nomenclature qualifiers), but this practice has been absent from the literature for the last 50 years (Hardy, 1969).

Table 1.1: Taxonomic constructs that apply to the 113 Australian Dacini species (Hancock and Drew, 2006*, De Meyer et al., 2015, Hancock and Drew, 2015*, Drew and Hancock, 2016*, Hancock and Drew, 2016*, 2017a*, 2017b*, 2017c*, Doorenweerd et al., 2018, San Jose et al., 2018). *Place the *Zeugodacus* group and *Zeugodacus* as a subgenus group and subgenus, respectively, of *Bactrocera.*

Genus	Subgeneric Group	Subgenus	No. of species
Bactrocera	*Bactrocera* Group	*Apodacus* Perkins	2
		Bactrocera Macquart	83
		Bulladacus Drew & Hancock	2
		Calodacus Hancock	1
	Melanodacus Group	*Hemizeugodacus* Hardy	1
		Neozeugodacus May	2
		Paratridacus Shiraki	1
		Parazeugodacus Shiraki	1
	Queenslandacus Group	*Queenslandacus* Drew	1
Dacus		*Callantra* Walker	1
		Mellesis Bezzi	2
		Neodacus Perkins	10
Zeugodacus	*Zeugodacus* Group	*Austrodacus* Perkins	1
		Diplodacus May	1
		Sinodacus Zia	1
		Zeugodacus Hendel	3

As molecular systematic studies are published on the Dacini, it becomes clear that these subgeneric classifications, based on morphological taxonomy, often do not represent monophyletic clades (San Jose et al., 2018). There have been multiple recent taxonomic revisions of Dacini subgenera to try and reconcile morphological

taxonomy and molecular systematics (Hancock and Drew, 2015, Hancock, 2015, Drew and Hancock, 2016, Hancock and Drew, 2016, Hancock and Drew, 2017a, Hancock and Drew, 2017b, Hancock and Drew, 2017c, Hancock and Drew, 2018a, Hancock and Drew, 2018b, Hancock and Drew, 2018c), but incongruence still remains (Dupuis et al., 2018, San Jose et al., 2018).

1.2.3.1. Issues at the genus level

The generic status of *Zeugodacus* continues to be a point of conflict between taxonomists and systematists. Once a subgenus within genus *Bactrocera,* molecular studies have found that *Zeugodacus* forms a distinct clade from *Bactrocera* and *Dacus,* and is more closely related to genus *Dacus* (Muraji and Nakahara, 2001, Zhang et al., 2010, Krosch et al., 2012, Virgilio et al., 2015, Dupuis et al., 2018). However, Hancock and Drew (2015) and Drew and Romig (2013, 2016) did not support this elevation, arguing that morphological traits are homoplasious among all three species groups, postulating that the subgenera *Bactrocera* and *Zeugodacus* share a common ancestor (i.e. both sit within the genus *Bactrocera*). While there is increasing international recognition of *Zeugodacus* as a separate genus (Clarke, 2019), molecular and morphological evidence for the placement of some native Australian *B.* (*Zeugodacus*) species, i.e. to remain within *Bactrocera* or to be elevated to genus, is yet to be provided.

1.2.3.2. Issues at the subgenus level

The assignment of subgenera among genera (in particular *Bactrocera* and *Zeugodacus*) is under constant criticism (Doorenweerd et al., 2018, San Jose et al., 2018) and formal revision (Hancock and Drew, 2006, Hancock and Drew, 2015, Drew and Hancock, 2016, Hancock and Drew, 2016, Hancock and Drew, 2017a, Hancock and Drew, 2017c). This is partly due to new descriptions of species, subsequent reclassification of defining characters (Drew and Hancock, 2016, Hancock and Drew, 2017b), and the ongoing disagreement and confusion that surrounds the elevation of *Zeugodacus* from subgeneric to generic status (Hancock and Drew, 2015, Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018, De

Meyer et al., 2015b, Drew and Romig, 2016, Elfekih et al., 2016, Li et al., 2017). These issues are discussed fully in Chapter 3.

1.2.3.3. Issues at the species complex level

There are numerous examples within the Dacini of species complexes that require investigation. The term 'species complex', as used in the tephritid literature, is confusing as it can (sometimes simultaneously) refer to a taxonomic species group (species that share a set of common morphological traits or geographical distribution), a cryptic species complex (where species are morphologically indistinct), or a group of sibling species (monophyletic clade) (Clarke and Schutze, 2014, Schutze et al., 2017). Because of the confounding of usage, different phylogenetic, systematic and taxonomic assumptions can be made of different taxa within a complex. Of particular interest to this thesis is the *B. tryoni* (Froggatt) species complex. Molecular phylogenetics has been unable to resolve species in the *B. tryoni* species complex (Morrow et al., 2000, Armstrong and Ball, 2005, Cameron et al., 2010, Blacket et al., 2012, Dupuis et al., 2018) and the species status of members *B. melas* (Perkins & May) and *B. aquilonis* (May) continues to be questioned (Hancock et al., 2000, Cameron et al., 2010, Clarke et al., 2011). Furthermore, *B. humilis* (Drew & Hancock) and *B. curvipennis* (Froggatt) have been considered for inclusion in the *B. tryoni* complex (Drew et al., 1981), but have never been formally placed within it. This is despite subsequent genetic evidence that suggests *B. curvipennis* falls within the complex (Smith et al., 2003, Armstrong and Ball, 2005, Blacket et al., 2012). These examples illustrate that relationships among close sister groups may require further investigation and subsequent taxonomic revision (Clarke et al., 2011).

I refer throughout this thesis to the terms 'species group' and 'species complex'. I will use the term 'species group' to refer to new taxonomic groups I have assigned or redescribed and will continue to use the term 'species complex' to refer to existing groups. In the discussion chapter of this thesis I will address the problems with the continued use of species complex.

1.2.3.4. Issues at the species level

Morphological variation exists within and among different Dacini species (Schutze et al., 2015b, Leblanc et al., 2015, Leblanc et al., 2013) and is a constant cause of confusion for morphological diagnosticians. As examples, Leblanc et al. (2015) documented intraspecific variation in *B. osbeckiae* Drew & Hancock (Fig. 1.3) and interspecific variation between *B. propinqua* (Hardy and Adachi) (Fig. 1.4) and *B. cacuminata* (Hering) (Fig. 1.5) which are three species from the *B. dorsalis* (Hendel) species complex. Notably, intraspecific variation in *B. osbeckiae* can be as wide ranging as the interspecific variation demonstrated to exist between two separate and geographically isolated species (*B. propinqua* and *B. cacuminata*). It is important to document this variation when delimiting and identifying species, as it is possible for a wide ranging species with high phenotypic variation to be described independently multiple times, causing taxonomic, systematic and pest management issues, as was the case with *B. dorsalis* (Schutze et al., 2015a). Chapters 6 and 7 explore these issues in greater detail.

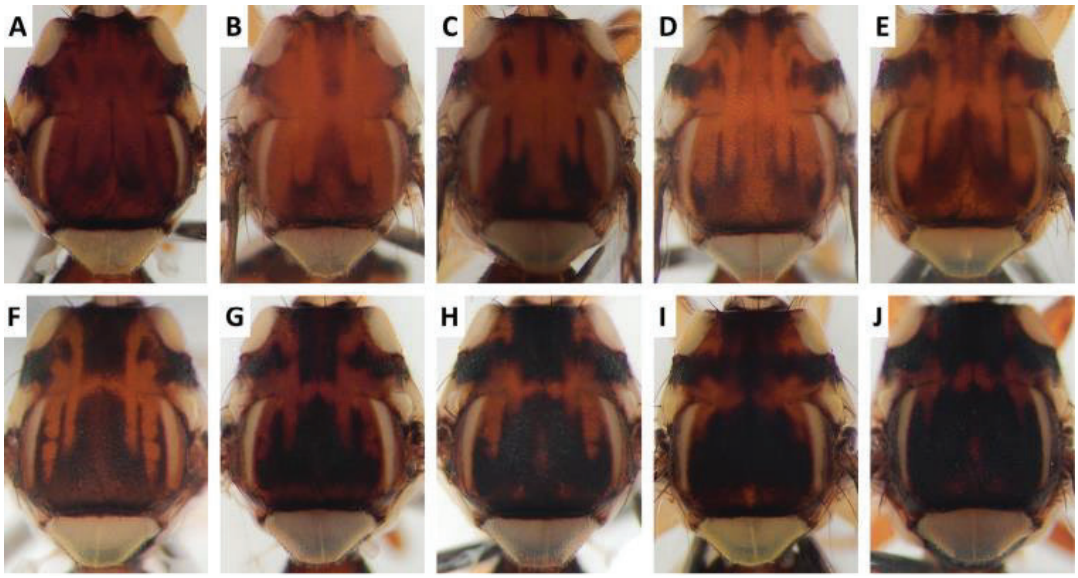


Figure 1.3: Examples of intraspecific scutum variation in *B. osbeckiae* (Leblanc et al., 2015).

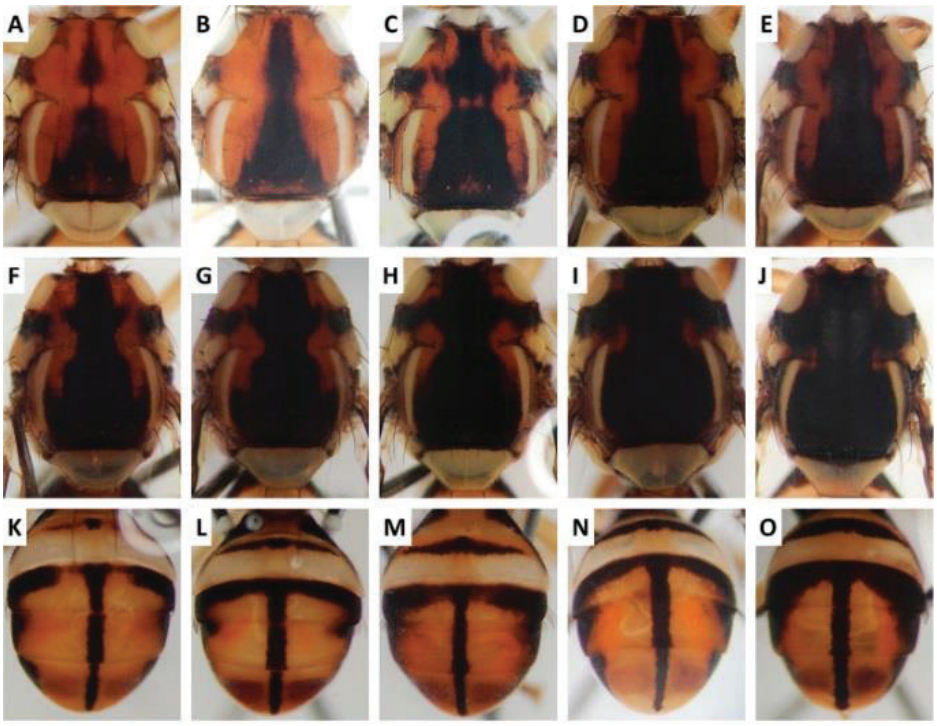


Figure 1.4: Examples of intraspecific scutum variation (A-J) and abdomen variation (K-O) in *B. propinqua* (Leblanc et al., 2015).

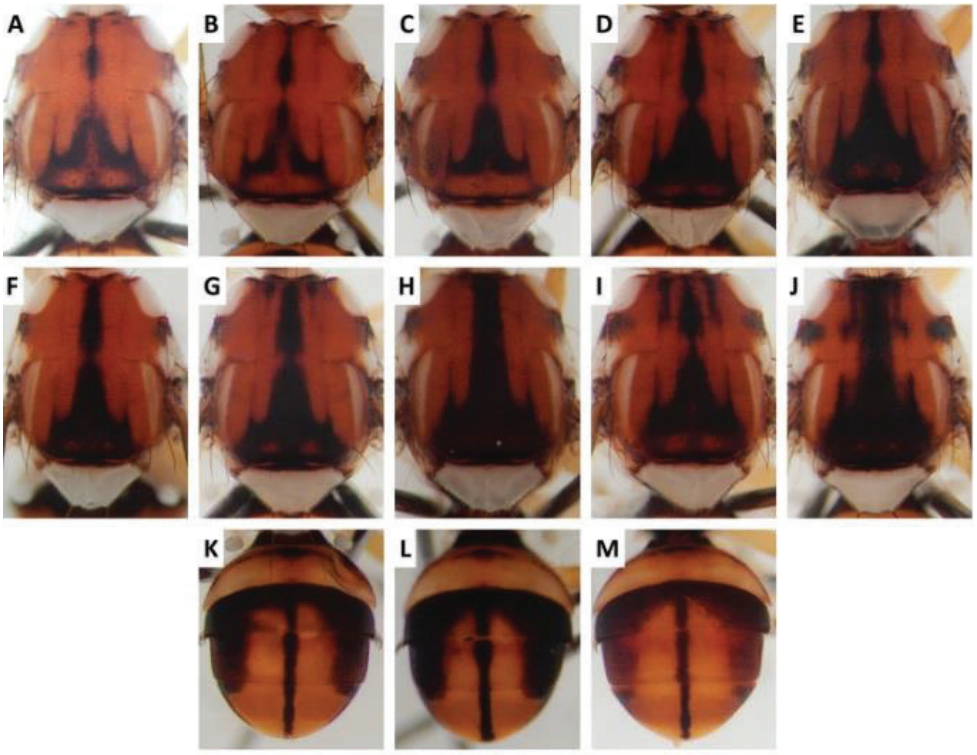


Figure 1.5: Examples of intraspecific scutum variation (A-J) and abdomen variation (K-M) in *B. cacuminata* (Leblanc et al., 2015).

1.2.1. The Unified Species Concept

When faced with difficult sibling species such as those dealt with in this thesis, deciding where the 'species line' is drawn can become difficult and making an *a priori* statement of the species concept being applied is recommended (Schlick-Steiner et al., 2010). Here, the Unified Species Concept (USC) is observed. de Queiroz (2007) proposed the USC as a basis for diagnosing species with the view that species are: *"…separately evolving metapopulation lineages…"*. This concept allows multiple different lines of evidence to contribute to the definition of a species and recognises that speciation is not the product of one defining biological change; changes may occur at any time and in any order (e.g. phenotypic, behavioural or genotypic) (de Queiroz, 2007).

Gaps in knowledge to be addressed

iii. Morphological characters have traditionally informed taxonomic classifications of species, but there remains a disconnect between the relationships proposed by taxonomy and those proposed via molecular phylogenetic analyses.

1.2.5. Morphology in phylogenetics

Only three morphological phylogenetic studies have been published on the Dacini tribe. These studies are now over two decades old and failed to resolve relationships at deeper nodes and at the species level (Michaux, 1996, Michaux and White, 1999). The most recent morphological phylogeny (Fig. 1.6) is presented, which was heavily weighted in order to resolve relationships (White and Hancock, 1997, Michaux and White, 1999, White, 2000). Despite heavy weighting, *B. distincta* remained unresolved and *Bactrocera* (*Bactrocera*) was paraphyletic. Comparing these results to more recent molecular phylogenies highlights many inconsistencies in the relationships resolved. As mentioned previously, genetics has suggested a close relationship between *B. tryoni* and *B. curvipennis* (Smith et al., 2003, Armstrong and Ball, 2005, Blacket et al., 2012) however, this is not resolved within the morphological tree (Michaux and White, 1999). Similarly, *B. trilineola* (Drew)*, B.*

caledoniensis Drew and *B. frauenfeldi* (Schiner) are near identical morphologically and exhibit close genetic affinity (Plant Health Australia, 2018a), but all are also polyphyletic across the morphological tree (Michaux and White, 1999). The issue that arises is that the utility of these morphological characters, though quite useful for diagnostics and species classifications, is largely unknown in a phylogenetic sense. These characters might be phenetically informative, but are not phylogenetically informative.

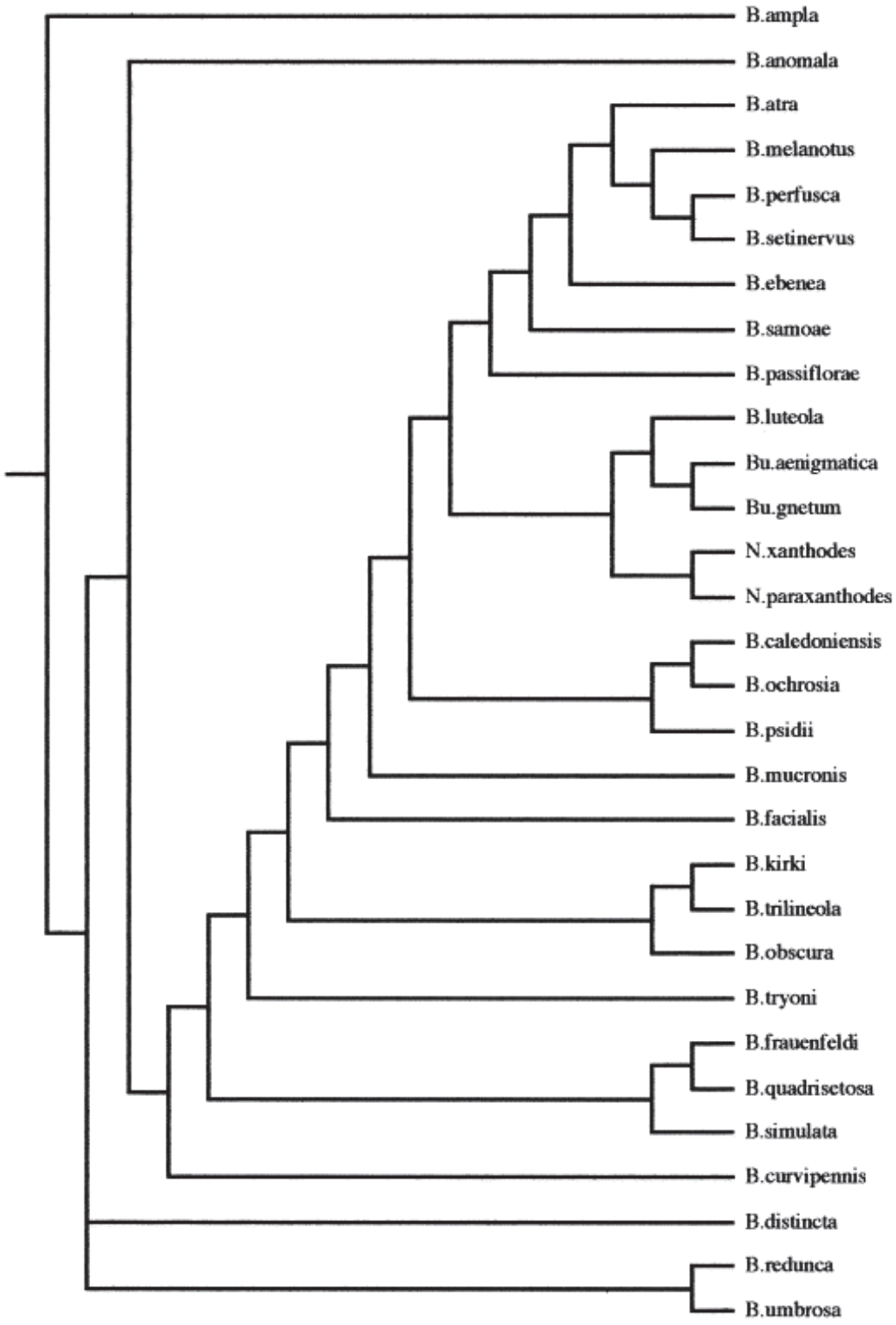


Figure 1.6: Strict consensus tree of 30 Dacini species based on weighted morphological characters (Michaux and White, 1999). Note: genus initials represent subgeneric placements (B. = *Bactrocera,* Bu. = *Bulladacus,* and N. = *Notodacus*), not generic placements (all species are within genus *Bactrocera*).

Gaps in knowledge to be addressed

iv. Morphological characters have been largely underutilised in Dacini morphological phylogenies. The usefulness of diagnostic and descriptive characters for phylogenetic inference has not been specifically tested before.

1.2.6. Current use of molecular phylogenies

In the last two decades there have been 13 molecular phylogenies published on the Dacini tribe, (all of which are discussed in greater detail in Chapter 3). Some of which have included over 160 Dacini species (San Jose et al., 2018). Despite this, one of the most common trends apparent across the majority of the existing Dacini phylogenies is the lack of concentrated taxonomic and geographic sampling. Sampling bias exists across most of the current phylogenetic studies on the tribe. This is primarily due to the aims of the study at the time, for example, some studies aimed to contribute toward better diagnostics and pest management (Boykin et al., 2013), but this has limited the applications of the phylogeny that has been produced. Another example of this is shown in one of the more recently published phylogenies on the tribe (Fig. 1.7) (San Jose et al., 2018). The authors included a large number of pest taxa (indicated by the red '!' next to the taxon names) as well as targeted coverage of subgeneric and species complexes. The approach of the study was to sample as many species as possible across many taxonomic groups (subgenera) in order to identify inconsistencies between the morphological taxonomy and the results from the molecular phylogeny (San Jose et al., 2018). While effective at achieving the aims set out, that study, like many others on the tribe, did not sample many Australian Dacini, and did not aim to answer any deeper evolutionary questions.

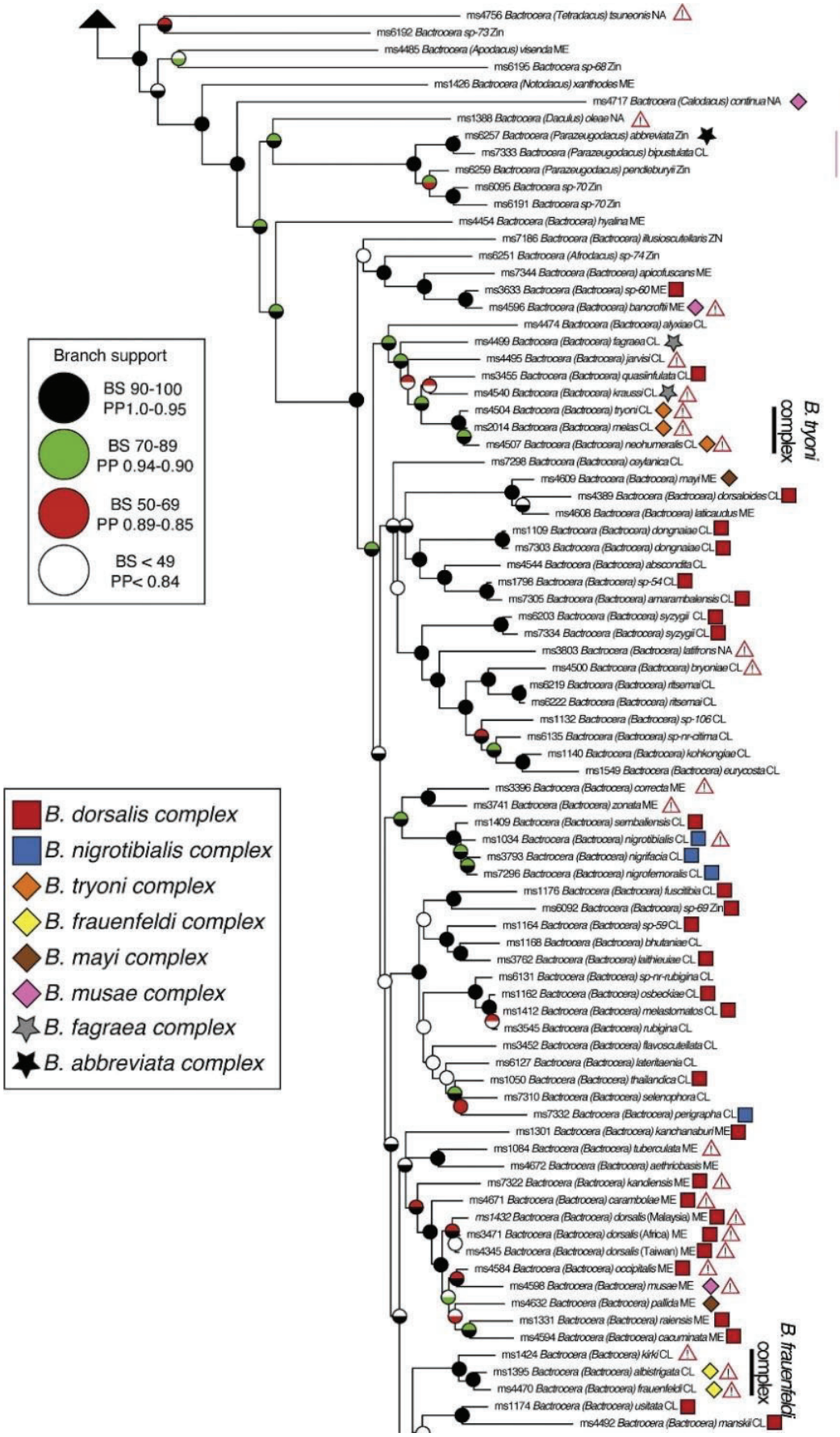


Figure 1.7: Extract from majority consensus Bayesian phylogeny of tribe Dacini using a partitioned dataset (San Jose et al., 2018). '!' illustrates pest species.

1.2.6.1. Phylogenies that answered evolutionary questions

There are two exceptions to the sparse sampling strategies I have outlined above. A good example of thorough geographic sampling is Virgilio et al. (2009) which provided a phylogeny of African *Dacus* species. Thorough sampling allowed the authors to investigate use of host plant families across the phylogeny (Virgilio et al., 2009). Similarly, Krosch et al. (2012) produced a dated phylogeny in order to investigate the influence of biogeography on speciation of the Dacini, and hypothesised the origins of the tribe as they moved off the rafting Indian plate. Both studies are excellent examples of what targeted sampling can achieve however, one issue remains the same: the Australian taxa are largely under sampled, therefore trait evolution and the influence of biogeography on species divergence of the Australian Dacini remains unknown.

Gaps in knowledge to be addressed

v. Existing molecular phylogenies have focussed on easy to collect pest and lure responsive species, and often do not have concentrated geographic sampling. Australian species have been largely neglected in existing studies.

vi. Previous phylogenies largely not aimed at applying their phylogenies to deeper evolutionary questions and those that have, have not had focussed geographic sampling of the Australian region.

1.2.7. Biogeography

Biogeography is the study of the distribution of species through geographic space and geological time (Cox and Moore, 2005). South-east Asia, the region with the highest fruit fly diversity (Drew, 2004), is also the region with the most complex geological history (Hall, 2001), being situated at the juncture of five geological plates (Turner et al., 2001). As a result, South-east Asia and the surrounding Australian and Pacific region has been subjected to sea level fluctuations, volcanism and rapid changes in topography (Hall, 2001). As mentioned previously, Krosch et al. (2012) conducted an in-depth analysis into the origins of the Dacini, suggesting

the tribe has origins in India. However, there have been no further studies to support these claims, and no studies focussed on the Australian and Pacific region; a region largely excluded from the previous analysis (Krosch et al., 2012).

1.2.7.1. Australian biogeography

On a smaller scale, there have been many biogeographic barriers that have been identified within Australia (Ebach et al., 2015, Bryant and Krosch, 2016). These barriers are predominantly dry lowland arid landscapes, that have restricted gene flow for many taxa that inhabit the adjoining wet closed forests (Bryant and Krosch, 2016). Bryant and Krosch (2016) noted that many barriers along the east coast are 'leaky' and some were more effective than others at restricting movement of different taxa. Despite data that has shown the influence of these barriers on birds (Toon et al., 2010), reptiles (Bell et al., 2010), mammals (Frankham et al., 2015) and insects (Krosch, 2011), the influence of these barriers on the Dacini has not been investigated in any capacity.

<u>Gaps in knowledge to be addressed</u>

vii. Despite being such a speciose group, very little is known of the biogeographic influences on the Dacini and their divergence pathways in the greater Pacific.

viii. Australian biogeographic barriers are known to have impacted the spread and speciation of other taxa, but the influence on the Dacini is remains unknown.

1.3. Thesis aims and structure

In this introductory chapter, while keeping the literature deliberately brief due to its comprehensive coverage in the research chapters, I have identified the following key issues that have not been adequately addressed in existing Dacini phylogenetics, systematics and taxonomy.

- There is a lack of deeper use of phylogenies for mapping evolution of traits such as lure response and host diet breadth.
- Morphological and molecular approaches have so far struggled to reach a unified consensus on the systematics and taxonomy of Dacini species.
- The utility of morphological characters for phylogenetic applications is largely unknown.
- Existing studies on the tribe have been limited in their utility by their sampling strategies which were sparse, and pest focussed.
- There has been minimal coverage in existing phylogenies of the Australian taxa and their divergence in the region.
- The influence of Australian biogeographic barriers on Dacini speciation has not been investigated.

The above issues can be addressed by adopting an overarching thesis aim, which will differ from previous approaches outlined above in its applications. This thesis aims to produce a well-sampled, Australian-focussed Dacini phylogeny and use this phylogeny to answer deeper evolutionary questions surrounding the evolution of traits such as lure response and host breadth; the utility of morphological characters; investigate historical movements of species in the Australian and Pacific region and address taxonomic inconsistencies.

Chapter 2 presents methodology and trapping data resulting from targeted collections undertaken for this thesis to try and reach comprehensive taxon coverage. In addition to this, collections were undertaken in remnant Gondwanan forests in order to gather species distributional data for the following biogeography analysis. The results presented in this chapter include new distribution records, male lure records, a new species description, and images of rare and new species.

Using specimens collected in Chapter 2, along with material from existing collections, Chapter 3 presents a multigene molecular phylogeny of the Australian Dacini and selected species from the Western Pacific and South-east Asia. Taxon coverage from outside of Australia is restricted, but sufficient to ensure the phylogeny could be logically fitted within the regional and global Dacini fauna. Fossils were used to calibrate nodes and produce divergence time estimates, and this

dated phylogeny was then used to investigate ancestral states and the evolution of the biological traits of male lure response and host breadth.

Chapter 4 investigates morphological character traits and their informativeness when applied to phylogenetic reconstruction. Structural characters and colour characters were scored as both are used in taxonomic descriptions. Morphological trees are compared against a subset of the molecular phylogeny produced in Chapter 3 in order to determine whether: (i) morphological character states produce phylogenetically informative trees on their own; and (ii) if they add phylogenetic signal when combined with molecular characters.

Subsets of the molecular phylogeny from Chapter 3 were again utilised in Chapter 5 in order to explore the influence that biogeographical barriers may have had on divergence and speciation of the Australian and Pacific Dacini fauna. Key dispersal pathways are explored and the suspected origins of the Australian and Pacific Dacini proposed. Additionally, the chapter also proposes changes to current hypothesised origins of the tribe.

Chapter 6 utilised findings from the results of Chapter 3 and proposed a new taxonomic definition of the *Bactrocera aglaiae* species group. Species descriptions and images are provided along with a likelihood mapping analysis that supported this clade as the basal clade to all *Bactrocera.*

The phylogeny of Chapter 3 resolved additional species as part of the *B. tryoni* species complex, as well as paraphyly of *B. neohumeralis* (Hardy)*,* a species that is part of this complex. Building on this result, Chapter 7 utilises morphological and additional molecular evidence to inform and undertake a taxonomic revision of an enlarged *B. tryoni* species group. Additionally, *B. melas* and *B. humilis* were synonymised with *B. tryoni* based on morphological and molecular evidence.

Based on the findings of each chapter within this thesis, Chapter 8 provides a general discussion of issues that remain to be addressed for the Dacini tribe. This includes key taxonomic changes to nomenclature and assignments within the Dacini, as well as a discussion on the future of systematics, taxonomy and diagnostics of this diverse group. I provide a case study to demonstrate practical applications of the findings of this thesis.

Chapter 2: Collections and new records

2.1. Introduction

A core aim of this thesis was to achieve comprehensive geographic and taxonomic coverage of Australian Dacini to allow for molecular and taxonomic study. In Australia, the Dacini are found along the eastern coast from the tip of Cape York down into Victoria (Hancock et al., 2000, Royer and Hancock, 2012), while also found in tropical regions along the coast of northern Western Australia, and the Northern Territory (Hancock et al., 2000). While some pest species have been able to expand their range into inland Queensland, New South Wales and the Northern Territory (Alice Springs) (Osborne et al., 1997), the majority of species are confined to the tropics (Hancock, 2013).

In order to gather fresh material, new field collections were necessary. Whilst significant usable material was already held at QUT and in other public collections, often species were rare or not appropriately preserved for molecular analysis. This chapter presents the methodology of these collections, which were successful in recovering the targeted specimens which are used in later chapters. Details of sampling events and methodologies are first provided, followed by new lure records and species distributions. Finally, a taxonomic component presenting images of new or rare species is included, along with a description of a new species record.

Because of disparity between the different sections, and to aid readability, each results section within this chapter incorporates its own discussion if required. This pattern only holds for this chapter: all subsequent research chapters are presented in a traditional chapter format.

2.2. Materials and methods

2.2.1. Trapping locations and rationale

Multiple collections were carried out over a period of three years along the east coast of Australia (Table 2.1 and Fig. 2.1). Early collections focussed on rare species which were required for Chapter 3. Later collections were site focussed, with locations chosen because they were previously under-sampled, or non-sampled old-

growth Gondwanan regions of high biogeographic interest for Chapter 5. The approach to trapping varied greatly due to the expected outcome. A combination of small-scale targeted collections, as well as larger distributional surveys and lure testing was undertaken. In some instances, traps were deployed in locations strictly for monitoring purposes only. This is reflected in the data, where count recordings and species identification were prioritised for certain locations.

Table 2.1: Australian trapping locations and survey dates for fruit fly collections undertaken in this thesis.

Location	Latitude and Longitude	Trapping dates
Daintree Rainforest Observatory, Cape Tribulation, QLD	-16.103983S, 145.449177E	30.iii-7.iv.2017
Sherwood Arboretum, QLD	-27.532151S, 152.974444E	xii.2017-vii.2018
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD	-17.272806S, 145.466E	15.i.2018-20.i.2018
Cairns Cemetery, QLD	-17.244127S, 145.480586E	15.i.2018-20.i.2018
Lake Morris Road, Cairns, QLD	-16.924971S, 145.718430E	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD	-16.860310S, 145.758040E	15.i.2018-20.i.2018
Tolga Scrub, Atherton, QLD	-17.244127S, 145.480586E	15.i.2018-20.i.2018
Woodford, QLD	-26.963027S, 152.784398E	ii.2018-x.2018
Tropical Fruit World, Duranbah, NSW	-28.284946S, 153.525139E	23.v.2018-19.ix.2018
Nambour, QLD	-26.636525S, 152.965024E	vi.2018-vi.2019
Border Ranges National Park, NSW	-28.408S, 153.034E	30.i.2019-12.iii.2019

Location	Latitude and Longitude	Trapping dates
Noosa National Park, QLD	-26.383129S, 153.100324E	12.ii.2019-2.iv.2019
Bulburin National Park, QLD	-24.511468S, 151.461447E	13.ii.2019-2.iv.2019
Dorrigo National Park, NSW	-30.357142S, 152.774391E	6.iii.2019-17.iv.2019
Barrington Tops National Park, NSW	-32.051S, 151.637E	3.iii.2019-16.iv.2019

Figure 2.1: Eight locations sampled for fresh material and distributional data along the east coast of Australia. NP = National Park.

2.2.2. Trapping

Trapping utilised the well-known Dacini male lures; methyl eugenol, cue-lure and zingerone, as well as more recently discovered lures; isoeugenol, methyl-isoeugenol

and dihydroeugenol, which have been shown to be effective at trapping previously non-lure responsive species (Fay, 2010, Royer, 2015). Protein based traps 'BioTrap fruit fly attractant gel' (BioTrap Australia Pty Ltd, Victoria, Australia), 'CeraTrap fruit fly attractant' (Barmac Pty Ltd, Blackstone, Australia) and dry protein sachets (Probodelt, Amposta, Spain) were also deployed for female flies and non-responsive species.

Male lures were prepared by dosing dental wicks at a rate of 1mL to 4mL of insecticide to male lure, respectively. Wicks were then secured in place through the lid of the Bugs-For-Bugs bucket fruit fly traps (Bugs-For-Bugs, Toowoomba, Australia). Approximately 15mL of propylene glycol was placed in the bottom of the traps to preserve specimens from desiccation. In the protein baited traps, BioTrap fruit fly attractant gel filled the bottom of the trap with a Dichlorvos (DDVP) cube (commercially sold as Killmaster zero pest strip) (Amgrow, Australia) used as a knock-down for flies entering the trap. Probodelt dry protein sachets were suspended on the plastic side cups of the trap with a DDVP cube on top and propylene glycol was placed in the bottom of the trap.

2.2.3. Fruit rearing for rare species

Fruits were collected from Tolga Scrub, a small stretch of rainforest located next to the Kennedy Highway near Atherton, Queensland. Fruits were collected from this location as a target species, *Bactrocera phaleriae* (May) was known from this location, and unlike most rainforest fruits, the fruits grow on small shrubs, so were easily accessible (Cooper and Cooper, 2013). Any and all other fruits that had fallen from trees and which appeared to have fruit fly oviposition marks were collected for rearing and were also transported back to QUT, Brisbane for incubation. Fruit rearing methods followed Allwood et al. (1999), Clarke et al. (2001) and White and Elson-Harris (1994). Infested fruits were placed on moist vermiculite inside plastic containers with the lids removed and covered in thick paper towel. They were placed in an incubator at 25°C at 70% humidity. After 14 days the pupae were sieved from the vermiculite, placed within an insect cage for emergence and transferred to the QUT Insectary under controlled conditions of 26°C and 70% humidity. Water and

sugar were provided for the emerging adults. Adults were killed after roughly a week for morphological identification.

2.2.4. Collection permits and permissions

Trapping in New South Wales National parks was undertaken with permission granted from the local ranger in charge. Field work in Queensland National Parks was undertaken under the Entomological Society of Queensland's permit no. WITK18701717.

2.2.5. Species identification and vouchers

All species used in this thesis were identified by the candidate using the taxonomic keys included in Drew (1989), Drew et al. (2011), Drew and Romig (2016) and the expanded online LUCID key (https://fruitflyidentification.org.au/identify-northern/, Plant Health Australia, 2018b). Identified specimens held in the Queensland Museum and QLD Department of Agriculture and Fisheries (QDAF) insect collections were used for referencing and validation. Voucher specimens are stored at QUT in ethanol at -20°C. Vouchers were assigned codes based on existing naming conventions at QUT: ABC123; where letters represent a species code and numbers correspond to the appropriate specimen number. Upon publication of new species descriptions (in this chapter, and subsequent chapters), these specimens will be lodged at the Queensland Museum (Brisbane) and the Australian Museum (Sydney, NSW). Initial taxonomic support was provided by Ms. Jacinta McMahon (QUT), Ms. Tara Wheatland (QDAF) and Ms. Jane Royer (QDAF).

2.3. Results and Discussion

2.3.1. Fruit rearing results

Species that were reared from these fruits are listed in Table 2.2, along with their collection and identification details. Fruits were identified using Cooper and Cooper (2013).

Table 2.2: Fruits collected in Tolga Scrub with number of individuals reared indicated in brackets.

Species	Common name	No. of fruits collected	Species reared
Phaleria octandra	Dwarf phaleria	50+	none
Aglaia sapindina	Boodyarra	3	none
unidentified *Syzygium* sp.		1	*B.* species near *aglaiae* (1)

2.3.2. New Collections

Over 8600 specimens across 35 species in three genera were collected during the course of the thesis. This included two new species, new lure records and new range expansions. Full specimen and collection data is provided in Appendix 1.

2.3.3. New Lure and Distribution Records

2.3.3.1. New lure records

New lure responses were recorded for *B. aberrans* (Hardy)*, B. mutabilis* (May) and a single *B. phaleriae* specimen, all of which responded to isoeugenol. Further trapping may be necessary to confirm level of attractiveness of *B. phaleriae* to isoeugenol.

Bactrocera (*Bactrocera*) *aberrans* (Hardy)

60 ♂ collected at isoeugenol, Tropical Fruit World, Duranbah (-28.284946S, 153.525139E), 23.v.2018-19.ix.2018. Previously two individuals recorded at isoeugenol (Royer, 2015).

Bactrocera (*Bactrocera*) *mutabilis* (May)

2 ♂ collected at isoeugenol, Bulburin National Park (-24.505010S, 151.449969E), central Queensland, 13.ii.2019-2.iv.2019. No previous lure response recorded.

Bactrocera (*Bactrocera*) *phaleriae* (May)

1 ♂ collected at isoeugenol, Tolga Scrub (-17.244127S, 145.480586E), near Atherton Tablelands, 15.i.2018-20.i.2018. No previous lure response recorded.

2.3.3.2. New distribution records

New distributions (geographic, altitudinal and/or climactic) were recorded for five species; *B. aberrans, B. brunnea* (Perkins & May)*, B. mutabilis, B. silvicola* (May) and *B. aurea* (May) (see Appendix 2 for maps).

Bactrocera (*Bactrocera*) *aberrans* (Hardy)

76 ♂ recorded at Tropical Fruit World, Duranbah (-28.284946S, 153.525139E), New South Wales, 23.v.2018-19.ix.2018. Previously known from south-eastern (Toowoomba, Mt Tamborine, Ashgrove) and northern Queensland (Atherton Tableland) (Hancock and Drew, 2017c). The new records are a southward range extension of approximately 80 kilometres.

Bactrocera (*Bactrocera*) *brunnea* (Perkins & May)

2 ♂ collected in Bulburin National Park (-24.505010S, 151.449969E), central Queensland, 13.ii.2019-2.iv.2019. Previously known from throughout South east and northern Queensland (Atherton), as far west as Toowoomba and Stanthorpe. New records are suggestive that this species occurs right down the Queensland east coast in isolated patches.

Bactrocera (*Bactrocera*) *mutabilis* (May)

3 ♂ collected in Bulburin National Park (-24.511468S, 151.461447E), central Queensland 13.ii.2019-2.iv.2019. Previously known from south-eastern and northern Queensland and had been suspected to be confined to higher altitudes (e.g. type locality, Toowoomba, elevation 691m). This record indicates this species may also be distributed along the coast and, here this species was also trapped at elevation (approximately 580m).

Bactrocera (*Bactrocera*) *silvicola* (May)

13 ♂ collected at Bulburin National Park, (-24.505010S, 151.449969E), central Queensland, 13.ii.2019-2.iv.2019. Previously known from northern Queensland; no further south than Mackay. The new records are a southward range extension of approximately 500 kilometres.

Bactrocera (*Hemizeugodacus*) *aurea* (May)

1 ♂ Barrington Tops National Park (-32.062S, 151.683E), New South Wales, 3.vi.2019-16.iv.2019; 94 ♂ Border Ranges National Park (-28.408S, 153.034E), northern New South Wales, 30.i.2019-12.iii.2019; 45 ♂ Bulburin National Park (-24.505010S, 151.449969E), central Queensland, 13.ii.2019-2.iv.2019; 2 ♂ Dorrigo National Park (-30.357142S, 152.774391E), central New South Wales., 6.iii.2019-17.iv.2019; 10 ♂ Noosa National Park (-26.383129S, 153.100324E), south east Queensland, 12.ii.2019-2.iv.2019. Known previously from Ravensbourne, south-eastern Queensland and Lockhart River far-northern Queensland. The new records are a southward range extension of approximately 670 kilometres.

2.3.4. Discussion of new lure and distribution records

Here I used a combination of new and traditional lures, protein baits and fruit rearing in order to collect rare species; succeeding in reaching approximately 80% coverage of the continental Australian Dacini species (Doorenweerd et al., 2018). The success of this work is predominantly due to targeted collection efforts which focussed on locating known host plants, used a wider range of lures, and sampled in rainforests which had largely not been surveyed previously. These efforts resulted in numerous range extensions, new male lure responses and a better understanding of native species' geographic distribution, climactic distributions and distribution at elevation.

The largest range expansions recorded were for *B. silvicola* and *B. aurea. Bactrocera silvicola* was trapped in Bulburin National Park, although historically this species hasn't been previously recorded south of Mackay (Royer and Hancock, 2012) and has not been detected as part of year-round monitoring in nearby coastal regions of Bundaberg or Gladstone (J. Royer, unpub. data). This strongly suggests that the *B. silvicola* population in Bulburin National Park may be isolated. Royer and Hancock (2012) noted that *B. silvicola* populations in Mackay and the Whitsundays appeared to have darker markings on the scutum and abdomen when compared with other northern populations which tend to be red-brown on the scutum. The *B. silvicola* specimens trapped here were observed with this same dark patterning.

With the new records presented here, *B. aurea* can be regarded as having one of the largest natural distribution ranges of any Australian Dacini fly (certainly of the non-pests). Royer (2015) trapped *B. aurea* in far north Queensland at Lockhart River (−12.79243S, 143.33411E), while the southern-most range was previously considered to be South-east Queensland (Hancock et al., 2000). New records indicate this species extends further south to the remnant Gondwanan rainforests of Barrington Tops, in central New South Wales. The one known host plant, *Alangium villosum* subsp. *tomentosum* (F. Muell.) Bloemb. (Hancock et al., 2000), has not been recorded south of Urunga, NSW (Atlas of Living Australia, 2019), suggesting either this host plant is present further south than currently known, or that *B. aurea* is utilising more than one host plant.

2.3.5. Photographs of rare and undescribed specimens

Trapping uncovered a previously undescribed species, referred to as *Bactrocera* species A and three rare species. The rare species, *B. brunnea* (Fig. 2.2), *B. mutabilis* (Fig. 2.3) and *B. phaleriae* (Fig. 2.4) were imaged as these species are represented in collections by as few as a single individual of greater than 60 years of age. A second undescribed species (*Bactrocera* species near *aglaiae*) is explored in greater detail in Chapter 6. Specimens photographed and sequenced in this section are listed in Appendix 3.

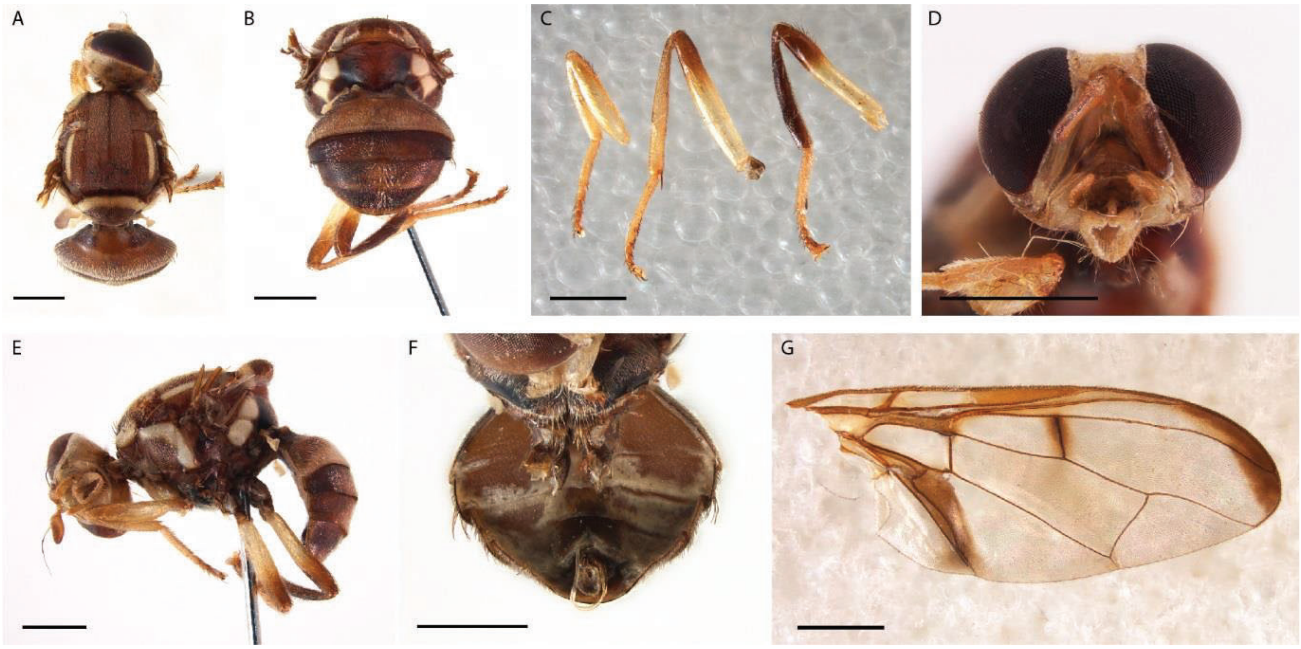


Figure 2.2: *Bactrocera brunnea* male (BRU001). A: scutum dorsal; B: abdomen dorsal; C: fore, mid and hind legs; D: head frontal; E: whole body lateral; F: abdomen ventral; and G: wing. Scale: 1mm.

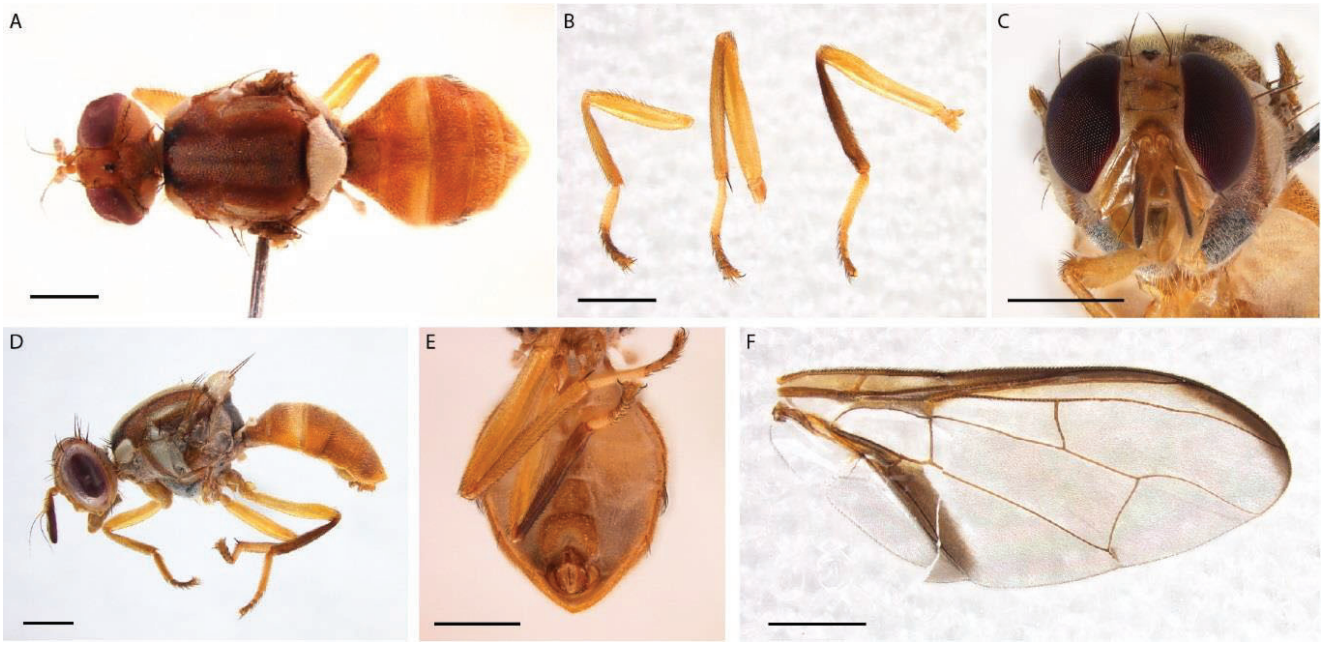


Figure 2.3: *Bactrocera mutabilis* male (MUT002). A: whole body dorsal; B: fore, mid and hind legs; C: head frontal; D: whole body lateral; E: abdomen ventral; and F: wing. Scale: 1mm.

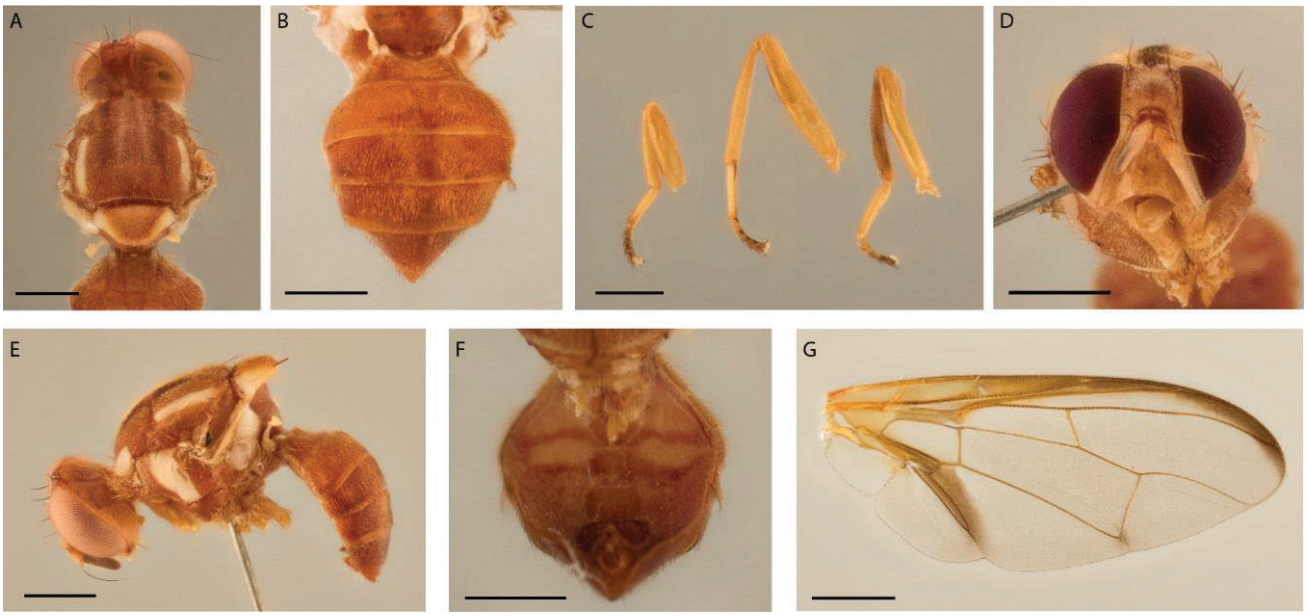


Figure 2.4: *Bactrocera phaleriae* male (PHA001). A: scutum dorsal; B: abdomen dorsal; C: fore, mid and hind legs; D: head frontal; E: whole body lateral; F: abdomen ventral; and G: wing. Scale: 1mm.

2.3.6. New species description

One new species, *Bactrocera* species A (Fig. 2.5) was described based on morphology after terminology used in Drew and Romig (2013) and Royer and Hancock (2012).

***Bactrocera* species A**

TYPE SPECIMENS

Holotype ♂, AUSTRALIA, Couchy Creek, New South Wales, (-28.277514S, 153.270616E), 24-27.i.2018, attracted to zingerone, coll. V. Varghese. Specimen held at QUT, Brisbane for lodging upon publication.

Paratypes 2 ♂ AUSTRALIA, Couchy Creek Nature Reserve, 1-4.v.2018, attracted to zingerone, coll. V. Varghese, 1 ♂, Border Ranges National Park, 30.i.2019-12.iii.2019, attracted to zingerone coll. F. Strutt & M. Starkie.

DIAGNOSIS

Medium-sized species, medium sized black facial spots present, humeral and notopleural calli yellow; scutum orange-brown, narrow mesopleural stripe, lateral postsutural vittae present, medial postsutural vittae absent, scutellum yellow except apical 1/3 pale fuscous; wing with a narrow dark fuscous costal band confluent with R2+3 and widening after reaching extremity of R2+3, and broad fuscous anal streak, costal cells fuscous with microtrichia covering all of the first and ¾ of the second costal cells; abdominal terga III-V orange-brown, with broad pale fuscous lateral longitudinal markings and a dark fuscous medial line which darkens posteriorly to end before base of tergum V.

DESCRIPTION OF MALE

HEAD

Fig. 2.4C. Head generally fulvous. Vertical length 1.2 mm. Frons of even width, length 1.25 times breadth; fulvous with pale fuscous around orbital bristles and on anteromedial hump; latter covered with short dark setae; orbital bristles black: 1 s.or., 3 i.or., lunule fuscous. Ocellar triangle black. Vertex fulvous. Face fulvous with

small to medium sized oval shaped black spots; length of face 0.4 mm. Genae fulvous, red-brown subocular spot present; strong genal bristle present. Occiput fuscous, fulvous along eye margins; occipital row with 4-5 bristles. Antennae with segments 1 and 2 fulvous, segment 3 fulvous with fuscous on apex and outer surface; a fuscous dorsal bristle on segment 2. Arista red-brown (fulvous basally); length of segments: 0.12 mm, 0.25 mm, 0.49 mm.

THORAX

Mesonotum and pleural areas uniformly orange-brown (Fig. 2.4A, D). Yellow markings as follows: postpronotal lobe; notopleura, narrow mesopleural stripe ¼ of the way between anterior npl. bristle and postpronotal lobe, anterior margin parallel with posterior margin, becoming convex towards base; upper hypopleural calli (posterior apices orange-brown); 2/3 lower hypopleural calli (remainder orange-brown); two narrow, lateral post-sutural vittae beginning at mesonotal suture and tapering posteriorly to end before upper p.sa bristle. Postnotum fuscous. Scutellum yellow with narrow fuscous basal band and apex 1/3 fuscous. Setae: sc. 2, prsc. 2, ia. 1, p.sa. 1, a.sa. absent, mpl. 1, npl. 2, scp. 4.

LEGS

Fig. 2.4C-D. Fore coxae fulvous, mid and hind coxae fuscous; fore femora fulvous; mid femora fulvous, darkening to pale fuscous apically; hind femora fulvous, apical ¼ fuscous; fore tibiae fulvous; mid tibiae fulvous with pale fuscous basally; hind tibiae fuscous; all tarsi pale fuscous; mid tibiae with apical black spur.

WING

Fig. 2.4E. Length 5.6 mm; wing slightly tinted with markings as follows: costal and subcostal cells fuscous, microtrichia covering all of second costal cell and ¾ of first costal cell; a narrow dark fuscous costal band confluent with R2+3 and widening after reaching extremity of R2+3, ending just before M vein; a broad dark fuscous anal streak ending at wing margin; A1+CuA2 covered in dense microtrichia; supernumerary lobe of weak development.

ABDOMEN

Fig. 2.4A. Oval, terga free, pecten present on tergite III. Tergum 1 wider than long. Terga I and II orange-brown with tergum II whitish posteriorly; terga III-V orange

brown with broad pale fuscous lateral longitudinal markings predominantly on terga III and IV. A dark fuscous medial line which is fuscous on tergum I and darkens posteriorly to end before base of tergum V. Posterior lobe of surstylus short, sternum V with slight concavity on posterior margin. Abdominal sterna orange-brown (Fig. 2.4B).

ATTRACTANT

Zingerone

HOSTS

No known record.

DISTRIBUTION

Known only from Couchy Creek Nature Reserve, (-28.277514S, 153.270616E, -28.271123, 153.276958, -28.274621, 153.271850), NSW and Border Ranges National Park, (-28.388S, 153.064E), NSW.

This species has only been trapped in temperate rainforest in northern New South Wales. Due to the restricted distribution of this species (not being detected in other locations) this species may only utilise a single host.

COMMENTS

Bactrocera species A is similar to *Bactrocera aurea* in that it possesses a yellow scutellum with 1/3 fuscous and general scutum and abdominal colouration. It differs in having only 2 scutellar bristles, more extensive hind femora markings, markings on the abdomen which consist of a black medial line which is more prominent on T4 and T5, a very thick dark wrap around costal band, and in not having the transverse band present on the wing.

Bactrocera species A was sequenced for the cytochrome c oxidase subunit I barcode region. A BLAST search (June, 2019) (Altschul et al., 1990), revealed a 15% sequence divergence from the nearest relative suggesting this specimen is a new species. This sequence was included in the phylogenetic analysis provided in later chapters.

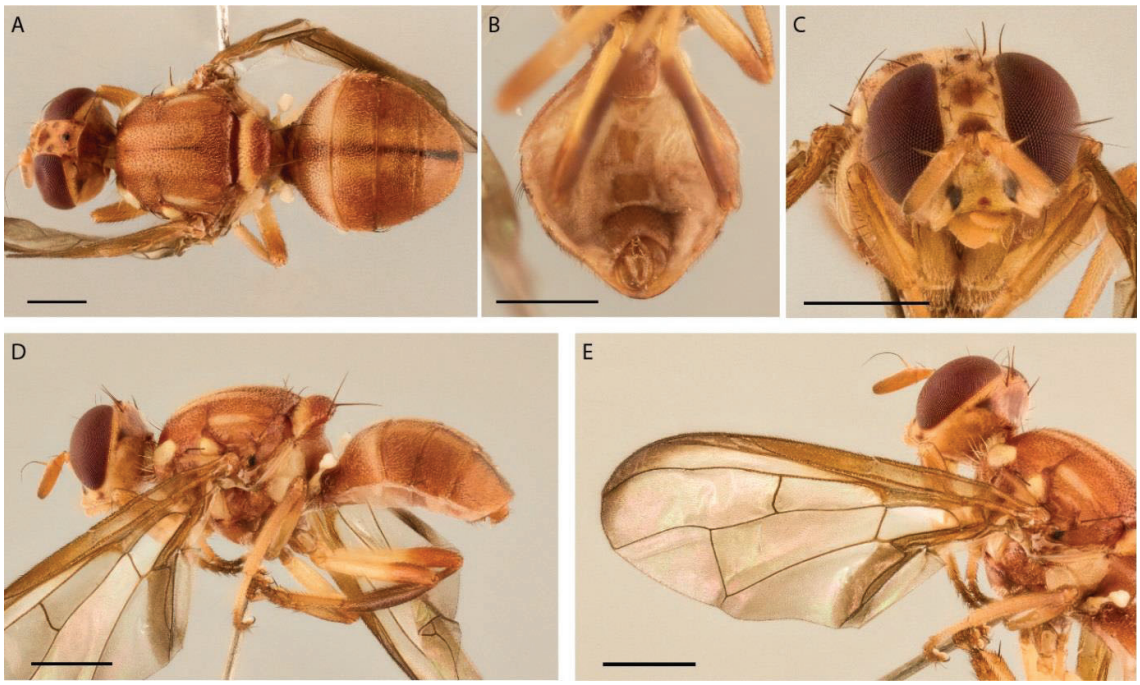


Figure 2.5: *Bactrocera* sp. A male (VFL001). A: whole body dorsal; B: abdomen ventral; C: head frontal; D: whole body lateral with legs; and E: wing. Scale: 1mm.

Chapter 3: A dated phylogeny of the Australian Dacini fruit flies with implications for trait evolution

3.1. Introduction

The Dacini are a tribe of fruit flies that have been the subject of many phylogenetic studies. As an introduction to this chapter, I review the last 20 years of dacine molecular phylogenetics; tracking the trends, progress and methodology through to the present day. Given the taxon coverage and very large genetic and genomic datasets that have been compiled to reconstruct Dacini phylogenies, some may assume further phylogenetic work would be redundant. However, when considering the work of previous authors, there are a number of identifiable gaps that need to be addressed. Firstly, despite a great body of work, there is still disagreement between systematic arrangements based on morphology and molecules; secondly, geographic and taxonomic coverage of species is patchy; thirdly, shifting methodology, and confidence in analysis methods remains a question; and finally, there has been minimal application of phylogenies to ecological and biogeographical research questions. Here I address each of these in detail, while recognising that some (notably morphology vs molecules, and application of phylogenies to ecology and biogeography) are addressed more fully in later chapters.

3.1.1. Summary of Dacini phylogenetics since 2000

The main themes identified in the Dacini phylogenetic literature are summarized in Table 3.1. Typically, Dacini phylogenies have been concerned with establishing monophyly of the tribe (Segura et al., 2006) and the genera within it (Krosch et al., 2012), with numerous paraphyletic relationships among the subgenera also identified (Nakahara and Muraji, 2008, Virgilio et al., 2009, San Jose et al., 2018). For example the molecular study by San Jose et al. (2018), found *Dacus* (*Dacus*), *D.* (*Mellesis*), *D.* (*Didacus*), *Zeugodacus* (*Parazeugodacus*), *Z.* (*Sinodacus*) and *Bactrocera* (*Calodacus*) were all paraphyletic, despite recent morphological revisions of these subgenera (Hancock, 2015, Hancock and Drew, 2015, Hancock and Drew, 2018b). The biggest taxonomic change that phylogenetics has prompted is the elevation of the subgenus *B.* (*Zeugodacus*) to generic status. Segura et al. (2006) first noted that

B. cucurbitae Coquillet (now *Z. cucurbitae*) was more closely related to the genus *Dacus* than it was to *Bactrocera,* with further evidence provided by Krosch et al. (2012). The official taxonomic elevation was later carried out by Virgilio et al. (2015) and De Meyer et al. (2015b), and then supported by San Jose et al. (2018), who also confirmed monophyly of the three genera. Allowing molecular phylogenetics to contribute to taxonomic decisions and resolving paraphyly within the tribe remains an important issue within this group. However, more comprehensive sampling is required in order to achieve increased accuracy, and greater resolution of species (Zwickl and Hillis, 2002, Pollock et al., 2002).

In addition to the systematic issues outlined above, Table 3.1 presents methodological approaches of previous Dacini phylogenies. Early Dacini phylogenies tended to have poor support at backbone nodes (perhaps due to loci selection), but reasonable support at terminal ones. Data sets have traditionally relied on a small number of genes (Muraji and Nakahara, 2001, Smith et al., 2005, Segura et al., 2006, Nakahara and Muraji, 2008), but two recent papers have utilised large genomic datasets (Dupuis et al., 2018, Catullo et al., 2019). Analysis methods have shifted from non-parametric and distance-based approaches (i.e. Smith et al. (2003)) to parametric methods (i.e. Krosch et al. (2012)). Taxonomic sampling is often biased towards pest species (i.e. Zhang et al. (2010)) and has as a result, has uneven coverage of geographic sampling ranges. Finally, when looking at the key aims of these phylogenetic studies, there is a clear focus on data generation, species delimitation and taxonomic/systematic resolution, with very minimal applications of these phylogenies to answering other questions surrounding the evolution of ecological and biological traits. These issues are all dealt with in greater detail in the following sections.

3.1.1.1. Node support and analysis methods

Early phylogenies of the Dacini tended to achieve high support for tip relationships, but struggled at the basal nodes (Muraji and Nakahara, 2001, Smith et al., 2002, Smith et al., 2003, Smith et al., 2005, Zhang et al., 2010). Over the last decade, a shift in analysis methods from Maximum Parsimony (MP) and Neighbour-Joining (NJ) to Maximum Likelihood (ML) and Bayseian Inference (BI) methods has

coincided with increased taxon sampling and the of more extensive molecular data sets, which has helped combat these issues. Earlier phylogenies incorporated mitochondrial protein-coding gene fragments (Zhang et al., 2010), and small additional flanking tRNA regions (Muraji and Nakahara, 2001, Smith et al., 2002, Smith et al., 2003, Segura et al., 2006), but the gradual inclusion of larger nuclear fragments (Virgilio et al., 2009) has continued, and publications over the last eight years contain nearly equal representation of mitochondrial and nuclear data (Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018). In addition, Dacini phylogenies have shifted from traditional non-parametric (Maximum Parsimony (MP)) and distance (Neighbour-Joining (NJ)) methods of phylogenetic inference (Muraji and Nakahara, 2001, Smith et al., 2002, Smith et al., 2003, Nakahara and Muraji, 2008, Zhang et al., 2010) to parametric methods (Maximum Likelihood (ML) and Bayesian inference analysis (BI)) (Virgilio et al., 2009, Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018). While there are advantages and disadvantages to each method, and different views on which method is more accurate (Holder and Lewis, 2003), one trend remains the same across these phylogenies; that with the exception of Virgilio et al. (2009) and Smith et al. (2005), studies continue to use at least two methods of reconstruction to reach a consensus (Segura et al., 2006, Krosch et al., 2012, San Jose et al., 2018, Dupuis et al., 2018). Therefore, a good selection and ratio of mitochondrial and nuclear data, combined with at least two methods of reconstruction, and thorough taxon sampling suggest this could be the key for well supported Dacini phylogenies generated with non-genomic datasets (a perfect example being San Jose et al. (2018)).

3.1.1.2. Next-generation and sanger sequencing datasets in Dacini phylogenetics

Phylogenetics is fast becoming a field dominated by large genomic datasets (Gatesy et al., 2007), but currently there are only two Dacini phylogenies based on genomic datasets (Dupuis et al., 2018, Catullo et al., 2019). For Dupuis et al. (2018), the genomic approach, while helping achieve high basal node support and efficiency in the laboratory, was still unable to resolve some important pest species complexes such as the *B. tryoni* species complex. Catullo et al. (2019) produced a phylogeny

based on SNP data, and achieved well resolved clades, however their sparse taxon sample size limited the conclusions they could draw. The application of large, genomic datasets to systematics does not automatically guarantee more strongly resolved trees. Rokas et al. (2003) investigated the minimum number of loci required in order to achieve highly supported branches across a tree. They found that with a subset of 20 genes they were able to reach the same resolution as their initial dataset, which consisted of 120 genes. For the Dacini, comparable phylogenetic results were obtained by the genomic dataset of Dupuis et al. (2018) and the seven loci dataset by San Jose et al. (2018). Thus, depending on the aims of the study, large genomic datasets for the Dacini may not be necessary as node support is not directly proportional to the number of informative molecular markers used in phylogenies; for example, increased loci sampling has resulted in partition conflicts in other groups due to recombination rates (Romiguier et al., 2013). While partition conflicts have plagued other Dacini phylogenies (Virgilio et al., 2015), utilising Dacini-specific loci which have been identified for biosecurity diagnostics (Krosch et al., 2019b, Plant Health Australia, 2020), and have good resolution capacity at the species level, is a good starting point for achieving better node support.

Table 3.1: Summary of the main findings of 13 Dacini molecular phylogenetic studies over the past 20 years in chronological order. *Pest status follows Doorenweerd et al. (2018). Excluded are morphological studies and studies that predominantly focused on a single species group, notably the *Bactrocera dorsalis* species complex. MP = maximum parsimony, NJ = neighbour-joining, ML = maximum likelihood, BI = Bayesian inference.

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
Muraji and Nakahara (2001)	Taxonomic resolution and assessment of the diagnostic utility of loci	19/48 (80)	Africa and Asia	12S 16S $tRNA^{val}$	1.6kb	MP NJ	Possible applications for PCR-RFLP analyses; unresolved *B. dorsalis* complex members; paraphyly of subgenera, tips well supported, deeper nodes moderately supported
Smith et al. (2002)	Wider sampling and utility of mitochondrial loci for phylogenetics	16/34 (75)	Australia, Asia, Europe, Africa, North America	12S 16S COII $tRNA_{Asp}$ $tRNA_{Lys}$	1.4kb	MP NJ	Monophyly of subfamilies (Tephritinae and Trypetinae), the Dacini tribe, subtribe Dacina, and the genera *Dacus* and *Bactrocera;* general

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
							congruence with morphology, tips and deeper nodes moderately supported
Smith et al. (2003)	Produce a phylogeny and investigate the evolutionary pathways of morphology and male lure response	27/29 (88)	Australia, Asia, Pacific Islands, Europe, Africa, North America	12S 16S COII $tRNA_{Asp}$ $tRNA_{Lys}$	841bp	MP NJ	Genus *Bactrocera* monophyletic; *B.* (*Zeugodacus*) paraphyletic; *B.* (*Bactrocera*) monophyletic; cue-lure is the ancestral response; *B.* (*Daculus*) sister to *B.* (*Bactrocera*)*;* tips and deeper nodes moderately supported, a few polytomies
Smith et al. (2005)	Investigate the evolutionary pathways of morphology,	40/41 (73)	Australia, Asia, Pacific Islands, Europe,	12S 16S COII	1.8kb	MP	Genus *Bactrocera* monophyletic; *B.* (*Zeugodacus*) polyphyletic; *B. dorsalis* complex polyphyletic; simple character evolution across the tree

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
	geographic distribution and male lure response		North America	NADH $tRNA_{Asp}$ $tRNA_{Lys}$			(gain/loss); *B.* (*Bactrocera*) exhibited clades that corresponded to geographic distribution; cue-lure considered ancestral lure response state; four clades correlate to geographic regions; tips well supported, deeper nodes moderately supported
Segura et al. (2006)	Phylogenetic utility of loci, systematics of the group	9/23 (100)	Australia, Africa, Europe, North America	cytb ND1 $tRNA^{Ser}$	308bp	ML MP NJ	Disagreement with the current taxonomy; subfamily Trypetinae not monophyletic; *B.* (*Z.*) *cucurbitae* closely related to *Dacus* clade, deeper nodes well supported, tips moderately supported, polytomies within *Bactrocera* clade

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
Nakahara and Muraji (2008)	Systematics of the group	27/70 (85)	Australia, Africa, Asia, Pacific Islands	COI COII $tRNA^{Leu}$	1.3kb	MP NJ	Disagreement with taxonomy; *B.* (*Bactrocera*) and *B.* (*Zeugodacus*) non-monophyletic, well supported across all nodes
Virgilio et al. (2009)	Produce phylogeny, investigate taxonomic relationships and evolution of host plant diet	35/71 (20)	Africa	16S COI period	1.7kb	BI MP	Disagreement with taxonomy; clear trends identified among host plant choice and phylogenetic clades, well supported across all nodes
Zhang et al. (2010)	Phylogenetic resolution, diagnostic utility	27/29 (93)	Afrotropical, Australasian, Holarctic,	16S COI	1kb	MP NJ	*B.* (*Bactrocera*) and *B.* (*Zeugodacus*) non-monophyletic; cucurbit feeders *B.* (*Zeugodacus*) and *B.* (*Austrodacus*)

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
	of loci, evaluate taxonomic assignments		Nearctic, Neotropical, Oriental, Palearctic				closely related to *B.* (*Afrodacus*) Bezzi*, B.* (*Bactrocera*)*,* and *B.* (*Gymnodacus*) Munro; *B.* (*Daculus*) Speiser potentially an ancestral lineage, tips well supported, deeper nodes within genera not well supported
Krosch et al. (2012)	Test hypotheses for the origin of the Dacini, produce phylogeny, map male-lure	105/125 (33)	Australia, Asia, Pacific Islands, Africa, Europe	16S COI COII White-eye	3kb	BI ML	Genus *Bactrocera* non-monophyletic; *Zeugodacus* group of subgenera sister to the genus *Dacus;* support for India being the origin of the Dacini, polytomies within genera, reasonably well supported deeper nodes; no

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
	response and host breadth						patterns for male lure response or host breadth evident
Virgilio et al. (2015)	Investigate taxonomic relationships and the status of *Zeugodacus*	92/157 (51)	Australia, Asia, Africa, North America	16S COI ND6 Period $tRNA_{pro}$	2.3kb	BI ML MP	Raised *Zeugodacus* to generic status based on monophyly of *Dacus, Bactrocera* and *Zeugodacus,* well resolved clades, but partition conflicts
San Jose et al. (2018)	Investigate systematic relationships, produce phylogeny, test current	167/172 (24)	N/A	Cad1 Cad5 COI Ef1a Period White Wingless	4.5kb	BI ML	Monophyly of the Dacini genera; paraphyly of the subgenera, good support across the tree

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
	taxonomic groups			PGD			
Dupuis et al. (2018)	Develop methodology for high-throughput sequencing and analysis, produce a phylogeny of pest species and close relatives	64/348 (46)	Australia, Asia, Africa, Pacific Islands, North America	Genomic	151.5kb	BI ML	Monophyly of the genera; resolution of species complexes still difficult; development of methodology for easily generating and processing large phylogenomic datasets, good support across the tree
Catullo et al. (2019)	Generate SNP data for phylogeny	14/14 (57)	Australia, Malaysia	Genomic	-	ML SVDQuartets	Disagreement with current taxonomy, no correlation between monophyletic clades and lure response, dispersal

Study	Main aims	No. Dacini species/total individuals and (% that are pests*)	Geographic origin of species	Genes	Length	Analysis methods	General findings and resolution of the phylogeny
	reconstruction and biogeographical implications						events identified between South east Asia and Australia, well supported nodes

3.1.1.3. Geographic and taxonomic sampling of species

While there is an increasing trend towards inclusion of rare and non-pest flies in dacine phylogenies, there is still a significant focus on pest species (Table 3.1). Because of their abundance, pests are easily collected and of interest for biosecurity and agricultural purposes, therefore it is not surprising that many larger phylogenetic studies have included a significant number of pests and their close relatives (Table 3.1). As a result, with the exception of Virgilio et al. (2009) which focussed on the African *Dacus*, many previous dacine phylogenies have had geographically scattered sampling coverage which often neglected Australian native and non-pest species.

A lack of comprehensive geographic sampling can result in the absence of key representative species for genera, subgenera and species complexes. The importance of this, of course, depends on the aim of the study. Project aims do vary, for example, Dupuis et al. (2018) note that they targeted their study toward developing methodology for high throughput sequencing and solving difficult relationships at the species level; while Krosch et al. (2012) aimed for resolution at deeper nodes with wider outgroup sampling for node dating. A lack of some representative taxonomic groups was not critical for either of these studies. In contrast, Virgilio et al. (2015) sampled widely at the genus level with an aim to achieve taxonomic resolution. Virgilio et al. (2009) extensively sampled *Dacus* species from a single region (Africa), allowing the authors to then apply their phylogeny to deeper ecological and biological questions surrounding the genus, for example determining ancestral host use of the genus. This level of analysis has not been implemented by any other Dacini phylogenetic study to date. At present, no study has included a complete range of subgenera, species complexes and other groupings from a single geographic region. Nevertheless, continental-wide sampling is integral when using phylogenies to accurately estimate species trees and then make biological inferences based on those phylogenies (Lecointre et al., 1993, Heath et al., 2008).

3.1.1.4. Phylogenetic divergence dating

In addition to investigating systematic issues within the Dacini, some phylogenetic studies incorporated other key aims; most notably, dating and trait mapping. Krosch et al. (2012) produced the only Dacini dated phylogeny based on fossil evidence to

test the hypothesis of Drew and Hancock (1999) that the Dacini radiated out of India. The dated phylogeny of Krosch et al. (2012) largely supported an out-of-India hypothesis, and dated the first Dacini ancestor arising around 79.6 mya (95% CI = 65.1-94.7). In this instance a dated phylogeny provided the authors with the opportunity to further investigate the evolution of traits (discussed in the next section). However, it must be noted that this date for Dacini evolution is in conflict with that proposed by other authors based on host plant radiations (White, 2006) or deeper dipteran evolution (Wiegmann et al., 2011), and is in need of reassessment (Clarke, 2019).

3.1.1.5. Applications of phylogenies to other questions

Male lure response

In addition to answering systematic questions, some Dacini studies have investigated trait evolution. Traits have included morphological characters such as the evolutionary gain/loss of the emargination on the fifth sternite of males (Smith et al., 2003); evolution of host species usage (Virgilio et al., 2009); and lure response and geographical distribution (Smith et al., 2005, Krosch et al., 2012, Catullo et al., 2019). The 'male lures' are a unique Dacini trait, being a small group of plant derived chemicals to which males respond strongly and positively (Clarke, 2019). The male lures are used widely in trapping and monitoring for pest dacines and so are of great economic importance (Vargas et al., 2008). Smith et al. (2003), Krosch et al. (2012) and Catullo et al. (2019) all investigated the evolution of lure response in fruit flies, with Smith et al. (2003) suggesting that the chemical cue-lure was the ancestral response and that independent evolution of methyl eugenol response was occurring across the tree. Subsequently, Krosch et al. (2012), with a significantly larger sample size, concluded there were no apparent evolutionary patterns present for lure response across the phylogeny. Additionally, Catullo et al. (2019) also found no patterns for lure response evolution within their dataset. In these earlier studies, cue-lure and methyl eugenol were the only two chemicals that were mapped onto the phylogeny (Smith et al., 2003, Krosch et al., 2012), with the addition of a single zingerone responsive species in Catullo et al. (2019). However, in the last decade, there has been increased interest in identifying lures for species that were previously

considered non-lure responsive, and an increased range of chemicals are now known to attract dacines (Fay, 2010, Fay, 2012, Royer et al., 2014, Royer, 2015, Royer et al., 2018, Royer et al., 2019). Incorporating these new lure records provides an opportunity for a more extensive evolutionary analysis of the male lure trait.

Biogeography

In addition to lure response, Smith et al. (2005), Krosch et al. (2012) and Catullo et al. (2019) also investigated the geographic distribution of clades with respect to systematic placement. Smith et al. (2005) identified four clades that correlated with geographic distribution, but in contrast, Krosch et al. (2012) did not find any major clades comprised of representatives from a single biogeographic region, with the exception of the African *Dacus*. Catullo et al. (2019) confirmed multiple incursions of Dacini into and out of Australia, but recognising the limitation of their own taxon coverage suggests a more in-depth study was needed to resolve this issue. This is a key element of Chapter 5.

Larval host range

Dacini fruit flies have diverse host ranges, with some species being monophagous (lay their eggs within the fruit of a single plant species), whereas other species are highly polyphagous (lay eggs in multiple host species across plant families) (Drew, 2004). The evolutionary drivers for the evolution of host use in dacines are still unclear (Clarke, 2017) and, to help address this question there have been some attempts to map these traits onto phylogenies. Krosch et al. (2012) mapped host breadth (specialist or generalist) onto their phylogeny and did not identify any evolutionary patterns. However like Smith et al. (2005) for biogeography and lure response, Krosch et al. (2012) did not conduct any formal analyses for trait evolution. Traits were simply mapped onto the phylogeny tips, with evolutionary conclusions hypothesized based on the presence (or alternatively, the absence) of obviously clustered patterns at terminal groups. In contrast, Virgilio et al. (2009) mapped host plant species on their phylogeny through an ancestral state reconstruction, finding distinct phylogenetic signal for host species choice for

African *Dacus* species. With the exception of Virgilio et al. (2009), there has been minimal use of phylogenies for in-depth analysis of research questions outside of taxonomy (such as ancestral trait evolution) and this is a large gap in the Dacini literature.

3.1.2. Chapter aims and hypotheses

In addition to producing a phylogeny (aims outlined below), two main hypotheses were tested: i) that male response to cue-lure was the ancestral lure response; and ii) that Dacini species evolved from generalist to specialist, with a large number remaining generalists or still undergoing the transition to specialisation. To test these hypotheses, this chapter has three main aims to progress our understanding of dacine systematics and evolution. Firstly, to produce the first comprehensive phylogenetic reconstruction of a regional dacine fauna, these being the Australian dacines, with sufficient taxonomic and geographic coverage of South-east Asian and Pacific representatives to inform broader systematic relationships. Secondly, to determine how these systematic relationships align with current taxonomic constructs. Finally, to date the phylogeny using fossil information to calibrate the molecular divergences and then use the tree to investigate the evolution of lure response and host breadth. The phylogeny produced in this chapter also becomes the 'master' phylogeny, which will be applied to downstream applications in subsequent chapters.

3.2. Materials and methods

3.2.1. Species selection

High priority was placed on collecting all known species of Australian Dacini in order to have complete continental coverage for analysis. Obtaining representative species from taxonomically recognised subgenera and species complexes from outside Australia was also given priority: this included species from New Guinea and the South Pacific (focused taxon coverage for clades with strong Australian/regional representation) as well as Dacini found in South-east Asia (minimal taxon coverage, as required for phylogenetic completeness). Where possible, I sequenced two geographically distant individuals for each species, to help confirm identifications

(the expectation being that two or more specimens of the same species should form a monophyletic group) and to account for geographic sequence variation if present. Sequences were compared by eye during alignment and also by comparing branch lengths once the phylogeny was produced. If variation was identified, the morphology was revisited to eliminate the possibility of misidentification. If the morphology was ambiguous, where possible, additional samples were sequenced and included in the dataset.

3.2.1.1. Material sources

Fresh material was collected for this project as outlined in Chapter 2. In addition, dry pinned specimens, and specimens in alcohol were provided from the following organisations (Appendix 3):

- Queensland University of Technology, Brisbane (alcohol specimens);
- Northern Australia Quarantine Strategy, [Commonwealth] Department of Agriculture, Water and the Environment, Cairns (pinned and alcohol specimens);
- Biosecurity Queensland, [Queensland] Department of Agriculture and Fisheries, Brisbane (including the Department of Agriculture and Fisheries Insect Collection), Brisbane (pinned and alcohol); and
- Operational Science Services, [Commonwealth] Department of Agriculture, Water and the Environment, Melbourne (pinned specimens).

3.2.1.2. Outgroup selection

Outgroups were selected in concordance with the fossils used for calibration. Species were selected from within the Tephritidae, Tephritoidea, Pallopteridae, Muscidae and Culicidae. 'Within-Dacini' outgroups such as those that are geographically distinct and unlikely to have evolved alongside present-day Australian and Pacific species (therefore would not be expected to share close genetic lineages or biogeographic heritage with them), and species that exhibited unusual characteristics from what is typically seen within *Bactrocera* (*Bactrocera*) (such as unusual wing patterning, oddly shaped medial vitta, dark pleural areas and scutellum) were also included to

test the phylogenetic placement of these species in relation to both Australian species and the currently recognised taxonomy.

3.2.1.3. Fossils

Three fossil taxa were used to provide minimum bounds for node calibrations in the phylogeny. These minimum bounds were determined by searching the literature for fossils and their ages based on the strata they were found within. The maximum bound ages were defined to cover the age of well sampled fossil faunas in which crown group members of the relevant clade were absent, but which sampled stem members. The age and authorities for each of the three calibrations used in this study are found in Table 3.2.

Table 3.2: Information regarding three fossil deposits used in the calibrations of the dated Dacini phylogeny generated in this thesis. Minimum bounds represent the predicted age of the oldest fossil based on geological estimates, while maximum bounds are provided based on the point in time at which the crown members are absent, but stem members are still present in the fossil record. '^' indicates geological age reference, and '*' indicates fossil reference.

Fossil	Representative nodes	Min. bound (mya)	Max. bound (mya)	Geological deposit	Reference
Phytomyzites spp.	Crown of Schizophora	64-64.7	163.5	Fort Union formation	Belt et al. (2004)^ and Winkler et al. (2010)*

Fossil	Representative nodes	Min. bound (mya)	Max. bound (mya)	Geological deposit	Reference
Family Pallopteridae *Glaesolonchaea electrica* Hennig *Morgea mcalpinei* Hennig *Pallopterites electrica* Hennig	Crown of Tephritoidea	37.2-33.9	100.5	Baltic amber	Powell (1992)^, Luterbacher et al. (2004)^ and Gentilini et al. (2006)*
Subfamily Blepharoneurinae *Ceratodacus priscus* Norrbom & Condon	Crown of Tephritidae	13.7-20.4	47.8	Dominican amber	Smith and Poinar (1992)^ and Norrbom and Condon (2000)*

3.2.2. DNA extraction, PCR and sequencing

3.2.2.1. DNA extraction

Genomic DNA was extracted from a total of 273 individuals. For some species, previously extracted genomic DNA was made available as the result of a concurrent diagnostics project led by Dr Mark Schutze at QUT (PBCRC: 2147) (discussed further below).

Genomic DNA was extracted from specimens using the DNeasy Blood and Tissue Kit for purification of DNA from insects (Qiagen Inc., Valencia, CA). DNA extraction of dried specimens also followed the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) protocol. Modifications to this protocol included: a 24hr incubation at 37°C during lysis; and a two-step elution, first into 5-10ul of buffer and then into 10-15ul. Both elution steps were incubated on a heat block for 10min at 56°C. Genomic DNA was stored at -20 ºC for later use.

3.2.2.2. Loci selection, amplification and sequencing

Six loci were amplified for all individuals: the mitochondrial cytochrome c oxidase subunit I (COI) (1482bp) and cytochrome c oxidase subunit II (COII) (748bp); the ribosomal loci 16S rRNA (542bp); and three nuclear loci: Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2 isoform X2 (DDOSTs2) (695bp), replication protein A 32 kDa subunit (RPA2) (525bp) and eukaryotic translation initiation factor 3 subunit L gene (EIF3L) (550bp). The three nuclear loci were chosen because they had been developed specifically for diagnostics of this tribe (Krosch et al., 2019b, Plant Health Australia, 2020). Primers used to amplify each loci are presented in Table 3.3, with mastermix recipes, thermocycler protocols and modifications provided in Appendices 4 & 5. PCR clean-up followed the ISOLATE II PCR and Gel Kit Bench-top protocol (Bioline UK). PCR sequencing clean-up followed a standard ethanol precipitation method (Applied Biosystems, USA), sequencing PCR conditions followed a standard protocol (Appendix 6). Sequencing was carried out on a 3500 Genetic Analyzer (Applied Biosystems, USA) at the Molecular Genetics Research Facility at QUT. As a result of later collections, one additional specimen was sequenced through Macrogen Inc. (Seoul, South Korea).

3.2.2.3. Pinned specimens

For the pinned specimens, mitochondrial DNA was targeted because it can remain amplifiable for longer than nuclear DNA (Lindahl, 1993). Dacine-specific nested primers designed for amplification of fragments from old specimens (Krosch et al., 2020b) were used.

3.2.2.4. Shared data with diagnostics project

Sequence data was shared between this project and a concurrently running fruit fly diagnostics project. At the culmination of the diagnostics project in 2018, some data generated for this chapter was made publicly available through the Plant Health Australia (2018a) website, on Genbank and contributed to a publication (Appendix

7). Likewise, some sequences that were not publicly available through the diagnostics project are used here for the first time in the phylogenetic analysis. The origins of sequences used here and Genbank accession numbers are provided in Appendix 8.

Table 3.3: Loci, primers and annealing temperatures used in gene amplification in this chapter. Note: multiple COI barcode primer pairs were required for species that were difficult to amplify due to age (Krosch et al., 2020b) or the presence of numts (Blacket et al., 2012).

Loci	Length	Primer name	Sequence (5'-3')	Tm (°C)	Reference
COI barcode	651bp	LCO1490	GGTCAACAAATCATAAAGATATTGG	50.5	Folmer et al. (1994)
		HCO2198	TGATTTTTTGGTCACCCTGAAGTTTA	55.3	Folmer et al. (1994)
	550bp	FFCOI-F	GGAGCATTAATYGGRGAYG	51.9	Blacket et al. (2012)
	307bp	LCO1490-mod	TYTCAACAAATCATAAAGATATTGG	48.9	Krosch et al. (2020b)
		Dac-COI-r	GAAAACGGRGCBGGTACAGGTTGAAC	62.0	Krosch et al. (2020b)
	407bp	Dac-COI-f	GCHTTCCCHCGAATAAATAATA	49.7	Krosch et al. (2020b)
		HCO2198-mod	TGATTYTTTGGWCACCCTGAAGTTTA	55.8	Krosch et al. (2020b)
COI	831bp	C1-J-2183	CAACATTTATTTGATTTTTTGG	45.9	Simon et al. (1994)
		TL2-N-3014	TCCAATGCACTAATCTGCCATATTA	54.3	Simon et al. (1994)
COII	748bp	TL2-J-3037	ATGGCAGATTAGTGCAATGG	53.2	Simon et al. (1994)
		TK-N-3785	GTTTAAGAGACCAGTACTTG	47.7	Simon et al. (1994)
16S	542bp	mtd32	CCGGTCTGAACTCA GATCACGT	58.6	Palumbi (1996)
		mtd34	CGCCTGTTTAACAAAAACAT	49.6	Palumbi (1996)
DDOSTs2	695bp	DDOSTs2-f	GTGGCAGATCGTGTTGAAGA	53.6	Krosch et al. (2019b)
		DDOSTs2-r	GGAACTTTAAAGGCCGATAATACTC	55.1	Krosch et al. (2019b)
RPA2	525bp	RPA2-f	ACAAATCTTATATTCGCBTGAGGG	54.3	Krosch et al. (2019b)

Loci	Length	Primer name	Sequence (5'-3')	Tm (°C)	Reference
		RPA2-r	AATTTTTDTTGCAAYTCTTTGCGG	53.6	Krosch et al. (2019b)
EIF3L	550bp	EIF3L-f	CCCAAGGAAAYGATCCYCAA	54.9	Plant Health Australia (2020)
		EIF3L-r	GCTGACGCACTTCATCCATA	55.0	Plant Health Australia (2020)

3.2.3. Sequence alignment and phylogenetic analysis

Sequences were compiled in BioEdit v7.2.5 (Hall, 2011) and then aligned in MEGA7 (Kumar et al., 2016) using MUSCLE (Edgar, 2004) and by eye where necessary. Sequence alignments were concatenated in GENEIOUS v9.1.8 (Biomatters, 2017). I attempted to reconstruct the phylogeny using, Bayesian, Neighbour Joining (NJ), Maximum Parsiomony (MP) and Maximum Likelihood (ML) methods, however, the Bayesian and Maximum Parsimony analyses did not converge. A NJ tree was produced using PAUP v4.0b10 (Swofford, 1998) to provide insight into the reliability of the ML tree in the absence of other comparable methods. Additionally, to account for a lack of Bayesian inference, I used two different ML models: the 'proportionally edge-linked' and 'edge-unlinked' models (from here on referred to as linked and unlinked respectively). The linked model allows proportional branch lengths to be calculated, with each partition having its own specific evolutionary rate, whereas the unlinked model allows each partition to have its own set of branch lengths (Minh et al., 2019).

The appropriate substitution models were determined for each ML partition using IQ-Tree ModelFinder (Kalyaanamoorthy et al., 2017) and are presented in Table 3.4. Based on the findings, mitochondrial partitions COI and COII were combined and partitioned by first, second and third codon positions. The other mitochondrial and nuclear loci were modelled as separate partitions. The two phylogenetic reconstructions were carried out using 1000 ultrafast bootstrap replications (Minh et al., 2013) in IQ-Tree V1.7 (Trifinopoulos et al., 2016). In addition to the ultrafast bootstrap analysis, I explored other methods in order to investigate the reliability of the bootstrap values. These were: (i) the SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al., 2010) and (ii) the partition and site resampling technique (IQ-Tree command: '-bsam GENESITE', still 1000 iterations run) (Nei et al., 2001, Gadagkar et al., 2005). All analyses were carried out on the high-performance computer cluster 'Snyder' (24 cores, two 2.60 GHz Sky Lake processors, and 384 GB of memory), maintained by Information Technology at Purdue University, Indiana, USA.

Table 3.4: Loci model selection and partitions used in the phylogenetic analysis as determined through IQ-Tree ModelFinder (Kalyaanamoorthy et al., 2017).

Loci	Model selection
COI+COII first codon	GTR+F+I+G4
COI+COII second codon	TPM2u+F+I+G4
COI+COII third codon	TIM+F+I+G4
16S	TIM2+F+I+G4
RPA2	TPM3u+F+I+G4
DDOSTs2	TN+F+I+G4
EIF3L	TIM2e+I+G4

3.2.4. Pinned collection material and undescribed species

I attempted to extract DNA from 16 pinned specimens (15 species) however due to the age (some over 40 years old) and preservation of the specimens there were only three species for which the sequences were reliable enough to be included (Table 3.5). In addition, there was also minimal sequence data available for *B.* species A (from Chapter 2) (Table 3.5). In order to estimate the phylogenetic placement of these species, IQ-Tree V1.7 was used to reconstruct the tree using only COI barcode data and 1000 ultrafast bootstraps, the topology from the multigene tree was used as a constraint to reconstruct this tree. The same models and partitions were used as stated in Table 3.4.

Table 3.5: *Bactrocera* species for which only minimal genetic data was available (COI barcode), that were added to the phylogenetic tree using a constrained topology approach.

Species	Code	Fragment size (bp)
B. species A	VFL001	372
B. mendosa (May)	MND001	601
B. nigrovittata (Drew)	NGV001	580
B. strigata (Perkins)	STR001	622

3.2.5. Node calibrations

Similar to Krosch et al. (2012), a Bayesian approach was used to estimate divergence times. However here, the topology was input into MCMCtree (Yang and Rannala, 2006, Rannala and Yang, 2007) in PAML version 4.8 (Yang, 2007) and different fossil calibrations were used at younger nodes in the tree. The three minimum and maximum bounds used are presented in Table 3.2. I used the GTR model with an independent clock rate, a burn-in of 20,000 iterations and 4000 samples every 50 generations. The root age was constrained to between 64-163.5 mya. Analysis was carried out on Purdue University's HPC. The dated phylogeny was visualised using R v3.6.2 (R Core Team, 2003) and RStudio v1.2.5001 (RStudio Team, 2019), and the package MCMCtreeR (Puttick and Title, 2019).

3.2.6. Ancestral state reconstruction

I mapped male lure response and host breadth onto the dated phylogenetic tree. See Appendix 9 for raw data used in the analyses. I used a subset of the dated tree and trimmed duplicate specimens (within a species) and outgroups from the tree. For each dataset there was a different number of species for which data was available (lure response = 144 species, host breadth = 84 species). However, the methodology for mapping both traits was identical. Traits were mapped and visualized using the RStudio statistical packages: ape (Paradis and Schliep, 2019), geiger (Pennell et al., 2014), ggtree (Yu et al., 2017, Yu et al., 2018), phyloch (Heibl, 2008), phylotate (Beer and Beer, 2019), phytools (Revell, 2012) and strap (Bell and Lloyd, 2014).

3.2.6.1. Lure response

Lure response data was taken from published records (Drew and Hooper, 1981, Drew, 1989, Amice and Sales, 1997, Hancock et al., 2000, Drew and Romig, 2001, Huxham and Hancock, 2002, Fay, 2012, Drew and Romig, 2013, Royer, 2015, Royer et al., 2018, Royer et al., 2019) and records obtained in Chapter 2 (Appendix 1) of this thesis. Some species are known to respond to more than one lure; this is common as some lures are analogues of cue-lure and ME (Royer, 2015). The species' strongest response to one of six chemicals: cue-lure, methyl eugenol, zingerone,

dihydroeugenol, methyl isoeugenol and isoeugenol[1] was scored based on data from Royer (2015) and abundance of records in the literature (i.e. if a record was only reported once, it may have been a case of contamination).

3.2.6.2. Host breadth

Two host breadth trees were produced using two different methods of categorising the data. For the first host breadth tree, data was scored as follows: monophagous (species where females lay into, and larvae feed upon, a single host plant species), narrowly oligophagous (species that lay within a single host genus), oligophagous (species that lay in a single family) and polyphagous (species that lay into more than one plant family). Data was filtered to remove incorrect host identifications and single records of larvae from a host which have not been confirmed by subsequent surveys. In addition, sampling for hosts has been quite extensive (list provided below), therefore there is confidence in the species being correctly assigned to breadth categories. For the second host breadth tree, the data was transformed into a binary dataset with monophagous (=specialist) and the other three traits; narrowly oligophagous, oligophagous and polyphagous combined into a single trait (=generalist). Definitions follow Wiklund (1982) and Novotny et al. (2002) for the first tree, and Loxdale et al. (2011) and Clarke (2017) for the second. The use of both trees for the diet breadth mapping was to help make any evolutionary signal more obvious (the evolution of two traits is easier to visualise than four), and because there is ongoing debate in the herbivory literature about diet-breadth categorization, and whether it should be considered multi-trait or binary (Loxdale and Balog, 2018, Loxdale et al., 2019). Host records were obtained from: May (1953), May (1957), May (1960), Hardy (1973), Allwood and Angeles (1979), Drew (1989), White and Elson-Harris (1992), Liang et al. (1993), Drew and Hancock (1994), Amice and Sales (1997), Allwood et al. (1999), Hancock et al. (2000), Athar (2005), Novotny et al. (2005), Leblanc et al. (2012), Drew and Romig (2013) and Vargas et al. (2015).

[1] Note: Dacine species generally only respond to one lure (Clarke, 2019). Where a species is known to respond to two or more lures, one lure is generally far more attractive than the other(s), e.g. *B. jarvisi* which is strongly attracted to zingerone but only weakly attracted to cue-lure (Fay, 2012).

3.2.6.3. Phylogenetic signal

In order to investigate the phylogenetic signal of host breadth and lure response, I converted both datasets into a binary trait format so that the D-statistic and Pagel's lambda could be calculated. Lures were reduced to two groups/traits based on their chemical structures following Royer (2015), with compounds containing the 2-butanone side-chain (zingerone and cue-lure) combined into a single group/trait with the second group containing the eugenol analogues which have propyl, allyl and propenyl side-chains (methyl eugenol, dihydroeugenol, methyl isoeugenol and isoeugenol). For host breadth, the 'specialist/generalist' tree (as described above) was used. I used R packages ade4 (Chessel et al., 2004, Dray and Dufour, 2007, Dray et al., 2007, Bougeard and Dray, 2018), adephylo (Jombart et al., 2017), caper (Orme et al., 2018), picante (Kembel et al., 2010), ape (Paradis and Schliep, 2019), phylobase (Hackathon et al., 2020), geiger (Pennell et al., 2014) and phytools (Revell, 2012) to test for phylogenetic signal.

3.2.6.4. Correlation between host breadth and lure response

The tree was trimmed to 80 species for which there was both host breadth and lure response data. The data was treated as binary with the same groupings of lures and host breadth as in the previous step. Tree data and trait data was input into BayesTraits v3.0.2 following the discrete Bayes factor analysis method (Pagel, 1994, Pagel and Meade, 2006) with 20 million iterations, a burn-in of 2 million, a sampling of 100 stones for 10,000 iterations, with all rate priors set to exponential with a mean of 10. I ran the discrete independent and discrete dependent models in order to calculate the log Bayes factor, which will evaluate the hypothesis that one trait is independent of the other.

3.3. Results

A total of 144 described Dacini species from three genera, including 76 of the 90 (83%) Australian mainland Dacini fauna were sampled for a total of 4332 bp (80% of sequence data was generated as part of this thesis, see Appendix 8 for more details), with 70% of species represented by more than one specimen. A total of 12

subgenera and 14 species complexes were represented in the dataset, along with six undescribed species (one described in Chapter 2, another in Chapter 6). A full list of collection information is provided in Appendix 1. The 14 missing species must be regarded as extremely rare, being represented in collections by very few, very old specimens (from which DNA extraction was either not allowed, or was not successful), and having been failed to be freshly captured despite targeted collection efforts (Chapter 2). Those missing from this dataset represent species that, based on taxonomic assignments, are expected to be scattered across the tree. Therefore, it is not expected that large clades would be entirely missing from this dataset. All subgenera are represented here, except for the subgenus *B.* (*Queenslandacus*) of which there is a single species found in Australia, *B. exigua* (May).

3.3.1. Tree reconstructions

Monophyly of the genera was congruent between the NJ tree and the ML trees with unlinked and edge-linked (proportional) branch lengths, with minimal incongruence occurring at the species level (Fig. 3.1). Based on recommendations from the IQ-Tree manual (Minh et al., 2019), comparison of the BIC scores found that the best fit model for ML reconstruction was the edge-linked model (BIC: 226705.65) over the edge-unlinked model (BIC: 243614.36); this model approach is also supported as the most appropriate fit by a recent comparative study (Duchene et al., 2020). One key difference observed between the NJ tree (Appendix 10) and the edge-linked tree was the clade resolved as sister to the rest of *Bactrocera.* In the NJ tree the clade resolved as sister to the remainder of *Bactrocera* consisted of *B. visenda* (Hardy)*, B. cheesmanae* (Perkins), *B. neocheesmanae* Drew, and the *B. aglaiae* species group, however, in the edge-linked tree, the clade only consisted of the *B. aglaiae* species group. For subsequent analyses here, and in the rest of this thesis, the edge-linked tree is used unless otherwise stated.

3.3.2. Maximum Likelihood tree

3.3.2.1. Genera and subgenera

All deeper nodes were resolved with high bootstrap support, except for the node that resolved *B.* (*Apodacus*) as sister to the other *Bactrocera* subgenera. The three ingroup genera, *Bactrocera, Zeugodacus* and *Dacus,* were resolved into monophyletic clades although notably, *Bactrocera* further resolved into two clades (referred to as Clade 1 and Clade 2) (Fig. 3.2). Current subgeneric groupings based on taxonomy are compared to results from this study based on molecular data in Table 3.6. Clade 1 consisted of the majority of *B.* (*Bactrocera*) and *B.* (*Calodacus*) *calophylli* (Perkins & May), while Clade 2 consisted of *B.* (*Apodacus*), *B.* (*Bulladacus*), *B.* (*Daculus*), *B.* (*Neozeugodacus*), *B.* (*Notodacus*) Perkins*, B.* (*Parazeugodacus*)*, B.* (*Tetradacus*) Miyake and a small clade of *B.* (*Bactrocera*) species. A total of seven out of 12 subgenera (of more than one representative) and 11 out of 14 species complexes (of more than one representative) included in this dataset were paraphyletic within the three genera. The subgenera which were paraphyletic were the *Dacus* subgenera *D.* (*Mellesis*), *D.* (*Neodacus*) and *D.* (*Callantra*), as were the *Zeugodacus* subgenera *Z.* (*Zeugodacus*), *Z.* (*Sinodacus*) and *Z.* (*Javadacus*) Hardy, and *Bactrocera* (*Bactrocera*). *Zeugodacus* (*Parasinodacus*) Drew and Romig was monophyletic, as were the *Bactrocera* subgenera *B.* (*Apodacus*), *B.* (*Notodacus*), *B.* (*Tetradacus*) and *B.* (*Parazeugodacus*). The subgenus *B.* (*Hemizeugodacus*) was sister to both Clade 1 and Clade 2.

Some subgenera included only a single representative. These were *Z.* (*Papuodacus*) *neopallescentis* (Drew)*,* which was sister to *Z.* (*Javadacus*) *sandaracinus* (Drew)*; B.* (*Hemizeugodacus*) *aglaiae* was nested within the new species (forming the new *aglaiae* species group discussed in Chapter 6) and was sister to the rest of *Bactrocera; B.* (*Daculus*) *oleae* (Gmelin) was sister to a clade containing *B.* (*Bactrocera*) *aberrans* and *B.* (*Bactrocera*) *speewahensis* (Fay & Hancock); *B.* (*Neozeugodacus*) *aurea* was sister to *B.* (*Bactrocera*) *brunnea; B.* (*Bulladacus*) *tigrina* (May) was sister to the *B.* (*Parazeugodacus*) clade*;* and *B.* (*Calodacus*) *calophylli* which was nested within the larger *B.* (*Bactrocera*) clade, was sister to *B.* (*Bactrocera*) *murrayi* (Perkins).

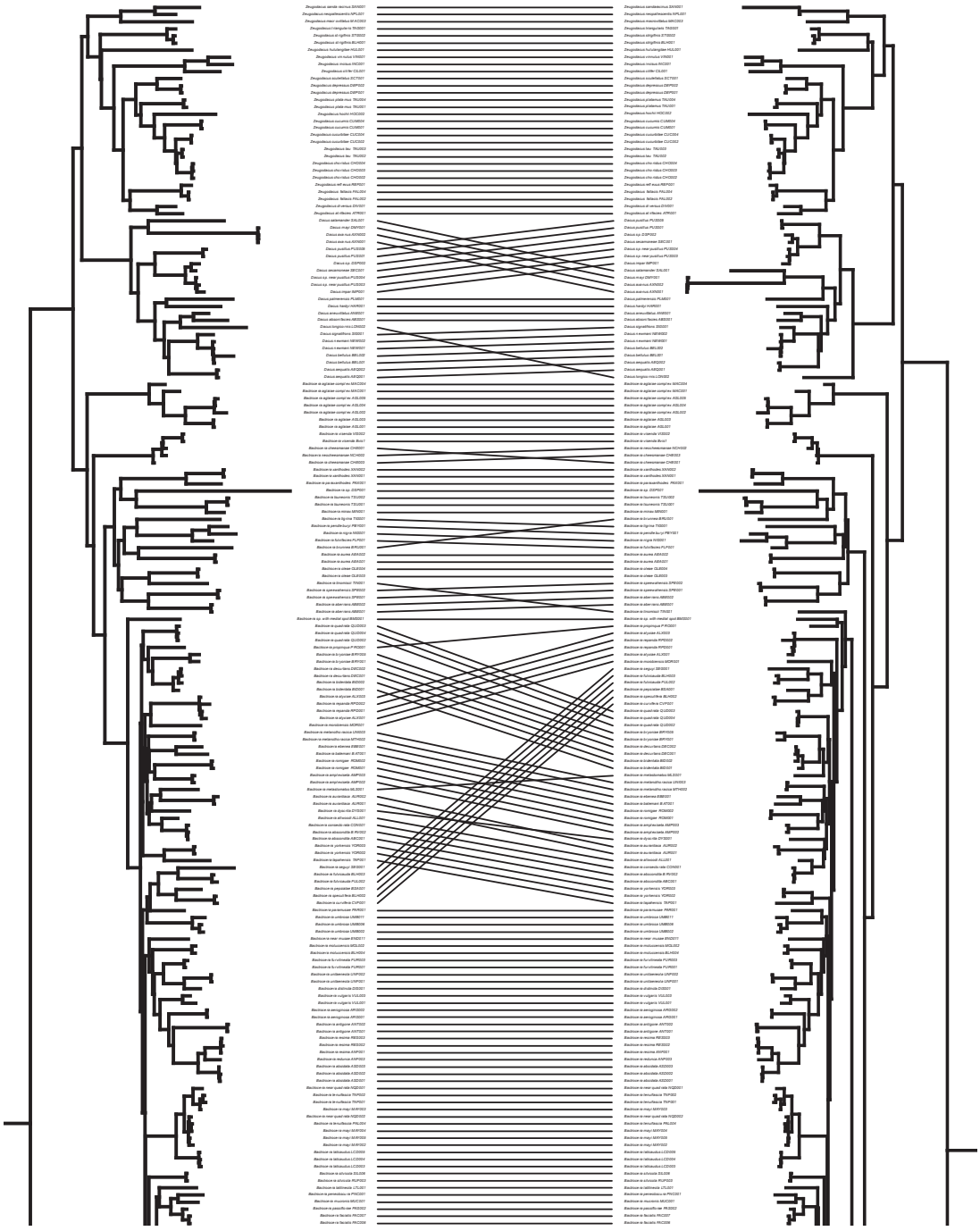
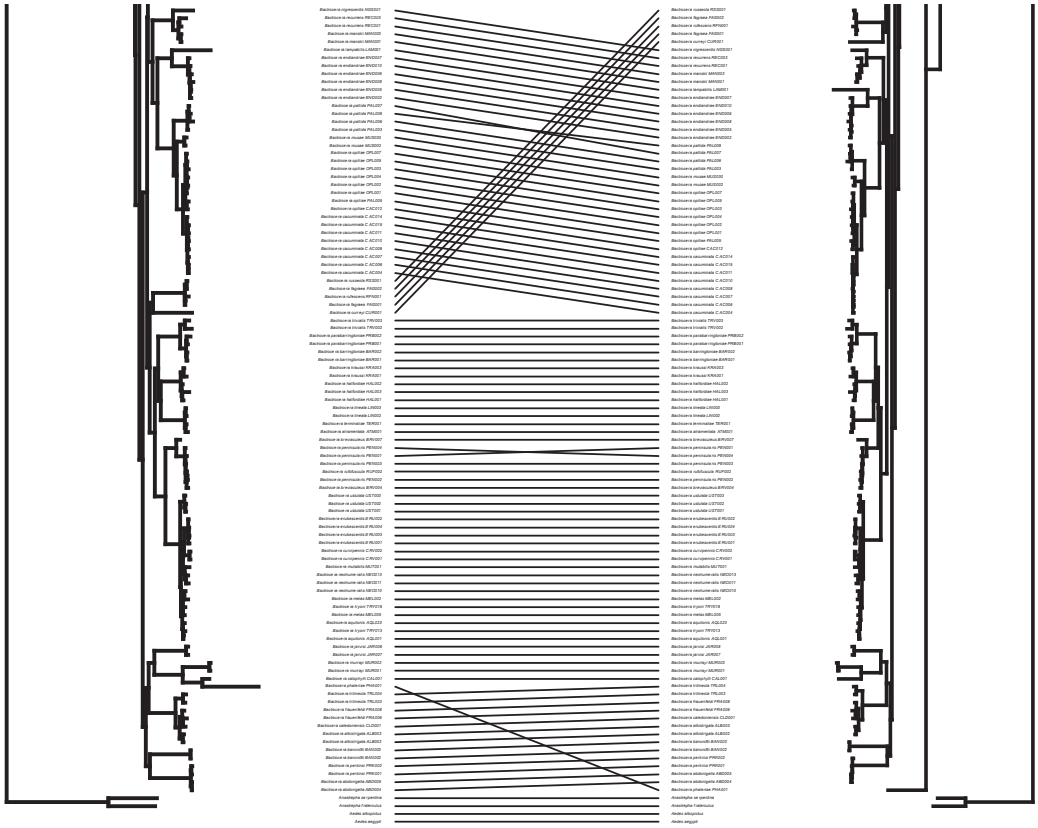


Figure 3.1 (continued next page): Tanglegram presenting the differences in topology between two Dacini phylogenies analysed using alternative branch-length models; the unlinked (left) and proportionally-linked (right) models. The tree was reconstructed using ML methods with seven partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L. Differences and

similarities in topology are represented by the connecting lines between the taxa. Outgroups were removed for presentation purposes.



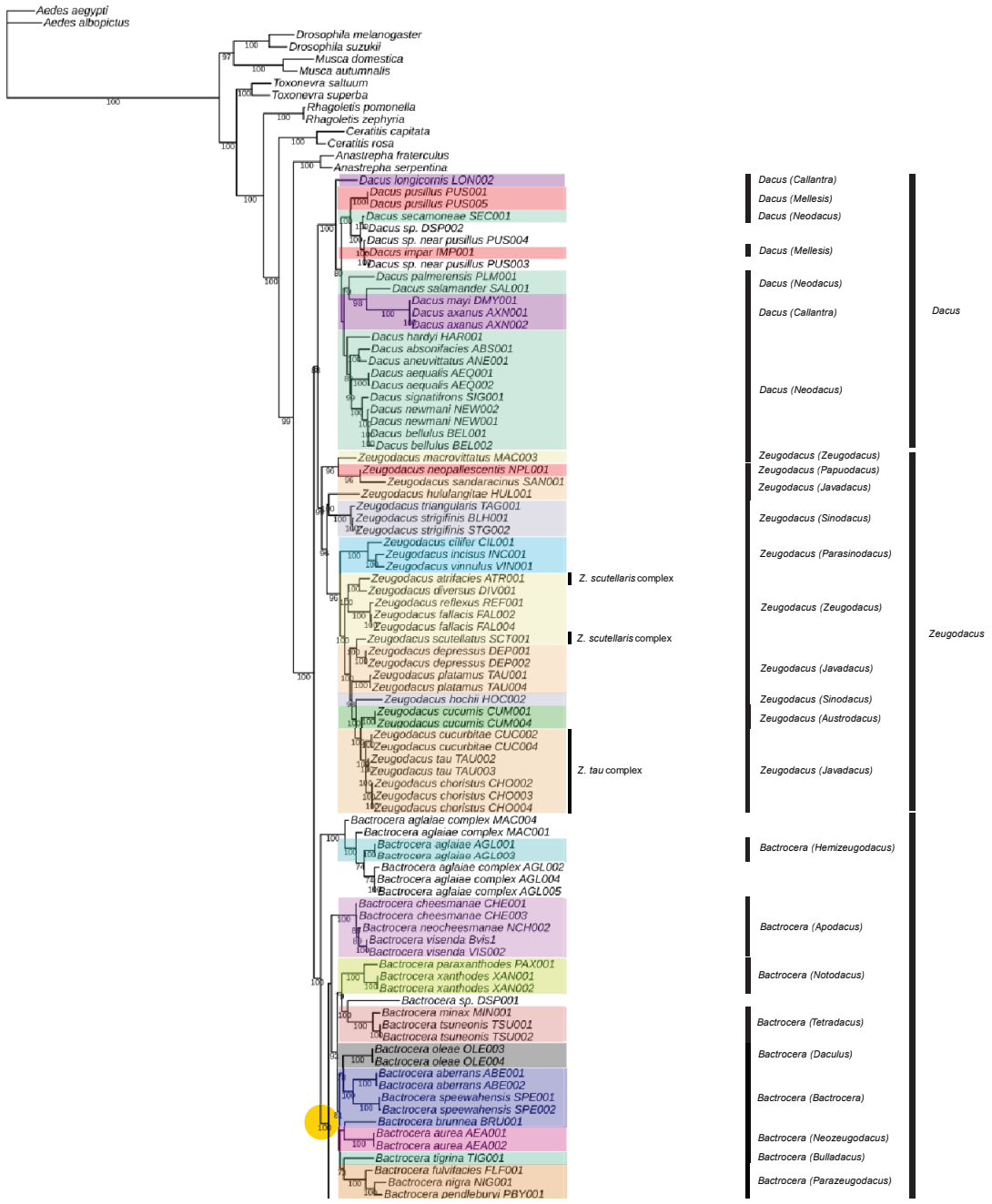
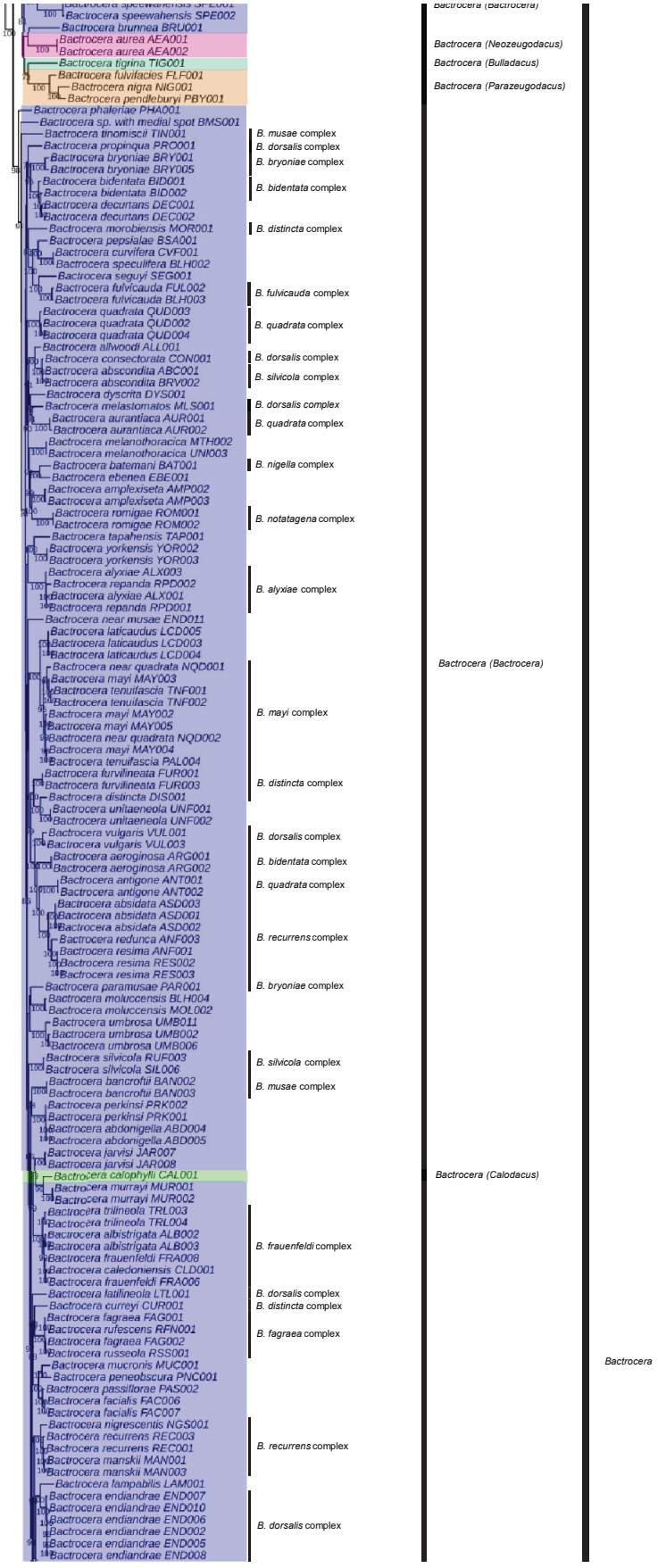


Figure 3.2 (continued next two pages): Proportionally linked ML phylogenetic tree of the Dacini, with a focus on Australian species, reconstructed from seven partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L. Maximum likelihood ultra-fast bootstrap values > 75 are shown at the nodes. Generic, subgeneric and species complex assignments are indicated, with species highlighted based on subgeneric classifications. Highlighted node denotes split into Clades 1 and 2. Species that are not highlighted have no subgeneric assignment.


Bactrocera speewahensis SPE002
Bactrocera brunnea BRU001
Bactrocera aurea AEA001
Bactrocera aurea AEA002
Bactrocera tigrina TIG001
Bactrocera fulvifacies FLF001
Bactrocera nigra NIG001
Bactrocera pendleburyi PBY001
Bactrocera phaleriae PHA001
Bactrocera sp. with medial spot BMS001
Bactrocera tinomiscii TIN001
Bactrocera propinqua PRO001
Bactrocera bryoniae BRY001
Bactrocera bryoniae BRY005
Bactrocera bidentata BID001
Bactrocera bidentata BID002
Bactrocera decurtans DEC001
Bactrocera decurtans DEC002
Bactrocera morobiensis MOR001
Bactrocera pepsilalae BSA001
Bactrocera curvifera CVF001
Bactrocera speculifera BLH002
Bactrocera seguyi SEG001
Bactrocera fulvicauda FUL002
Bactrocera fulvicauda BLH003
Bactrocera quadrata QUD003
Bactrocera quadrata QUD002
Bactrocera quadrata QUD004
Bactrocera allwoodi ALL001
Bactrocera consectorata CON001
Bactrocera abscondita ABC001
Bactrocera abscondita BRV002
Bactrocera dyscrita DYS001
Bactrocera melastomatos MLS001
Bactrocera aurantiaca AUR001
Bactrocera aurantiaca AUR002
Bactrocera melanothoracica MTH002
Bactrocera melanothoracica UNI003
Bactrocera batemani BAT001
Bactrocera ebenea EBE001
Bactrocera amplexiseta AMP002
Bactrocera amplexiseta AMP003
Bactrocera romigae ROM001
Bactrocera romigae ROM002
Bactrocera tapaiensis TAP001
Bactrocera yorkensis YOR002
Bactrocera yorkensis YOR003
Bactrocera alyxiae ALX003
Bactrocera repanda RPD002
Bactrocera alyxiae ALX001
Bactrocera repanda RPD001
Bactrocera near musae END011
Bactrocera laticaudus LCD005
Bactrocera laticaudus LCD003
Bactrocera laticaudus LCD004
Bactrocera near quadrata NQD001
Bactrocera mayi MAY003
Bactrocera tenuifascia TNF001
Bactrocera tenuifascia TNF002
Bactrocera mayi MAY002
Bactrocera mayi MAY005
Bactrocera near quadrata NQD002
Bactrocera mayi MAY004
Bactrocera tenuifascia PAL004
Bactrocera furvilineata FUR001
Bactrocera furvilineata FUR003
Bactrocera distincta DIS001
Bactrocera unitaeneola UNF001
Bactrocera unitaeneola UNF002
Bactrocera vulgaris VUL001
Bactrocera vulgaris VUL003
Bactrocera aeroginosa ARG001
Bactrocera aeroginosa ARG002
Bactrocera antigone ANT001
Bactrocera antigone ANT002
Bactrocera absidata ASD003
Bactrocera absidata ASD001
Bactrocera absidata ASD002
Bactrocera reduncа ANF003
Bactrocera resima ANF001
Bactrocera resima RES002
Bactrocera resima RES003
Bactrocera paramusae PAR001
Bactrocera moluccensis BLH004
Bactrocera moluccensis MOL002
Bactrocera umbrosa UMB011
Bactrocera umbrosa UMB002
Bactrocera umbrosa UMB006
Bactrocera silvicola RUF003
Bactrocera silvicola SIL006
Bactrocera bancroftii BAN002
Bactrocera bancroftii BAN003
Bactrocera perkinsi PRK002
Bactrocera perkinsi PRK001
Bactrocera abdonigella ABD004
Bactrocera abdonigella ABD005
Bactrocera jarvisi JAR007
Bactrocera jarvisi JAR008
Bactrocera calophylli CAL001
Bactrocera murrayi MUR001
Bactrocera murrayi MUR002
Bactrocera trilineola TRL003
Bactrocera trilineola TRL004
Bactrocera albistrigata ALB002
Bactrocera albistrigata ALB003
Bactrocera frauenfeldi FRA008
Bactrocera caledoniensis CLD001
Bactrocera frauenfeldi FRA006
Bactrocera latilineola LTL001
Bactrocera curreyi CUR001
Bactrocera fagraea FAG001
Bactrocera rufescens RFN001
Bactrocera fagraea FAG002
Bactrocera russeola RSS001
Bactrocera mucronis MUC001
Bactrocera peneobscura PNC001
Bactrocera passiflorae PAS002
Bactrocera facialis FAC006
Bactrocera facialis FAC007
Bactrocera nigrescentis NGS001
Bactrocera recurrens REC003
Bactrocera recurrens REC001
Bactrocera manskii MAN001
Bactrocera manskii MAN003
Bactrocera lampabilis LAM001
Bactrocera endiandrae END007
Bactrocera endiandrae END010
Bactrocera endiandrae END006
Bactrocera endiandrae END002
Bactrocera endiandrae END005
Bactrocera endiandrae END008
B. musae complex
B. dorsalis complex
B. bryoniae complex
B. bidentata complex
B. distincta complex
B. fulvicauda complex
B. quadrata complex
B. dorsalis complex
B. silvicola complex
B. dorsalis complex
B. quadrata complex
B. nigella complex
B. notatagena complex
B. alyxiae complex
B. mayi complex
B. distincta complex
B. dorsalis complex
B. bidentata complex
B. quadrata complex
B. recurrens complex
B. bryoniae complex
B. silvicola complex
B. musae complex
B. frauenfeldi complex
B. dorsalis complex
B. distincta complex
B. fagraea complex
B. recurrens complex
B. dorsalis complex
Bactrocera (Bactrocera)
Bactrocera (Neozeugodacus)
Bactrocera (Bulladacus)
Bactrocera (Parazeugodacus)
Bactrocera (Bactrocera)
Bactrocera (Calodacus)
Bactrocera

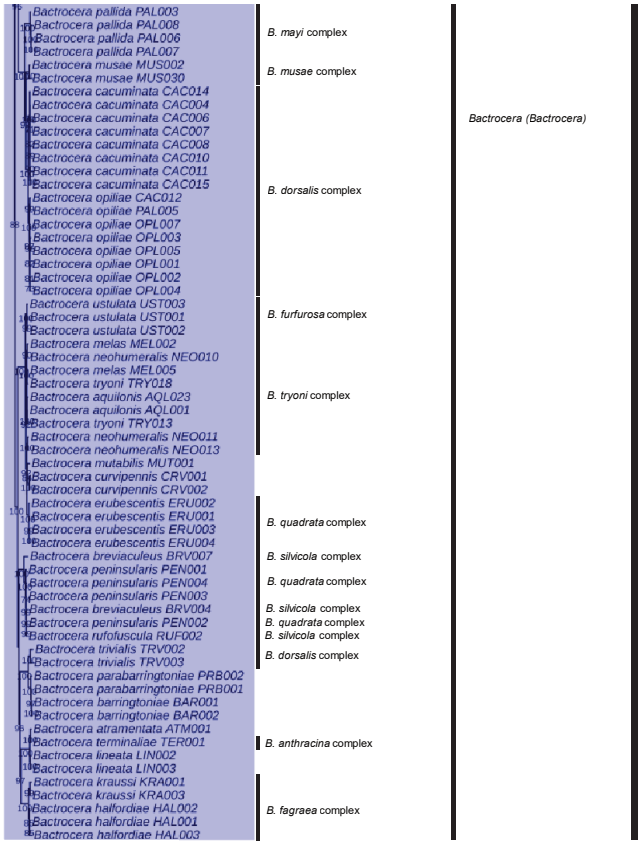


Table 3.6: Taxonomic groupings of Dacini subgenera (left) according to Hancock and Drew (2015, 2017b, 2018a), compared to the molecular groupings resolved in this chapter (right). Most groupings are within the genus *Bactrocera,* however the genus *Zeugodacus* is also included here along with the hypothesised ancestral subgenus *B.* (*Hemizeugodacus*) (Hancock and Drew, 2018a) and subgenus *B.* (*Tetradacus*) which has been placed within a grouping of its own as per Hancock and Drew (2018a). Bolded subgenera are those that were not resolved within their current taxonomic group, and instead were placed within another group based on the molecular analysis (or were paraphyletic as is the case of *B.* (*Bactrocera*)).

Current taxonomic classification	Molecular 'groupings'
Bactrocera **group of subgenera**	**genus** *Bactrocera* **(Clade 1)**
Apodacus	***Bactrocera***
Bactrocera	*Calodacus*
Bulladacus	
Calodacus	

Current taxonomic classification	Molecular 'groupings'
Melanodacus **group of subgenera**	**genus** *Bactrocera* **(Clade 2)**
Daculus	***Apodacus***
Hemizeugodacus	***Bactrocera***
Neozeugodacus	***Bulladacus***
Notodacus	*Daculus*
Parazeugodacus	*Neozeugodacus*
	Notodacus
	Parazeugodacus
	Tetradacus
Zeugodacus **group of subgenera**	**genus** *Zeugodacus*
Austrodacus	*Austrodacus*
Javadacus	*Javadacus*
Papuodacus Drew	*Papuodacus*
Parasinodacus	*Parasinodacus*
Sinodacus	*Sinodacus*
Zeugodacus	*Zeugodacus*
Considered 'ancestral/primitive' and sister to all *Bactrocera*	Sister to all *Bactrocera* (Clades 1 and 2)
Hemizeugodacus	***Hemizeugodacus***
Tetradacus **group**	
Tetradacus	

3.3.2.2. Species complexes

Within *Bactrocera* (*Bactrocera*), the *B. tryoni* complex, based on prior understanding (Drew 1989), is the most economically important species complex in Australia, containing and consisting of four of Australia's most important pest fruit fly species: *B. tryoni*, *B. neohumeralis*, *B. melas*, and *B. aquilonis*. In the phylogeny, the complex is shown to be larger than just these four species, with the addition of another four previously under-sampled species: *B. erubescentis* (Drew & Hancock), *B. mutabilis, B. curvipennis, B. ustulata*. These eight species sit within two main clades. The first clade consists of *B. ustulata* Drew as sister to an unresolved clade of the four original members of the complex. The second clade consists of *B.*

erubescentis, B. mutabilis, B. curvipennis and two *B. neohumeralis* specimens, all of which were well resolved. *B. neohumeralis* specimens are present in both clades.

Also within the genus *Bactrocera,* the *scutellaris* (Bezzi)*, musae* (Tryon)*, dorsalis, bryoniae* (Tryon)*, bidentata* (May)*, distincta* (Malloch)*, quadrata* (May)*, mayi* (Hardy)*, recurrens* (Hering)*, silvicola* and *fagraea* (Tryon) species complexes were all paraphyletic*,* calling into question the usefulness of these species groupings.

3.3.3. Other techniques for measuring and calculating node and branch support

The results of the SH-aLRT and site-resampling technique are presented in Appendices 11 & 12. There was very little difference between the bootstrap values and the SH-aLRT values, with the majority of branches receiving high support. In very few cases, the values were dramatically different, for example the branch connecting *Z. depressus* to the larger clade containing *Z. platamus, Z. hochii, Z. cucumis, Z. cucurbitae, Z. tau* and *Z. choristus* has an SH-aLRT value of only 28.9, but a bootstrap value of 98. The site-resampling technique also showed similar results to the bootstrap values obtained via regular UFboot sampling, with barely any difference in support values, in fact, some deeper nodes had higher support values using site-resampling. Overall, there were minimal differences between the three branch support methods calculated, indicating good support and confidence in the relationships presented in Fig. 3.2. Note, for the SH-aLRT approach, there are not yet internationally accepted ‘rules’ of what values constitute strong or weak node support, although previous studies have applied a position that branches are well supported if they have an SH-aLRT value > 80% (Labeda et al., 2017, Minh et al., 2013, Dupuis et al., 2018). However, how this 80% threshold was first determined is not clear in the literature.

3.3.4. Placement of rare pinned and undescribed species

Figure 3.3 shows the relevant portions of the re-constructed tree using just COI barcode data, with the addition of the pinned species and the newly described species from the previous chapter. *Bactrocera* sp. A (VFL001) was most closely related to

another undescribed *Bactrocera* species (DSP001) (Fig. 3.3A); *B. strigata* was sister to *B. silvicola* (Fig. 3.3B); *B. mendosa* was sister to the *B. laticaudus* (Hardy) + *B. mayi* complex clade (Fig. 3.3C); and *B. nigrovittata* was sister to *B. perkinsi* (Drew & Hancock) + *B. abdonigella* (Drew) clade (Fig. 3.3D).

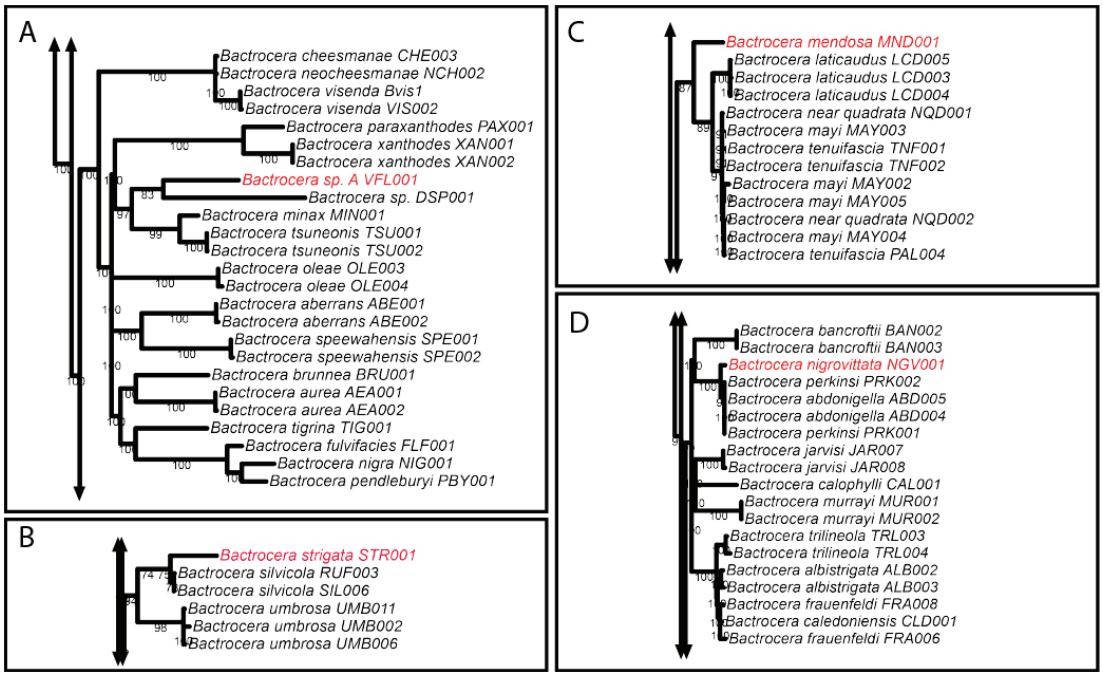


Figure 3.3: Relevant portions of the ML reconstructed tree estimated with COI barcode sequence data using the linked tree topology (Fig. 3.2) (based on mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L) as the contrained backbone. A: *Bactrocera* sp. A VFL001; B: *B. strigata* STR001; C: *B. mendosa* MND001; and D: *B. nigrovittata* NGV001. UFBoot values >74 are depicted on the nodes.

3.3.5. Dated phylogenetic tree

The dated phylogenetic tree is presented in Fig. 3.4. I found that *Bactrocera* split from *Dacus* + *Zeugodacus* approximately 41 mya (95% CI = 33.61-46.11) with *Dacus* and *Zeugodacus* splitting not long after that at approximately 40 mya (95% CI = 32.7-45.23). The majority of *B.* (*Bactrocera*) (Clade 1) split off approximately 37 mya (95% CI = 28.71-39.85), with diversification still occurring up until recent

history. Further discussion of the speciation of the Dacini will be undertaken in Chapter 5, which will be accompanied by a more in-depth analysis.

3.3.5.1. Australian radiations and speciation

The phylogeny shows that multiple Dacini incursions into Australia, followed by local radiations, have occurred through evolutionary history, the majority within the last 10 mya (avg. 95% CI = 3-18). This includes, but is not limited to, many species within Clade 2 (which includes multiple endemic subgenera), the *B. mayi* species complex + *B. laticaudus* clade, and an Australian clade of species from the *B. dorsalis* species complex. Additionally, the phylogeny identifies multiple incursions from Australia into and out of New Guinea and the Pacific, with clades such as the *B. tryoni* and *B. frauenfeldi* species complexes consisting of groups of species occurring in all three localities; Australia, New Guinea and the Pacific.

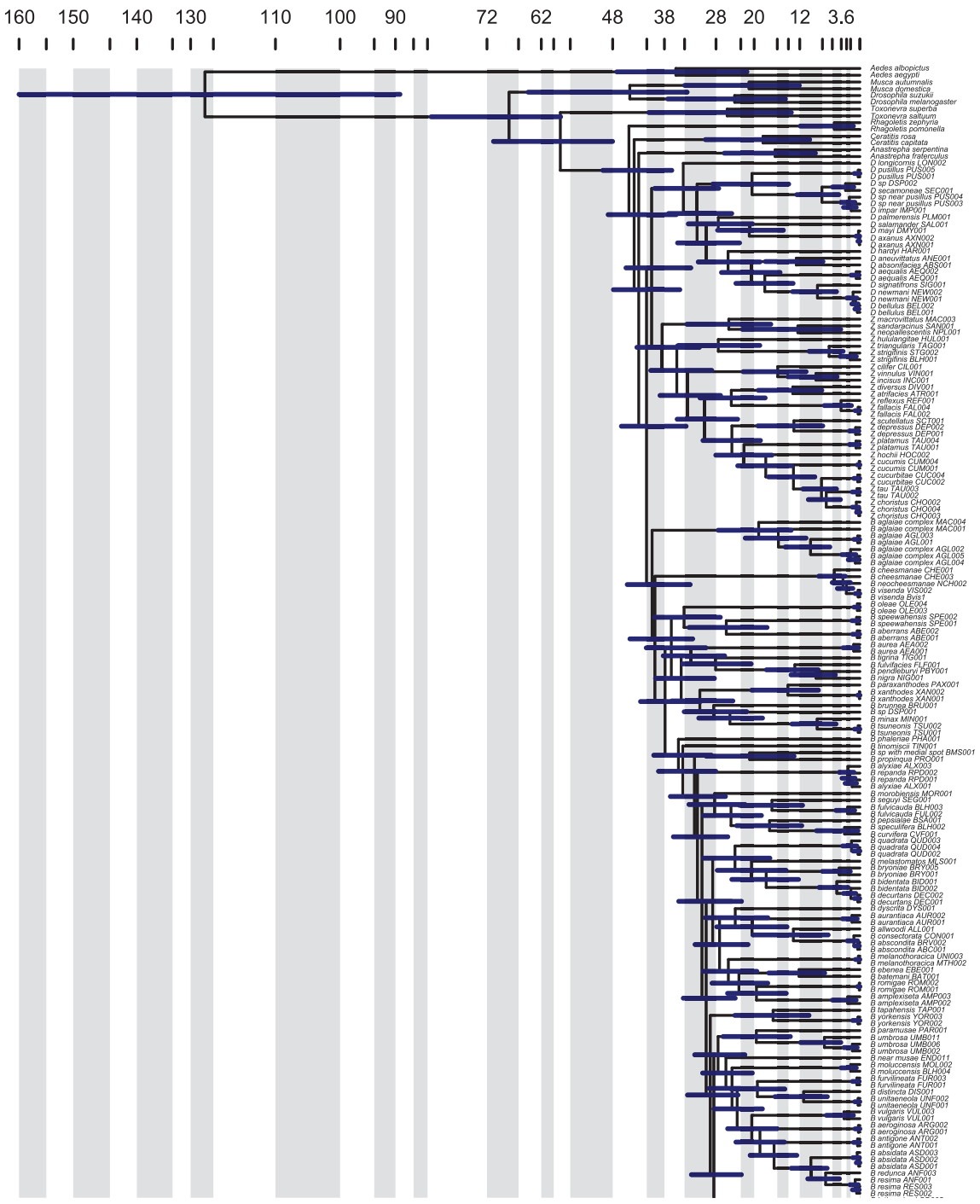
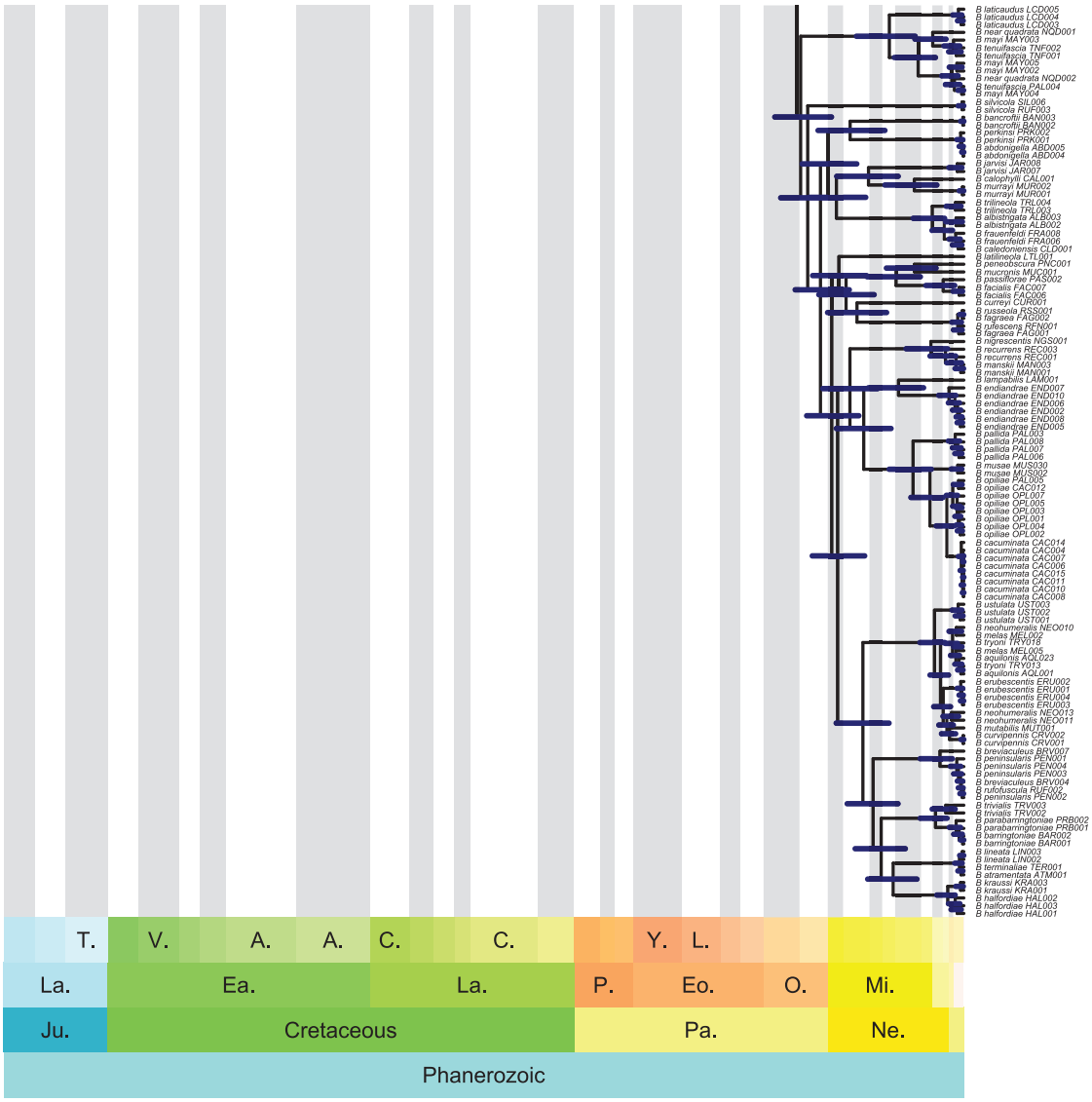


Figure 3.4 (continued next page): Bayesian inference chronogram for the Dacini reconstructed from the COI, COII, 16S, DDOSTs2, RPA2 and EIF3L partitioned molecular dataset and three fossil calibrations using MCMCTree. Geological time scales are presented along the x axis with millions of years illustrated above. 95% confidence intervals are represented by the blue bars on each node. Figure produced using R package MCMCtreeR (Puttick and Title, 2019).



3.3.6. Ancestral reconstructions of lure response and host breadth

3.3.6.1. Lure response

Figure 3.5 demonstrates the ancestral states at each node on the dated phylogeny. It is evident that the ancestral lure response is cue-lure, with multiple transitions to other lure responses over evolutionary time. In this case, there are at least four separate transitions from cue-lure to methyl eugenol responsiveness, and similarly, three separate transitions from cue lure to zingerone responsiveness. Transitions from cue-lure to isoeugenol appeared twice on this tree, and both occurred within the last 5my.

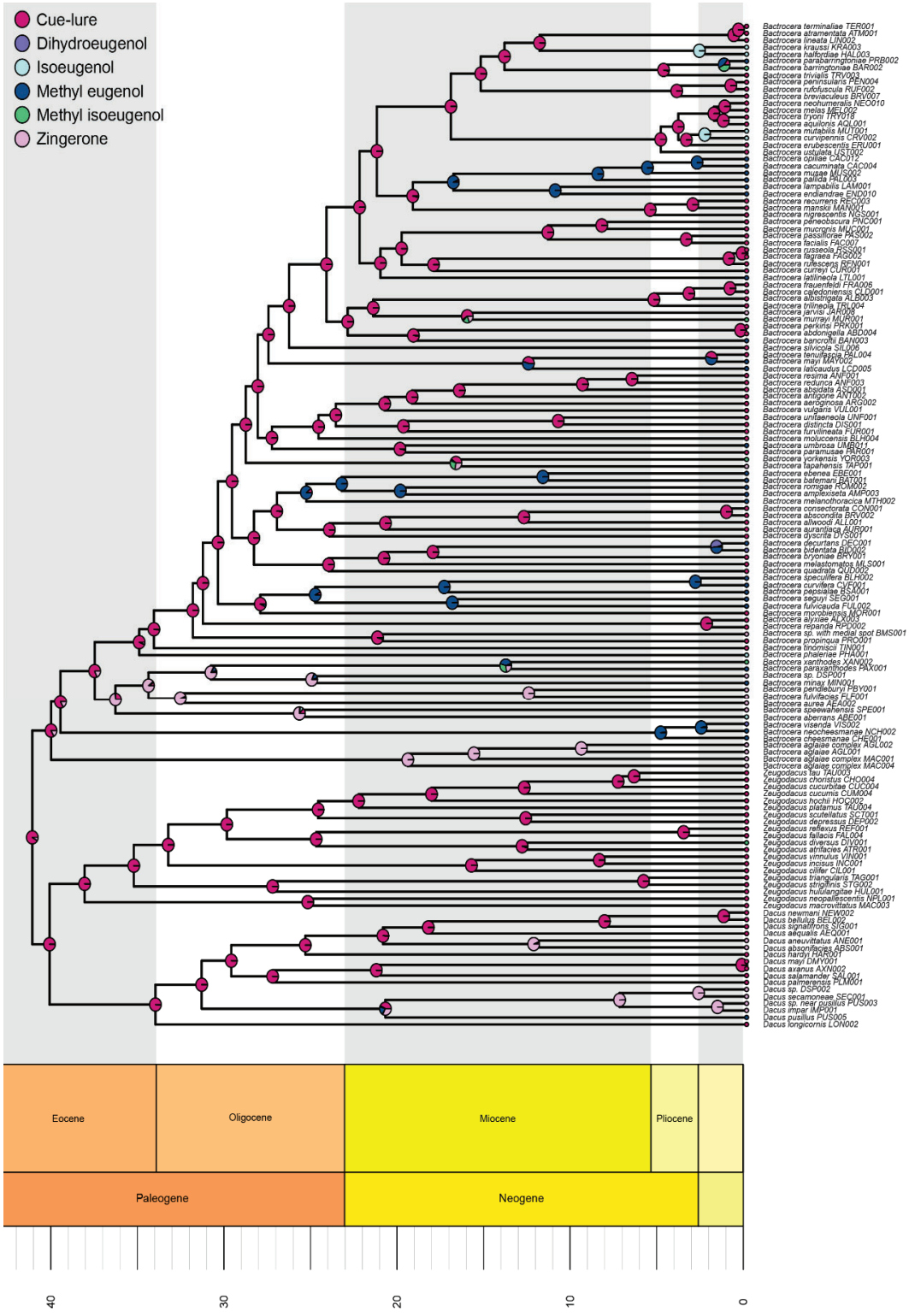


Figure 3.5: Ancestral state reconstruction of the lure response trait of a subset of Dacini fruit flies calculated in R (refer to methods for specific packages). Pie charts on nodes denote the likelihood of that ancestor exhibiting that trait. Tip circles represent the species' scored response.

3.3.6.2. Host breadth

The two host breadth trees provide very different insights. Figure 3.6 demonstrates a higher occurrence of monophagy towards the basal nodes, and a higher occurrence of polyphagy within *Bactrocera* (*Bactrocera*). All traits have arisen more than once, however not in a way that correlates with clades identified in the tree. Despite patterning at the nodes, traits appear to be randomly distributed at the tips. Additionally, the complexity of the pie charts illustrates a large amount of uncertainty of ancestral states in the Eocence and Oligocene, which may indicate a lack of sufficient sampling.

When the data was reduced to the binary classifications of specialist and generalist, a trend was more apparent (Fig. 3.7), with it being evident that the Dacini have evolved from generalist to specialist diet breadths, but with the transition from generalism to specialism occurring only infrequently. Dietary specialism is also more prominent within *Dacus* and *Zeugodacus* with a much smaller number of specialist species within *Bactrocera.*

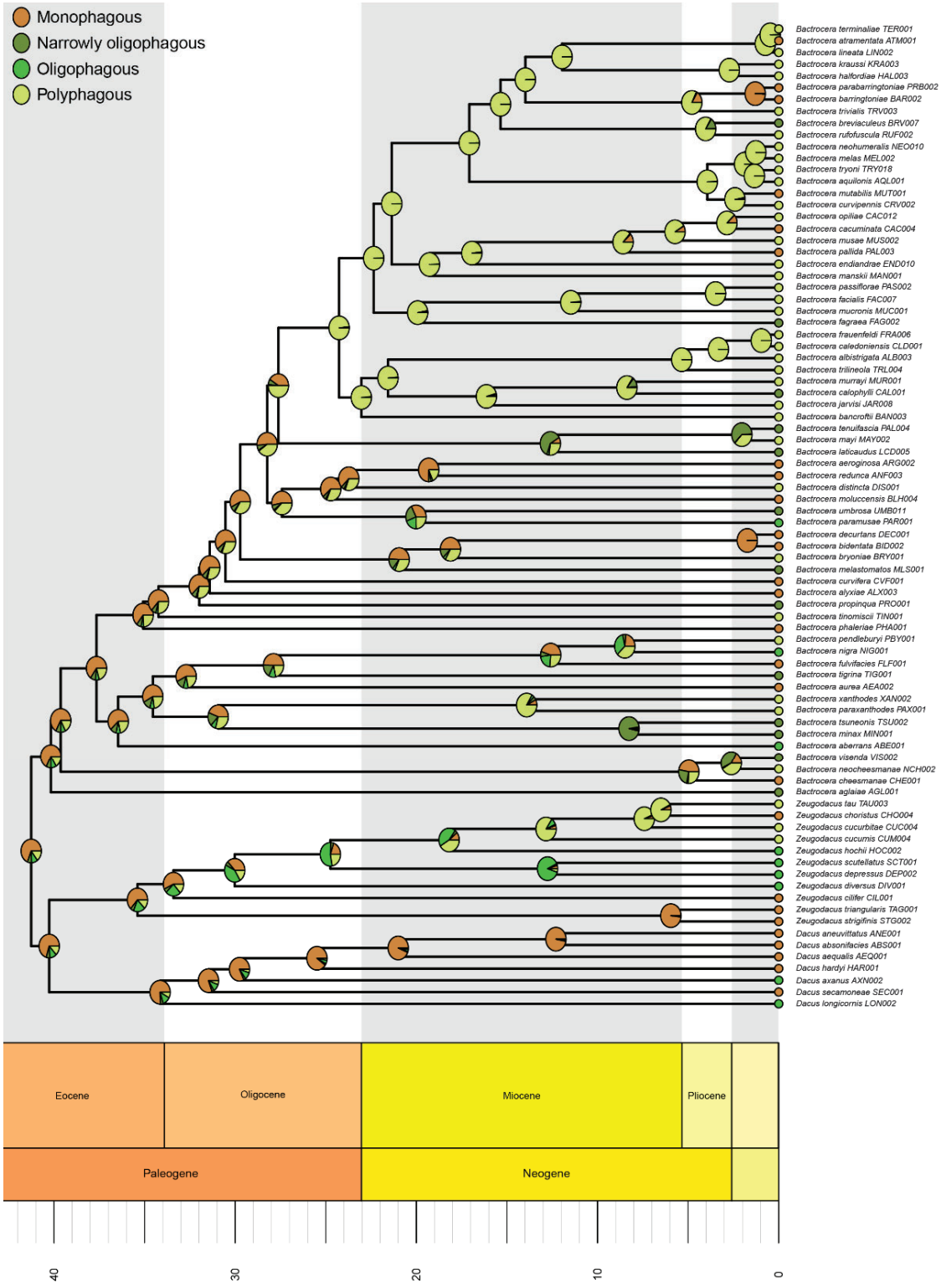


Figure 3.6: Ancestral state reconstruction of four host breadth traits of a subset of Dacini fruit flies calculated in R (refer to methods for specific packages). Pie charts on nodes denote the likelihood of that ancestor exhibiting that trait. Tip circles represent the species' scored host breadth.

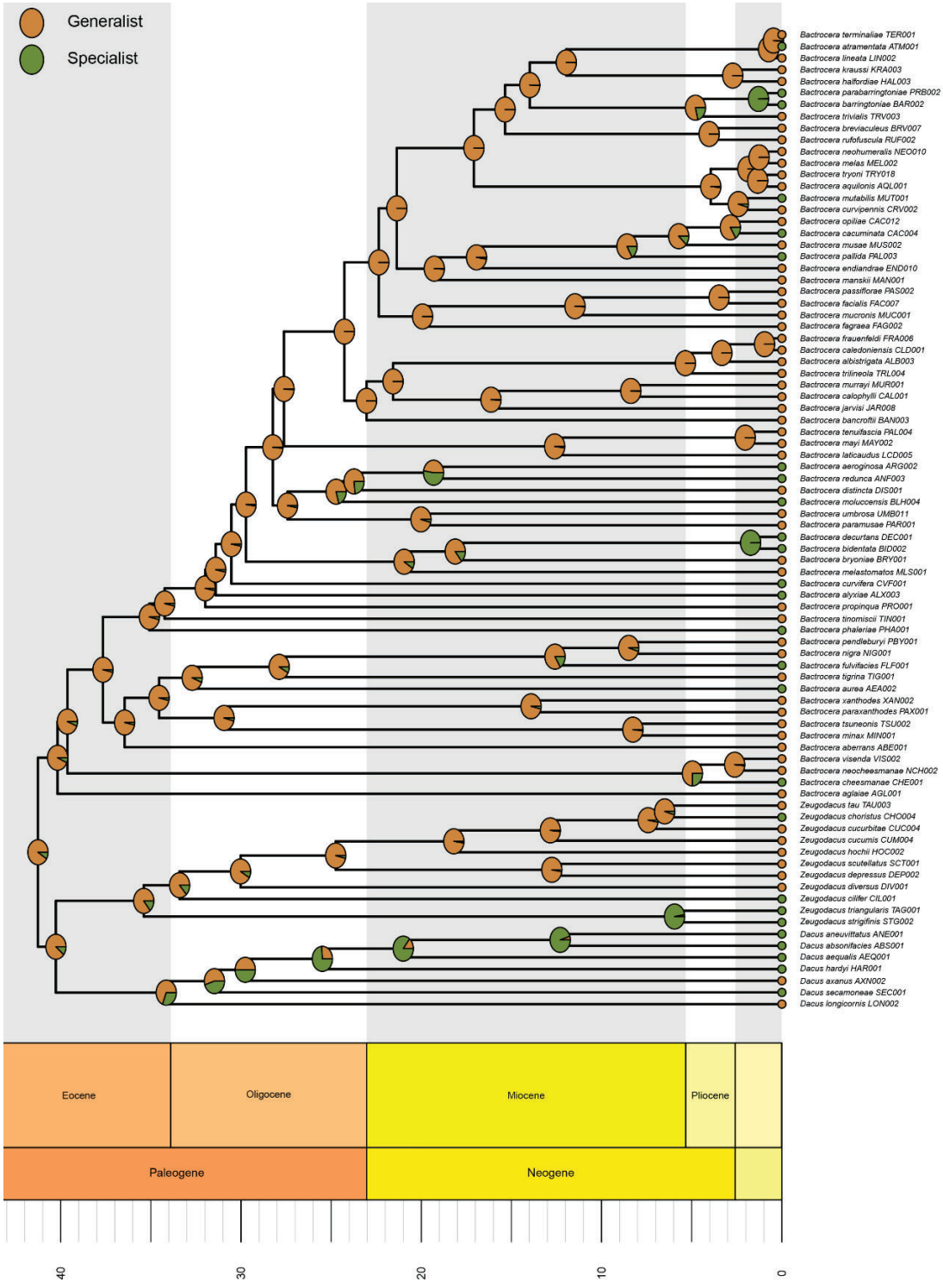


Figure 3.7: Ancestral state reconstruction of the combined host breadth traits (generalist and specialist) of a subset of Dacini fruit flies calculated in R (refer to methods for specific packages). Pie charts on nodes denote the likelihood of that ancestor exhibiting that trait. Tip circles represent the species' scored host breadth.

3.3.7. Phylogenetic signal

The results of the three statistical tests for phylogenetic signal across the two subset trees (lure response and binary host breadth) are presented in Table 3.7. On a scale that can range between <0 and >1, a low D-statistic indicates the traits are highly conserved, whereas a high D-statistic can indicate that the traits to not exhibit phylogenetic signal (Fritz and Purvis, 2010). Pagel's lambda indicates there is phylogenetic signal when the value is close to 1, and less signal as it approaches zero (Ewers et al., 2013). Both tests were consistent in demonstrating high to very high phylogenetic signal for lure response. However, phylogenetic signal was far weaker for diet breadth based on the D-statistic and Pagel's lambda.

Table 3.7: Summary statistics of D-statistic and Pagel's lambda calculated for phylogenetic signal for Dacini lure response and host breadth.

Lure response		Host breadth	
D-statistic	Pagel's lambda	D-statistic	Pagel's lambda
-0.18 Probability of E(D) resulting from no (random) phylogenetic structure: <0.0001	0.99 p-value <0.0001	0.45 Probability of E(D) resulting from no (random) phylogenetic structure: 0.009	0.40 p-value = 0.05

3.3.8. Correlation between host breadth and lure response

The log Bayes factor is calculated by the model: Log BF = 2 (log marginal likelihood complex model (dependent model) – log marginal likelihood simple model (independent model)). Fitting the values from the BayesTraits analysis, to test for correlation between host breadth and lure response: Log BF = 2 ((-104.31) - (-101.01)) = -6.6. Typically, a log Bayes factor approaching or surpassing +10 would indicate a correlation between two traits (Meade and Pagel, 2016). With a logBF

score of -6.6, no correlation between lure response and host breadth is considered to concur.

3.4. Discussion

My phylogeny is the first near-comprehensive, continent-wide phylogeny for the Dacini. As well as exceeding 80% coverage of the Australian fauna, I included closely related species from Papua New Guinea and the Pacific islands, with the addition of some South-east Asian taxa where relevant. Generally I found similar results to other large Dacini phylogenies, in that the three main genera are monophyletic, but with a large amount of paraphyly among subgeneric and species groupings (Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018, Dupuis et al., 2018). Support across the tree was corroborated with multiple topology and branch tests, indicating confidence in the construction. If nodes were supported by >75 bootstrap, I assumed that this was a well resolved relationship. When determining new species (for example those in the *B. aglaiae* species group – Chapter 6), several factors were taken into consideration: branch length, node support, sequence divergence at individual loci, as well as geographic, host use and morphological data. I recognise that many taxonomic species included in this phylogeny may not hold up to such strict standards (i.e., very small sequence differences and short branch lengths between *B. passiflorae* and *B. facialis*), and this would need to be revisited in greater detail for further taxonomic revision, but not here in this thesis. In this section I will discuss systematics, the results from the dating analysis and the implications of the ancestral state reconstructions.

3.4.1. Systematics

The relationships resolved in the phylogeny are in disagreement with current taxonomic classifications, with numerous instances of paraphyly of the subgenera and species complexes. Here I present a discussion of novel findings and their implications.

3.4.1.1. Outcomes from concentrated geographic sampling

As a result of thorough geographic coverage, my phylogeny sampled more subgenera than previous studies (San Jose et al., 2018, Krosch et al., 2012), which resulted in the identification of many paraphyletic relationships and two main clades within the genus *Bactrocera* (discussed in greater detail later). Improved sampling also provides greater confidence in my dates for this group, as divergence time estimates are greatly improved with increased taxa (Soares and Schrago, 2015). With that said, in order to get wider coverage, I did not sample as widely at the population level. I chose this approach because the intent was not to investigate species at the population level, but to look at deeper relationships. Given the approach for identification confirmation I outlined in the methods, my confidence in the results is high; especially considering I have sampled more individuals at the species level compared to other larger phylogenies on the tribe (Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018).

Due to this comprehensive taxon sampling and thus a subsequently higher likelihood that the species sampled are more closely related, I can be more confident that the time to the most recent common ancestor (mrca) for each species is more accurate than for the taxa sampled in Krosch et al. (2012). For example the mrca of *Bactrocera, Dacus* and *Zeugodacus* could be estimated as much younger if I weren't to include *B.* (*Hemizeugodacus*) and the numerous other subgeneric groupings that were not sampled by Krosch et al. (2012). In terms of character mapping, in particular host breadth, there were less species included in the tree due to the lack of available data. Nevertheless, if I was utilizing a different dataset comprised predominantly of the well-studied pest taxa (as has been the case for many dacine trees, Table 3.1), this tree may look drastically different, with the data biased toward polyphagy. A disadvantage of concentrated sampling may be encountered when applying this dated phylogeny to biogeographical questions, as the Australia/Pacific could be limiting. My coverage is not extensive enough that other regions (such as China, India and Africa) are equally represented in my dataset. These issues are discussed further in Chapter 5.

3.4.1.2. Clades within the Dacini

All three genera were resolved into monophyletic clades, which agreed with previous molecular studies (Krosch et al., 2012, Virgilio et al., 2015, Dupuis et al., 2018, San Jose et al., 2018). However, unexpectedly, two main clades were resolved within *Bactrocera.* The first clade consisted of *B.* (*Bactrocera*) and *B.* (*Calodacus*); and the second clade consisted of *B.* (*Parazeugodacus*), *B.* (*Bulladacus*), *B.* (*Neozeugodacus*)*, B.* (*Daculus*), *B.* (*Tetradacus*)*. B.* (*Notodacus*), *B.* (*Apodacus*) and some species from *B.* (*Bactrocera*) and *B.* (*Hemizeugodacus*) was sister to both clades. The two clades, which split 37 mya (95% CI = 30.82-42.41), each contain species endemic to Australia and New Guinea, but also to the greater South-east Asian and Pacific regions. This strongly infers multiple *Bactrocera* incursions into and out of Australia. Large speciation events have occurred within species clades that are largely Australian and Pacific in origin, for example, the *B. tryoni* species complex (discussed in greater detail later). Additionally, there are multiple occurrences throughout the tree of small isolated clades, within larger groups, that consist of Australian endemics (e.g. the Australian *B. dorsalis* species complex clade). Such clades in most cases are intermixed with species from New Guinea, indicating multiple to and from New Guinea-Australian incursions in the last 30my. The biogeographic movements of species into Australia will be investigated in greater detail in Chapter 5.

3.4.1.3. Subgeneric relationships

Most of the subgeneric groupings are paraphyletic in my phylogeny, which is in agreement with many previous studies. Muraji and Nakahara (2001) found that *B.* (*Gymnodacus*) *calophylli* Perkins and May (now transferred to subgenus *Calodacus*) fell within *Bactrocera* (*Bactrocera*), a finding supported by Zhang et al. (2010) and Krosch et al. (2012) and again here in my study. Within the genus *Zeugodacus, Z.* (*Sinodacus*) and *Z.* (*Zeugodacus*) are identified both here and in San Jose et al. (2018) as paraphyletic. There was also agreement between my study and San Jose et al. (2018) on the paraphyly of *Dacus* (*Mellesis*), despite the two studies sampling different species, taxon sampling was identical for the subgenus *Z.* (*Parasinodacus*) across my study and San Jose et al. (2018) with both studies agreeing on the

monophyly of the subgenus. With the addition of the results from my work, there is now an abundance of evidence that suggests that taxonomic classifications do not agree with molecular relationships (Virgilio et al., 2015, Dupuis et al., 2018, San Jose et al., 2018, Krosch et al., 2012); suggesting there is a need for a deeper review to resolve these relationships. The utility of Dacini morphological traits for systematics is examined in the next chapter.

3.4.1.4. Ancestral hypotheses

Previous authors have discussed the origin of the genus *Bactrocera,* with different hypotheses proposed. The subgenus *B.* (*Tetradacus*) was suggested to be the ancestral subgenus by Hancock and Drew (2015), due to shared characters between *Dacus* and *B.* (*Tetradacus*). However, after these authors reviewed molecular evidence which suggested *B.* (*Daculus*) and *B.* (*Parazeugodacus*) could be potential ancestral subgenera (San Jose et al., 2018), they hypothesised that due to the presence of medial vitta, that subgenus *B.* (*Hemizeugodacus*) may be the ancestral subgenus (Hancock and Drew, 2018a). San Jose et al. (2018) did not sample *B.* (*Hemizeugodacus*), but found that *B.* (*Apodacus*) *visenda* and *B.* (*Tetradacus*) *minax* (Enderlein) formed a clade that was sister to the remainder of all other *Bactrocera* species. Dupuis et al. (2018) (also missing *B.* (*Hemizeugodacus*)) had similar findings, however the addition of an undescribed (possibly *B.* (*Tetradacus*)) species resolved this species within a clade with *B. minax* (with *B. visenda* a sister clade) as sister to all remaining *Bactrocera.* My sampling included multiple species from *B.* (*Apodacus*) and *B.* (*Tetradacus*), and I found that the newly defined *B.* (*Hemizeugodacus*) *aglaiae* species group was well supported via bootstraps in the phylogeny as sister to all other *Bactrocera,* with *B.* (*Tetradacus*) and *B.* (*Apodacus*) well supported within Clade 1. I did not include any other species from within *B.* (*Hemizeugodacus*) so cannot comment on the taxonomy of this group, however, our results agreed with the hypothesis presented by Hancock and Drew (2018a) that *B.* (*Hemizeugodacus*) is the ancestral subgenus of *Bactrocera,* further explored in Chapter 6.

3.4.1.5. Species complexes

In addition to paraphyly of subgenera, a large majority of the species complexes were also not monophyletic. When comparing again to San Jose et al. (2018) (ten species complexes), my study (14 species complexes), also had very minimal success in resolving complexes as monophyletic clades. My only monophyletic clades were the *B. tryoni*, *B. frauenfeldi* and *B. alyxiae* (May) species complexes. Despite this, within all three complexes, species level resolution was not achieved; similar results were also reported for the *B. tryoni* and *B. frauenfeldi* complexes in the large phylogenomic study of Dupuis et al. (2018). This suggests that integrative taxonomic efforts, targeted for each species complex, such as those employed by Schutze et al. (2015a) for a very small number of species within the *B. dorsalis* species complex, would be required for resolution and species delimitation of difficult groups.

3.4.1.6. Bactrocera tryoni *species complex*

The *B. tryoni* complex, previously considered to include just *B. tryoni, B. neohumeralis, B. melas* and *B. aquilonis* (Drew, 1989), was found to contain an additional four species: *B. erubescentis, B. mutabilis, B. curvipennis* and *B. ustulata*, phylogenetically placed within two clades. Previous studies have resolved *B. curvipennis* within the complex (Smith et al., 2003, Krosch et al., 2012), and the results of this analysis support this placement. It is noteworthy that this newly expanded 'complex' now contains species that respond to different lures and are geographically spread over a wider range (*B. curvipennis* is from New Caledonia while *B. ustulata* is from New Guinea). *Bactrocera neohumeralis* specimens were present in both clades and while polyphyly of *B. neohumeralis* has been shown in other phylogenetic studies, the original four members of the complex are also always polyphyletic (Blacket et al., 2012, Dupuis et al., 2018). With the addition of these other closely related species, there is better resolution of the relationships and it is clear that some *B. neohumeralis* specimens are genetically different from others, at the population, or potentially species level. As the most important fruit fly species complex in Australia; the taxonomic resolution of this group will be investigated further in Chapter 7.

3.4.2. Dated phylogeny

I found that the sequence of events for the evolution of the Dacini follow the same chronology as that obtained by Krosch et al. (2012), however there was a pattern of younger node dates observed throughout the cladogram. For example, key splits such as *Dacus* + *Zeugodacus* from *Bactrocera* were estimated by Krosch et al. (2012) to have occurred approximately 72.2 mya (95% CI = 59.3-86.3) whereas my findings indicate this occurred around 41 mya (95% CI = 33.61-46.11) . This may have been due to different methods of analysis and fossil calibrations. Furthermore, my fossils are more closely related to each other than those used by Krosch et al. (2012), which in comparison to my study spanned deeper nodes of their phylogeny. My dates for the origins of the Tephritidae are closer to those obtained by Kitto (1983), who used biochemical techniques (microcomplement fixation) to measure changes in the enzyme α-glycerophosphate dehydrogenase, which evolves at a constant rate, and is not plagued with issues such as homoplasy (a problem commonly encountered in Dacini morphology). Kitto (1983) found that the ancestors of the Tephritidae arose roughly 55 mya, which is slightly older than my results which predicted 44 mya (95% CI = 36.37-49.69); additionally Kitto's predictions place the origins of the Tephritoidea somewhere between 70-90 mya and overlap only slightly with my findings of 58 mya (95% CI = 47.82–70.83), however there are no confidence intervals provided by Kitto in any of this work for direct comparison of ranges. Wiegmann et al. (2011) and Han and Ro (2016) produced higher level phylogenies of the Diptera and Schizophora respectively. Despite both studies placing hard constraints on the Schizophora at 70 mya, my cladogram agrees with their findings, suggesting the mrca of the Schizophora arose 67 mya (95% CI = 57.87-82.89); this was achieved without imposing hard constraints. Han and Ro (2016) estimated the Tephritoidea arose 58 mya (95% CI = 23.69-93.80), which is a wider confidence interval than my interval estimate of 47.82–70.83 mya, however this in agreement. A similar trend was evident when comparing dates for the Tephritidae at 51.24 mya (95% CI = 20.59-81.89) (Han and Ro, 2016) compared to my estimate of 36.37-49.69 mya.

3.4.3. Ancestral states and phylogenetic signal – lure response

I found that cue-lure response was the ancestral male lure for the Dacini fruit flies in my subset tree. This is in agreement with other studies that have hypothesised cue-lure (or raspberry ketone – the hydrolysed form found in nature) is the ancestral lure type based on biogeography, trait mapping and ancestral morphology (Metcalf, 1990, Smith et al., 2005, Smith et al., 2003, Smith et al., 2002). Lure response exhibited strong phylogenetic signal throughout the tree, suggesting there was a genetic driver of the response, which may be driven by selection for fitter males. Numerous authors have postulated a link between male lures and sexual selection in dacines (Shelly, 2017, Tan and Nishida, 2000). For example Kumaran et al. (2013) and Kumaran and Clarke (2014) found that *B. tryoni* males that had fed on cue-lure and zingerone had higher mating success and their offspring were more likely to respond to these lures; suggesting a possible increase in fitness for the next generation.

3.4.4. Ancestral states and phylogenetic signal – host breadth

When the host breadth trait was treated as binary, there was phylogenetic signal across that subset tree. The overall trend suggests that species are evolving from generalist to specialist, an evolutionary pathway supported by many theoreticians (Loxdale et al., 2011, Loxdale and Harvey, 2016, Loxdale et al., 2019). However, the number of generalists is still far greater than specialists, with a high rate of polyphagy observed within *Bactrocera*. This has been discussed by (Clarke, 2017), who identified four drivers, based on environmental factors, that could explain why specialism (= monophagy), exhibited by >90% of insect herbivores, is relatively infrequent within *Bactrocera,* compared to generalism (= polyphagy) which is common. This suggests that perhaps there are other influences (or lack thereof) that drive specialisation in the Dacini.

3.4.5. Utility of dated phylogeny

Here, comprehensive sampling allowed for deep investigation of trait evolution in the Australian and Pacific fruit flies. This concentrated sampling will be utilised for

the following chapters in order to test the utility of morphology, and investigate the influences of biogeography on movement of species in the region.

Chapter 4: Morphology in a phylogenetic context

4.1. Introduction

Morphological analyses of the tephritids have informed behavioural ecology (Dodson, 2000), supported investigations of species' distribution (Kubota et al., 2007) and even informed olfaction research (Dodson, 1978, Dickens et al., 1988, Arzuffi et al., 2008, Hu et al., 2010). However, there are very few morphological analyses that have focussed on resolving evolutionary relationships among species within the Dacini tribe, and those that have are lacking in resolution (Michaux, 1996, Michaux and White, 1999, White, 2000). Here I will briefly review the few existing Dacini phylogenies based on morphology, outline some of the important issues associated with incorporating morphological characters into phylogenetics (notably, the importance of identifying whether characters are phenetically informative, phylogenetically informative, or both), and summarise some Dacini-specific morphology issues such as the use of colour and genitalic character traits.

4.1.1. Morphological phylogenetics of the tribe Dacini

Only four studies have focussed on phylogenetic reconstruction of the Dacini (or parts thereof) based solely on morphological character traits (Michaux, 1996, Michaux and White, 1999, Drew and Hancock, 1999, White, 2000). Drew and Hancock (1999) used morphology to produce a tree-like diagram of their hypothesised phylogenetic relationships within the Dacini at the generic and subgeneric levels, with implications for biogeography and systematics also discussed. While drawing on their expert knowledge of Dacini morphology, there was no phylogenetic analysis provided by Drew and Hancock (1999), which leaves only three studies that have formally investigated morphological phylogenetics of the Dacini. Morphological phylogenetic analysis on eight characters within 21 subgenera within *Bactrocera*, found that morphological character traits produced a tree with high support at the basal nodes, but with many clades left unresolved (Michaux, 1996). Michaux and White (1999) produced a morphological phylogeny based on 74 characters of 30 South West Pacific *Bactrocera* species, but could not obtain resolution without weighting characters. This study had representatives from the Pacific islands but did not sample widely from Australia. White (2000) also produced

a phylogeny based on 38 characters and 45 pest species scattered throughout SE Asia and the Pacific region. This study had similar results; a phylogeny with poor resolution, with multiple polytomies across the tree (White, 2000). These studies give clues that either morphological characters are not phylogenetically informative, or that their methods were not appropriate for the character traits used. This prompted the focus of this chapter: investigating the utility of morphological characters in Dacini phylogenetics.

4.1.2. Key terms: phenetics versus cladistics

Phenetics, the basic premise of traditional taxonomy, can be defined as the attempt to classify species based on overall similarity (Sokal, 1986). Similarity is determined using morphological and other observable traits, without regard for their phylogenetic or evolutionary relationships. In literature, the term 'phenetics' is commonly used to refer to taxonomic character trait analyses which uses phenotypic distance to investigate the degree of similarity between taxa based on the character traits analysed (Lidicker, 1973, Sokal, 1986). In contrast, cladistic analyses are concerned with determining the evolutionary relationships and derived traits between the taxa; i.e. their ancestral lineages (Doyen and Tschinkel, 1982). Morphological taxonomic characters can be used in both phenetic and cladistic analyses, as can other character traits such as behaviour and molecular data. However, morphological characters may not be informative for both, the premise of this chapter is to evaluate the use of morphological characters for inferring cladistic relationships rather than phenetic relationships.

4.1.2.1. Issues with using taxonomic characters in phylogenetics

Taxonomic characters are used for the identification of taxa down to the species or sub-species level and are generally utilised via dichotomous keys or multi-access keys (Hagedorn et al., 2012). Whereas phylogenies ideally use all available information in an attempt to resolve evolutionary relationships among three or more species (Huelsenbeck et al., 1996), dichotomous keys use character differences to separate two groups of species, repeating the process with different characters until

left with a single pair of species, which are then themselves split (Osborne, 1963). This means that the same character states may be used to distinguish between species at different points in the same dichotomous key, indicating possible homoplasy of those characters (White, 2000). Multi-access keys use character state data in a matrix formation, rather than dichotomous format, but nevertheless still use shared or different character states to increasingly split groups of taxa until only one is left (Hagedorn et al., 2012). Morphological taxonomic keys may not be able to narrow the results of identification to genetically related species, unless the character states used in the key have phylogenetic signal.

4.1.3. Morphological character states used in Dacini taxonomy

4.1.3.1. Commonly used taxonomic characters

There are numerous character states that have been used to describe and diagnose Dacini species. Some of the most diagnostically useful characters for the Australian Dacini include; on the wing (Fig. 4.1): (A) the presence of microtrichia and colouration in the first and second costal cells; (B) costal band width; (C) width of the anal streak; (D) changes in the width of the costal band; and (E) presence of wing patterning. On the thorax (Fig. 4.2): (A) colouration of the postpronotal lobes; (B) length and number of vittae; and (C) colouration on the scutellum. Additional characters of the thorax include (Fig. 4.3): (A) presence of the supra-alar bristle; and (B) width of the mesopleural stripe. Key abdominal characters include (Fig. 4.4): (A) the presence of pecten on terga III; (B) the shape of the abdomen; and (C) abdominal markings (Plant Health Australia, 2018b).

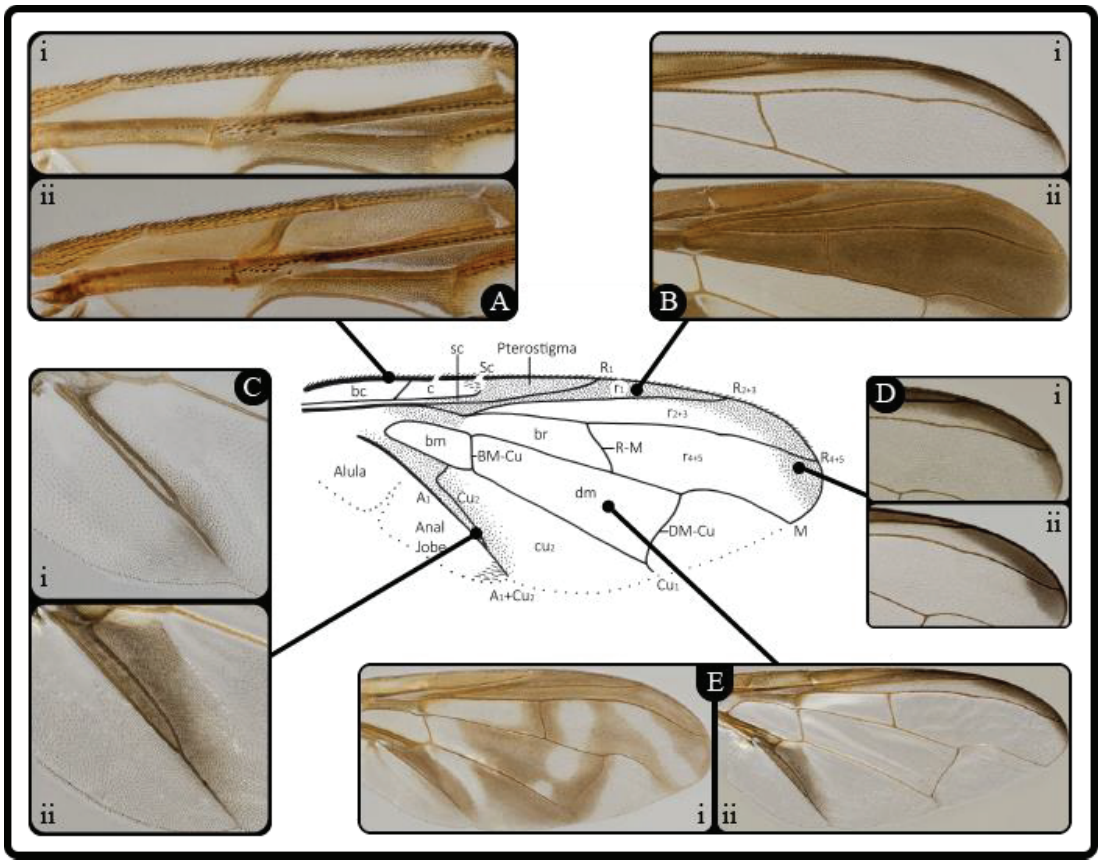


Figure 4.1: Commonly used taxonomic characters for diagnosis and description of Australian Dacini species. A: (i) costal cells without microtrichia or tinting; (ii) costal cells with microtrichia and tinting; B: (i) thin costal band; (ii) wide costal band; C: (i) no anal streak, (ii) wide anal streak; D: (i) costal band of even width; (ii) costal band widening; and E: (i) patterning on the wing; (ii) no patterning on the wing. Central diagram by A. Carmichael; images from Plant Health Australia (2018b).

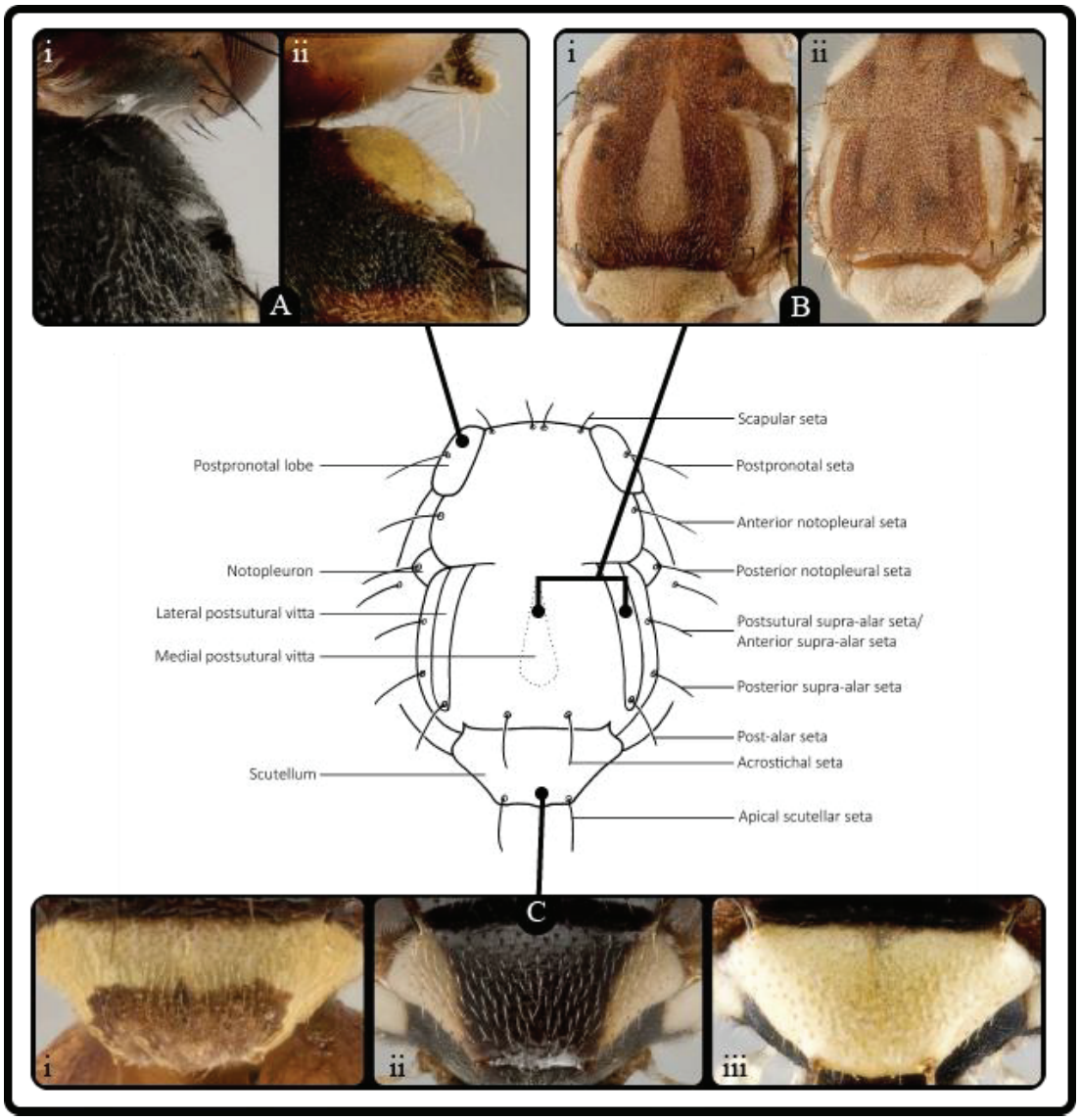


Figure 4.2: Commonly used thoracic taxonomic characters for diagnosis and description of Australian Dacini species. A: (i-ii) colouration of the postpronotal lobes; B: (i) two lateral postsutural vittae and a medial vitta, as well as markings anterior to the mesonotal suture; (ii) two lateral postsutural vittae; and C: (i-ii) common markings present on the scutellum, and (iii) absence of markings on the scutellum. Central diagram by A. Carmichael; images from Plant Health Australia (2018b).

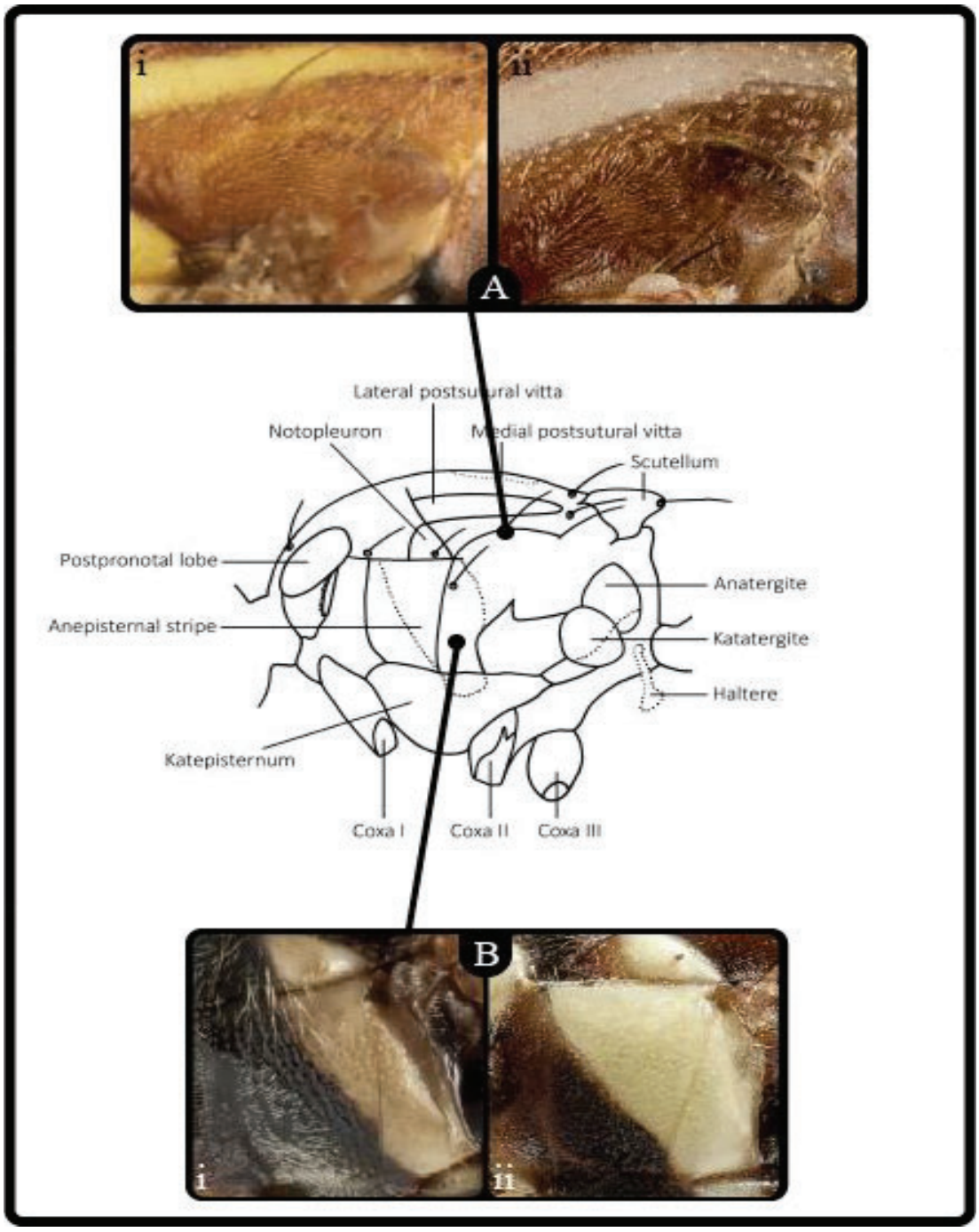


Figure 4.3: Commonly used lateral thoracic taxonomic characters for diagnosis and description of Australian Dacini species. A: (i) dark colouration of the postpronotal lobes, (ii) pale colouration of the postpronotal lobes; B: (i) two lateral postsutural vittae and a medial vittae, as well as markings anterior to the mesonotal suture, (ii) two lateral postsutural vittae; and C: (i-iii) common markings present on the scutellum. Central diagram by A. Carmichael; images from Plant Health Australia (2018b).

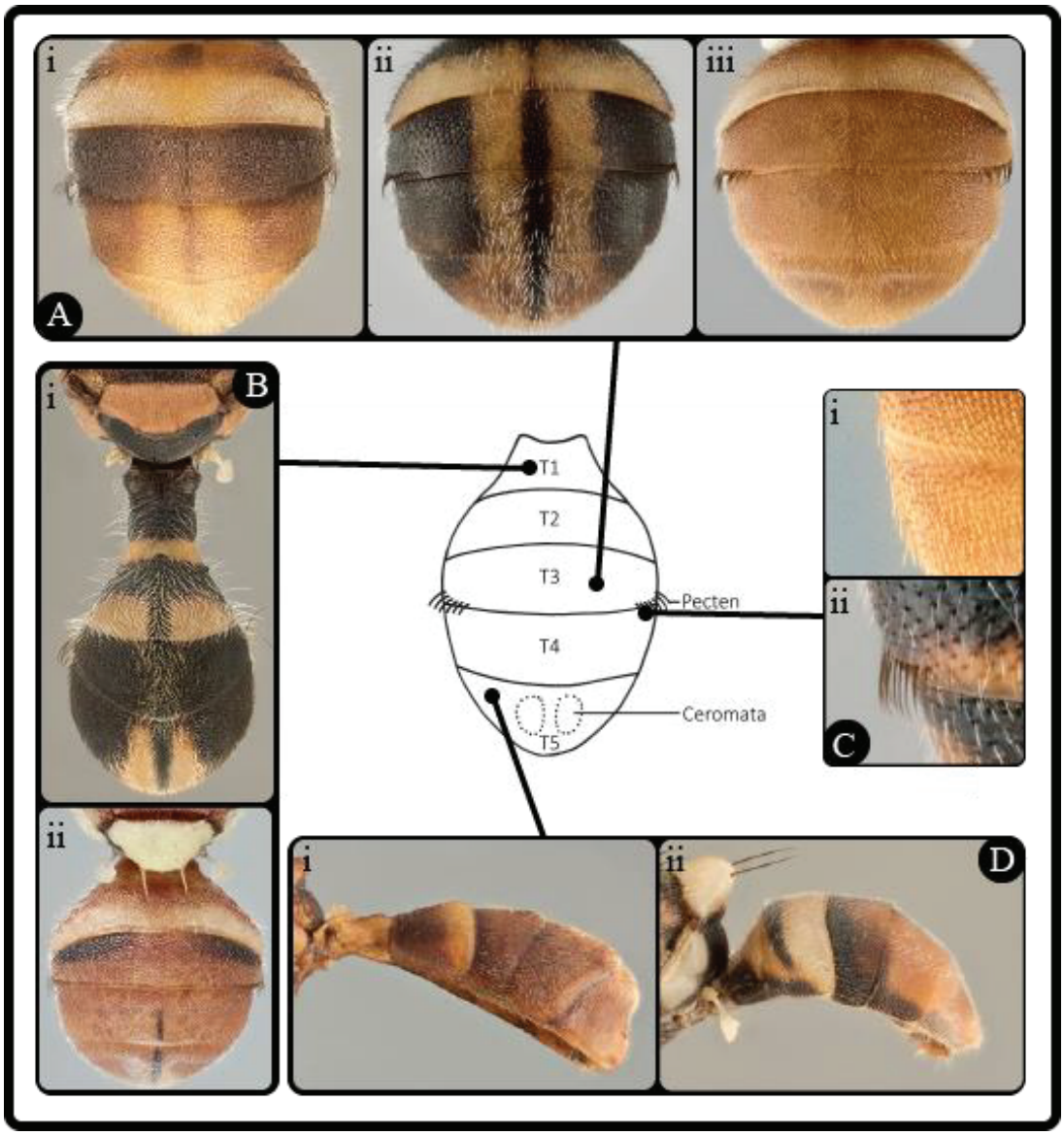


Figure 4.4: Commonly used abdominal taxonomic characters for diagnosis and description of Australian Dacini species. A: (i-iii) patterning on the abdomen; B: (i) elongated abdomen, (ii) oval abdomen; C: (i) pecten on terga III absent and (ii) present; and D: (i) club shaped abdomen, (ii) petiolate shaped abdomen. Central diagram by A. Carmichael; images from Plant Health Australia (2018b).

4.1.3.2. Problematic characters: colour and genitalia

Colour (e.g. red-brown, brown, black) and colour patterns (e.g. abdominal terga with or without a 'T' pattern) have been used as key taxonomic character to describe major pest species (Drew et al., 2005) and remain standard components of species descriptions (Drew and Romig, 2013). Nevertheless, while used extensively in

Dacini taxonomy, colour and colour patterns are widely recognised as highly variable character states within a species (Drew et al., 2005, San Jose et al., 2013, Leblanc et al., 2015, Nair et al., 2017). However, the colour variation within Dacini species is known to be influenced by the age of a specimen when collected, the larval host the specimen was reared from (May, 1953, May, 1963), the geographic location of collection (Schutze et al., 2015b) and the curatorial age and post-collection handling of a specimen (Lawson et al., 2003).

Male and female genitalia are often used in dipteran taxonomy and diagnostics (Eberhard, 2010), but they are not used as extensively in the Dacini. May (1963) found that different populations of the same species were highly variable in female aculei measurements and concluded that there was no way of differentiating among species using this method. The presence and absence of lobes on the preapical margin of the female aculeus was found to be useful for differentiating between a limited number of species (Hardy, 1951), however, further investigation found this character may be homoplasious (Drew, 1972).

Drew (1969, 1972) similarly considered male genitalic characters to be of no taxonomic value. Studies on the male reproductive system found that with age the endoskeleton changed shape and he warned that the use of these characters could pose issues for taxonomists (Drew, 1969). However, Drew and Hancock (1994) subsequently used male aedeagus length as a taxonomic character separating *B. dorsalis* from (it's now synonymised) sibling *B. papayae,* and again Drew et al. (2008) used male genitalia to support the (then) existing taxonomy of the *B. dorsalis* species complex. Nevertheless, population-level studies have found that variation in aedeagal length in *B. dorsalis sensu lato* was clinal in South-east Asia (Krosch et al., 2013). Similarly, Iwahashi (1999a) found that aedeagal characters were not able to distinguish between *B. carambolae* (Drew & Hancock) and *B. dorsalis* (then *B. papayae)* alone, and other characters were needed.

4.1.4. Chapter aims and hypothesis

It was hypothesised that morphological characters would not be able to outperform a molecular phylogeny. To assess this, the aim of the chapter was to evaluate Dacini taxonomic characters for their phylogenetic utility. Researchers have used taxonomic

characters in formal phylogenetic studies (Michaux, 1996, Michaux and White, 1999, White, 2000), and informally taxonomists use characters to create taxonomic groupings (species complexes, sub-genera) which they believe contain evolutionarily related species (Drew and Hancock, 1999, Hancock and Drew, 2015, Drew and Hancock, 2016, Hancock and Drew, 2016, Hancock and Drew, 2017b, Hancock and Drew, 2017c, Hancock and Drew, 2017a, Hancock and Drew, 2018a, Hancock and Drew, 2018b, Hancock and Drew, 2018c). All such usage assumes that the taxonomic characters provide useful phylogenetic signal, but the value of taxonomic characters in providing informative phylogenetic signal is something that has never been formally tested for the Dacini.

Since the last published Dacini morphological phylogeny by White (2000), there has been a multitude of molecular phylogenetic studies published on the Dacini (Krosch et al., 2012, Virgilio et al., 2015, Dupuis et al., 2018, San Jose et al., 2018) which, based on the widely recognised utility of molecular phylogenetics (Yang and Rannala, 2012, Hajibabaei et al., 2007), should offer a reliable insight into the true relationships of the tribe. The results of these studies, along with the results of my previous chapter, provide a benchmark by which to compare the results of a morphological phylogenetic study.

Specifically, I will test if taxonomic characters can provide phylogenetic resolution which matches currently accepted molecular species relationships (the results from Chapter 3) and when used together with molecular data, can improve phylogenetic resolution. This chapter differs from morphological studies before it in that both taxonomically close, and distant species that share character traits have been deliberately chosen for analysis in order to test the utility of the characters (i.e. will actual closely related species resolve together or will all species with one character be grouped together regardless?) Here, scoring also follows a contrasting methodology. If morphological data can accurately resolve species relationships alone, it could offer the opportunity to incorporate species for which molecular data may not be available, such as rare or fossil taxa. Recommendations for the future use of morphology in Dacini phylogenetics will be provided.

4.2. Methodology

4.2.1. General approach

The methodology consists of formally comparing phylogenies generated using morphological data to a subset of the larger phylogeny created in Chapter 3. I make the explicit assumption that a well-supported, multi-locus molecular phylogeny will more accurately represent the evolutionary history of the taxa under study than would morphology, a view generally (but not universally, e.g. Hancock and Drew (2018c)) supported in the literature (Clarke et al., 2005). Species were selected to maximise the coverage of different taxonomic groupings from genera down to species complexes, and character matrices were created based on published descriptions and specimen examination. Due to the ongoing debate surrounding the usefulness of colours in Dacini taxonomy, I created separate data matrices for 'colour patterns' and 'other characters' to evaluate which combination(s) of data, if any, are the most phylogenetically informative. Following this, I tested the dataset to see whether it was possible to correctly place species if there was only morphological data available.

4.2.2. Species selection

To test the utility of morphology in resolving phylogenetic placements, species were selected which incorporated taxa from across several genera, subgenera and species complexes (Table 4.1.). *Ceratitis capitata* (Wiedemann) was chosen as an outgroup species as it is morphologically distinct from species within the tribe Dacini.

Table 4.1: Taxa used in this study for assessing the phylogenetic signal of morphological character states used for Dacini taxonomy and their taxonomic groupings. *Denotes species that have been taxonomically reassigned by others since beginning this study. *Bactrocera aurea* was moved from *B.* (*Hemizeugodacus*) to *B.* (*Neozeugodacus*) (Hancock and Drew, 2018a) and *B. melanothoracica* Drew and *B. aberrans* were moved from *B.* (*Javadacus*) to *B.* (*Bactrocera*) (Hancock and Drew, 2017c).

Species	Justification for inclusion
Bactrocera bidentata *Bactrocera aeroginosa* (Drew & Hancock)	Complete *B. bidentata* species complex
Bactrocera tryoni *Bactrocera neohumeralis* *Bactrocera aquilonis* *Bactrocera melas*	Complete *B. tryoni* species complex (as currently defined in the literature)
Bactrocera aglaiae	Subgenus *Hemizeugodacus*
Bactrocera aurea	Subgenus *Neozeugodacus**
Bactrocera melanothoracica *Bactrocera aberrans*	Subgenus *Bactrocera**
Zeugodacus choristus (May) *Zeugodacus cucumis* (French)	Genus *Zeugodacus*
Bactrocera cacuminata *Bactrocera opiliae* (Drew & Hardy) *Bactrocera endiandrae* (Perkins & May)	*B. dorsalis* species complex
Bactrocera bancroftii (Tryon) *Bactrocera musae*	*B. musae* species complex
Bactrocera abscondita (Drew & Hancock) *Bactrocera silvicola* *Bactrocera breviaculeus* (Hardy) *Bactrocera rufofuscula* (Drew & Hancock)	*B. silvicola* species complex

Species	Justification for inclusion
Bactrocera antigone (Drew & Hancock)	Complete *B. quadrata* species complex
Bactrocera aurantiaca (Drew & Hancock)	
Bactrocera erubescentis	
Bactrocera peninsularis (Drew & Hancock)	
Bactrocera quadrata	
Dacus pusillus	Genus *Dacus* (*Callantra*)
Dacus axanus	
Ceratitis capitata	Outgroup

4.2.3. Morphological character selection and matrix

I scored 47 structural characters and 27 colour pattern characters for the 29 species in Table 4.1. All morphological characters were scored as unordered and multistate. There is disagreement as to which methods are appropriate for scoring (Hauser and Presch, 1991, Kluge, 1991, Scotland et al., 2003, Grand et al., 2013), but here I adopted an unordered method because this can allow for a wider range of characters to be incorporated that do not fit traditional morphoclines (Gerber, 2019). Two matrices were compiled in order to test the phylogenetic signal of colour patterns and structural characters, and the impact of homoplasy on phylogenetic signal. The first matrix (= character matrix) included only structural characters (e.g. presence or absence of setae), while the second matrix (=colour and pattern matrix) included only colours and patterns.

The morphological characters used, and their states are provided in Table 4.2. The characters selected were based upon those used in published descriptions and diagnoses of the selected species (Drew et al., 1978, Drew et al., 1981, Drew, 1989, De Meyer, 2000). Characters used here are representative of all available characters (diagnostic and descriptive). This was done to avoid bias in the results by choosing characters that had been suggested to be “diagnostic”. Therefore, some diagnostic characters are included, but they are treated equally with other descriptive characters.

Information drawn from written descriptions were validated against named specimens in the Queensland Department of Agriculture and Fisheries Insect

Collection. Where there were discrepancies between published descriptions and specimen data, the specimen data was used. If there was an instance of all species sharing the same character state, this state was removed from the dataset.

Table 4.2: Structural characters and character states scored for 29 Dacini fruit flies and one outgroup species.

Character no.	Character	State
1	Frons	**0:** of even width, **1:** narrowing slightly posteriorly
2	Superior fronto-orbital bristles	**0:** 1 pair, **1:** 2 s.or. pairs
3	Interior fronto-orbital bristles	**0:** 2 pairs, **1:** 3 i.or.
4	Facial spots	**0:** absent, **1:** present
5	Facial spots	**0:** small, **1:** medium, **2:** large
6	Sub-ocular spot	**0:** absent, **1:** sometimes present, **2:** present
7	Number of rows of occipital bristles	**0:** 1 row, **1:** 2 rows
8	Arista with hairs	**0:** absent, **1:** present
9	Width of mesopleural stripe	**0:** narrow, **1:** medium, **2:** reaching bristle, **3:** reaching humeral calli
10	Mesopleural stripe	**0:** not extending to sternopleuron, **1:** extending to sternopleuron as a stripe

Character no.	Character	State
		or transverse spot, **2:** indistinct, no stripe-like features
11	Anterior margin of mesopleural stripe	**0:** concave, **1:** straight, **2:** slightly convex, **3:** convex
12	Triangle along anterior margin of mesonotal suture	**0:** absent, **1:** present
13	Lateral post-sutural vittae	**0:** absent, **1:** present
14	Lateral post-sutural vittae extending anteriorly to suture (sometimes as a spot)	**0:** absent, **1:** present
15	Shape of vittae	**0:** parallel sided, **1:** tapering/narrowing slightly, **2:** triangular
16	Lateral vittae ending	**0:** before posterior supra-alar, **1:** at p.sa, **2:** after p.sa
17	Medial vitta	**0:** absent, **1:** present
18	Medial vitta	**0:** beginning at mesonotal suture, **1:** beginning anterior to mesonotal suture
19	Medial vitta	**0:** ending before upper p.a. bristles, **1:** ending at prsc. bristles
20	Scutellum markings	**0:** narrow basal band, **1:** broad basal band, **2:** apical 1/3 of scutellum pale

Character no.	Character	State
		fuscous, **3:** two dark spots (separate or narrowly touching) covering 1/3 - 1/4 of scutellum from base
21	Scutellar bristles	**0:** 2 total, **1:** 4 total
22	Prescutellar bristles	**0:** no bristles, **1:** 2 bristles
23	Posterior supra-alar bristles	**0:** no bristles, **1:** 2 pairs (including the intra-alar bristles)
24	Supra-alar bristles	**0:** no bristles **1:** 1 pair of bristles
25	Mesopleural bristles	**0:** no bristles, **1:** 1 bristle per side
26	Dorsocentral bristles	**0:** absent, **1:** 1 pair
27	Bristles on post-pronotal lobe	**0:** absent, **1:** 1 per lobe
28	Notopleural bristles	**0:** absent, **1:** 2 bristles per side
29	Scapular bristles	**0:** absent, **1:** 4 bristles total
30	Mid tibia with apical spur	**0:** absent, **1:** present
31	Microtrichia	**0:** absent, **1:** in 2nd cell only, **2:** in both 1st and 2nd cell
32	Costal band	**0:** absent, **1:** present
33	Costal band length	**0:** not extending basally to costal cells, **1:** extending basally to costal cells (long)

Character no.	Character	State
34	Costal band width	**0:** not overlapping R2+3 (narrow), **1:** overlapping R2+3 but not reaching R4+5, **2:** confluent with R4+5 (broad), **3:** between R4+5 and M vein 4; to M vein
35	Costal band shape	**0:** narrowing towards apex, **1:** remaining similar width, **2:** widening in apex
36	Costal band length apically	**0:** short, at R4+5, **1:** 1/4 between R4+5 and M vein, **2:** medium 1/2 way between R4+5 and M vein, **3:** long 3/4, **4:** at M vein
37	Anal streak	**0:** absent, **1:** present
38	Anal streak	**0:** narrow, **1:** broad
39	Anal streak ending	**0:** before wing margin, **1:** at wing margin
40	Dense aggregation of microtrichia around CuA+1A	**0:** absent, **1:** present
41	Supernumerary lobe	**0:** very weak, **1:** weak, **2:** of medium development, **3:** very strong
42	Abdomen shape	**0:** oval, **1:** club shaped
43	Abdominal terga	**0:** not fused, **1:** fused

Character no.	Character	State
44	Pecten on T3	**0:** absent, **1:** present
45	Posterior lobe of surstylus	**0:** short, **1:** long
46	Abdominal sternum V	**0:** concave, **1:** deeply concave
47	Aculeus shape	**0:** acute, **1:** bifid, **2:** trilobed, **3:** 3 pairs of subapical keels, **4:** 6 lobes, **5:** obtuse (larger bulb shape), **6:** 2 small blunt lobes near apex

Colours and patterns were scored relative to an anatomical body part (Table 4.3), but simply scoring the colour(s) of individual body parts was avoided where possible due to high levels of variation. Rather, I scored colours as patterns of contrast. For example, Fig. 4.5 shows a 'stripe of contrast' along the side of the fly created by the similar or contrasting colours of the upper and lower hypopleural calli and the postnotum. These types of characters remain apparent even if the general colouring of a particular specimen is fresh or faded, or if it is a 'light-bodied' versus 'dark-bodied' individual within a species. Following the work of Drew et al. (1978): *"The main colours referred to in species keys are fuscous (a plain mixture of black and red), fulvous (tawny or brownish yellow), black (dull or glossy), yellow, orange-brown, red-brown or brown."* When scoring for straight colour characters (and not patterns) these seven colour descriptors were used, ordered from lightest to darkest from 1-7 respectively (with the addition of white as 0) (Fig. 4.6).

Table 4.3: Colours and patterns and their character states scored for 29 Dacini fruit flies and one outgroup species.

Character no.	Character	State
1	Lunule	**0:** lighter than head, **1:** same colour as head, **2:** darker than head
2	Ocellar triangle dark and distinguishable from head colour	**0:** not distinguishable, **1:** distinguishable
3	Face colour	**0:** lighter than head, **1:** same as rest of head, **2:** darker than head
4	General ventral occiput colour	**0:** significantly lighter than head, **1:** same colour as head, **2:** darker patches, **3:** significantly darker than head
5	Arista	**0:** entirely one colour (fuscous/red brown), **1:** two distinct colours (dark, fulvous/dark fulvous basally)
6	Upper hypopleural calli and lower hypopleural calli 'stripe' with respect to scutellum (very similar colour to scutellum)	**0:** absent (not same colour as scutellum), **1:** short (UHC pale only), **2:** long (both pale), **3:** disjunct (LHC only)
7	Humeral calli colour	**0:** paler than scutum, **1:** same as scutum, **2:** darker than scutum
8	Humeral calli pattern	**0:** absent, **1:** distinct spot

Character no.	Character	State
9	Notopleural calli	**0:** paler than pleural areas, **1:** same as pleural areas, **2:** darker than pleural areas
10	Leg markings	**0:** no markings, **1:** markings
11	Leg markings (of significant contrast)	**0:** hind tibia dark, **1:** mid tibia dark, **2:** fore tibia dark
12	Femora of significant contrast	**0:** hind, **1:** mid, **2:** fore
13	Scutellum	**0:** paler than scutum, **1:** same as scutum, **2:** darker than scutum
14	Wing pattern (other than costal band and anal streak)	**0:** absent, **1:** present
15	Wing pattern	**0:** three wide bands on wing, **1:** infuscation on cua1, **2:** one band on wing not touching costal band, **3:** infuscation on dm-cu vein
16	Mesonotum pattern	**0:** no pattern, **1:** pattern
17	Predominant pleural colouration	**0:** lighter than dominant mesonotum, **1:** same as, **2:** darker
18	Spots/ceromata	**0:** absent, **1:** lighter than rest of T5, **2:** same as rest of T5, **3**: darker than rest of T5

Character no.	Character	State
19	Sternite colouration	**0:** same colour as rest of "underneath" **1:** contrasting colour to rest
20	Sternites	**0:** all the same colour, **1:** different
21	Tergum 1 dominant colour	**0:** significantly lighter in colour than postnotum, **1:** same/not distinguishably different in colour from postnotum, **2:** noticeably darker than postnotum
22	Tergum 2 dominant colour	**0:** significantly lighter in colour than T1, **1:** same/not distinguishably different in colour from T1, **2:** noticeably darker than T1
23	Tergum 3 ground colour	**0:** white **1:** yellow, **2**: fulvous, **3:** orange-brown, **4:** red-brown, **5:** brown, **6:** fuscous, **7:** black
24	Tergum 4 ground colour	**0:** white **1:** yellow, **2**: fulvous, **3:** orange-brown, **4:** red-brown, **5:** brown, **6:** fuscous, **7:** black
25	Tergum 5 ground colour	**0:** white **1:** yellow, **2**: fulvous, **3:** orange-brown, **4:** red-brown, **5:** brown, **6:** fuscous, **7:** black
26	Terga 3-5	**0:** no markings or patterns, **1:** with patterns

Character no.	Character	State
27	Markings and patterns T3-5	**0:** medial line incomplete or complete on any or all three terga, **1:** transverse marking completing 't' on t3, **2:** oval orange-brown spot medially across intersegmental line of T4 and T5 connected to posterior margin of T5 by narrow medial longitudinal band, **3:** silver and yellow bands alternating down all terga, **4:** lateral bands on terga 3-5, some wide, some narrow

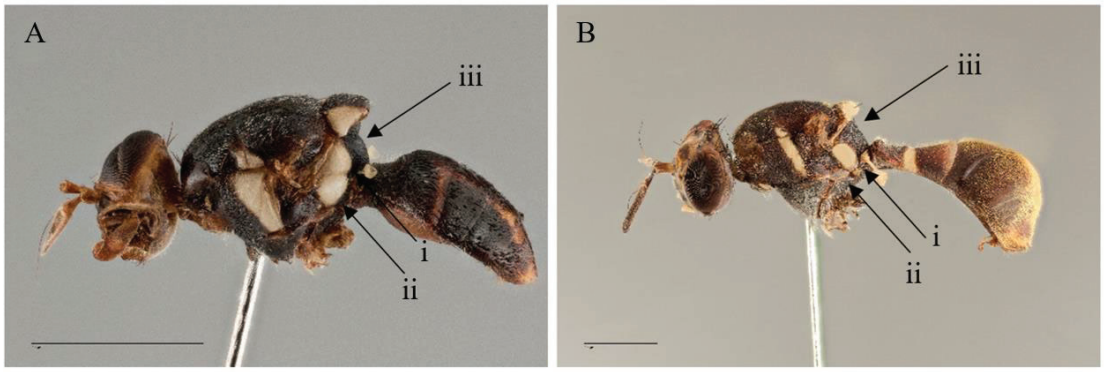


Figure 4.5: Demonstration of differences in colour contrast on the body of two different species. A: *Bactrocera trilineola*; and B: *Dacus axanus.* Arrows indicate the (i) upper hypopleural calli, (ii) the lower hypopleural calli, and (iii) the postnotum, a colour pattern which differs between the two species. Images taken from Plant Health Australia (2018a). Scale: 2mm.

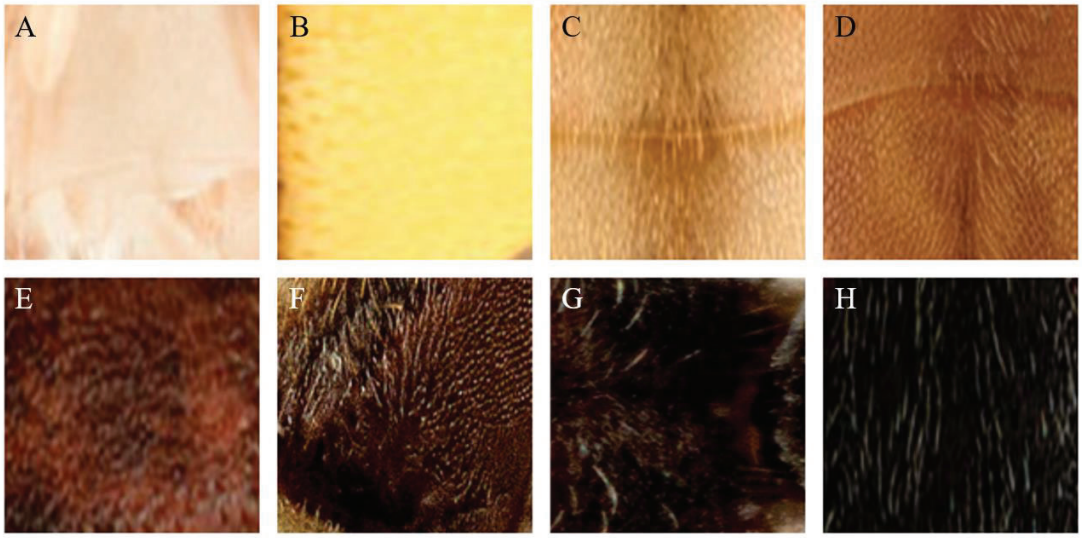


Figure 4.6: Dacini integument colours used in colour scoring, as described in Drew et al. (1978) and Drew and Romig (2016). A: white; B: yellow; C: fulvous; D: orange-brown; E: red-brown; F: brown; G: fuscous; and H: black. Colours taken from images available at Plant Health Australia (2018a).

4.2.4. Sequence alignment and phylogenetic reconstruction

Trees were reconstructed using MrBayes v3.2.2 on XSEDE (Huelsenbeck and Ronquist, 2001, Ronquist and Huelsenbeck, 2003) through the CIPRES science gateway (Miller et al., 2010). MrBayes was chosen because it allows the branch lengths to be unlinked between the morphological and molecular data but linked within. A total of six different phylogenetic trees were constructed; (i) molecular only, (ii) molecular + structural characters, (iii) molecular + colour patterns, (iv) molecular + structural characters + colour patterns, (v) structural characters + colour patterns and (vi) structural characters only. All trees were reconstructed using the same methodology; 1 million generations, with a sampling frequency of 2000, for a total of two runs on three chains at a temperature of 0.1. The molecular data was compiled as outlined in Chapter 3. Molecular data for the outgroup taxon *Ceratitis capitata* was taken from Genbank (see Appendix 8). Models used for each molecular partition are presented in Table 4.4. Morphological data was analysed using the MKY model for discrete morphological characters (Lewis, 2001).

Table 4.4: Molecular model selection and partitions used in the molecular phylogenetic analysis as determined through IQ-Tree ModelFinder (Kalyaanamoorthy et al., 2017). When moving from IQ-Tree models, the next most simple model for use in MrBayes was chosen.

Loci	Model selection
COI+COII first codon	GTR+I+G
COI+COII second codon	GTR+I+G
COI+COII third codon	GTR+I+G
16S	GTR+G
RPA2	HKY+G
DDOSTs2	GTR+I
EIF3L	HKY+G

4.2.5. Statistical analysis

Output files were viewed in Tracer v1.7.1 (Rambaut et al., 2018), to evaluate convergence and to assess the summary statistics. To test for the best topology, I ran the Approximately Unbiased (AU) test on IQ-Tree V1.7 (Trifinopoulos et al., 2016) on Purdue's HPC "Snyder", which is a method of hypothesis testing of tree regions in order to reduce bias (Shimodaira, 2002). Using the topology produced by the molecular dataset as a standard, all six topologies were tested against the molecular topology using the AU test (Shimodaira, 2002) for 2000 replicates. Using PAUP v4.0b10 (Swofford, 1998) the Homoplasy Index (HI) was calculated for each dataset against the overall topology (calculated on molecular + structural characters + colours and patterns) to see how the HI could be influenced by different partitions.

I made the explicit assumption that the molecular phylogeny was more reliable than the morphological phylogenies when comparing them using the AU test. This position is justified by not only a comparison of the posterior probabilities of the molecular and morphological trees, but also based on a large body of work by many other Dacini researchers who have repeatedly found similar relationships before me (Krosch et al., 2012, San Jose et al., 2018, Doorenweerd et al., 2018, Dupuis et al., 2018, Virgilio et al., 2015). Only a very small number of traditional Dacini taxonomists still argue against the results of molecular systematics (e.g. Drew and

Romig (2016)), but that view seems to be slowly changing with a recognition that "*while molecular evidence is not always reliable, phylogenies have become increasingly more informative"* (Hancock and Drew, 2018c).

4.2.6. Character testing

If the morphological characters proved to have phylogenetic signal, a potential application of this dataset would be to combine both morphological and molecular data in order to incorporate species for which there is only morphological data available, such as fossil data. To further examine the phylogenetic efficacy of the morphological data, I ran a combined molecular + morphological analysis, but with the molecular data removed for all individuals of two species (*B. peninsularis* and *B. bancroftii*) and compared their inferred placements with trees reconstructed with their morphological data also included. I chose *B. bancroftii* because it is well resolved in the tree, while conversely I chose *B. peninsularis* because the two individuals of this species were not monophyletic within the tree and I hoped to achieve better resolution of this species. Methods for tree reconstruction were the same as for previous methods.

4.3. Results

4.3.1. Scoring and matrices

I scored 47 structural characters and 27 colours and patterns for 29 species and from this created the structural character matrix (Table 4.5.) and colour and pattern matrix (Table 4.6).

Table 4.5: Scored structural character matrix for 47 morphological characters of 29 *Bactrocera, Dacus* and *Zeugodacus* species (plus *Ceratitis capitata* as an outgroup). The character numbers and states are detailed in Table 4.3.

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Species																																																	
C. capitata	1	0	0	0	0	1	1	3	2	0	0	0	0	-	-	0	-	-	3	1	1	1	1	1	1	1	1	1	2	0	-	-	-	1	0	-	0	3	0	0	0	-	-				0
B. bidentata	0	0	0	2	0	1	0	1	1	1	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	2	1	1	2	0	4	1	1	2	0	1	2	0	1	0	0	3
B. aeroginosa	1	0	0	2	2	1	0	2	1	1	0	1	0	0	1, 2	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1	1	0	4	1	1	2	0	1	2	0	1	0	0	-
B. tryoni	0	0	0	2	2	1	0	1	1	2	0	1	0	2	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	2	2	1	1	2	0	4	1	1	2	0	1	1	0	1	1	0	0
B. neohumeralis	0	0	0	2	2	1	0	1	1	2	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	2	2	1	1	1	0	4	1	1	1	0	1	2	0	1	1	0	0
B. aquilonis	0	0	0	3	2	1	0	2	1	2	0	1	0	1	1	0	-	-	0	0	1	1	1	1	0	0	1	1	1	2	2	1	1	2	0	4	1	1	2	0	1	2	0	1	1	0	0
B. melas	0	0	0	2	2	1	0	1	1	3	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	2	2	1	1	2	0	4	1	1	2	0	1	2	0	1	1	0	0
B. aglaiae	0	0	0	2	2	1	0	1	1	1	0	1	0	0	1	1	0	1	0	1	1	1	1	1	0	0	1	1	1	1	1	0	0	1	0	1	0	1	1	0	1	3	0	0	0	0	0
B. aurea	0	0	1	1	0	1	0	3	1	0, 3	0	1	0	0	0	0	-	-	2	1	1	1	0	1	0	0	1	1	1	2	2	0	1	2	3	4	1	1	1	0	1	2	0	0	1	0	-
B. melanothoracica	0	0	0	2	2	1	0	2	1	2	0	1	0	1	0	0	-	-	0	0	1	1	0	1	0	0	1	1	1	1	1	1	1	2	0	4	1	1	2	0	1	2	1	1	1	0	-

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
B. aberrans	0	0	0	1,2	0	1	0	1	1	1	0	1	0	1	0	0	-	-	0	0	1	0	0	1	0	0	1	1	1	1	1	0	2	2	0	4	1	1	2	0	1	2	1	0	0	0	0
Z. choristus	0	0	1	2	2	1	0	2	1	3	0	1	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	2	2	4	4	1	1	3	0	1	2	1	0	1	0	0
Z. cucumis	1	0	1	1	0	2	0	1	1	3	0	1	1	0	2	1	0	0	0	1	0	1	0	1	0	0	1	1	1	1	1	0	1	2	2	1	0	0	0	0	0	2	1	0	1	0	6
B. cacuminata	1	0	0	2	2	1	0	1	1	3	0	1	0	1	0,1	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	2	2	0	1	1	1	2	0	1	2	0	1	1	0	0
B. opiliae	1	0	0	1,2	2	1	0	1	1	1	0	1	0	0	2	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	2	0	1	0	1	2	0	1	2	0	1	1	0	0
B. endiandrae	1	0	0	1	2	1	0	1	1	2	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	2	0	4	0	1	2	0	1	2	0	1	1	0	0
B. bancroftii	1	0	0	2	2	1	0	1	1	1	0	1	0	0	1	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	4	1	1	2	0	1	2	0	1	0	0	0
B. musae	1	0	0	2	2	1	0	1	1	1,2	0	1	0	1	1	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	2	0	1	0	1	2	0	1	3	0	1	1	0	0
B. abscondita	0	0	0	3	2	1	0	2	1	3	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	2	0	4	1	1	2	0	1	3	0	1	0	0	-
B. silvicola	0	0	0	2	2	1	0	1	1	2	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	2	0	4	0	1	2	0	1	2	0	1	0	0	-
B. breviaculeus	0	0	0	2	2	1	0	1	1	2	0	1	0	0,1	1	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1	0	4	1	1	2	0	1	2	0	1	0	1	0

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
B. rufofuscula	0	0	0	3	2	1	0	1	1	1,3	0	1	0	1	2	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	2	0	4	1	1	2	0	1	3	0	1	1	0	-
B. antigone	0	0	0	2	0	1	0	2	1	1	0	1	0	0	1,2	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	0	4	1	1	2	0	1	2,3	0	1	0	0	-
B. aurantiaca	0	0	0	2	2	1	0	1	1	1	0	1	0	1	0	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	1	0	1	2	0	1	2,3	0	1	0	0	-
B. erubescentis	0	0	0	2	2	1	0	1	1	2	0	1	0	1	1	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	2	0	4	1	1	2	0	1	2	0	1	0	0	-
B. peninsularis	1	0	0	1,2	2	1	0	1	1	3	0	1	0	0	1,2	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	0	4	1	1	2	0	1	2	0	1	0	0	-
B. quadrata	1	0	0	3	2	1	0	1	1	1	0	1	-	0	2	0	-	-	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1	2	0	1	2	0	1	1	0	-
D. pusillus	0	0	0	3	2	1	0	1	1	1	1	0	0	-	-	0	-	-	0	0	0	1	1	1	0	0	1	1	1	2	2	2	1	1	0	0	0	1	1	1	1	2	1	0	1	0	-
D. axanus	0	0	0	3	2	2	0	0,1	0	1	1	0	0	-	-	0	-	-	1	0	0	1	0	1	0	0	-	-	1	2	2	3	1	4	0	4	1	1	1	1	1	2,3	1	0	1	0	0

Table 4.6: Scored matrix for 27 colour and pattern characters of 29 *Bactrocera, Dacus* and *Zeugodacus* species (plus *Ceratitis capitata* as an outgroup). The character numbers and states are detailed in Table 4.3.

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Species																											
C. capitata	1	1	0	2	0	0	1	1	1	0	-	-	1	1	0	1	0	0	-	-	-	-	-	1	1	0	3
B. bidentata	1	1	1	3	1	2	0	0	0	1	0	-	0	0	-	0	0	2	0	0	2	0	3	3	3	1	0, 1
B. aeroginosa	1	1	1	1	1	2	0	0	0	0	-	-	0	0	-	1	0	2	0	0	1	1	3	3	3	1	0
B. tryoni	2	1	1	2	1	2	0	0	0	1	0	-	0	0	-	1	0	1	1	0	1	0	4	4	4	1	0, 1, 4
B. neohumeralis	1	1	1	2	1	2	0, 1	0	0	1	0	-	0	0	-	1	1, 2	2	1	0	1	0	4, 5, 6	4	4	1	0, 4
B. aquilonis	1	1	1	2	1	2	0	0	0	1	0	-	0	0	-	1	0	2	1	0	0, 1	0	3	3	3	1	0, 1, 4

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
B. melas	1, 2	1	1	3	1	2	0	0	0	1	0	-	0	0	-	1	2	2	1	0	1	0	4, 5, 6	4, 5, 6	4, 5, 6	1	0, 1, 4
B. aglaiae	1	1	1	1	1	2	0	0	0	0	-	-	0	0	-	0	0	3	0	0	1	0	2	2	2	1	0, 4
B. aurea	1	1	1	1	1	2	0	0	0	1	0	-	0	1	2	0	0	2	1	0	0	1	2	2	2	1	0, 4
B. melanothoracica	1	1	1	1	1	2	0	0	0	1	0	-	0	0	-	1	2	2	1	0	1	0	3	3	3	1	0, 1, 4
B. aberrans		1	1	1	1	2	0	0	0	1	0	-	0	0	-	1	1	2	0	0	1	0	3	3	3	1	0, 1, 4
Z. choristus	2	1	1	1	1	2	0	0	0	1	0	-	0	1	3	1	1	2	1	0	0	1	2	2	2	1	0, 1, 4

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Z. cucumis	1	1	1	1	1	2	0	0	0	0	-	-	0	1	1	0	1	2	1	0	0	1	2	2	2	1	0, 1, 4
B. cacuminata	1, 2	1	1	1	1	2	0	0	0	1	0	-	0	0	-	1	2	2	1	0	1	0	3	3	3	1	0, 4
B. opiliae	1	1	1	1	1	2	0	0	0	1	0	-	0	0	-	1	0, 1	2	1	0	1	0	2	3	3	1	0, 1, 4
B. endiandrae	2	1	1	1	1	2	0	0	0	1	0	-	0	0	-	1	0	2	1	0	1	0	3	3	3	1	0, 4
B. bancroftii	1	1	1	3	1	2	0	0	0	0	-	-	0	0	-	1	0	2	0	0	0	1	3	3	3	0	0, 1
B. musae	1	1	1	3	1	2	0	0	0	1	0, 1, 2	-	0	0	-	1	0, 1	3	1	0	1	0	3	3	3	0, 1	1, 4

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
B. abscondita	2	1	1	3	1	2	0	0	0	1	0	-	0	0	-	1	0	3	0	0	1	0	3	3	3	1	0, 1, 4
B. silvicola	2	1	1	2	1	2	0	0	0	1	0, 2	-	0	0	-	1	2	2	0	0	1	0	3	3	3	1	0, 4
B. breviaculeus	1	1	1	2	1	2	0	0	0	1	0	-	0	0	-	1	2	2	0	1	1	0	3	3	3	1	0, 1, 4
B. rufofuscula	1	1	1	2	1	2	0	0	0	1	0	-	0	0	-	1	2	3	1	0	1	0	3	3	3	1	0, 4
B. antigone	1	1	1	1	1	2	0	0	0	0	-	-	0	0	-	1	1	2, 3	0	0	1	0	3	3	3	1	0, 4
B. aurantiaca	1	1	1	1	1	2	0	0	0	0	-	-	0	0	-	0, 1	2	2, 3	0	0	1	0	2	2	2	1	0, 1, 4

Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
B. erubescentis	1	1	1	1	1	2	0	0	0	1	0	-	0	0	-	1	1	2	0	0	1	0	3	3	3	1	0, 1, 4
B. peninsularis	1	1	1	1	1	2	0	0	0	0	-	-	0	0	-	0	1	2	0	0	1	0	3	3	3	1	0, 4
B. quadrata	1	1	1	3	1	2	0	0	0	1	0	-	0	0	-	0	2	2	1	0	1	0	3	3	3	1	0, 1, 4
D. pusillus	1	1	1	3	1	2	0, 1	0	0	1	0	-	0, 1	0	-	1	2	2	1	0	1	0	3	3	3	1	0 4
D. axanus	2	1	1	3	1	1	0	0	0	-	0	0, 1, 2	0, 1	0	-	1	2	2, 3	1	0	1	0	6	6	3, 6	1	2, 4

4.3.2. Phylogenetic reconstructions

The results of the AU testing found that trees generated from structural characters alone (Table 4.5) and structural characters + colours and patterns (Table 4.6) were rejected when tested against the phylogeny generated from the molecular data (Fig. 4.7) (Table 4.7). In addition, these phylogenies had extremely poor branch supports when compared to the trees that contained molecular data. The molecular topologies (Figs. 4.7-10) were all accepted with very similar p-values and log likelihood values (Table 4.7). The dataset that contained the combination of molecular data and colour traits (Fig. 4.9) was slightly more informative however, the values are so similar among all four accepted topologies that I conclude that the addition of morphological characters to molecular characters has not made a significant difference to the resolution of the Dacini phylogeny.

Table 4.7: Summary statistics for the six different phylogenetic reconstructions. ESS = effective sample size; stdev = standard deviation of split frequencies; and HPD = 95% highest posterior density interval.

Tree	ESS	Stdev	HPD
Molecular (Fig. 4.7)	1562.8	0.01	0.424, 0.494
Molecular + structural characters (Table 4.5) (Fig. 4.8)	778.2	10.0541	-27952.5, -27913.23
Molecular + colours and patterns (Table 4.6) (Fig. 4.9)	780.1	9.9403	-27801.75, -27732.99
Molecular + structural characters (Table 4.5) + colours and patterns (Table 4.6) (Fig. 4.10)	782.7	10.7334	-28.339.93, -28299.1
Structural characters (Table 4.5) + colours and patterns (Table 4.6) (Fig. 4.11)	951.9	9.3867	-899.1666, -863.596
Structural characters (Table 4.5) (Fig. 4.12)	885.9	9.4637	-560.56, -523.4657

Table 4.8: Results from the AU test for six different topologies of Dacini species relationships; (i) molecular characters, (ii) molecular and structural characters, (iii) molecular and colour and pattern characters, (iv) molecular, structural and colour and pattern characters, (v) Structural and colour and pattern characters, and (v) structural characters. When tested against the molecular topology "+" indicates an accepted hypothesis of good fit based on the p-value, while "-" indicates a rejected hypothesis.

Tree	LogL	p-AU
Molecular (Fig. 4.7)	-26799.097	0.423+
Molecular + structural characters (Table 4.5) (Fig. 4.8)	-26798.351	0.482+
Molecular + colours and patterns (Table 4.6) (Fig. 4.9)	-26797.409	0.587+
Molecular + structural characters (Table 4.5) + colours and patterns (Table 4.6) (Fig. 4.10)	-26800.105	0.433+
Structural characters (Table 4.5) + colours and patterns (Table 4.6) (Fig. 4.11)	-28918.649	0-
Structural characters (Table 4.5) (Fig. 4.12)	-28920.436	0-

The HI index was calculated for all datasets against the tree topology that was reconstructed using all partitions (Table 4.9). The HI was lower for the datasets that included molecular and morphological data, than those that contained just molecular data, or just morphological data alone.

Table 4.9: Comparison of Homoplasy Index (HI) of six datasets compared against the best tree topology as determined by the AU test.

Data	Tree	HI
Molecular	Molecular + structural characters + colours and patterns	0.547
Molecular + structural characters	Molecular + structural characters + colours and patterns	0.519
Molecular + colours and patterns	Molecular + structural characters + colours and patterns	0.519

Data	Tree	HI
Molecular + structural characters + colours and patterns	Molecular + structural characters + colours and patterns	0.521
Structural characters + colours and patterns	Molecular + structural characters + colours and patterns	0.629
Structural characters	Molecular + structural characters + colours and patterns	0.634

The trees that contained only morphological data failed to resolve key clades, including the genera *Bactrocera, Dacus* and *Zeugodacus.* For example, the character-only tree (Fig. 4.12) resolved *B. neohumeralis* in a clade with *D. axanus* and *D. pusillus.* When colours and patterns were added to the character data (Fig. 4.11), the genus *Dacus* was resolved as sister to *B. aquilonis,* and nested within the *B. tryoni* species complex.

The morphological characters, when used alone, were only able to resolve a few species into their currently classified species group/complex. The structural characters dataset was able to resolve *B. aquilonis* and *B. tryoni* in a clade, however other members of the complex; *B. melas* and *B. neohumeralis* were resolved as sister to all remaining *Bactrocera* species and sister to the genus *Dacus* respectively. Another taxonomic relationship represented by the character tree is the clade containing *B. rufofuscula* and *B. abscondita,* which is not a genetically close relationship (Fig. 4.7). When the taxonomic characters were added to the molecular dataset, there was no additional resolution of species within *B. tryoni* species complex, a notoriously difficult to resolve group.

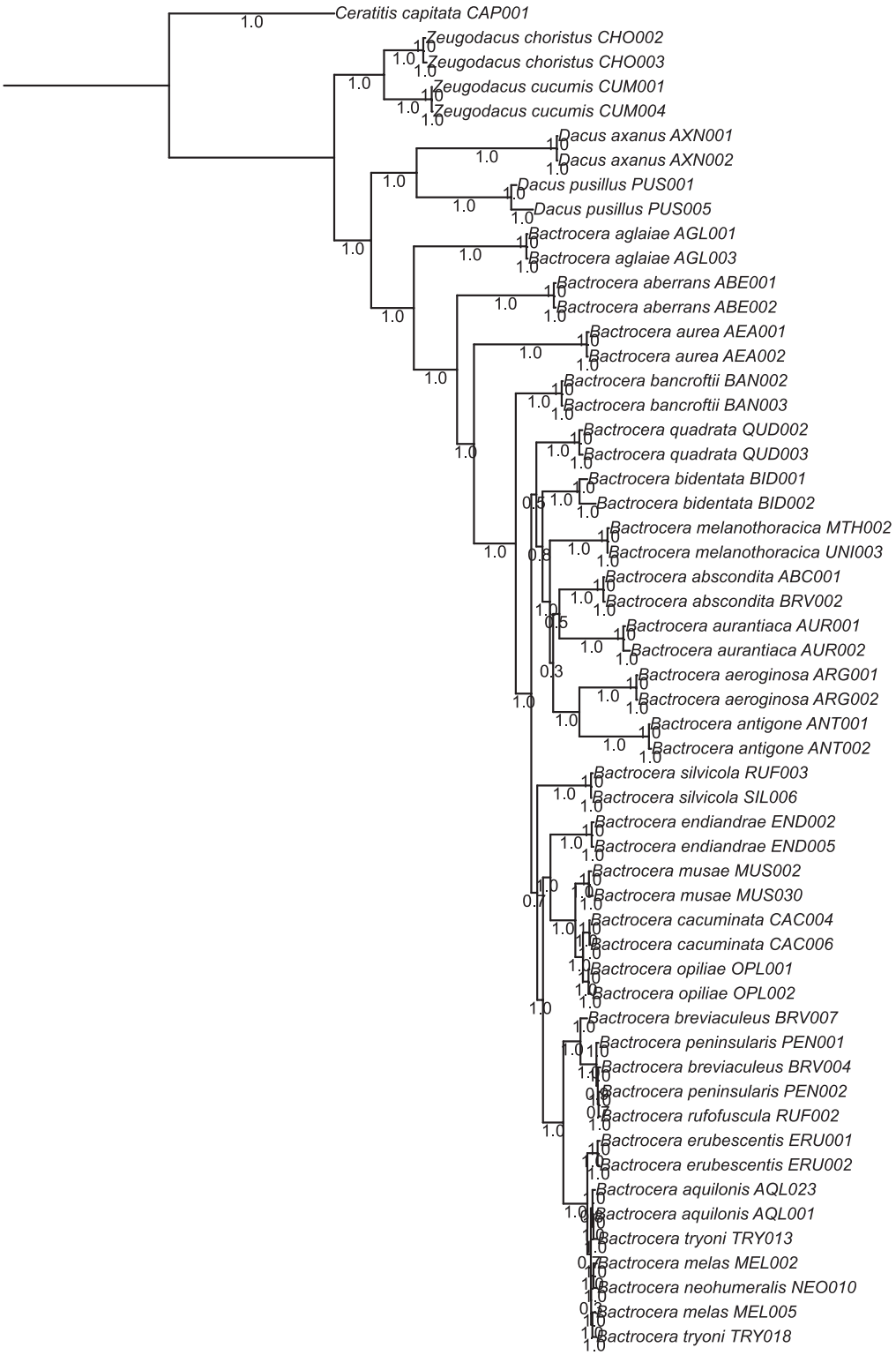


Figure 4.7: Bayesian inference tree topology of Dacini species reconstructed from seven partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L. Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

Figure 4.8: Bayesian inference tree topology of Dacini species reconstructed from eight partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L and one partition of morphological character state data: structural characters. Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

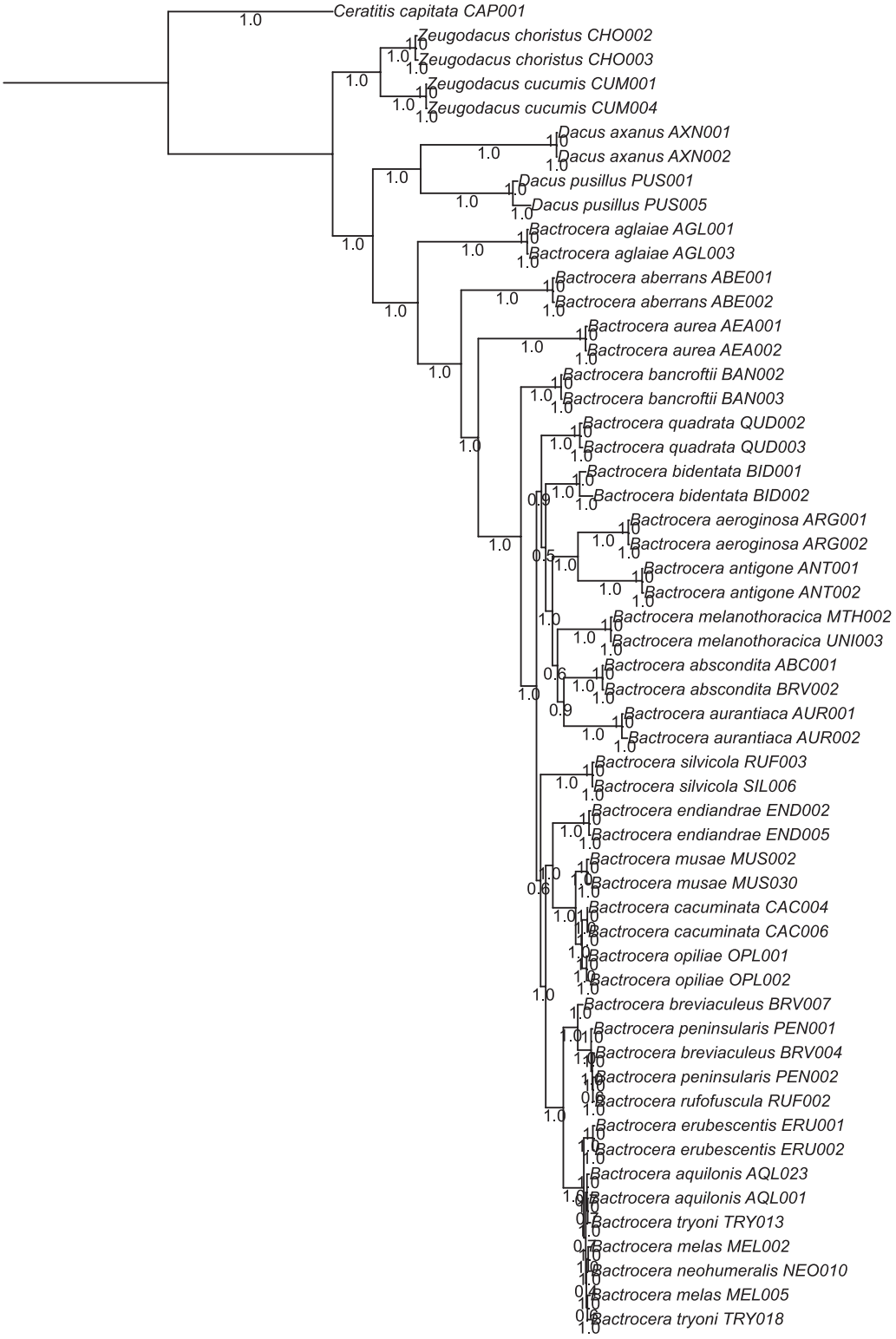


Figure 4.9: Bayesian inference tree topology of Dacini species reconstructed from eight partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L and one partition of morphological character state data: colours and patterns. Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

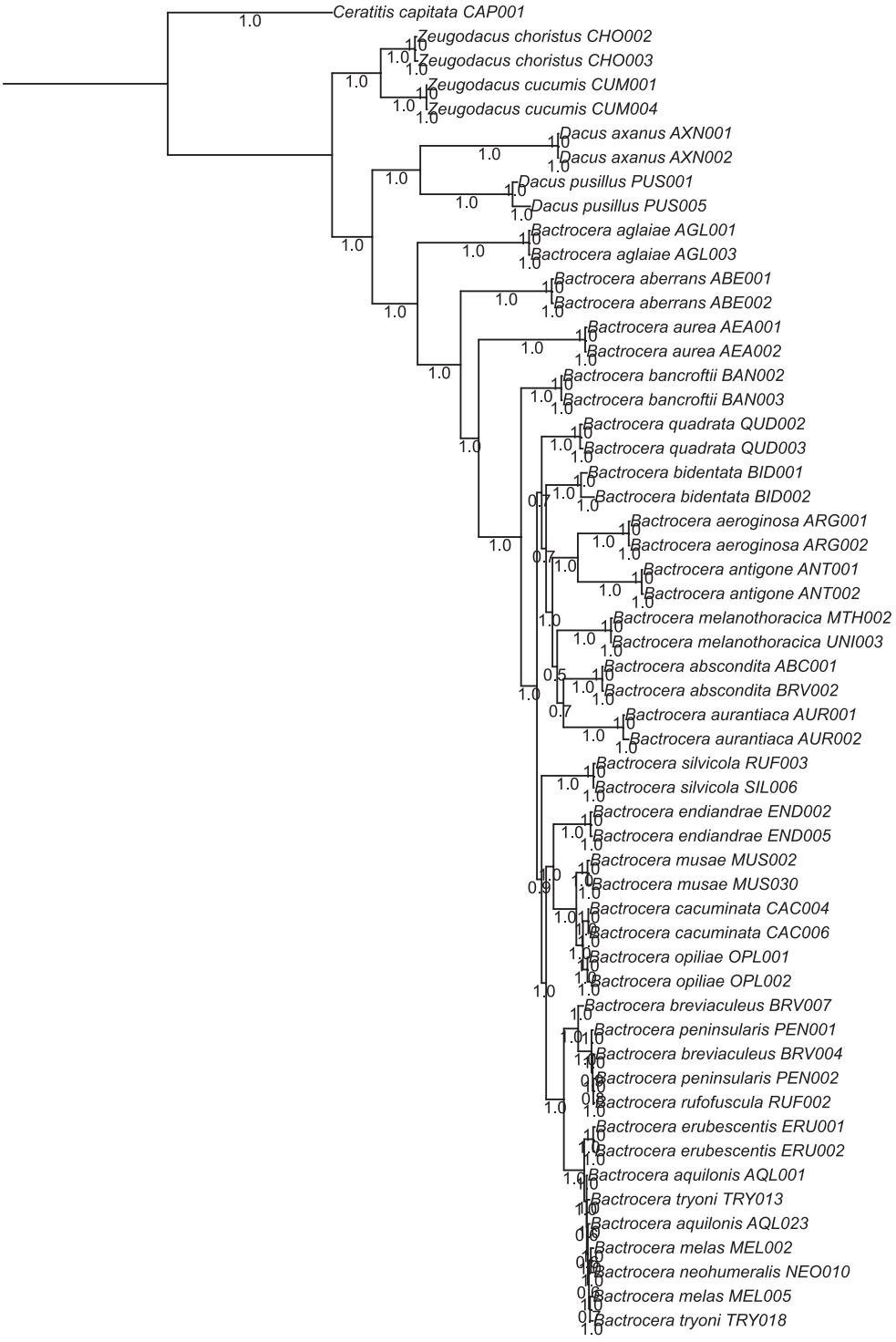


Figure 4.10: Bayesian inference tree topology of Dacini species reconstructed from nine partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L and two partitions of morphological character state data: structural characters; and colours and patterns. Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

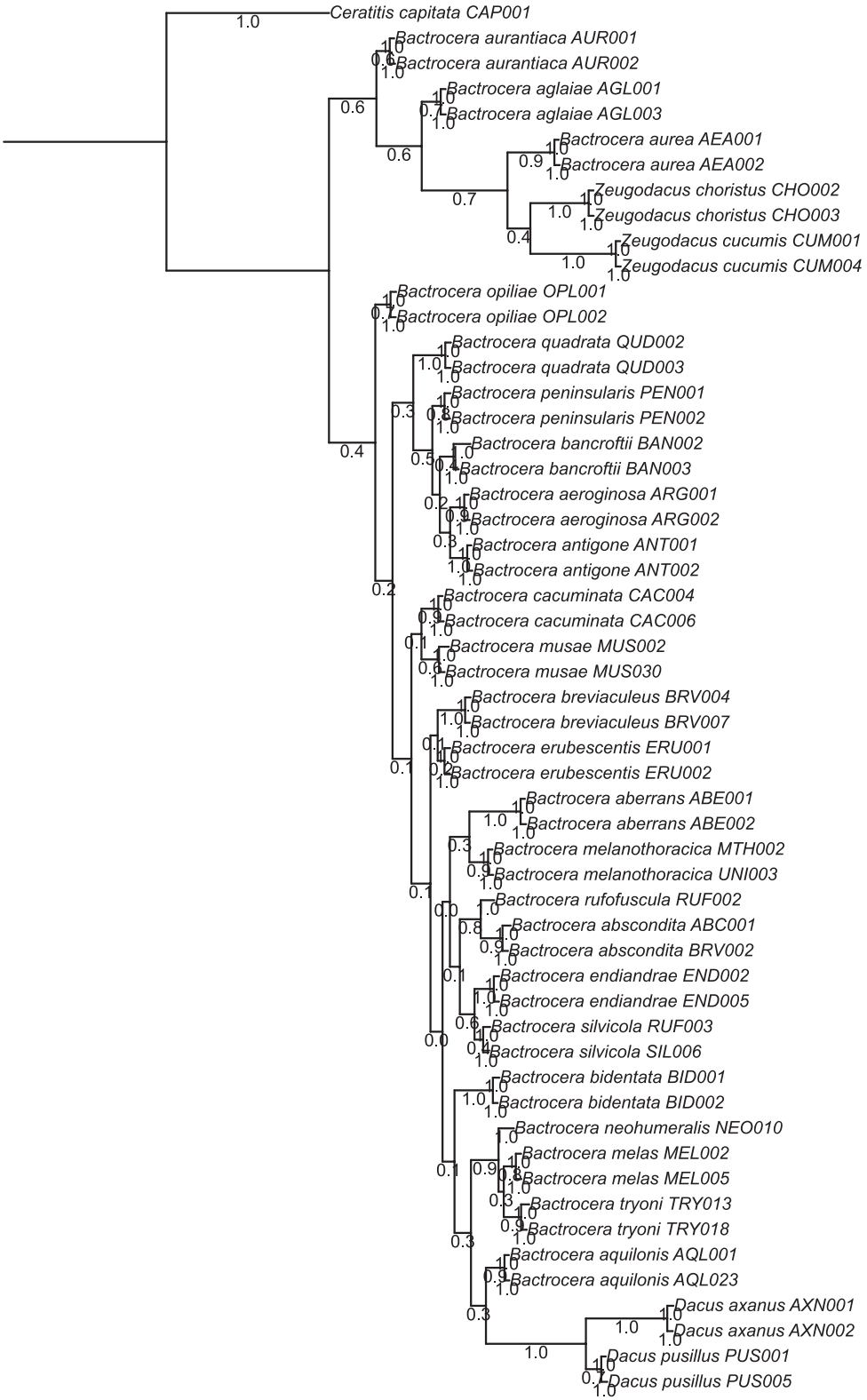


Figure 4.11: Bayesian inference tree topology of Dacini species reconstructed from two partitions of morphological character state data: structural characters; and colours and patterns. Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

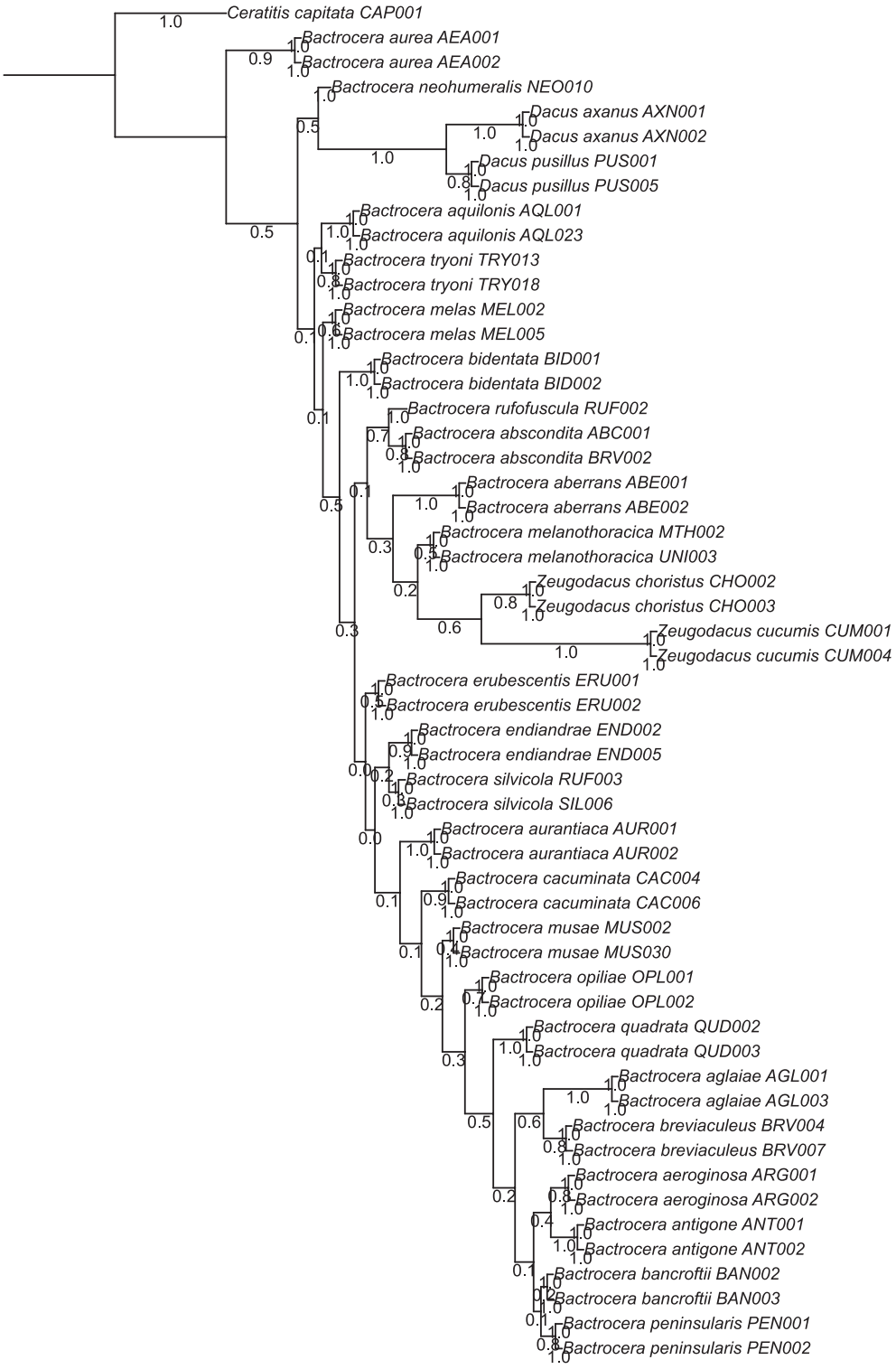


Figure 4.12: Bayesian inference tree topology of Dacini species reconstructed from one partition of morphological character state data: structural characters. Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

4.3.3. Character testing

The results of the character testing are presented in Fig. 4.13. The placement of *B. bancroftii* and *B. peninsularis* were not resolved into the same relationships as in the previous trees where molecular data was included for these species. When molecular data was included for *B. peninsularis* this species was placed in an unresolved clade with *B. breviaculeus* and *B. rufofuscula*, however, when only morphological data was included, it was resolved as sister to *B. quadrata* (both species are members of the *B. quadrata* species complex). Similarly, *B. bancroftii,* was sister to all of *Bactrocera* (*Bactrocera*) when molecular characters were included for this species, however, it was placed as sister to *B. aeroginosa,* a member of the *B. bidentata* species complex when morphological characters were used alone.

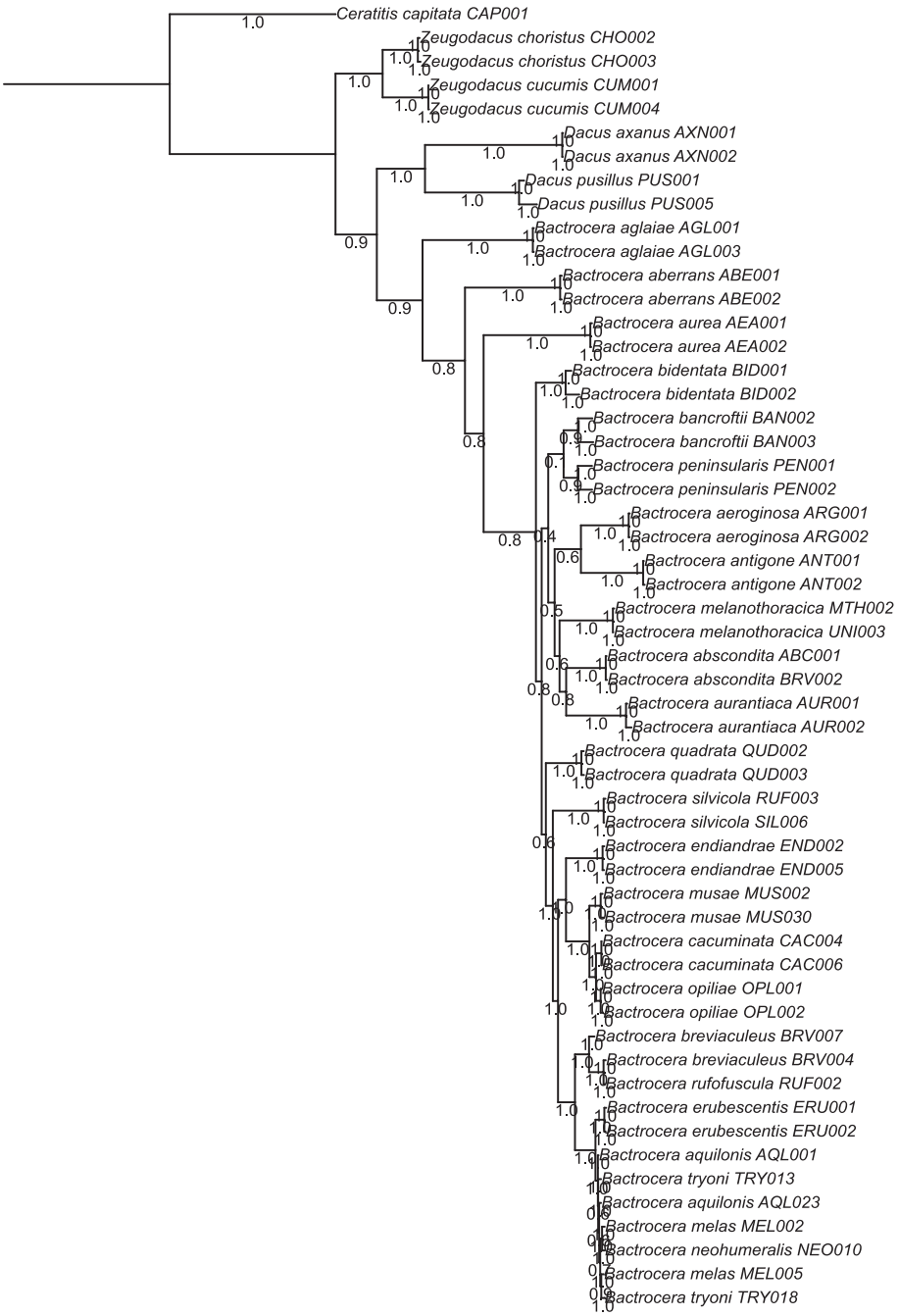


Figure 4.13: Bayesian inference tree topology of Dacini species reconstructed from nine partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L and two partitions of morphological character state data: structural characters; and colours and patterns with molecular data removed for *B. peninsularis* (PEN001 and PEN002) and *B. bancroftii* (BAN002 and BAN003). Bayesian posterior probabilities are shown at the nodes. Tree rooted with *Ceratitis capitata* as outgroup.

4.4. Discussion

I produced matrices for 29 Dacini species consisting of 47 structural characters and 27 colours and patterns in order to test the phylogenetic utility of these taxonomic characters against a subset of the molecular phylogeny from Chapter 3. I found that when morphology alone was used in phylogenetic reconstruction, that relationships were not well resolved. The best phylogeny was achieved using molecular + colour data; however, the addition of morphology (characters and/or colour) did not greatly improve the results, while the relationships found via morphological methods were not well supported and did not reflect current taxonomic or systematic relationships.

4.4.1. Signal provided by morphology in the phylogenies

Examining the relationships recovered using the morphology alone, members of the *B. tryoni* species complex were not monophyletic, similarly the *B. breviaculeus, B. peninsularis* and *B. rufofuscula* group was also not resolved at the species level. When molecular data was removed for *B. peninsularis* and *B. bancroftii*, the resultant phylogeny did not agree with the molecular phylogeny, indicating that phenetic traits are not useful in a phylogenetic context. The current taxonomic relationships that were supported in the morphological phylogenies were some members of the *B. tryoni* species complex, and some members of the *B. silvicola* species complex that were resolved into respective clades. No other traditional taxonomic relationships were resolved. The posterior probability values were so low at the deeper nodes that the relationships obtained by the morphological trees should be considered unreliable.

The lower HI that was obtained for the datasets that included molecular and morphological data should be addressed here. The HI is not a direct measure of the utility of a character in phylogenetics, and is influenced by the frequency and independence of characters (Archie, 1996). Additionally, autapomorphies and missing data can also impact upon the calculations of the HI by incorrectly assuming low, or no homoplasy (Brooks et al., 1986, Sanderson and Donoghue, 1989). While the HI was found to be lower for the combined datasets (i.e. morphological and molecular characters), when compared to the molecular dataset alone, this could also be explained by the limited number of character state changes that are present within

molecular data; only four, when compared to the morphological data which is capable of many more (Mishler et al., 1988).

4.4.1.1. Colour and pattern

Due to the consistent use of colour in Dacini taxonomy (Drew, 1989, White and Hancock, 1997), I investigated the phylogenetic utility of these characters and found that it was minimal, even when combined with molecular data. For dacines, colour and colour patterning is highly variable between individuals (Hardy, 1969, Leblanc et al., 2015) and what are now recognised as single biological species (Schutze et al., 2015a) were described as different taxonomic species based predominantly on colour variation and aedeagal measurements (Drew et al., 2005, Drew and Hancock, 1994). Recent publications have found that colour variation in flies is influenced by gene expression levels. Bai et al. (2019) found in *B. dorsalis*, that expression of the *white eye* gene was associated with melanin pigmentation in the compound eye and for spots located at the base of bristles between the eyes. Expression of *yellow,* a gene involved in melanin biosynthesis was lower in flies with less pigmentation. In *Drosophila* Fallén species, *yellow* was found to be one of the genes responsible for encoding a protein that deposits black melanin in the cuticle of flies (Wittkopp et al., 2002). Jeong et al. (2008) found that two closely related species, *Drosophila santomea* Lachaise & Harry and *D. yakuba* Burla, exhibited morphological divergence resulting from the loss of expression of *tan* and *yellow* pigment genes, thus influencing colour and patterning. Whilst underlying mechanisms will not be explored further here, it is evident that intraspecific colour variation between fruit flies can be explained by gene expression levels. These colour patterns should therefore be used with caution in taxonomy, and perhaps replaced with more stable characters such as wing patterning and sub-cuticular yellow markings (for example the vittae, scutellum, mesopleuron and notopleuron), as outlined in Drew (1972).

4.4.2. Recommendations

Based on the findings of this chapter, it seems appropriate to offer recommendations for future use of morphological characters in Dacini phylogenetics. I achieved

similar results to those who have attempted morphological phylogenies before me (Michaux, 1996, Michaux and White, 1999, White, 2000), who all had difficulty resolving meaningful relationships and had multiple polytomies. It is clear, as others have discussed before me (Scotland et al., 2003), that while not all morphological and molecular characters provide phylogenetic signal, molecular data still provided more signal (and had more informative characters) than informative sites present in the morphological data. The ease of collecting large volumes of molecular data also provides a greater chance of discovering informative nucleotides, where there may be only a limited pool of characters that can be scored for morphology that will ever be informative for phylogenetics.

For these reasons I conclude that these characters are "phenetic" or purely "taxonomic characters" and not "phylogenetic characters". For the Dacini, morphology remains useful for taxonomic purposes, and certainly at the species level there have been multiple instances where it has been invaluable for helping to resolve systematic questions at the species complex level (Krosch et al., 2013, Iwahashi and Routhier, 2001, Iwahashi, 1999a, Iwahashi, 1999b, Iwaizumi et al., 1997). Morphology may, indeed, be linked with mate recognition; for example, White (2000) suggested that a lack of wing patterning in the Dacini may indicate other colour patterns are indicators of mate recognition signals. My primary recommendation would be to use morphology as a component of integrative taxonomy sensu Schutze et al. (2017), for systematics only at the species level; and to answer questions surrounding species delimitation, population differentiation and ecology. At any systematic level, I suggest that colours and patterns be used with extreme caution because, as demonstrated here, they exhibit high homoplasy which has resulted in poorly resolved relationships.

Chapter 5: Biogeographic influences on the evolution and geographic distribution of the Australo-Pacific Dacini

5.1. Introduction

The tribe Dacini is predominantly found in the Afrotropical region, South-east Asia, the Western Pacific and Northeast Australia (Drew and Hancock, 1999). The method by which these species became so widespread can be linked to historical geology and biogeography. However, the very limited research on the topic has resulted in a biogeographical hypothesis which conflicts with the findings of Chapter 3 of this thesis. The dates proposed for the divergence of the early Dacini, and their biogeographical movements were proposed by Krosch et al. (2012), but given that my dates are approximately 30 my younger, this requires further investigation.

This introduction will provide a background on the biogeography of Southern Hemisphere continents and their movements post-Gondwanan breakup, as well as an overview of the distribution and diversity of the Dacini and how this resulted in the current hypothesis for the origins of the tribe. Due to the comprehensive sampling of Australian taxa for this dataset, this region was the primary focus of this chapter.

5.1.1. Geological history of the Southern Hemisphere

Gondwana was an ancient Southern Hemisphere supercontinent that consisted of the present-day landmasses of: Australia, South America, Africa, Antarctica, New Zealand, New Caledonia, New Guinea, India and Madagascar (Fig. 5.1) (McLoughlin, 2001). Approximately 167 mya, Gondwana began to fracture into smaller landmasses, beginning with the separation of West Gondwana (South America and Africa) from East Gondwana (Australia, India, Madagascar and Antarctica) (Chatterjee et al., 2013). India + Madagascar proceeded to separate from Australia + Antarctica approximately 120-130 mya (Gaina et al., 2007), as did Africa from South America approximately 80-100 mya (Pletsch et al., 2001) however, southern land connections between South America, Antarctica and Australia remained (Li and Powell, 2001). Approximately 90 mya India separated from Madagascar (Raval and Veeraswamy, 2003) and Australia + PNG separated from Antarctica (Li and Powell, 2001).

Figure 5.1: Simplified representation of Gondwana and the present-day landmasses of which it consisted. Taken from Karori Sanctuary Trust (2016).

5.1.1.1. Australia and the Pacific

Following breakup of Gondwana, the Australian plate, with part of present-day New Guinea as the leading edge, began to move northwards (Hall, 2001). Australia and New Guinea were joined by a land bridge for millions of years until the Pleistocene (2.6-0.011 mya) when the land bridge entered a period of intermittent submergence (Doutch, 1972). Collision with the Asian plate in the Late Oligocene (23-33 mya) caused uplift of New Guinea (Axelrod and Raven, 1982) and scattered islands such as Fiji to form during the Eocene (47-56 mya) which are now present on the outer arc of the plates (Karig, 1974). Other island groups, such as the Solomon Islands and New Hebrides are later archipelago formations (suspected to span Eocene-Pleistocene, 2-23 mya) (Carney and MacFarlane, 1982, Coleman and Packham, 1976), for a more in-depth review see Raven and Axelrod (1972).

5.1.1.2. South-east Asia

South-east Asia has a complex geological history. This region formed as a result of the collision of multiple plates: the Pacific, Australian, Indian, Philippine and Eurasian plates, and its history is still debated in the literature (Turner et al., 2001). Factors such as volcanic activity, plate collisions, sea floor spreading and sea level fluctuations have resulted in rapid changes in topography in this region (Hall, 2001). Many parts of South-east Asia are Gondwanan in origin; in fact many fragments were once part of the Australian plate that drifted northwards (Burrett et al., 1991). West Malesia (or the West Malaysian archipelago/area west of Wallace's Line, (discussed later)) consists of early fragments that broke off the Australian plate approximately 100-200 mya (Audley-Charles, 1987, Metcalfe, 1998, Morley, 2000), reaching their present day position approximately 50 mya (van Welzen et al., 2011). High sea levels in the Late Eocene are thought to have submerged this region numerous times (Hall, 2009, Hall, 2001), although the dates are still debated. East Malesia (or the area east of Wallace's Line) consists of much later fragments from the Australian plate, that broke off around 15 mya (van Welzen et al., 2003) however, subsequent authors have suggested their age is as early as 50 mya (Audley-Charles, 1987). Most of these areas remained submerged until approximately 5-10 mya (van Welzen et al., 2005, Hall, 2009), however van Welzen et al. (2011) provides evidence that there are conflicting arguments from authors on this topic, further highlighting how little is known about this region.

5.1.1.3. Rafting India

Of all of the continental movements that took place after the breakup of Gondwana, India's movements were the fastest and most complex (Chatterjee et al., 2013). After breakup with Madagascar, India is suspected to have had contact with Greater Somalia between 70-75 mya (Chatterjee and Scotese, 1999) and the Oman-Kohistan-Dras island arc, which is hypothesised to have spanned the Indian Ocean between Asia and Africa approximately 66 mya (Chatterjee and Scotese, 2010). First direct contact between the Indian plate and South-east Asia is estimated to have occurred approximately 50-57 mya via a land bridge with Sumatra (Grismer et al., 2016)

before hard collision with Eurasia 20-25 mya (van Hinsbergen et al., 2012, Aitchison et al., 2007).

5.1.2. Proposed origins of the Dacini

Only a few hypotheses have been proposed for the geographic origin of the Dacini. Drew and Hancock (1999) provided arguments in favour of an 'out-of-India' hypothesis for the Dacini. This hypothesis was subsequently supported by molecular analysis which provided a hypothesised sequence of events (Fig. 5.2) based on the divergence times then estimated for the group (Krosch et al., 2012). Drew and Hancock (1999) hypothesised based on morphological and distributional data, that after India split from Gondwana, that the origins of the first Dacini began to evolve as the Indian plate drifted north, most notably with the genus *Dacus* moving into savannah regions and diversifying earlier in the mid to late Cretaceous (Krosch et al., 2012). After India collided with Laurasia (the northern landmass) *Bactrocera* began to diversify, specialising predominantly in rainforest plants; eventually dispersing into Asia and the South Pacific region. However, White (2006) rejected Drew and Hancock (1999), stating that *Dacus* species could not have originated on the India-Madagascar plate because the host plants did not exist when India and Madagascar were in contact and that for this hypothesis to be correct, the unlikely event of all Asian and African *Dacus* simultaneously undergoing host plant shifts to the same three plant families (Cucurbitaceae, Passifloraceae and Apocynaceae), independently of each other, would have had to have occurred. White (2006) provides an alternate hypothesis, but acknowledged more investigation is needed: *"..an initial spread, subsequent evolution of* Dacus *in Africa, and a later climatically filtered spread of dry-tolerant* Dacus *subgenera into Asia, is at this stage very speculative."* While hesitant to suggest an origin of the Dacini, White (2006) highlighted an issue that has still not been resolved today; that there is not universal agreement on the origins of the Dacini, and there have been minimal further studies on the subject since. Despite Krosch et al. (2012) providing evidence in support of the out-of-India hypothesis, they did not resolve any questions surrounding the origins of the Dacini in South-east Asia, Australia and the Pacific, and as identified in Chapter 3, the estimated dates of Krosch et al. (2012) for the evolution of the Dacini are likely too old by 20-30my.

Such a significant time gap is important when interpreting the evolution of the clade with respect to regional plate movements and positions.

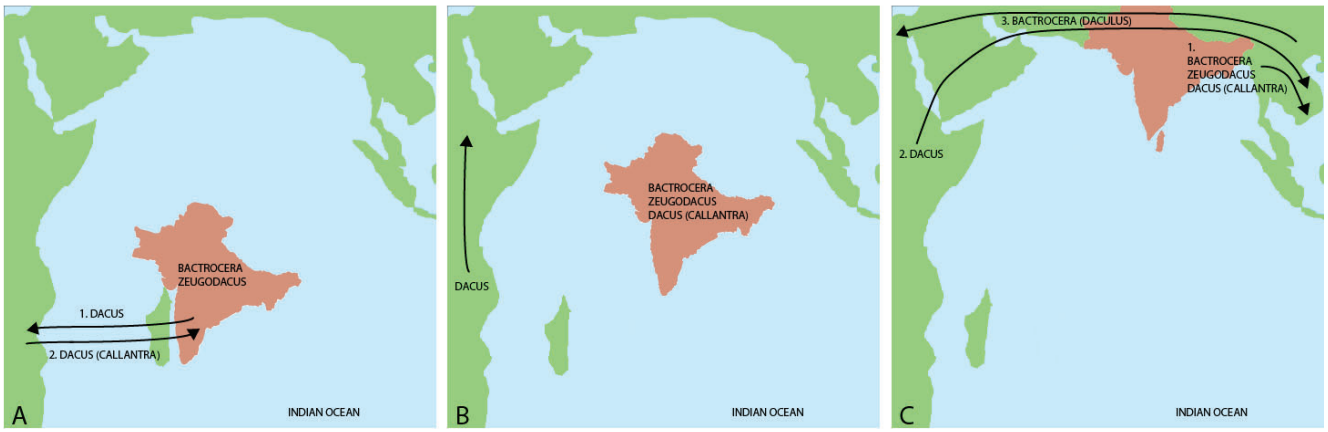


Figure 5.2: The out of India hypothesis first postulated by Drew and Hancock (1999) and subsequently modified by Krosch et al. (2012) showing the hypothesised movements of the three main Dacini genera; (*Dacus, Zeugodacus* and *Bactrocera*), over geological time as well as movements of key subgenera *Dacus* (*Callantra*) and *Bactrocera* (*Daculus*). A: 1. 65-80 mya; 2. 63 mya; B: 57-63 mya; and C: 1. 35-57 mya, 2. 45 mya and 3. 18 mya. Figure adapted from Krosch et al. (2012).

5.1.3. Biogeographic zones and Dacini evolution

The movement of geological plates, collisions and separations, local uplifts, and seaway inundation or drying are all factors that can drive development of regionally unique fauna and flora and create what we now regard as biogeographic regions (Raven and Axelrod, 1974, Glor and Warren, 2010). The biogeographic history of Asia and the Western Pacific is particularly complex (van Welzen et al., 2003), and this section introduces the regional and Australian biogeographic zones before discussing the relationships of the regional Dacini with those zones. For a more detailed summary of biogeographic zones in South-east Asia, see van Welzen et al. (2011)) and for detail on zones in Australia, see Ebach et al. (2015) and Bryant and Krosch (2016).

5.1.3.1. Biogeography of South-east Asia and the West Pacific

Due to the complex geological movements discussed above, understanding the formation and timing of biogeographic barriers in the Asia-Pacific is not straightforward. Malesia, consisting of New Guinea, the Malay Peninsula, the Philippines and smaller surrounding islands is considered the source area of most taxa found in the tropical Pacific (van Balgooy, 1971). Because of the early arrival of West Malesia, taxa in this region are considered South-east Asian in origin, whereas East Malesian taxa are considered to constitute mostly Australian fauna (Whitmore, 1982, Turner et al., 2001). Hall (2001) argues that there is no easy explanation as to how Australian and Asian taxa have 'mixed' in this region. Because this is a region where taxa of different origins overlap (or do not overlap) this has prompted investigations of potential biogeographic zones in this region and the development of numerous biogeographic "lines" by various authors (Fig. 5.3) (Hall, 2001, van Welzen et al., 2011). Many of these biogeographic regions are defined by faunal distributions (van Welzen et al., 2011), and Whitmore (1982) argues these lines are subject to factors such as dispersibility of the group and adds that rainforest plants in this region have permeated these proposed barriers (in this instance the author refers to Wallace's Line). These zones, such as those bordered by Wallace's Line and the Isthmus of Kra (a barrier across the Malaysian archipelago) have been shown to restrict movement of several insect species (Kitching et al., 2001, Beck et al., 2006),

which prompts the need for further investigation into how the Dacini speciated in this region.

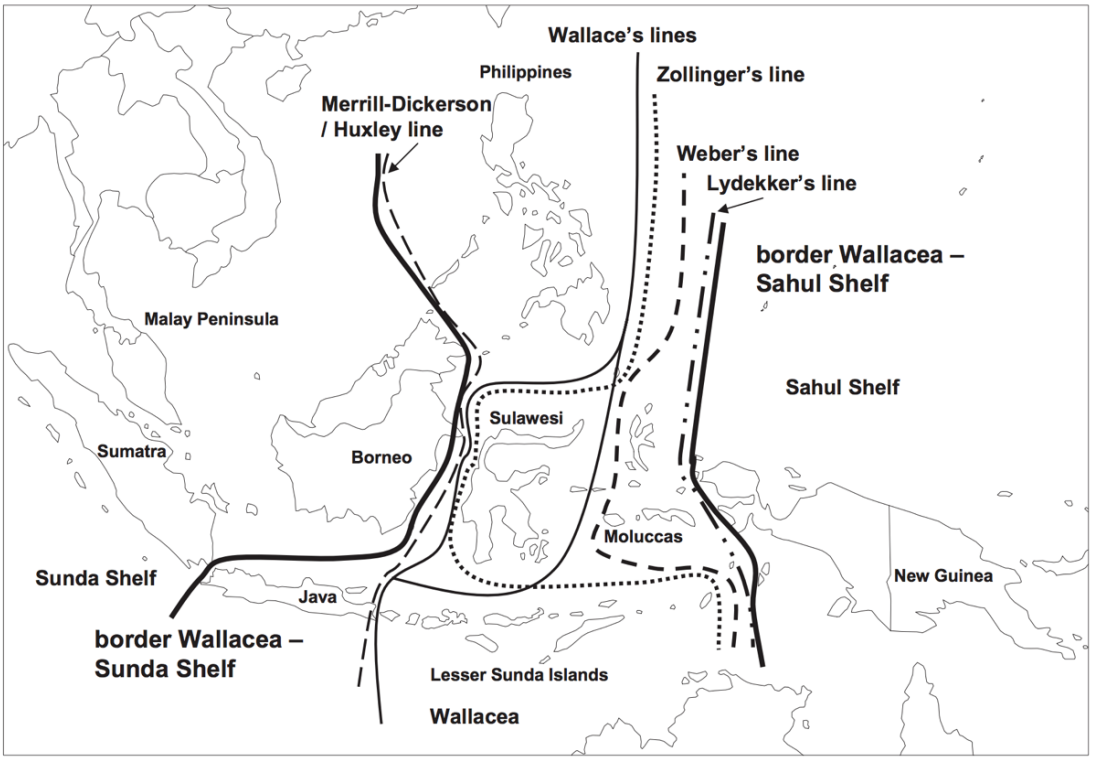


Figure 5.3: The numerous biogeographical lines that have been proposed as barriers to dispersal in the Malesian region of South-east Asia. Figure taken from van Welzen et al. (2011).

5.1.3.2. Australia and New Guinea

Australia and New Guinea, while currently separated by the Torres Strait and the Arafura Sea, have a long geological association (Hall, 2001) and, as demonstrated later in the results of this chapter, have much commonality in their Dacini fauna. For those reasons they are dealt with here together.

New Guinea is considered to have one of the highest levels of faunal and floral diversity in the region (Diamond, 1984). With roughly 60% of species considered to be endemic to the island, New Guinea is home to 6-8% of the world's biodiversity (Papua New Guinea Department of Conservation, 2017). New Guinea consists of three major geological elements, the leading edge of the Australian craton in the

south, and the intermixed New Guinea orogon and the accreted New Guinea terranes in the centre and north (Heads, 2001). Various groups of plants, vertebrates and invertebrates show geographic clustering based on their association with the Australian craton or the New Guinea orogon + the accreted New Guinea terranes (Polhemus and Polhemus, 1998, Parsons, 1999, Heads, 2002a, Heads, 2002b).

Biogeographical boundaries have been identified along the northern and eastern coastlines of Australia (Fig. 5.4) (Ebach et al., 2015, Bryant and Krosch, 2016) and are thought to represent barriers to dispersal for many taxa (Bell et al., 2007, Bryant and Fuller, 2014). These barriers constitute significant changes in habitat for example, rainforest systems separated by dry arid zones (Bryant and Krosch, 2016). Scattered along the eastern coast, and commonly intersected by these biogeographical barriers are old Gondwanan rainforests (Kershaw, 1994, Williams and Pearson, 1997, Nicholls and Austin, 2005). Weber et al. (2014) investigated these isolated patches and found that there are several centres of endemism that are predicted to have remained as stable and highly diverse rainforest systems for the past 120,000 years. These isolated habitats may have acted as refuge for species isolated by these barriers.

Figure 5.4: Identified biogeographical boundaries around the northern and eastern coastlines of Australia. Figure adapted from Bryant and Krosch (2016) and Ebach et al. (2015).

5.1.4. Impact of biogeography on distribution and diversity of the Dacini in Australasia

The South-east Asian and New Guinean region is considered to be the centre of Dacini diversity (Drew and Hancock, 1999, Drew, 2004) with *Bactrocera, Zeugodacus* and *Monachrostichus* most prevalent in the Asia-Pacific, whereas *Dacus* is concentrated in Africa (Drew and Hancock, 1999, Doorenweerd et al., 2018). Drew (2004) recorded that Dacini species numbers decline as you move west from South-east Asia, and east and south-east of New Guinea. In fact, the greatest species diversity in a single country occurs in Papua New Guinea (Drew, 2004), with particularly high levels of endemism recorded in the Morobe and Central provinces (Clarke et al., 2004, Novotny et al., 2005). Drew (2004) argues that high levels of endemism in the major distribution regions (i.e. India, South-east Asia, New Guinea,

Australia and the Pacific Islands), indicate the Dacini have speciated in isolation for a long period of time due to biogeographic barriers.

Hancock and Drew (2015) identified six biogeographic zones in South-east Asia and the Pacific based on levels of Dacini endemism (Fig. 5.5). The authors note that species from the *Bactrocera* subgenus *Parazeugodacus* Shiraki are present in all six regions that they identified, suggesting these species diversified due to vicariance (Hancock and Drew, 2015). Despite this, there was no formal biogeographic analysis of species distributions provided in this study, and therefore the implications of this subgeneric distribution will remain unknown.

Drew (2004) identified a strong disjunction between the Dacini species of South-east Asia and the Western Pacific, noting that the line of demarcation between Asian and Pacific endemics in New Guinea and those in South-east Asia is to the east of Wallace's Line (Fig. 5.3). Species-level studies have investigated the influence of biogeographic barriers on distribution and population structure. Krosch et al. (2019a) found that *B. umbrosa* F. is native to the West Pacific but has colonised west of Wallace's Line via a single incursion event; independent of human introduction. In that study, the major split (both genetically and morphologically) between Asian and Pacific populations was found to be associated with Lydekker's Line (Fig. 5.3). The Isthmus of Kra, located on peninsula Thailand, and a known biogeographic barrier that separates Asian and Sunda flora and fauna, was found to have minimal impact on population structuring of (now) *B. dorsalis* (Krosch et al., 2013) and *Z. cucurbitae* (Boontop et al., 2017). Despite this, differentiation in climate, vegetation and terrain along the Thai peninsula was found to significantly influence population structuring of *Z. cucurbitae* (Boontop et al., 2017), adding greater complexity to fruit fly biogeography.

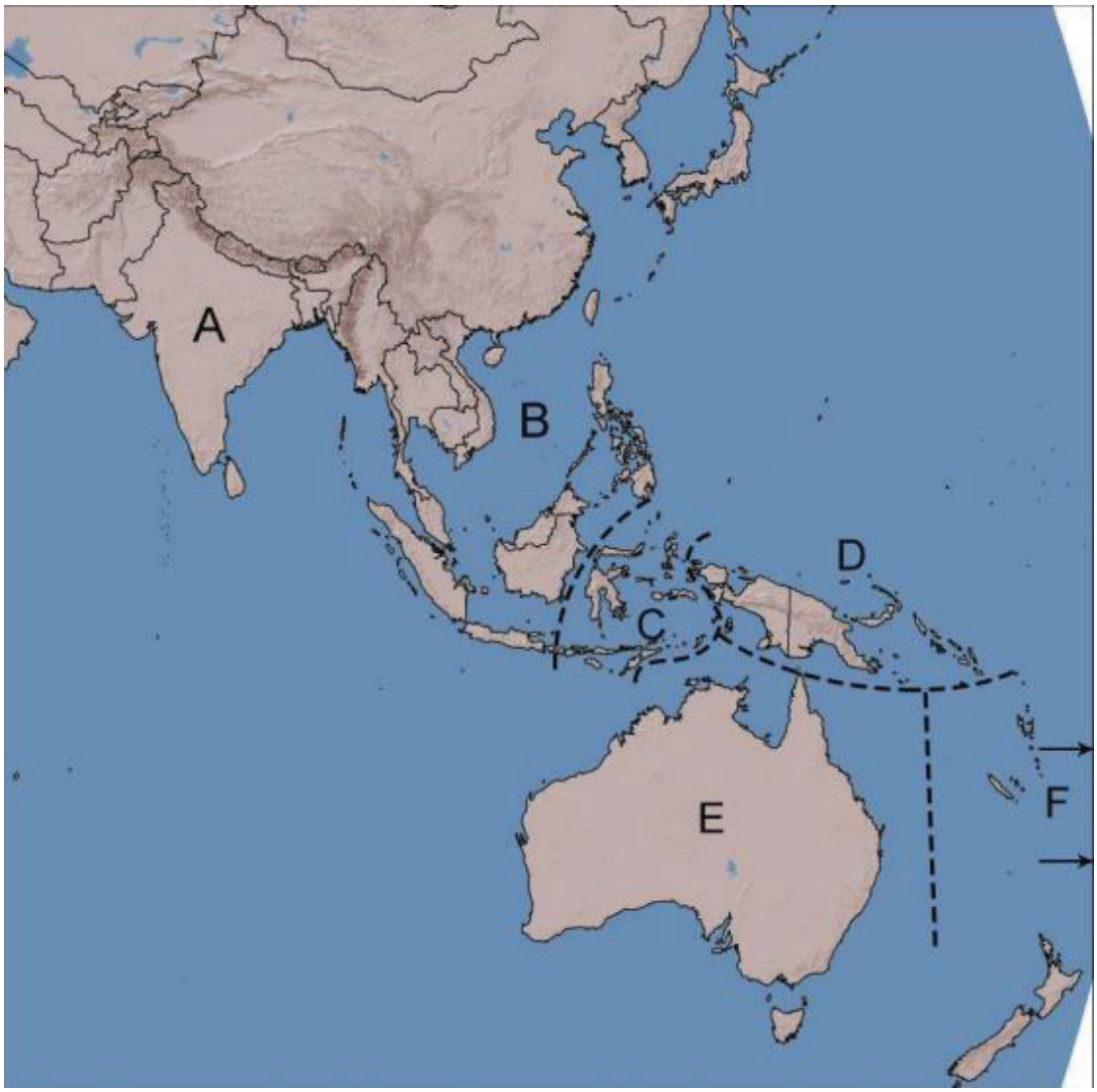


Figure 5.5: Biogeographic zones of Dacini endemism identified by Hancock and Drew (2015). A: India; B: South-east Asia; C: Wallacea; D: New Guinea; E: Australia; and F: South Pacific. Figure taken from Hancock and Drew (2015).

Australian species diversity is highest in the Cape York Peninsula, and decreases with higher latitudes (Hancock et al., 2000, Huxham and Hancock, 2002, Huxham et al., 2006, Royer and Hancock, 2012). Species are not found in dry arid regions (such as the Carpentaria Basin that separates northern NT and Queensland (Hancock et al., 2000), therefore barriers such as this are expected to have influenced speciation in the region. Yu et al. (2001) investigated the population genetics of *B. tryoni* in Australia over five years. The study found population structuring due to geographic distance, and Queensland populations were found to have moved into southern

regions in later collection years. This is an example of a prolific pest species that is incredibly good at dispersing and can occupy urban areas in high numbers (Raghu et al., 2000). There is little known of how biogeographic barriers in Australia have influenced other Dacini species which may have limited host ranges and live in niche habitats (such as those identified in Chapter 2). The factors which have influenced these narrow ranges are yet to be investigated.

Based on morphological characters, Michaux and White (1999) produced an area cladogram for the Dacini of the Southwest Pacific (Fig. 5.6). They found the species in the outer Pacific islands formed a sister clade to species in the Southern Melanesian arc islands clade; Vanuatuan species were sister to both of these clades; with Micronesia and New Caledonia forming a clade that was then sister to these. However, the authors concluded that further work was needed to fully test Pacific relationships. Additionally, Michaux and White found the Australian Dacini form close relationships with those from the Solomon Islands, Bismarck Archipelago and mainland New Guinea (the cratonic margin). After more thorough sampling of the New Guinea provinces, Clarke et al. (2004) concluded that there is far greater Dacini species diversity in the accreted terranes of New Guinea and the offshore islands (such as Bougainville and the Bismarck Archipelago) than the southern part of New Guinea which was the leading edge of the Australian plate (before plate collision). The close relationships between the Dacini taxa from Australia and New Guinea has been recognised (Drew, 1989, Michaux and White, 1999), but the pathways and origins of these species has never been explored in detail. However, the relationships which are already known provide clues that New Guinea could be the site of species transfer between Australian and Asian plates.

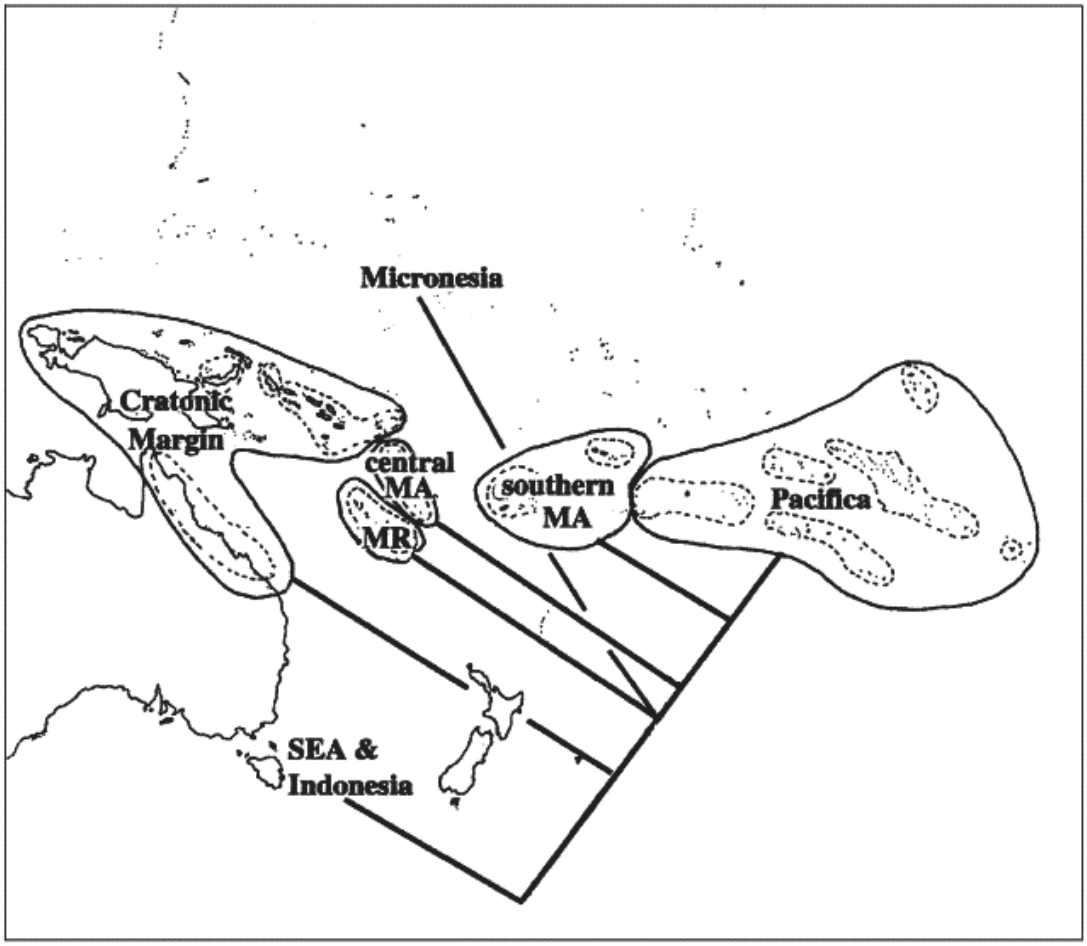


Figure 5.6: Area cladogram relationships imposed upon the Southwest Pacific by Michaux and White (1999). Central MA: central Melanesian arc; southern MA: southern Melanesian arc; and MR: Melanesian rift. Figure taken from Michaux and White (1999).

5.1.5. Host plant associations

Large bursts of radiation have been identified within the Dacini, in particular within *Bactrocera* in the Gondwanan rainforests of South-east Asia (Drew and Romig, 2013) and New Guinea (Drew, 2004). Drew (2004) suggested that dacines have coevolved alongside these plants during the Tertiary Period. An example is provided by Drew and Hancock (1999) who identified the relationships between the distribution of hosts within the plant family Asclepiadaceae and the genus *Dacus* which are both found in higher numbers in Africa. However, countering a close co-speciation between plants and the Dacini is evidence that most *Bactrocera* are not host specific (Drew, 2004, Novotny et al., 2005) and that recent radiations in

Bactrocera have tended to involve polyphagous species. While I recognise the important role that host plants are suspected to play in the evolution of the Dacini, this chapter will focus only on exploring the role of biogeographical influences on regional species movement and diversification.

5.1.6. Chapter aims and hypotheses

I have provided a background and review of the biogeographic factors that have influenced the evolutionary history of the Dacini, which has informed the aims of this chapter. This chapter aims to investigate the movement of the Dacini species through South-east Asia into Australia and the Pacific by testing four key hypotheses; (i) that the movement of the Dacini into the Pacific through Australia and New Guinea was unidirectional; (ii) that movement of the Dacini into Australia was predominantly via a land bridge between New Guinea; and (iii) that Australian species have been restricted by identified biogeographical barriers. While the main aim of this chapter is to investigate movement and dispersal of the Dacini in Australia and the Pacific, the dated analysis (Chapter 3) provides evidence that may require modification of the current 'out of India' hypothesis on the origins of the Dacini.

5.2. Methodology

5.2.1. General methods

The three analyses followed the same general methodology using the RStudio v3.6.3 (RStudio Team, 2019) package BioGeoBEARS (Matzke, 2013a) and supporting packages; ape (Paradis and Schliep, 2019), cladoRcpp (Matzke, 2013b), devtools (Wickham et al., 2020), gdata (Warnes et al., 2017), gtools (Warnes et al., 2020), optimx (Nash and Varadhan, 2011), phylobase (Hackathon et al., 2020), plotrix (Lemon, 2006), Rcpp (Eddelbuettel and Balamuta, 2018), RccpArmadillo (Eddelbuettel and Sanderson, 2014), rexpokit (Matzke and Sidje, 2013), snow (Tierney et al., 2018), SparseM (Koenker and Ng, 2019), vegan (Oksanen et al., 2019), xtable (Dahl et al., 2019). The input included dated tree topologies (subsets from Chapter 3) and a text file that consisted of the native range of the taxa. Trees

were trimmed so that all outgroups, undescribed species and additional multiples of species were removed. I also utilised the non-adjacent ranges script (Matzke, 2013a), to ease the computational burden and remove impractical movements between ranges. Six models were run on each dataset and the results from the best AIC score was presented in the results.

5.2.2. Geographic range data

I scored ranges for 143 species in three genera. Range data was collated from Smith et al., (1988), Drew, (1989), Osborne et al., (1997), Drew et al., (1999), Raganath and Veenakumari, (1999), White and Evenhuis, (1999), Hancock et al., (2000), Huxham and Hancock, (2002), Hollingsworth et al., (2003), Drew et al., (2011), Leblanc et al., (2012), Royer and Hancock, (2012), Drew and Romig, (2013), Leblanc et al., (2014), Royer, (2015), Hancock and Drew, (2017c) Linda et al., (2018) and Royer, (2018). I only included the native ranges for taxa and removed any ranges considered to be human introductions, e.g. the case of *B. frauenfeldi* which is invasive to Australia (Royer et al., 2016) or recent expansions such as *B. umbrosa* from the Western Pacific in South-east Asia (Krosch et al., 2019a). Species such as *B. oleae,* which has a native range in Africa, were also removed due to the Australasian focus of this dataset. The scored ranges and species included for each analysis are provided in Tables 5.1-5.3.

There are limitations to this data that must be mentioned. Firstly, species ranges may not be fully captured in the literature, particularly for species which are not lure responsive such as *B. tigrina* which has been reared only a handful of times (Drew, 1989). “New records” and “invasive records” have also been confused for Dacini species in the past (Clarke et al., 2019) and this may impact on a small number of my native records. It is also acknowledged that pre-history human mediated transport may be responsible for present-day distributions. However, some Dacini have been recorded flying up to 13km in a 24 hour period (Remund et al., 1976); suggesting wide ranges are more likely explained by the dispersal capabilities of the flies; i.e. *B. tryoni* is an extremely good disperser (Macfarlane et al., 1987) and it could be argued that it has the potential to move further than humans may have been able to travel at that time.

5.2.2.1. Pacific analysis

Here I tested the following hypothesis: that there was unidirectional movement into the Pacific (New Caledonia, Melanesia, Polynesia, but not including Papua New Guinea) via two main pathways through Papua New Guinea and/or Australia, with no movement back into Australia or Papua New Guinea. Six areas were utilised for this analysis (Table 5.1), with regions grouped based on their geography. New Caledonia and Papua New Guinea were kept as individual regions so that they were not lost amongst the rest of the dataset and specific movement pathways could be tested. Papua New Guinea was kept separate as it is Australia's nearest neighbour, so a suspected entry point for species (Drew, 1989). The Torres Strait islands were grouped with Papua New Guinea because of the large number of species that are either endemic to the Torres Strait or share distributions with Papua New Guinea and not Australia (Hancock, 2013). New Caledonia was kept separate because of its unique geological history (being part of Zealandia, and not an accreted terrane like surrounding island arcs (Mortimer et al., 2017)), and because it is an area of high endemism with sufficient taxon coverage (Drew, 2004). The combination of smaller islands into "Polynesia" and "Melanesia" also avoided biases in the data. The main aim of this analysis was to determine the role the Pacific Islands have played in the dispersal and speciation of Australia's native species.

Table 5.1: Scored range states for 143 Dacini species for six regions used in the Pacific biogeographic analysis. *North and west of Wallacea* = Borneo, Java, Sumatra, Christmas Island, Malay Peninsula (south of Isthmus of Kra), Philippines, the China-Tibetan region, Japan, Indo-Asia (north of Isthmus of Kra and South China), the Andaman and Nicobar Islands, Molluccas, Sulawesi and Sunda Islands; *New Guinea* = Papua New Guinea, West Papua and the Torres Strait Islands; *Melanesia* = the Bismarck Archipelago, Solomon Islands, Vanuatu and Fiji; and *Polynesia* = Niue Island, Samoa, Pitcairn Islands, Tonga, Wallis and Futuna, French Polynesia, Cook Islands.

Species	North and west of Wallacea	Australia	New Guinea	Melanesia	New Caledonia	Polynesia
B. abdonigella	1	0	1	0	0	0
B. aberrans	0	1	0	0	0	0
B. abscondita	0	1	0	0	0	0
B. absidata	0	0	1	0	0	0
B. aeroginosa	0	1	0	0	0	0
B. aglaiae	0	1	1	0	0	0
B. albistrigata	1	0	0	0	0	0
B. allwoodi	0	1	0	0	0	0
B. alyxiae	0	1	1	0	0	0
B. amplexiseta	0	1	0	0	0	0
B. antigone	0	1	0	0	0	0
B. aquilonis	0	1	0	0	0	0
B. atramentata	0	0	0	1	0	0
B. aurantiaca	0	1	1	0	0	0
B. aurea	0	1	0	0	0	0
B. bancroftii	0	1	1	1	0	0
B. barringtoniae	0	1	1	0	0	0
B. batemani	0	1	0	0	0	0
B. bidentata	0	1	0	0	0	0
B. breviaculeus	0	1	1	0	0	0
B. bryoniae	0	1	1	1	0	0
B. brunnea	0	1	0	0	0	0
B. cacuminata	0	1	0	0	0	0
B. caledoniensis	0	0	0	0	1	0
B. calophylli	1	1	0	0	0	0
B. cheesmanae	0	0	1	0	0	0
B. consectorata	0	0	1	1	0	0

Species	North and west of Wallacea	Australia	New Guinea	Melanesia	New Caledonia	Polynesia
B. curreyi	0	0	1	0	0	0
B. curvifera	1	0	1	1	0	0
B. curvipennis	0	0	0	0	1	0
B. decurtans	0	1	1	0	0	0
B. distincta	0	0	0	1	0	1
B. dyscrita	0	0	0	1	0	0
B. ebenea	0	0	0	0	1	0
B. endiandrae	0	1	1	0	0	0
B. erubescentis	0	1	1	0	0	0
B. facialis	0	0	0	0	0	1
B. fagraea	0	1	0	0	0	0
B. frauenfeldi	1	0	1	1	0	0
B. fulvicauda	0	0	1	0	0	0
B. fulvifacies	0	0	0	0	1	0
B. furvilineata	0	0	1	0	0	0
B. halfordiae	0	1	0	0	0	0
B. jarvisi	0	1	1	0	0	0
B. kraussi	0	1	1	0	0	0
B. lampabilis	0	0	0	1	0	0
B. laticaudus	0	1	0	0	0	0
B. latilineola	1	0	0	0	0	0
B. lineata	0	0	1	0	0	0
B. manskii	0	1	1	0	0	0
B. mayi	0	1	1	0	0	0
B. melanothoracica	0	1	1	0	0	0
B. melas	0	1	0	0	0	0
B. melastomatos	1	0	0	0	0	0
B. minax	1	0	0	0	0	0
B. moluccensis	1	0	1	1	0	0
B. morobiensis	0	0	1	0	0	0
B. mucronis	0	0	0	0	1	0
B. murrayi	0	1	1	0	0	0
B. musae	0	1	1	1	0	0
B. mutabilis	0	1	0	0	0	0
B. neocheesmanae	0	0	1	0	0	0
B. neohumeralis	0	1	1	0	0	0
B. nigra	0	1	0	0	0	0
B. nigrescentis	0	0	0	1	0	0

Species	North and west of Wallacea	Australia	New Guinea	Melanesia	New Caledonia	Polynesia
B. opiliae	0	1	0	0	0	0
B. pallida	0	1	1	0	0	0
B. parabarringtoniae	0	0	1	0	0	0
B. paramusae	0	0	1	0	0	0
B. paraxanthodes	0	0	0	0	1	0
B. passiflorae	0	0	0	1	0	1
B. pendleburyi	1	0	0	0	0	0
B. peneobscura	0	0	0	1	0	0
B. peninsularis	0	1	1	0	0	0
B. pepsialae	0	0	0	1	0	0
B. perkinsi	0	1	1	0	0	0
B. phaleriae	0	1	0	0	0	0
B. propinqua	1	0	0	0	0	0
B. quadrata	0	1	1	1	0	0
B. recurrens	1	0	1	0	0	0
B. redunca	0	0	1	1	0	0
B. repanda	0	0	1	0	0	0
B. resima	1	0	1	0	0	0
B. romigae	0	1	0	0	0	0
B. rufescens	0	1	0	0	0	0
B. rufofuscula	0	1	1	0	0	0
B. russeola	0	1	0	0	0	0
B. seguyi	0	0	1	1	0	0
B. silvicola	0	1	1	0	0	0
B. speculifera	0	0	1	1	0	0
B. speewahensis	0	1	0	0	0	0
B. tapahensis	1	0	0	0	0	0
B. tenuifascia	0	1	0	0	0	0
B. terminaliae	0	0	1	0	0	0
B. tigrina	0	1	0	0	0	0
B. tinomiscii	0	0	1	0	0	0
B. trilineola	0	0	0	1	0	0
B. trivialis	0	0	1	0	0	0
B. tryoni	0	1	1	0	0	0
B. tsuneonis	1	0	0	0	0	0
B. umbrosa	0	0	1	1	1	0
B. unitaeneola	0	0	0	1	0	0
B. ustulata	0	0	1	0	0	0

Species	North and west of Wallacea	Australia	New Guinea	Melanesia	New Caledonia	Polynesia
B. visenda	0	1	1	0	0	0
B. vulgaris	0	0	1	0	0	0
B. xanthodes	0	0	0	1	0	1
B. yorkensis	0	1	0	0	0	0
D. absonifacies	0	1	0	0	0	0
D. aequalis	0	1	0	0	0	0
D. aneuvittatus	0	0	0	0	1	0
D. axanus	0	1	1	1	0	0
D. bellulus	0	1	1	0	0	0
D. hardyi	0	1	1	0	0	0
D. impar	0	0	1	0	0	0
D. longicornis	1	0	0	0	0	0
D. mayi	0	0	1	0	0	0
D. newmani	0	1	0	0	0	0
D. palmerensis	0	1	0	0	0	0
D. pusillus	0	1	1	0	0	0
D. salamander	0	1	1	0	0	0
D. secamoneae	0	1	0	0	0	0
D. signatifrons	0	1	0	0	0	0
Z. atrifacies	1	0	0	0	0	0
Z. choristus	0	1	1	0	0	0
Z. cilifer	1	0	0	0	0	0
Z. cucumis	0	1	1	0	0	0
Z. cucurbitae	1	0	0	0	0	0
Z. depressus	1	0	0	0	0	0
Z. diversus	1	0	0	0	0	0
Z. fallacis	0	1	0	0	0	0
Z. hochii	1	0	0	0	0	0
Z. hululangitae	1	0	0	0	0	0
Z. incisus	1	0	0	0	0	0
Z. macrovittatus	0	0	1	0	0	0
Z. neopallescentis	0	0	1	0	0	0
Z. platamus	1	0	0	0	0	0
Z. reflexus	0	0	0	1	0	0
Z. sandaracinus	0	0	1	0	0	0
Z. scutellatus	1	0	0	0	0	0
Z. strigifinis	0	1	1	1	0	0
Z. tau	1	0	0	0	0	0
Z. triangularis	0	0	0	1	0	0

Species	North and west of Wallacea	Australia	New Guinea	Melanesia	New Caledonia	Polynesia
Z. vinnulus	1	0	0	0	0	0

5.2.2.2. Broad scale analysis

This analysis investigated the origins and movement of the ancestors of Australia's native species using a subset of the tree produced for the Pacific analysis (5.3.1). The previous analysis revealed species dispersal was unidirectional from West to East in the Pacific and therefore the removal of these species would have no impact on the patterns of dispersal into and within Australia. Therefore, species that were endemic to the Pacific (Melanesia, Polynesia and New Caledonia) were removed and the Pacific range data was removed. I identified six ranges for this analysis (Table 5.2), based on the hypothesis that species dispersed into Australia only via New Guinea. The alternate (or additional) hypothesis would be entry via Wallacea, therefore this region was also kept independent.

Table 5.2: Scored range states for 122 Dacini species for six regions used in the broad scale biogeographic analysis. *Indo-Asia* = mainland Asia (north of Isthmus of Kra), South China and the Andaman and Nicobar Islands; *China-Tibet-Japan* = North China, Japan and north of Himalayas; *Sunda* = Borneo, Java, Sumatra, Christmas Island, Malay Peninsula (south of Isthmus of Kra) and the Phillipines; *Wallacea* = Molluccas, Sulawesi and Sunda Islands; and *New Guinea* = Papua New Guinea, West Papua and the Torres Strait Islands.

Species	Indo-Asia	Australia	China-Tibet-Japan	Sunda	Wallacea	New Guinea
B. abdonigella	0	0	0	0	1	1
B. aberrans	0	1	0	0	0	0
B. abscondita	0	1	0	0	0	0
B. absidata	0	0	0	0	0	1
B. aeroginosa	0	1	0	0	0	0
B. aglaiae	0	1	0	0	0	1
B. albistrigata	1	0	0	1	1	0
B. allwoodi	0	1	0	0	0	0

Species	Indo-Asia	Australia	China-Tibet-Japan	Sunda	Wallacea	New Guinea
B. alyxiae	0	1	0	0	0	1
B. amplexiseta	0	1	0	0	0	0
B. antigone	0	1	0	0	0	0
B. aquilonis	0	1	0	0	0	0
B. aurantiaca	0	1	0	0	0	1
B. aurea	0	1	0	0	0	0
B. bancroftii	0	1	0	0	0	1
B. barringtoniae	0	1	0	0	0	1
B. batemani	0	1	0	0	0	0
B. bidentata	0	1	0	0	0	0
B. breviaculeus	0	1	0	0	0	1
B. bryoniae	0	1	0	0	0	1
B. brunnea	0	1	0	0	0	0
B. cacuminata	0	1	0	0	0	0
B. calophylli	1	1	0	1	0	0
B. cheesmanae	0	0	0	0	0	1
B. consectorata	0	0	0	0	0	1
B. curreyi	0	0	0	0	0	1
B. curvifera	0	0	0	0	1	1
B. decurtans	0	1	0	0	0	1
B. endiandrae	0	1	0	0	0	1
B. erubescentis	0	1	0	0	0	1
B. fagraea	0	1	0	0	0	0
B. frauenfeldi	0	0	0	0	1	1
B. fulvicauda	0	0	0	0	0	1
B. furvilineata	0	0	0	0	0	1
B. halfordiae	0	1	0	0	0	0
B. jarvisi	0	1	0	0	0	1
B. kraussi	0	1	0	0	0	1
B. laticaudus	0	1	0	0	0	0
B. latilineola	1	0	0	1	0	0
B. lineata	0	0	0	0	0	1
B. manskii	0	1	0	0	0	1
B. mayi	0	1	0	0	0	1
B. melanothoracica	0	1	0	0	0	1
B. melas	0	1	0	0	0	0
B. melastomatos	1	0	0	1	0	0
B. minax	1	0	1	0	0	0
B. moluccensis	0	0	0	0	1	0
B. morobiensis	0	0	0	0	0	1

Species	Indo-Asia	Australia	China-Tibet-Japan	Sunda	Wallacea	New Guinea
B. murrayi	0	1	0	0	0	1
B. musae	0	1	0	0	0	1
B. mutabilis	0	1	0	0	0	0
B. neocheesmanae	0	0	0	0	0	1
B. neohumeralis	0	1	0	0	0	1
B. nigra	0	1	0	0	0	0
B. opiliae	0	1	0	0	0	0
B. pallida	0	1	0	0	0	1
B. parabarringtoniae	0	0	0	0	0	1
B. paramusae	0	0	0	0	0	1
B. pendleburyi	1	0	0	1	0	0
B. peninsularis	0	1	0	0	0	1
B. perkinsi	0	1	0	0	0	1
B. phaleriae	0	1	0	0	0	0
B. propinqua	1	0	0	1	0	0
B. quadrata	0	1	0	0	0	1
B. recurrens	0	0	0	0	1	1
B. redunca	0	0	0	0	0	1
B. repanda	0	0	0	0	0	1
B. resima	0	0	0	1	0	1
B. romigae	0	1	0	0	0	0
B. rufescens	0	1	0	0	0	0
B. rufofuscula	0	1	0	0	0	1
B. russeola	0	1	0	0	0	0
B. seguyi	0	0	0	0	0	1
B. silvicola	0	1	0	0	0	1
B. speculifera	0	0	0	0	0	1
B. speewahensis	0	1	0	0	0	0
B. tapahensis	0	0	0	1	0	0
B. tenuifascia	0	1	0	0	0	0
B. terminaliae	0	0	0	0	0	1
B. tigrina	0	1	0	0	0	0
B. tinomiscii	0	0	0	0	0	1
B. trivialis	0	0	0	0	0	1
B. tryoni	0	1	0	0	0	1
B. tsuneonis	0	0	1	0	0	0
B. umbrosa	0	0	0	0	0	1
B. ustulata	0	0	0	0	0	1
B. visenda	0	1	0	0	0	1

Species	Indo-Asia	Australia	China-Tibet-Japan	Sunda	Wallacea	New Guinea
B. vulgaris	0	0	0	0	0	1
B. yorkensis	0	1	0	0	0	0
D. absonifacies	0	1	0	0	0	0
D. aequalis	0	1	0	0	0	0
D. axanus	0	1	0	0	0	1
D. bellulus	0	1	0	0	0	1
D. hardyi	0	1	0	0	0	1
D. impar	0	0	0	0	0	1
D. longicornis	1	0	0	1	0	0
D. mayi	0	0	0	0	0	1
D. newmani	0	1	0	0	0	0
D. palmerensis	0	1	0	0	0	0
D. pusillus	0	1	0	0	0	1
D. salamander	0	1	0	0	0	1
D. secamoneae	0	1	0	0	0	0
D. signatifrons	0	1	0	0	0	0
Z. atrifacies	1	0	0	1	0	0
Z. choristus	0	1	0	0	0	1
Z. cilifer	1	0	1	1	0	0
Z. cucumis	0	1	0	0	0	1
Z. cucurbitae	1	0	0	1	0	0
Z. depressus	0	0	1	0	0	0
Z. diversus	1	0	1	0	0	0
Z. fallacis	0	1	0	0	0	0
Z. hochii	1	0	1	1	0	0
Z. hululangitae	0	0	0	1	0	0
Z. incisus	1	0	1	1	0	0
Z. macrovittatus	0	0	0	0	0	1
Z. neopallescentis	0	0	0	0	0	1
Z. platamus	1	0	0	1	0	0
Z. sandaracinus	0	0	0	0	0	1
Z. scutellatus	1	0	1	0	0	0
Z. strigifinis	0	1	0	0	1	1
Z. tau	1	0	1	1	0	0
Z. vinnulus	0	0	0	1	0	0

5.2.2.3. Australian analysis

The same subset tree used in the broad scale analysis was used here (Table 5.3). The seven areas combined all non-Australian regions into a single region in order to gain a better understanding of how the biogeographical barriers within Australian have influenced species distribution and speciation. The regions identified by Bryant and Krosch (2016) and Ebach et al. (2015) (Table 5.3, Fig. 5.4) were used in this analysis to test whether they have restricted the movement of Australian Dacini. Three regions were combined here: The McPherson-Macleay overlap, the Southern Transition Zone and Eastern Queensland. This was done for two reasons: firstly, the majority of species that are found in these regions are pest species and their distribution in these regions is likely due to the presence of suitable hosts (crops); secondly, species that were considered to be non-pest forest dwellers were present in all three locations.

Table 5.3: Scored range states for 122 Dacini species for seven regions used in the refined Australian biogeographic analysis. Eastern QLD, NSW and VIC = Includes Eastern Queensland, McPherson-Macleay Overlap and Southern Transition Zone.

Species	Outside Australia	Cape York Peninsula	Atherton Plateau	Eastern QLD, NSW and VIC	Kimberley Plateau	Arnhem Land
B. abdonigella	1	0	0	0	0	0
B. aberrans	0	0	1	1	0	0
B. abscondita	0	1	1	0	0	0
B. absidata	1	0	0	0	0	0
B. aeroginosa	0	1	1	0	0	0
B. aglaiae	1	0	1	0	0	0
B. albistrigata	1	0	0	0	0	0
B. allwoodi	0	0	0	0	0	1
B. alyxiae	1	1	1	1	0	0
B. amplexiseta	0	0	1	0	0	0
B. antigone	0	1	0	0	0	0
B. aquilonis	0	0	0	0	1	1
B. aurantiaca	1	1	0	0	0	0
B. aurea	0	1	1	1	0	0
B. bancroftii	1	1	1	1	0	0

Species	Outside Australia	Cape York Peninsula	Atherton Plateau	Eastern QLD, NSW and VIC	Kimberley Plateau	Arnhem Land
B. barringtoniae	1	1	1	0	0	0
B. batemani	0	1	1	1	0	0
B. bidentata	0	0	1	1	0	0
B. breviaculeus	1	1	1	1	0	0
B. brunnea	0	0	1	1	0	0
B. bryoniae	1	1	1	1	1	1
B. cacuminata	0	1	1	1	0	0
B. calophylli	1	0	1	0	0	0
B. cheesmanae	1	0	0	0	0	0
B. consectorata	1	0	0	0	0	0
B. curreyi	1	0	0	0	0	0
B. curvifera	1	0	0	0	0	0
B. decurtans	1	1	1	0	1	1
B. endiandrae	1	1	1	1	0	0
B. erubescentis	1	1	0	0	0	0
B. fagraea	0	0	1	1	0	0
B. frauenfeldi	1	0	0	0	0	0
B. fulvicauda	1	0	0	0	0	0
B. furvilineata	1	0	0	0	0	0
B. halfordiae	0	0	1	1	0	0
B. jarvisi	1	1	1	1	1	1
B. kraussi	1	1	1	0	0	0
B. laticaudus	0	1	1	1	0	0
B. latilineola	1	0	0	0	0	0
B. lineata	1	0	0	0	0	0
B. manskii	1	1	1	0	0	0
B. mayi	1	1	1	1	0	0
B. melanothoracica	1	1	1	0	0	0
B. melas	0	0	1	1	0	0
B. melastomatos	1	0	0	0	0	0
B. minax	1	0	0	0	0	0
B. moluccensis	1	0	0	0	0	0
B. morobiensis	1	0	0	0	0	0
B. murrayi	1	1	1	0	0	0
B. musae	1	1	1	0	0	0
B. mutabilis	0	0	1	1	0	0
B. neocheesmanae	1	0	0	0	0	0

Species	Outside Australia	Cape York Peninsula	Atherton Plateau	Eastern QLD, NSW and VIC	Kimberley Plateau	Arnhem Land
B. neohumeralis	1	1	1	1	0	0
B. nigra	0	0	1	1	0	0
B. opiliae	0	1	1	0	1	1
B. pallida	1	1	1	0	1	1
B. parabarringtoniae	1	0	0	0	0	0
B. paramusae	1	0	0	0	0	0
B. pendleburyi	1	0	0	0	0	0
B. peninsularis	1	1	0	0	0	0
B. perkinsi	1	1	0	0	0	0
B. phaleriae	0	0	1	0	0	0
B. propinqua	1	0	0	0	0	0
B. quadrata	1	1	1	1	0	0
B. recurrens	1	0	0	0	0	0
B. redunca	1	0	0	0	0	0
B. repanda	1	0	0	0	0	0
B. resima	1	0	0	0	0	0
B. romigae	0	1	0	0	0	0
B. rufescens	0	0	1	1	0	0
B. rufofuscula	1	1	1	0	0	0
B. russeola	0	0	1	1	0	0
B. seguyi	1	0	0	0	0	0
B. silvicola	1	0	1	1	0	0
B. speculifera	1	0	0	0	0	0
B. speewahensis	0	1	1	0	0	0
B. tapahensis	1	0	0	0	0	0
B. tenuifascia	0	0	0	0	1	1
B. terminaliae	1	0	0	0	0	0
B. tigrina	0	1	1	0	0	0
B. tinomiscii	1	0	0	0	0	0
B. trivialis	1	0	0	0	0	0
B. tryoni	1	1	1	1	0	0
B. tsuneonis	1	0	0	0	0	0
B. umbrosa	1	0	0	0	0	0
B. ustulata	1	0	0	0	0	0
B. visenda	1	1	1	0	0	0
B. vulgaris	1	0	0	0	0	0
B. yorkensis	0	1	1	0	0	0

Species	Outside Australia	Cape York Peninsula	Atherton Plateau	Eastern QLD, NSW and VIC	Kimberley Plateau	Arnhem Land
D. absonifacies	0	0	1	1	0	0
D. aequalis	0	1	1	1	0	0
D. axanus	1	1	1	1	1	1
D. bellulus	1	1	1	0	0	1
D. hardyi	1	1	1	0	0	1
D. impar	1	0	0	0	0	0
D. longicornis	1	0	0	0	0	0
D. mayi	1	0	0	0	0	0
D. newmani	0	0	1	1	0	1
D. palmerensis	0	1	1	0	0	0
D. pusillus	1	1	1	0	0	0
D. salamander	1	1	1	0	0	0
D. secamoneae	0	1	1	0	0	1
D. signatifrons	0	0	0	1	0	0
Z. atrifacies	1	0	0	0	0	0
Z. choristus	1	1	1	1	0	0
Z. cilifer	1	0	0	0	0	0
Z. cucumis	1	1	1	1	0	1
Z. cucurbitae	1	0	0	0	0	0
Z. depressus	1	0	0	0	0	0
Z. diversus	1	0	0	0	0	0
Z. fallacis	0	1	0	0	0	0
Z. hochii	1	0	0	0	0	0
Z. hululangitae	1	0	0	0	0	0
Z. incisus	1	0	0	0	0	0
Z. macrovittatus	1	0	0	0	0	0
Z. neopallescentis	1	0	0	0	0	0
Z. platamus	1	0	0	0	0	0
Z. sandaracinus	1	0	0	0	0	0
Z. scutellatus	1	0	0	0	0	0
Z. strigifinis	1	1	1	0	0	0
Z. tau	1	0	0	0	0	0
Z. vinnulus	1	0	0	0	0	0

5.3. Results

5.3.1. Pacific biogeographic analysis

Movement of species into the Pacific Islands was unidirectional from west to east however, there were multiple pathways of entry predominantly via Australia and New Guinea (Fig. 5.7). Each island/region in the Pacific is discussed individually here.

5.3.1.1. New Caledonia

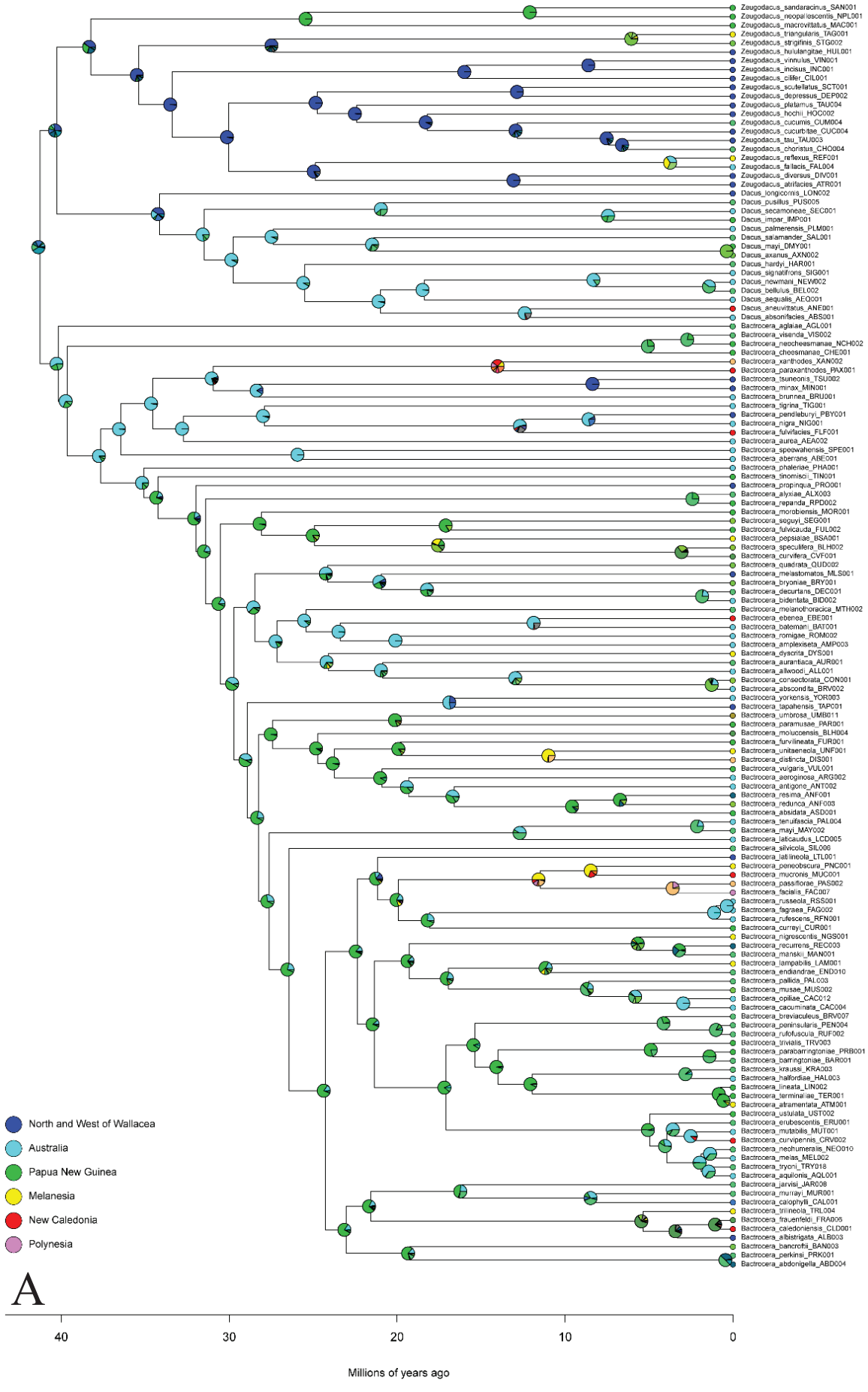
The New Caledonian fauna represents multiple radiations onto the landmass from both Australia and New Guinea, via the Melanesian islands. Divergence between New Caledonian and Australian species occurred between 6-20 mya (estimate represents avg. 95% CI). Species with New Guinean and Melanesian origins such as *B. caledoniensis* Drew*, B. mucronis* (Drew) and *B. curvipennis* diverged far more recently (1-13 mya, estimate represents avg. 95% CI), however *B. umbrosa* is a much older New Guinea lineage that diverged approximately 13-26 mya (estimate represents avg. 95% CI). The difference between *B. umbrosa* and the other endemic New Caledonian species is that *B. umbrosa* is also considered native to New Guinea and Melanesia, therefore it is possible that this species is not native to New Caledonia.

5.3.1.2. Polynesia

There were only four species native to Polynesia included in this analysis; *B. passiflorae* (Froggatt)*, B. xanthodes* (Broun)*, B. distincta* and *B. facialis* (Coquillet)*. Bactrocera passiflorae* and *B. facialis* formed a monophyletic clade, with stem groups present in New Guinea and Melanesia. *Bactrocera xanthodes* and *B. distincta* formed clades with their sister species from New Caledonia and Melanesia respectively, providing evidence towards an eastern radiation of species in the Pacific.

5.3.1.3. Melanesia

Melanesian species are polyphyletic across the tree, suggesting multiple lineages have moved into the region over time (majority being 5-15 mya, estimate represents avg. 95% CI)). This region has not been a priority of early sampling efforts that established range records in other areas, therefore there are limitations to these findings. For example, the timing of the divergence of *Z. reflexus* is unreliable as there is a lack of geographic and taxonomic sampling that has impacted this part of the tree. Greater sampling of more closely related species could resolve this. Nevertheless, there are still clear trends present in more densely sampled regions of the tree. There is clear evidence that the origin of Melanesian species' ancestors is New Guinea. There are very young species such as *B. atramentata* (Hering) and *Z. triangularis* which arose within the last 0-9 mya (estimate represents avg. 95% CI). Additionally, there are clades across the tree that contain species endemic to Melanesia and other eastern adjacent regions. For example, this includes but is not limited to a clade containing *B. unitaeniola* Drew and Romig and *B. distincta* which are present in Melanesia and Polynesia. This is a common trend across the dataset which suggests that some ancestors moved easterly to outer Pacific islands, while other sister species remained and diversified in Melanesia. There is no evidence of eastern Pacific species moving westward. This is further evidence toward eastern radiation of the Dacini in the Pacific.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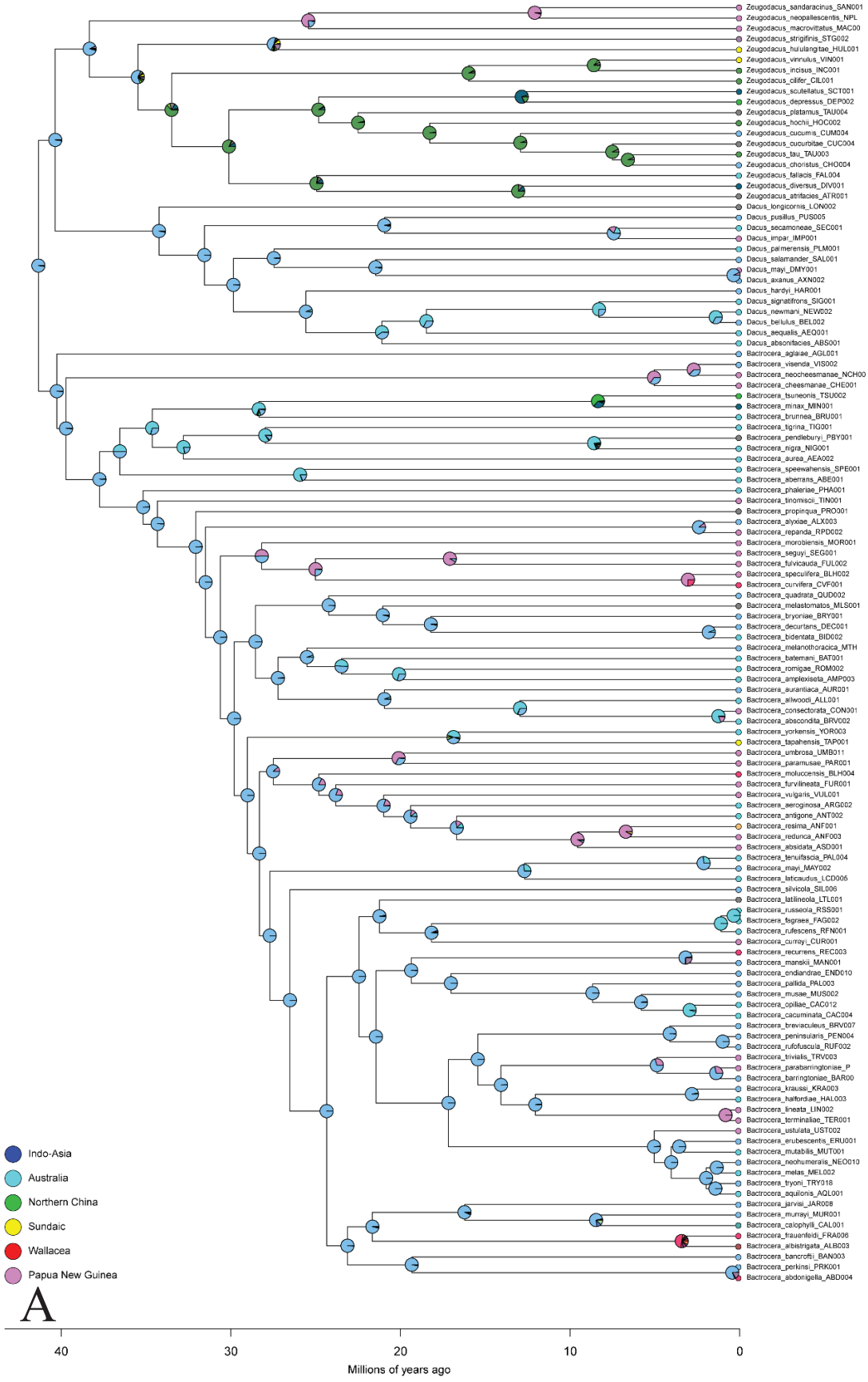
Zeugodacus_neopallescentis_NPL001
Zeugodacus_macrovittatus_MAC001
Zeugodacus_triangularis_TAG001
Zeugodacus_strigifrons_STG002
Zeugodacus_hululangitae_HUL001
Zeugodacus_vinnulus_VIN001
Zeugodacus_incisus_INC001
Zeugodacus_cilifer_CIL001
Zeugodacus_scutellatus_SCT001
Zeugodacus_depressus_DEP002
Zeugodacus_platamus_TAU004
Zeugodacus_hochii_HOC002
Zeugodacus_cucumis_CUM004
Zeugodacus_cucurbitae_CUC004
Zeugodacus_tau_TAU003
Zeugodacus_choristus_CHO004
Zeugodacus_reflexus_REF001
Zeugodacus_fallacis_FAL004
Zeugodacus_diversus_DIV001
Zeugodacus_atrifacies_ATR001
Dacus_longicornis_LON002
Dacus_pusillus_PUS005
Dacus_secamoneae_SEC001
Dacus_impar_IMP001
Dacus_palmerensis_PLM001
Dacus_salamander_SAL001
Dacus_mayi_DMY001
Dacus_axanus_AXN002
Dacus_hardyi_HAR001
Dacus_signatifrons_SIG001
Dacus_newmani_NEW002
Dacus_bellulus_BEL002
Dacus_aequalis_AEQ001
Dacus_aneuvittatus_ANE001
Dacus_absonifacies_ABS001
Bactrocera_aglaiae_AGL001
Bactrocera_visenda_VIS002
Bactrocera_neocheesmanae_NCH002
Bactrocera_cheesmanae_CHE001
Bactrocera_xanthodes_XAN002
Bactrocera_paroxanthodes_PAX001
Bactrocera_tsuneonis_TSU002
Bactrocera_minax_MIN001
Bactrocera_brunnea_BRU001
Bactrocera_tigrina_TIG001
Bactrocera_pendleburyi_PBY001
Bactrocera_nigra_NIG001
Bactrocera_fulvifacies_FLF001
Bactrocera_aurea_AEA002
Bactrocera_speewahensis_SPE001
Bactrocera_aberrans_ABE001
Bactrocera_phaleriae_PHA001
Bactrocera_tinomisci_TIN001
Bactrocera_propinqua_PRO001
Bactrocera_alyxiae_ALX003
Bactrocera_repanda_RPD002
Bactrocera_morobiensis_MOR001
Bactrocera_seguyi_SEG001
Bactrocera_fulvicauda_FUL002
Bactrocera_pepsiaiae_BSA001
Bactrocera_speculifera_BLH002
Bactrocera_curvifera_CVF001
Bactrocera_quadrata_QUD002
Bactrocera_melastomatos_MLS001
Bactrocera_bryoniae_BRY001
Bactrocera_decurtans_DEC001
Bactrocera_bidentata_BID002
Bactrocera_melanothoracica_MTH002
Bactrocera_ebenea_EBE001
Bactrocera_bateman_BAT001
Bactrocera_romigae_ROM002
Bactrocera_amplexiseta_AMP003
Bactrocera_dysoxia_DYS001
Bactrocera_aurantiaca_AUR001
Bactrocera_allwoodi_ALL001
Bactrocera_consectorata_CON001
Bactrocera_abscondita_BRV002
Bactrocera_yorkensis_YOR003
Bactrocera_tapaiensis_TAP001
Bactrocera_umbrosa_UMB011
Bactrocera_paramusae_PAR001
Bactrocera_moluccensis_BLH004
Bactrocera_furvilineata_FUR001
Bactrocera_unitaeneola_UNF001
Bactrocera_distincta_DIS001
Bactrocera_vulgaris_VUL001
Bactrocera_aeroginosa_ARG002
Bactrocera_antigone_ANT002
Bactrocera_resima_ANF001
Bactrocera_reducta_ANF003
Bactrocera_absidata_ASD001
Bactrocera_tenuifascia_PAL004
Bactrocera_mayi_MAY002
Bactrocera_laticaudus_LCD005
Bactrocera_silvicola_SIL006
Bactrocera_latilineola_LTL001
Bactrocera_peneobscura_PNC001
Bactrocera_mucronis_MUC001
Bactrocera_passiflorae_PAS002
Bactrocera_facialis_FAC007
Bactrocera_russeola_RSS001
Bactrocera_fagraea_FAG002
Bactrocera_rufescens_RFN001
Bactrocera_curreyi_CUR001
Bactrocera_nigrescentis_NGS001
Bactrocera_recurrens_REC003
Bactrocera_manskii_MAN001
Bactrocera_lampabilis_LAM001
Bactrocera_endiandrae_END010
Bactrocera_pallida_PAL003
Bactrocera_musae_MUS002
Bactrocera_opiliae_CAC012
Bactrocera_cacuminata_CAC004
Bactrocera_breviaculeus_BRV007
Bactrocera_peninsularis_PEN004
Bactrocera_rufofuscula_RUF002
Bactrocera_trivialis_TRV003
Bactrocera_parabarringtoniae_PRB001
Bactrocera_barringtoniae_BAR001
Bactrocera_kraussi_KRA003
Bactrocera_halfordiae_HAL003
Bactrocera_lineata_LIN002
Bactrocera_terminaliae_TER001
Bactrocera_atramentata_ATM001
Bactrocera_ustulata_UST002
Bactrocera_erubescentis_ERU001
Bactrocera_mutabilis_MUT001
Bactrocera_curvipennis_CRV002
Bactrocera_neohumeralis_NEO010
Bactrocera_melas_MEL002
Bactrocera_tryoni_TRY018
Bactrocera_aquilonis_AQL001
Bactrocera_jarvisi_JAR008
Bactrocera_murrayi_MUR001
Bactrocera_calophylli_CAL001
Bactrocera_trilineola_TRL004
Bactrocera_frauenfeldi_FRA005
Bactrocera_caledoniensis_CLD001
Bactrocera_albistrigata_ALB003
Bactrocera_bancroftii_BAN003
Bactrocera_perkinsi_PRK001
Bactrocera_abdonigella_ABD004
North and West of Wallacea
Australia
Papua New Guinea
Melanesia
New Caledonia
Polynesia
A
40
30
20
10
0
Millions of years ago

Figure 5.7 (continued from previous page): Results from the Pacific analysis. A: Dated Pacific biogeographical cladogram produced using the BioGeoBEARS DEC+J model. Pies on nodes represent the maximum likelihood of that ancestor inhabiting a region; and B: coloured map legend of six scored regions in this analysis. Note: if a species is present in more than one range, this is represented by a third colour i.e. presence in New Caledonia (red) and Melanesia (yellow) would be represented as orange in the ML pie. This is also applied if a species is present in multiple regions.

5.3.2. Broad scale biogeographic analysis

There are distinct Australian clades present across the tree (Fig. 5.8), and it is clear there have been multiple waves of incursions into Australia. The genus *Dacus* forms a monophyletic clade of Australia and New Guinea species. However, this region is home to a small percentage of this genus, so greater sampling would improve knowledge of dispersal pathways. The *Zeugodacus* clade also forms reasonably monophyletic geographic clades, with most species present in one or all of Indochina, Chinese-Tibet and Sunda, with a small clade restricted to New Guinea (*Z. sandaracinus, Z. neopallescentis* and *Z. macrovittatus*) and the three Australian

species are polyphyletic within this clade. Within the genus *Bactrocera,* the majority of species present in Australia and New Guinea occur on both landmasses, with endemic Australian and New Guinean species emerging at varying node ages in the tree. For example, endemic Australian species *B. cacuminata* and *B. opiliae* diverged approximately 3 mya (95% CI = 1.6-4.6), and similarly New Guinean endemics *B. lineata* (Perkins)*,* and *B. terminaliae* Drew also diverged within the last 9 my (95% CI = 7.9-16.2). In contrast to this, other Australian endemics *B. batemani* Drew*, B. romigae* (Drew & Hancock) and *B. amplexiseta* (May) and New Guinean endemics *B. umbrosa* and *B. paramusae* Drew*,* all diverged approximately 20 mya (avg. 95% CI = 13-25). These differing dates indicate multiple incursions between Australia and Papua New Guinea throughout their evolutionary history, with no evidence in this dataset of movement from Indonesia. Additionally, there are also much younger clades, such as the *B. tryoni* species complex, which contain very closely related species in one or both of these regions.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Zeugodacus_vinnulus_VIN001
Zeugodacus_incisus_INC001
Zeugodacus_cilifer_CIL001
Zeugodacus_scutellatus_SCT001
Zeugodacus_depressus_DEP002
Zeugodacus_platamus_TAU004
Zeugodacus_hochii_HOC002
Zeugodacus_cucumis_CUM004
Zeugodacus_cucurbitae_CUC004
Zeugodacus_tau_TAU003
Zeugodacus_choristus_CHO004
Zeugodacus_fallacis_FAL004
Zeugodacus_diversus_DIV001
Zeugodacus_atrifacies_ATR001
Dacus_longicornis_LON002
Dacus_pusillus_PUS005
Dacus_secamoneae_SEC001
Dacus_impar_IMP001
Dacus_palmerensis_PLM001
Dacus_salamander_SAL001
Dacus_mayi_DMY001
Dacus_axanus_AXN002
Dacus_hardyi_HAR001
Dacus_signatifrons_SIG001
Dacus_newmani_NEW002
Dacus_bellulus_BEL002
Dacus_aequalis_AEQ001
Dacus_absonifacies_ABS001
Bactrocera_aglaiae_AGL001
Bactrocera_visenda_VIS002
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Bactrocera_tsuneonis_TSU002
Bactrocera_minax_MIN001
Bactrocera_brunnea_BRU001
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Bactrocera_pendleburyi_PBY001
Bactrocera_nigra_NIG001
Bactrocera_aurea_AEA002
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Bactrocera_aberrans_ABE001
Bactrocera_phaleriae_PHA001
Bactrocera_tinomiscii_TIN001
Bactrocera_propinqua_PRO001
Bactrocera_alyxiae_ALX003
Bactrocera_repanda_RPD002
Bactrocera_morobiensis_MOR001
Bactrocera_seguyi_SEG001
Bactrocera_fulvicauda_FUL002
Bactrocera_speculifera_BLH002
Bactrocera_curvifera_CVF001
Bactrocera_quadrata_QUD002
Bactrocera_melastomatos_MLS001
Bactrocera_bryoniae_BRY001
Bactrocera_decurtans_DEC001
Bactrocera_bidentata_BID002
Bactrocera_melanothoracica_MTH
Bactrocera_batemani_BAT001
Bactrocera_romigae_ROM002
Bactrocera_amplexiseta_AMP003
Bactrocera_aurantiaca_AUR001
Bactrocera_allwoodi_ALL001
Bactrocera_consectorata_CON001
Bactrocera_abscondita_BRV002
Bactrocera_yorkensis_YOR003
Bactrocera_tapaiensis_TAP001
Bactrocera_umbrosa_UMB011
Bactrocera_paramusae_PAR001
Bactrocera_moluccensis_BLH004
Bactrocera_furvilineata_FUR001
Bactrocera_vulgaris_VUL001
Bactrocera_aeroginosa_ARG002
Bactrocera_antigone_ANT002
Bactrocera_resima_ANF001
Bactrocera_redunca_ANF003
Bactrocera_absidata_ASD001
Bactrocera_tenuifascia_PAL004
Bactrocera_mayi_MAY002
Bactrocera_laticaudus_LCD005
Bactrocera_silvicola_SIL006
Bactrocera_latilineola_LTL001
Bactrocera_russeola_RSS001
Bactrocera_fegraea_FAG002
Bactrocera_rufescens_RFN001
Bactrocera_curryi_CUR001
Bactrocera_recurrens_REC003
Bactrocera_manskii_MAN001
Bactrocera_endiandrae_END010
Bactrocera_pallida_PAL003
Bactrocera_musae_MUS002
Bactrocera_opiliae_CAC012
Bactrocera_cacuminata_CAC004
Bactrocera_breviaculeus_BRV007
Bactrocera_peninsularis_PEN004
Bactrocera_rufofuscula_RUF002
Bactrocera_trivialis_TRV003
Bactrocera_parabarringtoniae_P
Bactrocera_barringtoniae_BAR00
Bactrocera_kraussi_KRA003
Bactrocera_halfordiae_HAL003
Bactrocera_lineata_LIN002
Bactrocera_terminaliae_TER001
Bactrocera_ustulata_UST002
Bactrocera_erubescentis_ERU001
Bactrocera_mutabilis_MUT001
Bactrocera_neohumeralis_NEO010
Bactrocera_melas_MEL002
Bactrocera_tryoni_TRY018
Bactrocera_aquilonis_AQL001
Bactrocera_jarvisi_JAR008
Bactrocera_murrayi_MUR001
Bactrocera_calophylli_CAL001
Bactrocera_frauenfeldi_FRA006
Bactrocera_albistrigata_ALB003
Bactrocera_bancroftii_BAN003
Bactrocera_perkinsi_PRK001
Bactrocera_abdonigella_ABD004
Indo-Asia
Australia
Northern China
Sundaic
Wallacea
Papua New Guinea
A
40
30
20
10
0
Millions of years ago

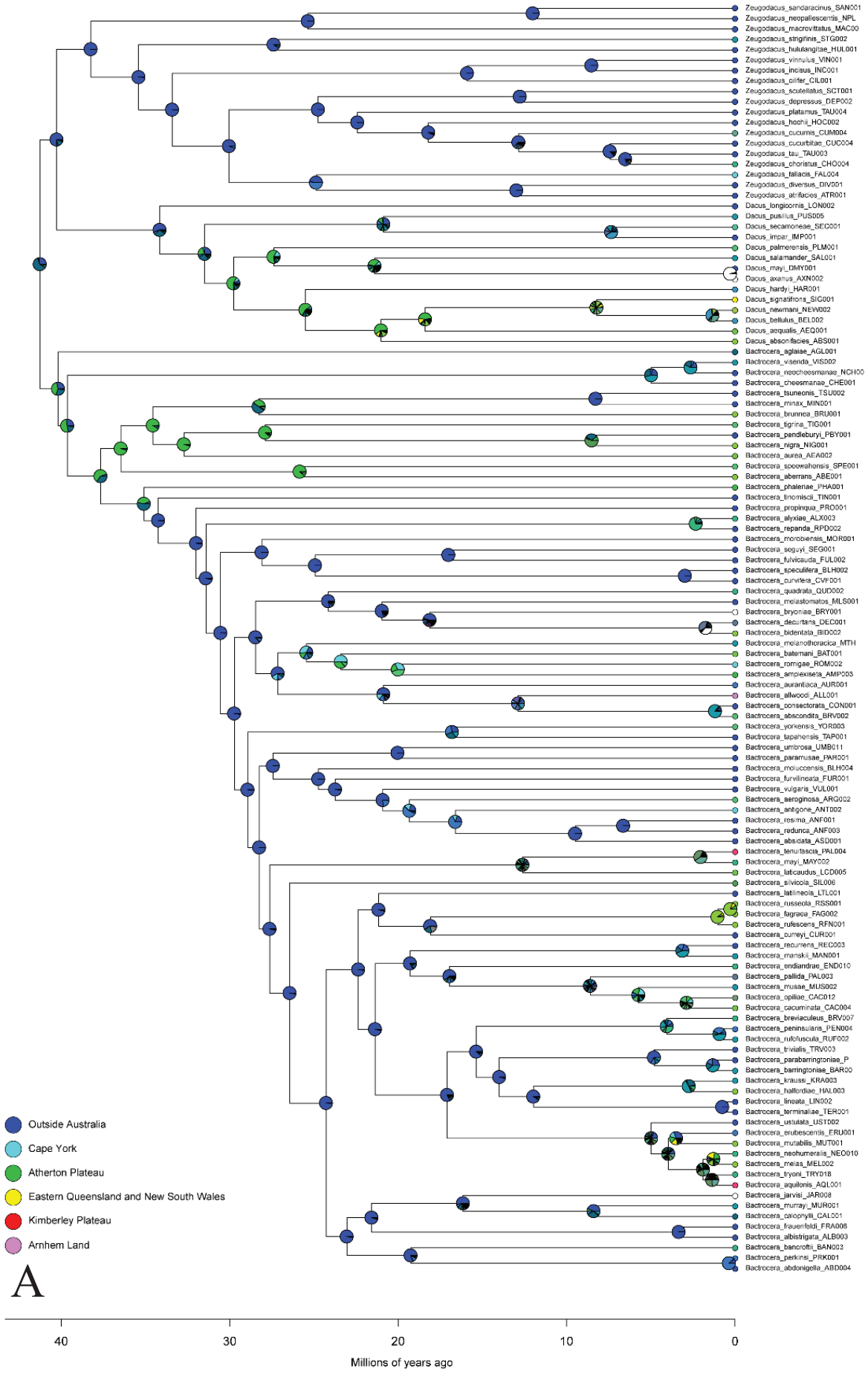
Figure 5.8 (contiuned from previous page): Results from the broad scale biogeographic analysis. A: Dated broad scale biogeographical cladogram produced using the BioGeoBEARS BAYAREALIKE+J model on a subset Dacini phylogeny. Likelihood pies at nodes represent the likelihood of that ancestor occupying that range. B: coloured map legend of six scored regions in this analysis. Note: if a species is present in more than one range, this is represented by a third colour i.e. here the dominant pale blue represents the likelihood a species is present in PNG and Australia at the same time. This is also applied if a species is present in multiple regions.

5.3.3. Australian biogeographic analysis

There is evidence of multiple incursions of species into Australia via Papua New Guinea (Fig. 5.9). Species have dispersed across the Carpentarian barrier from either Cape York, Atherton, or both (*D. bellulus* and *D. newmani* are good examples). Species endemic to eastern Queensland, and those found in Atherton, the Kimberley Plateau and Arnhem Land have undergone very recent speciation within the last 5my. There does not appear to be any clades of species that are restricted to a single

biogeographic region within Australia, instead there are species that are endemic to these regions which are polyphyletic across the tree. The biogeographical barriers down the eastern coast of Australia do not appear to have had a significant impact on speciation and movement of the Dacini.

In light of newly identified pathways, of note, is the species *B. calophylli*. *Bactrocera calophylli* is currently known from Atherton and west of Wallacea. This is a highly unusual distribution and could suggest that the population present "west of Wallacea" could be a sister species and needs to be examined further through morphology. Alternate explanations could include human introductions into one of these regions, or that *B. calophylli* is more widespread than currently recognised, but has remained undetected because it does not respond to any currently employed lures.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Zeugodacus_neopallescentis_NPL
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Zeugodacus_cilifer_CIL001
Zeugodacus_scutellatus_SCT001
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Zeugodacus_platamus_TAU004
Zeugodacus_hochii_HOC002
Zeugodacus_cucumis_CUM004
Zeugodacus_cucurbitae_CUC004
Zeugodacus_tau_TAU003
Zeugodacus_choristus_CHO004
Zeugodacus_fallacis_FAL004
Zeugodacus_diversus_DIV001
Zeugodacus_atrifacies_ATR001
Dacus_longicornis_LON002
Dacus_pusillus_PUS005
Dacus_secamoneae_SEC001
Dacus_impar_IMP001
Dacus_palmerensis_PLM001
Dacus_salamander_SAL001
Dacus_mayi_DMY001
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Bactrocera_minax_MIN001
Bactrocera_brunnea_BRU001
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Bactrocera_seguyi_SEG001
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Bactrocera_curvifera_CVF001
Bactrocera_quadrata_QUD002
Bactrocera_melastomatos_MLS001
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Bactrocera_decurtans_DEC001
Bactrocera_bidentata_BID002
Bactrocera_melanothoracica_MTH
Bactrocera_baternani_BAT001
Bactrocera_romigae_ROM002
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Bactrocera_aurantiaca_AUR001
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Bactrocera_consectorata_CON001
Bactrocera_abscondita_BRV002
Bactrocera_yorkensis_YOR003
Bactrocera_tapaiensis_TAP001
Bactrocera_umbrosa_UMB011
Bactrocera_paramusae_PAR001
Bactrocera_moluccensis_BLH004
Bactrocera_furvilineata_FUR001
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Bactrocera_mutabilis_MUT001
Bactrocera_neohumeralis_NEO010
Bactrocera_melas_MEL002
Bactrocera_tryoni_TRY018
Bactrocera_aquilonis_AQL001
Bactrocera_jarvisi_JAR008
Bactrocera_murrayi_MUR001
Bactrocera_caliphylli_CAL001
Bactrocera_frauenfeldi_FRA006
Bactrocera_albistrigata_ALB003
Bactrocera_bancroftii_BAN003
Bactrocera_perkinsi_PRK001
Bactrocera_abdonigella_ABD004
Outside Australia
Cape York
Atherton Plateau
Eastern Queensland and New South Wales
Kimberley Plateau
Arnhem Land
A
40
30
20
10
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Millions of years ago

Figure 5.9 (continued from previous page): Results from Australian biogeographic analysis. A: Dated Australian biogeographical cladogram produced using the BioGeoBEARS DEC+J model on a subset Dacini phylogeny; and B: coloured map legend of six scored regions in this analysis If a species is present in more than one range, this is represented by a third colour i.e. here the dominant blue colour represents the likelihood a species is present outside of Australia and in Cape York and Atherton at the same time. This is also applied if a species is present in multiple regions.

5.4. Discussion

I undertook three main analyses in this chapter in order to investigate; the dispersal pathways of the Pacific dacines; the Australian dacines; and on a smaller scale, the role of Australian biogeographic barriers on Australian dacines. I scored native ranges for 143 species in three genera and identified unidirectional movement of Dacini species into the Pacific through two main pathways via New Guinea and Australia. There was no movement of species back into Australia from the Pacific.

Australia appears to have been colonised exclusively via New Guinea and there were multiple occurrences of species moving back and forth between the two landmasses over historical time. The only entry point of the ancestors of Australian species was via Cape York, with species subsequently spreading west into Arnhem Land and the Kimberley Plateau as well as south along the east coast of Queensland and New South Wales. Cape York and New Guinea share a large number of species in common, adding further evidence to this region being the main point of entry for the ancestors of modern-day species. Additionally, there is little evidence to suggest that biogeographic boundaries within Australia have contributed to speciation of the Dacini in the region. The arguments for these conclusions are outlined below.

5.4.1. Origins of the Pacific Dacini

The fruit fly fauna in the Pacific appears to have been colonised from both New Guinea and Australia. Species found in greater Melanesia and Polynesia appear to have radiated from New Guinea, whereas species found in New Caledonia have sister taxa in both Australia and New Guinea.

5.4.1.1. Melanesian island arcs between New Guinea and New Caledonia

New Caledonia is considered to harbour high levels of endemic fauna (Bauer and Sadlier, 1993), and this is especially true for the Dacini (Michaux and White, 1999). I identified a radiation from New Guinea and Melanesia into New Caledonia with final divergence around 1-13 mya (representative of avg. 95% CI), with the exception being *B. umbrosa* which diverged 6 mya (95% CI = 13.45-26.46). Radiations through Melanesia are apparent across multiple parts of the phylogeny where some species have remained and diversified within Melanesia, while others have diversified into New Caledonia and Polynesia. Using the lineages of the *B. unitaeniola* and *B. distincta* clade, as well as the clade containing *B. peneobscura* Drew and Romig*, B. mucronis, B. passiflorae* and *B. facialis* as examples*,* the divergence of New Guinean species would have likely begun around 16-29 mya (avg. 95% CI). These dates coincide with those proposed for the existence of several

Melanesian island arcs between New Guinea and New Caledonia during the Eocene-Oligocene (Heads, 2010). These island arcs not only serve as explanations of dispersal patterns seen here in the dacines, but also lichen (Galloway, 2007), fruit bats (Simmons, 2005) and plant species (Govaerts et al., 2001, Swenson et al., 2007).

5.4.1.2. Australian and New Caledonian links

My analysis indicates that divergence dates between Australian and New Caledonian species are much younger than geological events of that time might suggest. Using the New Caledonian species *B. ebenea* (Drew) and *D. aneuvittatus* as examples, these species diverged from Australian fauna approximately 6-18 mya (avg. 95% CI). This is consistent with many theories that have been postulated for the radiation of other species from Australia into New Caledonia and New Zealand (for in-depth discussion see Condamine et al. (2017)), this is of particular interest because New Caledonia was submerged until approximately 33-38 mya (Murienne et al., 2005). Lucky (2011) investigated the biogeography of spider ants and found similar divergence times (4-10 mya) for Australian and New Caledonian taxa as found here and suggested species may have reached New Caledonia via long distance dispersal. Similarly, transoceanic dispersal was considered to be the primary explanation for colonisation of New Caledonia by chironomids from Australia, with divergence occurring around 8 mya (95% CI = 1.5-13) (Krosch et al., 2020a). Alternatively, Condamine et al. (2017) found that conifer diversification in the Pacific is best explained by the existence of ancient Pacific islands which acted as refugia for species during the Oligocene and Miocene, which allowed for species to move from Australia to New Caledonia and New Zealand. Either of these explanations are plausible; more investigation may be needed into this emerging trend of Oligocene-Miocene dispersal of insects into New Caledonia.

5.4.2. Is *Bactrocera umbrosa* native to New Caledonia?

Given that there has been a recent publication that investigated the movements and potential native range of *B. umbrosa* (Krosch et al., 2019a) (studies are lacking on other species that were included here), it offers a good opportunity to compare results

from this study. It is possible that *B. umbrosa,* a species documented to have spread widely in South east Asia and the Pacific (Krosch et al., 2019a), is not native to New Caledonia. Recent evidence suggests that *B. umbrosa* is not native to regions west of Papua New Guinea (Krosch et al., 2019a), but colonisation of Pacific islands by humans and their cultivation of *B. umbrosa* host plants *Artocarpus* spp. has facilitated its spread (Zerega et al., 2004). Therefore, it is not unreasonable to hypothesise that *B. umbrosa* diverged in the New Guinea and/or Melanesian region in the radiations that have been discussed above, and later invaded New Caledonia via human mediated pathways.

5.4.3. Movement of species between New Guinea and Australia

The data developed here indicates that there were multiple movements of species between Australia and Papua New Guinea at various time periods. The analysis suggests that there was a single point of entry for species into Australia, and this was Cape York via Papua New Guinea. A land bridge connecting Cape York and the Northern Territory in Australia with Papua New Guinea in the north has been supported by many biogeographers (Burbidge, 1960, Hall, 2001, Mirams et al., 2011). This land bridge was present for millions of years, forming sometime around the Carboniferous (358 mya) and was submerged intermittently throughout the Pleistocene (0.012-2.58 mya) (Doutch, 1972). The land connection was severed for the final time around 7,000 years ago (Voris, 2000, Reeves et al., 2008). Some species, such as the Australian *Dacus* clade, have moved between the two landmasses reasonably early (25-30 mya) corresponding with earlier land bridge connections, and the collision of Australia + southern Papua New Guinea with the accreted terranes of norther Papua New Guinea. Additionally, other species such as those in the *B. tryoni* species complex, have clearly undergone very recent exchange due to their close genetic affinity to each other and geographic polyphyly. The absence of Australian species at basal nodes of the tree, combined with an isolated geological history and the suggestion that species colonised from Papua New Guinea, adds to the mounting evidence that Australia is not the origin of the Dacini tribe.

5.4.4. Australian biogeographic barriers

The Australian analysis sought to investigate the influence of barriers within Australia on Dacini speciation. Some species were restricted to Cape York and the Atherton Plateau regions, which might suggest that the barrier between these regions (the Laura Basin) has not restricted movement. Additionally, it could be argued that the barrier identified south of the Atherton Plateau (Burdekin Gap) has restricted southern movement of some species such as *B. aurantiaca, B. barringtoniae* and *B. antigone* (to name a few), however this could be due to other factors such as climate and host plant distribution (Drew, 2004). On the other hand, species such as *B. tryoni, B. jarvisi* and *B. aurea* have been found inhabiting Atherton, Eastern Queensland and South of the MacPherson-Macleay Overlap, which suggests that they were not limited by the Burdekin Gap. It is unclear if these barriers are 'leaky barriers' allowing the movement of species, or if present-day distributions are a result of human-mediated movement and/or agricultural expansion.

5.4.5. Implications for existing hypotheses

This chapter did not aim to infer the geographic origins of the Dacini, and the lack of basal Asian taxa limits my ability to draw conclusions on these deeper nodes. However, my study found the Dacini were 30my younger than previously proposed by Krosch et al. (2012) and therefore I have to reject the dispersal pathways outlined by the out of India hypothesis (5.1.2). There is no evidence to suggest that species did not originate in India, however the pathways of entry into Africa and South-east Asia are slightly different and the dates obtained in this analysis can help refine the current hypothesis. Here I offer two alternate hypotheses based on these dates that are supported by geological plate movements and will provide plausible pathways for movement and dispersal of the ancestors of the extant Dacini.

5.4.5.1. Out of India?

If the Dacini are indeed younger than the dates proposed by Krosch et al. (2012), it is less likely that *Dacus* moved onto the African continent via Madagascar due to the location of rafting India at the time of divergence of the *Dacus*. Despite this, it is still

possible that the Dacini originated on the Indian plate, with divergence occurring much later via other dispersal mechanisms. I propose that before India collided with Eurasia, that taxa were transferred to and from Asia via multiple land bridges between Sumatra approximately 50-57mya (since revised to 48mya) (Grismer et al., 2016) and a second land bridge between India and the Thai-Malay Peninsula between approximately 34-55mya (Acton, 1999, Aitchison et al., 2007, Ali and Aitchison, 2008) (Fig. 5.10) before India's hard collision with Eurasia; estimated to be 20-25mya by some (van Hinsbergen et al., 2012, Aitchison et al., 2007) and pre-Oligocene by others (Tripathy-Lang et al., 2013). Early dispersal before hard collision is a pathway that has been established for other taxa such as crabs, frogs and lizards (Klaus et al., 2010, Li et al., 2013, Grismer et al., 2016), and would adequately explain movement of Dacini species between the two regions around the mid-Eocene (45mya). This would coincide with the divergence of *Bactrocera* from *Dacus* and *Zeugodacus* (33-46mya) and then the subsequent split of *Dacus* and *Zeugodacus* (32-45mya). The intermittent connection of Asia and India due to land bridge connections could explain the paraphyly of African *Dacus* taxa that was observed by Krosch et al. (2012), where some species were more closely related to Asian taxa, and others formed a monophyletic clade of only African taxa.

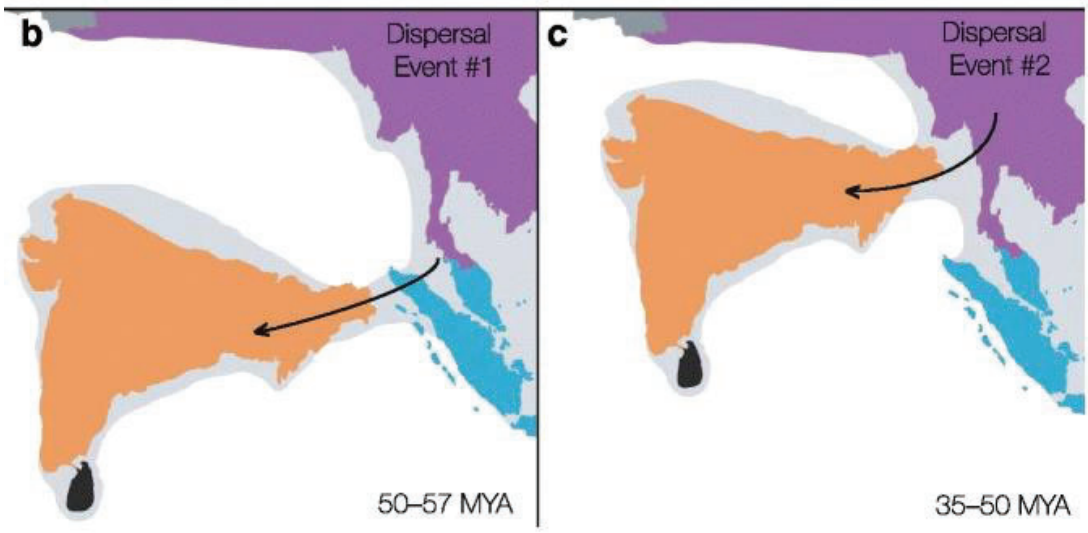


Figure 5.10: Proposed dispersal events between the Indian plate (orange) and Asia (purple) based on divergence time estimates of Dragon lizards. Figure taken from Grismer et al. (2016).

5.4.5.2. Out of South-east Asia?

It is quite possible that based on my findings, the origin of the Dacini is somewhere in South-east Asia or New Guinea (or a combination of these) rather than India. If this is the case, the land bridge theory presented above could still have played a significant role in divergence and dispersal of the Dacini. Here I offer an alternate hypothesis as to the origin of these species. Species may have been present in Asia up until the collision of the Indian plate with Sumatra (Fig. 5.10), which could have facilitated early movement of species onto the plate before hard collision of India with Eurasia. After breakup from Gondwana, Australia and New Guinea (part of which is the northernmost edge of the Australian plate) moved north towards the Pacific plate; and during this time New Guinea collided with Sepik and Papuan ophiolites which resulted in uplift and the formation of present day New Guinea (Hall, 2001). It seems unlikely that species were present in both Asia and the Australian plate due to the very late arrival of the Australian plate; the landmasses reached their present-day positions approximately 30mya (Hall, 2001). It is more likely that the Dacini may have arisen in Asia, dispersed westward into Europe and Africa via the pre-docked Indian plate (35-50mya) (Grismer et al., 2016) (using the land-bridge mechanism discussed above), and eastward through Wallacea and into

Australia and the Western Pacific after Australian plate collision (23-33 mya) (Axelrod and Raven, 1982). The northern half of New Guinea may have already harboured species before this collision, which could have facilitated early dispersal of Dacini into Australia.

Hancock (1986) considered genus *Ichneumenopsis* to be a basal genus of subfamily Dacinae. This genus is part of the Gastrozonini tribe, the majority of which breed in grasses (Kovac et al., 2013). The Gastrozonini are dominant in the oriental region, with some species also present in the Palearctic, Afrotropical and Australasian regions (Kovac et al., 2013). The inclusion of basal tribes from South-east Asia could help unravel the true origins of the Dacini.

Chapter 6: Taxonomy and systematics of the Australian members of the *Bactrocera aglaiae* species group

6.1. Introduction

Bactrocera aglaiae is a native Australian species, known only from north Queensland (Royer, 2015). This species inhabits rainforest regions and has only been recorded from two species of Meliaceae; *Aglaia sapindina* and *Aglaia ferruginea* (Hancock et al., 2000). However, because of its non-pest status, there has been very little coverage of this species in the literature. Taxonomically, this species has been classified within the subgenus *B.* (*Hemizeugodacus*) (Drew, 1989), however, there remain some taxonomic inconsistencies and there has been no inclusion of species from this subgenus in any molecular phylogenetics studies to date to support this position. Additionally, results from Chapter 3 identified a species group, consisting of additional species distributed not only in north Queensland, but also in Papua New Guinea. This species group resolved as sister to the rest of *Bactrocera,* which has been proposed by previous taxonomists in the field (Hancock and Drew, 2018a). Identifying one of the oldest diverging sister groups to *Bactrocera* could provide further support to biogeographic hypotheses and the evolution of morphological character traits (Hancock and Drew, 2018a).

6.1.1. Taxonomic inconsistencies

Hardy (1951) first described *B. aglaiae* from the Atherton Tablelands as *Dacus* (*Hemizeugodacus*) *aglaiae*. Following this, the species was assigned to the subgenus *Neozeugodacus* by May (1953) and later, Drew (1989) provided a re-description and transferred the species to the genus *Bactrocera.* Drew's redescriptions were from paratypes and stored specimens at the (then) Queensland Department of Primary Industries (DPI) collections and his re-description differs in taxonomically important characters from Hardy's description of the holotype. The most notable discrepancies being that Hardy's holotype specimen did not have anterior supra-alar bristles, had dark tinted costal cells and a prominent dark medial line on the abdomen covering all terga (Hardy, 1951). In comparison, Drew's description had supra-alar bristles present, a medial line on the abdomen that was much paler (red-brown) and covering

only TIII-V, and costal cells fulvous (Drew, 1989). Hardy also notes that there are no preapical setae present on the aculeus of the female (Hardy, 1951), which also disagrees with the SEM images provided by Drew (1989). In light of the findings from Chapter 3 which identified genetically diverging individuals within this group, and the taxonomic inconsistencies presented above, there is a definite need for revision of this species group.

6.1.2. Basal lineage?

A recent taxonomic revision (Hancock and Drew, 2018a) recognised only three species within the subgenus *B.* (*Hemizeugodacus*): *B. aglaiae, B. fulvosterna* Drew & Romig and *B. tetrachaeta* (Bezzi) (Hancock and Drew, 2018a). The revision concluded that, based on the presence of medial vitta, that this subgenus was likely a basal group within *Bactrocera,* a finding congruent with the large phylogeny presented in Chapter 3. Understanding the basal clades(s) of a genus is integral to understanding the movement and speciation of other species in the group (Drew, 2004, Hancock and Drew, 2018a) and so confirming or denying *B.* (*Hemizeugodacus*) as sister to *Bactrocera* is important in understanding the evolution of this very large genus.

6.1.2. Chapter aims and hypothesis

The results of Chapter 3 identified multiple species present within what I am referring to here as the *Bactrocera aglaiae* species group, a group formed based on morphological and genetic similarities which will be outlined further in this chapter. This included cryptic species from the greater Papua New Guinean region as well as additional species present within Australia. There was support for this subgenus being the basal clade, but the extent to which this is supported should be investigated further. In this chapter, I test the hypothesis that the phylogenetic placement of the *Bactrocera aglaiae* species group is a sister clade to the rest of *Bactrocera.* I am not conducting a full taxonomic revision of the *B.* (*Hemizeugodacus*) subgenus as other members of this complex are scattered throughout South-east Asia, and were not sampled as part of this thesis as the focus is on the Australian and Pacific region. In

addition, a revision of the taxonomic group is provided, along with a new species description and a redescription based on previously occupied names.

6.2. Methodology

6.2.1. Samples used in this chapter

Additional individuals were sequenced for COI barcode and morphologically examined to confirm the presence of additional or cryptic species in this species group (Table 6.1 with full details in Appendix 3). Four species were identified to exist within this group, but only the two species distributed in Australia are dealt with in this chapter.

Table 6.1: Individuals sampled in this chapter for COI barcode sequencing, morphological examination and photography.

Genus	Species	Code	Country	Location
Bactrocera	*aglaiae*	AGL006	Australia	Lake Eacham, Queensland
Bactrocera	*aglaiae*	AGL007	Australia	Lake Eacham, Queensland
Bactrocera	*aglaiae*	AGL009	Australia	Julatten, Queensland
Bactrocera	*aglaiae*	AGL001	Australia	Lake Eacham, Queensland
Bactrocera	*aglaiae*	AGL003	Australia	Atherton Tablelands, Winfield Park, Queensland
Bactrocera	*aglaiae*	AGL011	Australia	Cow Bay, Queensland
Bactrocera	*aglaiae* complex	AGL010	Australia	Cow Bay, Queensland
Bactrocera	*aglaiae* complex	AGL012	Australia	Cow Bay, Queensland
Bactrocera	*aglaiae* complex	AGL013	Australia	Lockhart River dump, Queensland
Bactrocera	*aglaiae* complex	AGL005	Papua New Guinea	Baitabag, Madang Province
Bactrocera	*aglaiae* complex	AGL002	Australia	Lockhart River dump, Queensland
Bactrocera	*aglaiae* complex	AGL004	Australia	Tolga Scrub, Queensland
Bactrocera	*near aglaiae*	AGL008	Australia	Julatten, Queensland
Bactrocera	*aglaiae* complex	MAC001	Papua New Guinea	Madang Province
Bactrocera	*aglaiae complex*	MAC004	Papua New Guinea	Baitabag, Madang Province

6.2.2. Morphological examinations and taxonomic revision

Bactrocera aglaiae specimens held at the Queensland Department of Agriculture and Fisheries Insect Collection were compared and contrasted with specimens that were sequenced for this chapter. Images of the holotype were also provided for examination of key morphological characters.

6.2.3. Likelihood mapping

This group was chosen for further analysis as its phylogenetic placement is taxonomically important as a putative basal group. To test the robustness of these placements, a likelihood mapping analysis was carried out in IQ-Tree v1.7 with 10,000 quartets drawn and four clusters which are outlined in Table 6.1. All outgroups were removed from the analysis.

Table 6.2: Clusters of species used in the IQ-Tree likelihood mapping analysis to explore the placement of *Bactrocera aglaiae* and the *B. aglaiae* species group in relation to the three main genera within the Dacini.

Cluster	Species included
1	*Bactrocera_aglaiae*_AGL001
	*Bactrocera_aglaiae*_AGL003
	*Bactrocera_aglaiae*_species_group_AGL002
	*Bactrocera_aglaiae*_species_group_AGL004
	*Bactrocera_aglaiae*_ species_group _AGL005
	*Bactrocera_aglaiae*_ species_group _MAC001
	*Bactrocera_aglaiae*_species_group _MAC004
2	All *Dacus* spp.
3	All *Zeugodacus* spp.
4	All remaining *Bactrocera* spp.

6.3. Results

6.3.1. Likelihood mapping

Three possible relationship combinations were tested using the likelihood mapping approach for four clusters: *B. aglaiae* species group, *Dacus, Zeugodacus* and all remaining *Bactrocera*. The combinations that were tested and the likelihood of each relationship is represented in Fig. 6.1. While there is some support for the *B. aglaiae* species group being sister to *Zeugodacus,* there is much higher support for a sister relationship with *Bactrocera* and a closer relationship between *Dacus* and *Zeugodacus.* This agrees with the results presented in the main phylogeny (Fig. 3.2).

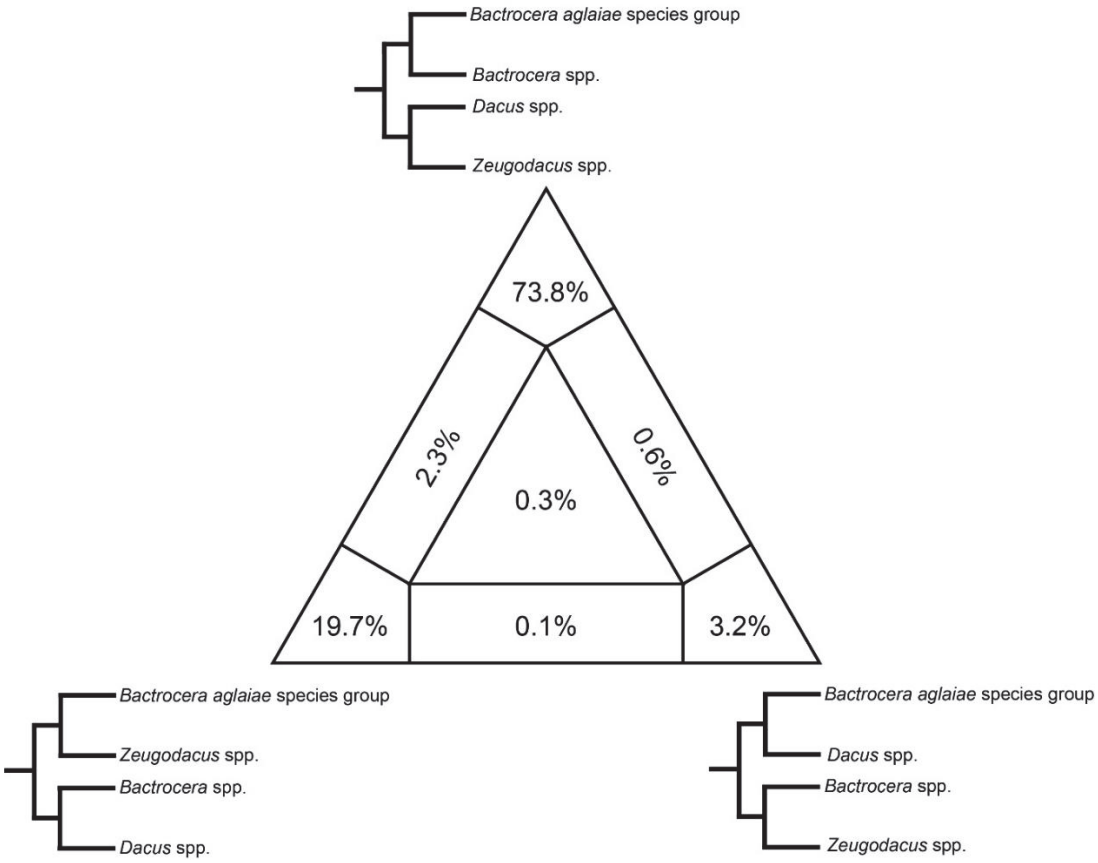


Figure 6.1: Likelihood mapping analysis of *Bactrocera aglaiae* species group relationships with the three possible relationships between the four species groupings represented at each point of the triangle. Percentage likelihood of each topology is represented in seven quartiles.

6.3.2. Revision of the *Bactrocera aglaiae* species group

This section provides descriptions and images of new species and a redescription of *Bactrocera aglaiae sensu stricto.* This group will now be referred to as the *Bactrocera aglaiae* species group. I refrain from using the term "species complex" here and will address this in greater detail in the Discussion chapter of this thesis. Here I describe species found only in Australia, but recognise that another author will be describing other members of the *B. aglaiae* species group from Papua New Guinea in the near future (R. Drew., pers comm.). When those species are published, a comprehensive revision of the entire group can be presented.

In chapter 3, I identified multiple genetic lineages within this group. I consider, upon further morphological investigation of these species and the descriptions mentioned previously, that the description of Hardy (1951), and the subsequent redescription of Drew (1989), are in fact referring to different species. Taxonomically, specimens that match the description of the holotype (Fig. 6.2) provided by Hardy should remain as *B. aglaiae* (and are referred to in this thesis as such), while the specimens that Drew described require a new name.

Figure 6.2: *Bactrocera aglaiae* holotype. A: whole body dorsal; B: abdomen dorsal; and C: whole body lateral. Images kindly provided by A. Seemann and A. Norrbom, Smithsonian Institute, USA.

Definition of *Bactrocera aglaiae* species group

Bactrocera species with narrow costal band (confluent with R2+3) and no markings on the wing except for a wide anal streak, some species with a tinted wing membrane, costal cells tinted with microtrichia in outer corner of second costal cell, lateral and medial postsutural vittae present, scutellum yellow with narrow dark basal band, scutum orange-brown, abdominal terga fulvous with variable patterns laterally and a medial line on the abdomen on some or all terga. Males attracted to zingerone.

Bactrocera* sp. near *aglaiae

Bactrocera (*Hemizeugodacus*) *aglaiae* Drew, 1989 (occupied name)

TYPE SPECIMEN

Holotype ♂, AUSTRALIA, Cow Bay, Queensland, coll. at zingerone, 1-3.x.2016, M. Krosch. Specimen held at QUT, Brisbane for lodging upon publication.

Described from a series of specimens: 2 ♂ AUSTRALIA, Lockhart River dump, Queensland, coll. 13.x.2014, J. Royer; 2 ♂ AUSTRALIA, 1 ♂ Cow Bay, Queensland, coll. 1-3.x.2016, M. Krosch; 1 ♂ PAPUA NEW GUINEA, Baitabag, Madang, coll. 5-11.xi.2016, R. Opasa and F. Philip; and 1 ♀ AUSTRALIA, Tolga Scrub, Atherton Queensland, emerged ii.2018 from *Szygium* sp. coll. J Royer and M. Starkie.

DIAGNOSIS

Medium-sized species, medium sized oval shaped black spots, humeral and notopleural calli yellow; scutum orange-brown, medium sized mesopleural stripe, lateral postsutural vittae present, medial postsutural vittae present, scutellum yellow; wing with microtrichia in outer corner of second costal cell; a thin pale fuscous costal band confluent with R2+3 and remaining thin after reaching extremity of R2+3, ending ½ way between R2+3 and M vein; a medium fuscous anal streak, costal cells colourless, abdominal terga III-V fulvous, with narrow to broad pale fuscous lateral longitudinal markings predominantly on terga III and IV and a fuscous medial line covering all terga tending to be darker on tergum V.

DESCRIPTION OF MALE

HEAD

Fig. 6.3C. Head generally fulvous. Vertical length 1.4 mm. Frons of even width, length 1.35 times breadth; fulvous with pale fuscous around orbital bristles and on anteromedial hump; latter covered with short dark setae; orbital bristles fuscous: 1 s.or., 2 i.or., lunule fuscous. Ocellar triangle fuscous. Vertex fulvous. Face fulvous with medium sized oval shaped black spots; length of face 0.58 mm. Genae fulvous, red-brown subocular spot present; strong genal bristle present. Occiput fulvous; occipital row with 5-6 bristles. Antennae with segments 1 and 2 fulvous, segment 3 fulvous with fuscous on apex and outer surface; a fuscous dorsal bristle on segment 2. Arista red-brown (fulvous basally); length of segments: 0.19mm, 0.34mm, 0.77mm.

THORAX

Fig. 6.3A, D and variation Fig. 6.4A-E. Mesonotum and pleural areas uniformly orange-brown. Yellow markings as follows: postpronotal lobe; notopleura, medium sized mesopleural stripe ½ of the way between anterior npl. bristle and postpronotal lobe, anterior margin convex; upper hypopleural calli (posterior apices orange-brown); 2/3 lower hypopleural calli (remainder orange-brown); two narrow, parallel sided, blunt ended lateral post-sutural vittae beginning at mesonotal suture and enclosing i.a. bristle; a broad medial longitudinal vitta rounded into a point at level of prsc. bristles and narrowing to a point anteriorly to end at mesonotal suture. Postnotum orange-brown. Scutellum yellow with narrow fuscous basal band. Setae: sc. 4, prsc. 2, ia. 1, p.sa. 1, a.sa. 1, mpl. 1, npl. 2, scp. 4.

LEGS

Fig. 6.3B. All coxae fulvous; all femora fulvous; fore and mid tibiae fulvous; hind tibiae ¼ fuscous basally; all tarsi pale fuscous; mid tibiae with apical black spur.

WING

Fig. 6.3F. Length 6.56 mm; with markings as follows: costal cells colourless, microtrichia in outer corner of second costal cell; a thin pale fuscous costal band confluent with R2+3 and remaining thin after reaching extremity of R2+3, ending ½ way between R2+3 and M vein; a medium fuscous anal streak ending before wing

margin; A1+CuA2 covered in dense microtrichia; supernumerary lobe of weak development.

ABDOMEN

Fig. 6.3A and variation Fig. 6.5A-D. Elongate-oval, terga free, pecten present on tergite III. Tergum 1 wider than long. Terga I and II fulvous with fuscous lateral margins with tergum II whitish posteriorly; terga III-V fulvous with variations from narrow to broad pale fuscous lateral longitudinal markings predominantly on terga III and IV. A fuscous medial line beginning as a larger spot basally on tergum I and covering all terga and darkening on tergum V. A pair of oval ceromata on tergum V. Posterior lobe of surstylus short, sternum V with slight concavity on posterior margin. Abdominal sterna orange-brown, see Fig. 6.3E.

DESCRIPTION OF FEMALE

As for male except no dense aggregation of microtrichia around A1+CuA2; supernumerary lobe weak; no pecten present on abdominal tergum III.

OVIPOSITOR

Basal segment orange-brown, dorsoventrally compressed and tapering posteriorly in dorsal view. See figures 6.6A-F for SEM photographs of the ovipositor.

ATTRACTANT

Males attracted to zingerone.

DISTRIBUTION

Known from far north Queensland (Cow Bay, Lockhart, Tolga Scrub) and Madang Province, Papua New Guinea.

COMMENTS

Bactrocera species near *aglaiae* is extremely similar to *Bactrocera aglaiae* but differs in having an a.sa. bristle and generally paler markings on the abdomen (usually a red-brown or fuscous marking) whereas *B. aglaiae* tends toward a black medial line.

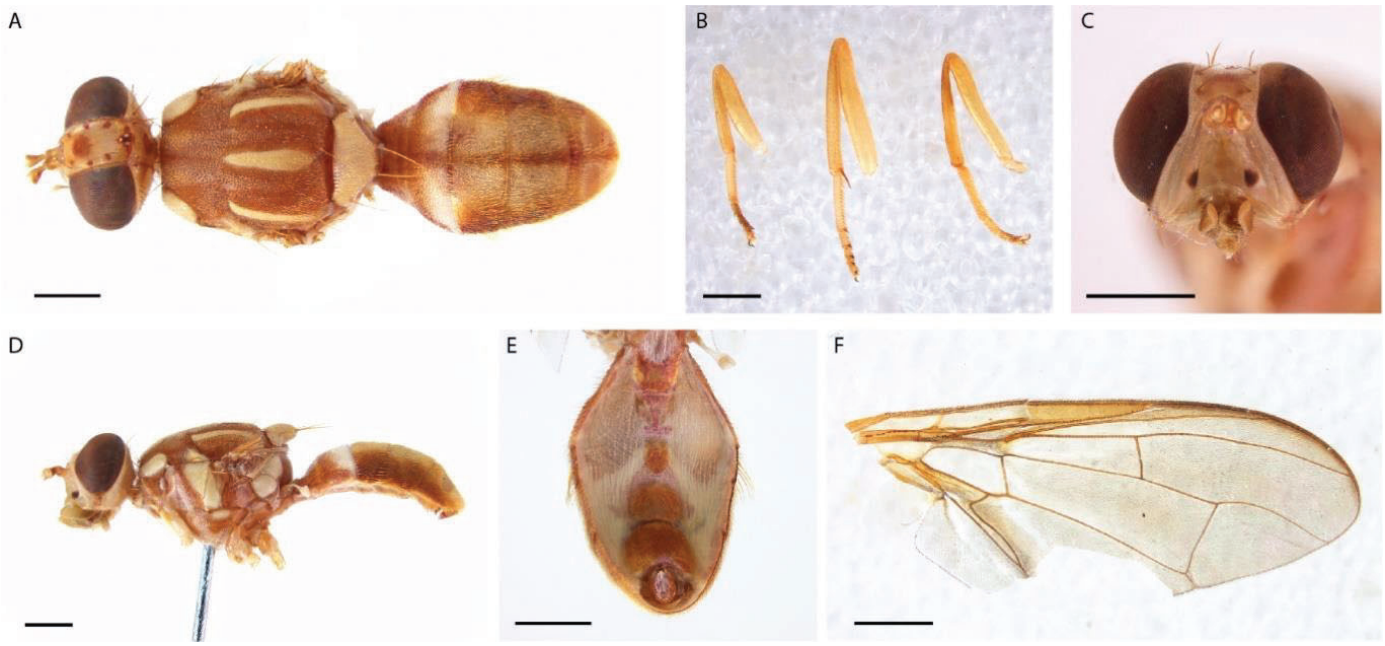


Figure 6.3: *Bactrocera* sp. near *aglaiae* male (AGL002). A: whole body dorsal; B: fore, mid and hind legs; C: head frontal, D: whole body lateral, E: abdomen ventral; and F: wing. Scale: 1mm.

Figure 6.4: Scutum variation in *Bactrocera* sp. near *aglaiae.* A: AGL008, Julatten, Queensland; B: AGL012, Cow Bay, Queensland; C: AGL010, Cow Bay, Queensland; D: AGL005, Baiatabag, Papua New Guinea; and E: AGL013, Lockhart River, Queensland.

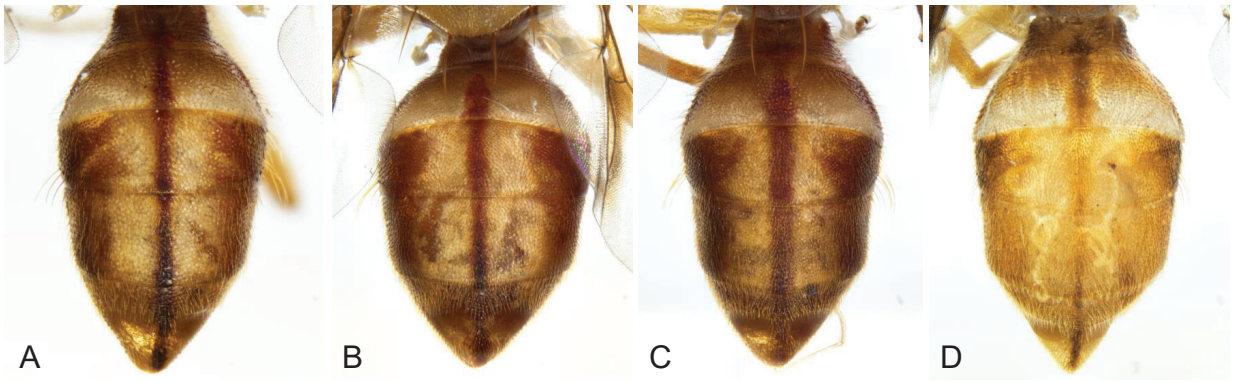


Figure 6.5: Abdomen variation in *Bactrocera* sp. near *aglaiae.* A: AGL008, Julatten, Queensland; B: AGL012, Cow Bay, Queensland; C: AGL010, Cow Bay, Queensland; and D: AGL005, Baitabag, Papua New Guinea.

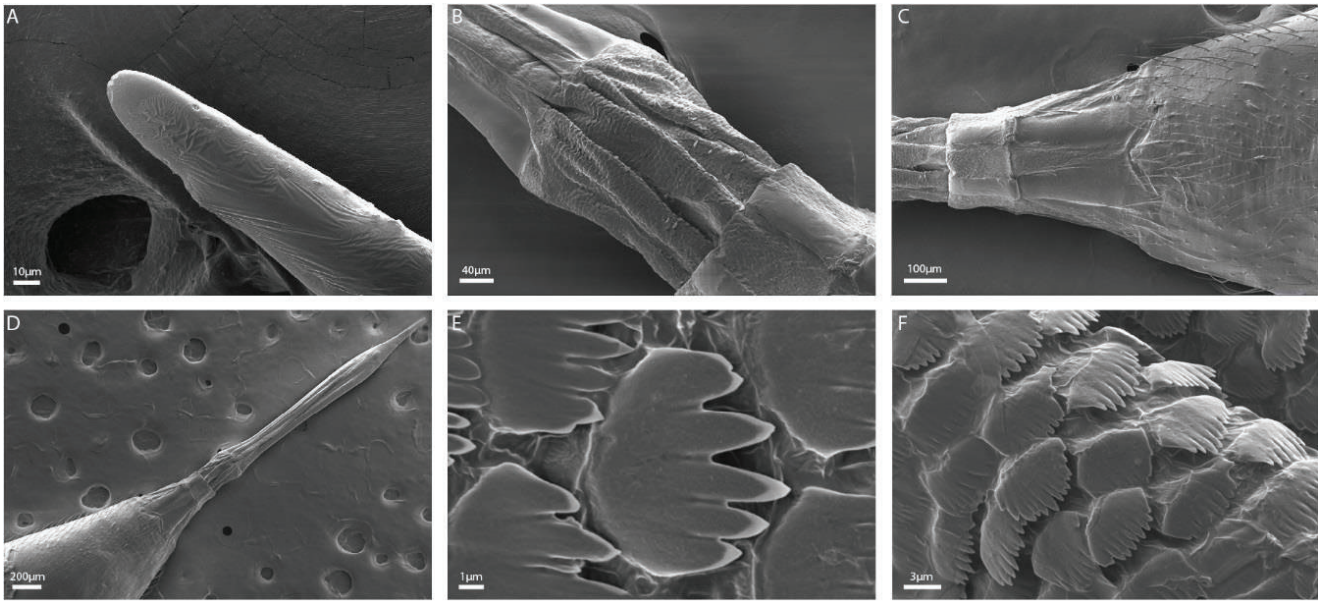


Figure 6.6: *Bactrocera* sp. near *aglaiae* female ovipositor (AGL004). A: tip of ovipositor; B: ovipositor sheath; C: base of ovipositor; D: ovipositor ventral; E and F: ovipositor scales. Scale indicated on each image. Images by: C. Cooper, CARF, QUT.

***Bactrocera* (*Hemizeugodacus*) *aglaiae* Hardy (1951) re-description**

Dacus (*Hemizeugodacus*) *algaiae* Hardy, 1951: 131-134 (description)

Dacus (*Hemizeugodacus*) *aglaiae* Hardy, 1952: 365 (name correction)

Neozeugodacus aglaiae May, 1953: 48 (transfer)

Neozeugodacus aglaiae May, 1963: 50 (maintained in key)

Bactrocera (*Hemizeugodacus*) *aglaiae* Drew, 1989: 180-181 (redescribed and transferred)

MATERIAL EXAMINED

Holotype ♂, AUSTRALIA, Atherton Tableland, Queensland, coll. xi.1949, E. Hardy.

Described from a series of specimens: 3 ♂ AUSTRALIA, Lake Eacham, Queensland, coll. 8-11.x.2016, M. Krosch; 1 ♂ AUSTRALIA, Cow Bay, Queensland, coll. 1-3.x.2016, M. Krosch; 1 ♂ AUSTRALIA, Julatten, Queensland, coll. 5-7.x.2016, M. Krosch; and 1 ♂ AUSTRALIA, Winfield Park, Queensland, coll. 3.iv.2013, J. Royer.

DIAGNOSIS

Medium-sized species, medium sized elongate oval to oval shaped black spots, humeral and notopleural calli yellow; scutum orange-brown, narrow mesopleural stripe, lateral postsutural vittae present, medial postsutural vittae present, scutellum yellow; wing with microtrichia in outer corner of second costal cell and scattered along upper margin of first costal cell; a thin pale fuscous costal band confluent with R2+3 and remaining thin after reaching extremity of R2+3 ending just beyond R2+3; a medium fuscous anal streak, costal cells pale fuscous; abdominal terga III-V fulvous, with broad fuscous lateral longitudinal markings predominantly on terga III and IV and a fuscous to black medial line covering TI-V, often darker on tergum V.

DESCRIPTION OF MALE

HEAD

Fig. 6.7D. Head generally fulvous. Vertical length 1.47 mm. Frons of even width, length 1.38 times breadth; fulvous with pale fuscous around orbital bristles and on anteromedial hump; latter covered with short dark setae; orbital bristles fuscous to black: 1 s.or., 2 i.or., lunule fuscous. Ocellar triangle fuscous. Vertex fulvous. Face fulvous with medium sized elongate oval to oval shaped black spots; length of face 0.37 mm. Genae fulvous, fuscous subocular spot present; strong genal bristle present. Occiput fulvous; occipital row with 4-6 bristles. Antennae with segments 1 and 2 fulvous, segment 3 fulvous with dark fuscous on apex and outer surface; a pale weak dorsal bristle on segment 2. Arista red-brown (fulvous basally); length of segments: 0.15mm, 0.31mm, 0.77mm.

THORAX

Fig. 6.7A, E and variation in Fig. 6.8A-C. Mesonotum and pleural areas uniformly orange-brown. Yellow markings as follows: postpronotal lobe; notopleura reaching anterior npl. bristle, narrow mesopleural stripe reaching anterior npl. bristle, anterior margin slightly concave; upper hypopleural calli (posterior apices orange-brown); 2/3 lower hypopleural calli (remainder orange-brown); two narrow, parallel sided, blunt ended lateral post-sutural vittae beginning at mesonotal suture and enclosing i.a. bristle; a broad medial longitudinal vitta rounded into a point at or above level of prsc. bristles and narrowing to a point anteriorly to end at mesonotal suture. Postnotum fulvous to orange-brown. Scutellum yellow with narrow fuscous basal band. Setae: sc. 4, prsc. 2, ia. 1, p.sa. 1, mpl. 1, npl. 2, scp. 4.

LEGS

Fig. 6.7D. All coxae fulvous; all femora and tibiae fulvous; fore tarsi pale fuscous, mid and hind tarsi fulvous; mid tibiae with apical black spur.

WING

Fig. 6.7C. Length 6.8mm; wing membrane tinted with markings as follows: costal cells pale fuscous, microtrichia in outer corner of second costal cell and scattered along upper margin of first costal cell; a thin fuscous costal band confluent with R2+3 and remaining thin after reaching extremity of R2+3, ending just beyond

R2+3; a medium fuscous anal streak ending before wing margin; A1+CuA2 covered in dense microtrichia; supernumerary lobe of weak development.

ABDOMEN

Fig. 6.7B and variation in Fig. 6.9A-D. Elongate-oval, terga free, pecten present on tergite III. Tergum 1 wider than long. Tergum I fuscous; Tergum II fuscous anteriorly extending to lateral margins and whitish posteriorly; terga III-V fulvous with broad fuscous lateral longitudinal markings predominantly on terga III and IV. A fuscous to black medial line covering TI-V, often darker on tergum V. A pair of oval ceromata on tergum V. Posterior lobe of surstylus short, sternum V with slight concavity on posterior margin. Abdominal sterna orange-brown, see Fig. 6.7F.

ATTRACTANT

Males attracted to zingerone.

DISTRIBUTION

Known previously from the Atherton Tablelands, far north Queensland, and newly recorded in other locations in far north Queensland; Lake Eacham, Winfield Park, Cow Bay and Julatten.

COMMENTS

Bactrocera aglaiae is similar to *B.* species near *aglaiae* but differs in having no a.sa. bristle and generally paler colouration on the legs, and a medial line that tends towards black on the abdomen. COI barcode data has confirmed fixed differences between the two species.

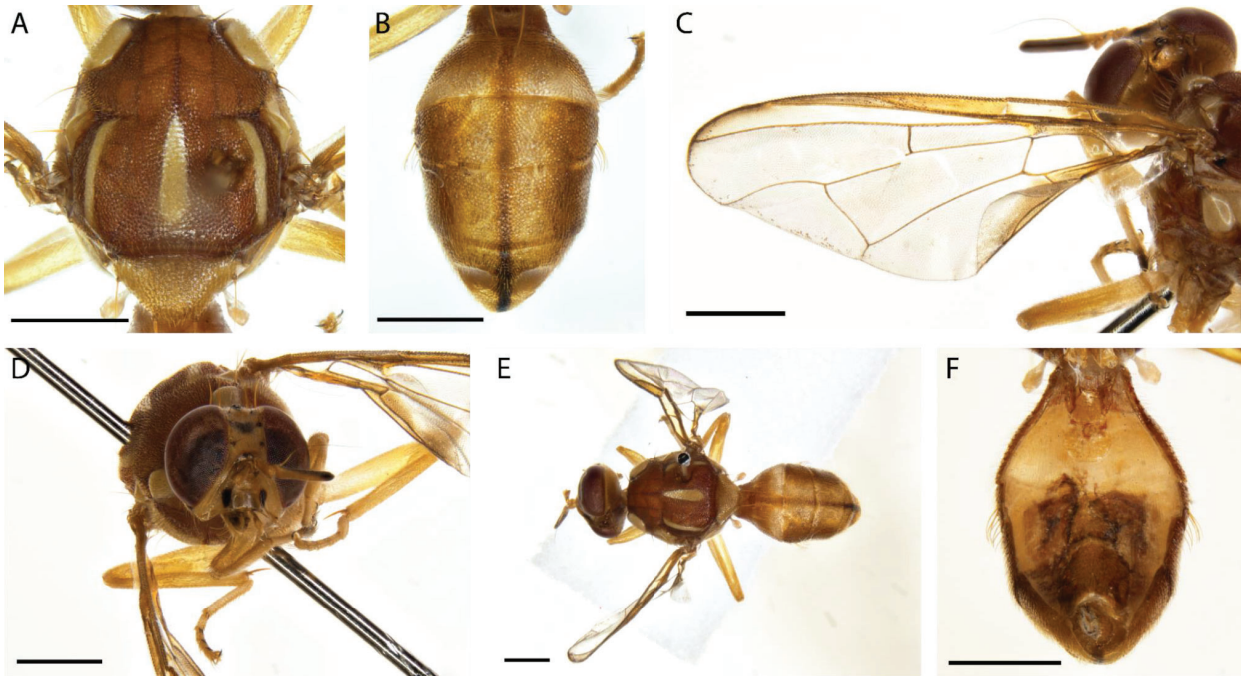


Figure 6.7: *Bactrocera aglaiae* male (AGL011). A: scutum dorsal; B: abdomen dorsal; C: wing; D: head; E: whole body dorsal; and F: abdomen ventral. Scale: 1mm.

Figure 6.8: Scutum variation in *Bactrocera aglaiae.* A: AGL001, Lake Eacham, Queensland; B: AGL007, Lake Eacham, Queensland; and C: AGL006, Lake Eacham, Queensland.

Figure 6.9: Abdomen variation in *Bactrocera aglaiae.* A: AGL001, Lake Eacham, Queensland; B: AGL007, Lake Eacham, Queensland; C: AGL009, Julatten, Queensland; and D: AGL006, Lake Eacham, Queensland.

6.4. Discussion

I have provided a taxonomic revision of the Australian members of the *B. aglaiae* species group based on multilocus sequence data from Chapter 3 and morphological examination. A formal species name for *B.* species near *aglaiae* has not been provided because of potential nomenclature confusion associated with naming a species in an unpublished thesis, versus a published document. Evidence was in support of the *B. aglaiae* species group being the basal clade to all of *Bactrocera,* as was previously suggested by Hancock and Drew (2018a) based on morphology. A recent molecular analysis resolved *B.* (*Daculus*) and *B.* (*Tetradacus*) as basal *Bactrocera* clades (San Jose et al., 2018). However, in my study, I sampled representatives from all three subgenera: *B.* (*Daculus*), *B.* (*Tetradacus*) and *B.* (*Hemizeugodacus*) and found that *B.* (*Hemizeugodacus*) was the ancestral clade and the likelihood mapping analysis further supported this placement. Determining the basal lineage offers clues as to the evolution of morphological and ecological traits, as well as divergence patterns within the genus.

The *B. aglaiae* species group was resolved here as a group of four species found in Australia and Papua New Guinea (here I deal with only two found in Australia). In addition, *B. fulvosterna* and *B. tetrachaeta* are also classified within *B.* (*Hemizeugodacus*) based on morphology and are native to eastern Malaysia and the Philippines, respectively. The widespread geographic nature of this subgenus is unusual and given new species are still being discovered within this group, there may be more species yet to be described or classified within this subgenus. Currently, no molecular analyses have included these two species, but further sampling could provide resolution as to whether the origins of the Australian and Pacific Dacini is South-east Asia or the Indian subcontinent.

Chapter 7: Taxonomy and systematics of the *Bactrocera tryoni* species group

7.1. Introduction

The *Bactrocera tryoni* complex is a sibling species complex which is considered to contain four morphologically and molecularly similar species (Drew, 1989, Morrow et al., 2000): the Queensland fruit fly, *B. tryoni*, the lesser Queensland fruit fly *B. neohumeralis*, the Northern Territory fruit fly *B. aquilonis*, and *B. melas*. A group native to Australia, all four species have wide host ranges and are of economic concern to agriculture either internationally, domestically or both (Clarke et al., 2011).

The results of Chapter 3 identified the *B. tryoni* species complex to be much larger than traditionally treated above, with *B. ustulata* (*B. furfurosa* species complex)*, B. erubescentis* (*B. quadrata* species complex)*, B. mutabilis* and *B. curvipennis* all falling within the complex. In addition to this, some "northern" *B. neohumeralis* individuals were resolved in a clade separate from *B. tryoni, B. aquilonis, B. melas* and other *B. neohumeralis* individuals. These northern *B. neohumeralis* specimens are more closely related to species that are geographically scattered such as *B. mutabilis* and *B. curvipennis,* indicating perhaps a different divergence pathway than that of the first clade that consists of members of the *B. tryoni* species complex *sensu stricto.*

In this chapter, I extend the work of Chapter 3 to focus further on the *B. tryoni* species complex to develop a better systematic and taxonomic definition of the group. The outcomes of this work is also extremely important for biosecurity interceptions and trade exports as information provided here can improve our understanding of this species complex, and for example, whether those distributed throughout Papua New Guinea, pose a threat to surrounding agricultural markets if they were to spread. This introduction discusses the distribution, taxonomy and phylogenetics of the group to set up the subsequent research.

7.1.1. Distribution

Members of the *B. tryoni* complex have different, but sometimes overlapping distributions in Australia (Fig. 7.1). The most widespread species in the complex is *B. tryoni,* inhabiting the east coast in sympatry with *B. melas* and *B. neohumeralis* (Drew et al., 1978). Additionally, *B. tryoni* has also been recorded as an invasive pest in New Caledonia, French Polynesia, and the Pitcairn Islands (Leblanc et al., 2012). There are records of *B. tryoni* from Papua New Guinea (Drew, 1974, Drew, 1989, Sar et al., 2000) however, these reports should be treated cautiously as, if present, it must be very rare as significant trapping and host-rearing over the last 20 years has not detected this species (Fletcher, 1998, Leblanc et al., 2001, Clarke et al., 2004, Novotny et al., 2005, Royer et al., 2018). Notably Drew (1989), while reporting the species from Papua New Guinea, also noted that *"it is most doubtful that this species is established there."*

Bactrocera neohumeralis has also been detected in Papua New Guinea and is more regularly reported (Drew, 1989, Sar et al., 2000, Clarke et al., 2004, Royer et al., 2018). However, the identity of this species has been questioned due to the differences in host use of this species when compared to host use in Australia (Leblanc et al., 2001). In Papua New Guinea, *B. neohumeralis* has occasionally been recorded from guava but is generally regarded as a non-pest (Leblanc et al., 2001, Leblanc et al., 2012), whereas in Australia, the species infests approximately 160 host species (Hancock et al., 2000).

Figure 7.1: Australian distribution of species in the *Bactrocera tryoni* species complex. A: *B. aquilonis*; B: *B. melas*; C: *B. neohumeralis*; and D: *B. tryoni* (May, 1963, Drew, 1989, Meats, 2006, Dominiak and Mapson, 2017).

7.1.2. Taxonomy

It is evident from examining the literature that the taxonomy of the *B. tryoni* species complex has never been straightforward. The history of the complex is difficult to untangle, with multiple taxonomic changes having taken place over the last 130 years.

In 1889 Henry Tryon discussed the ongoing damage that had impacted fruit orchards in Toowoomba, Queensland, and other parts of the surrounding colony by an undescribed fruit fly (Tryon, 1889). He provided accounts of the species behaviour and seasonality, and placed this species in the genus *Tephritis* (Tryon, 1889).

Froggatt (1897) later described this species after Tryon as *Tephritis tryoni* and, following this, Tryon (1927) identified three variants of the species: *juglandis, musa* and *sarcocephali.* These variants, which were yet to hold species status, were the topic of much discussion in taxonomic papers that followed. Hardy (1951) examined Tryon's specimens and concluded that var. *musa* was typical *B. tryoni,* and that var. *juglandis* was an intermediate between *B. tryoni* and var. *sarcocephali;* and synonymised these variants. Hardy (1951) also commented that var. *sarcocephali* may be a melanic variant of *B. tryoni* and appeared very similar to the (then) recently described *B. melas* (Perkins and May, 1949); suggesting that they may be the same species. In this same publication, Hardy (1951) described *B. melas* and *B. neohumeralis* as variants of *B. tryoni*, effectively synonymising the two, citing a lack of distinguishing structural characters. Following this, Drew et al. (1978) reinstated both of these variants as species and provided new descriptions of the species. Drew noted that most *B. melas* specimens he examined matched typical *B. tryoni* morphology, while others that were more "typically *B. melas"* appeared to be melanic forms of *B. tryoni;* with the main morphological difference being the dark markings on the mesonotum (Drew et al., 1978). Drew strongly questioned the status of this species by concluding that it was sympatric with *B. tryoni* and had been bred from the same hosts (Drew et al., 1978). However, Drew (1989) later designated lectotypes for *B. melas*, taxonomically declaring it a valid species. There has been no further mention of the status of this species in the subsequent taxonomic or systematic literature and it remains a specie of concern to those involved in negotiating horticultural market access as a potential quarantine pest (Clarke et al., 2011).

Bactrocera aquilonis was first trapped in the Northern Territory in 1961 (Austwick, 1961). Specimens were initially identified as *B. tryoni* variants with the main distinguishing characters being the size and colouration of the wing veins (Austwick, 1961). In 1965 May described *B. aquilonis* and differentiated this fly from *B. tryoni* as having overall paler colouration, minimal orbital spots on the frons, no markings on the scutum and longer, blunter lateral postsutural vittae that reach the upper p.sa bristle (May, 1965). He also noted a wider costal band, abdominal terga 3-5 with uniform colouration and differences in genitalic structures (May, 1965). Subsequent authors have found these characters to be variable in both *B. tryoni* and *B. aquilonis*

with overlap identified between the two (Drew, 1969, Drew, 1972, Drew and Lambert, 1986). Population genetic studies of *B. aquilonis* have not helped to resolve its species status, with results showing that samples collected from the northern Territory (which are regarded as *B. aquilonis* based on distribution) are divergent from east coast *B. tryoni* but not as divergent as closely related *B. neohumeralis,* therefore the species status of *B. aquilonis* remains unchanged (Cameron et al., 2010, Popa-Baez et al., 2020).

In 1989 *B. aquilonis, B. melas, B. neohumeralis* and *B. tryoni* were placed in a taxonomic species complex (Drew, 1989). Drew (1989) provided a definition of the *B. tryoni* complex as follows: "*Bactrocera species with clear wing membrane except for narrow costal band (not confluent with R4+5) and anal streak, costal cells fulvous or fuscous and generally covered with microtrichia, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow with narrow dark basal band, mesonotum red-brown (with or without dark colour patterns), abdominal terga generally red-brown with variable dark colour patterns, males attracted to cue lure".*

7.1.3. Species complexes

Here I have referred several times to the term 'species complex'. This is a somewhat arbitrary term used to describe a group of closely related species (Rooney et al., 2009); the definition of which is often difficult to discern. Definitions differ not only between tephritid workers and other taxonomists (Fegan and Prior, 2005), but even within the tephritid community multiple usages of the term exist (Clarke and Schutze, 2014, Schutze et al., 2017).

Clarke and Schutze (2014) and Schutze et al. (2017) recognised the term "species complex" is applied to three types of complexes within the tephritid literature; cryptic, sibling and taxonomic, but that it is often unclear which specific meaning is being applied. A cryptic species complex consists of species that are near-identical morphologically, but need not infer genetic relatedness. In contrast, a sibling species complex consists of species that are monophyletic, and may or may not be taxonomically cryptic with the others (Walter, 2005, Vanickova et al., 2015). The

existence of cryptic species complexes can cause confusion amongst taxonomists as the true number of species and groupings can be underestimated (Michaux and White, 1999). Taxonomic species complexes consist of species that are assigned to a group based on shared characters, such as morphology or lure response, but the species within the complex need not be cryptic with each other (Drew et al., 2011). There are many examples in Dacini taxonomy of species that have been placed in taxonomic species complexes, but are not supported as monophyletic by genetic analyses (Virgilio et al., 2009, Virgilio et al., 2015). An example is the *B. bryoniae* species complex which consists of species that share multiple morphological similarities (Drew, 1989). Despite this, molecular analyses of some members of the *B. bryoniae* species complex found that these species are not closely related (Krosch et al., 2012), and share closer relationships with species from outside the complex (Dupuis et al., 2018).

7.1.3.1. The B. tryoni *species complex*

To provide context, the *B. tryoni* species complex technically fits all three definitions of a species complex (Clarke and Schutze, 2014). Species within the complex are morphologically cryptic, form a monophyletic clade and share key taxonomic identifiers. However, the results of Chapter 3 present challenges to the 'complex' construct that was established by Drew (1989), and justifies further evaluation of this group.

7.1.3.2. Additional species

Some of the defining characters of the *B. tryoni* species complex are shared by a handful of other species that have not been placed in the complex. These characters include the combination of a clear wing membrane, tinted costal cells and microtrichia present in both cells (Drew, 1989) which are considered to be the important diagnostic characters of the *B. tryoni* species complex (Plant Health Australia, 2018a).

Bactrocera humilis and *B. mutabilis* are two species that might belong in the *B. tryoni* species complex. Drew et al. (1981) notes that using traditional taxonomic

keys and defining characters, that *B. humilis* would belong in the complex; however, a key distinction keeping it from the complex is that all current members of the complex respond to cue-lure, whereas *B. humilis* responds to methyl eugenol (Drew and Hooper, 1981). However, *B. humilis* was described from only a single specimen collected at methyl eugenol (Drew et al., 1981), and this species remains extremely rare; only a single individual has been trapped in over 20 years of state-wide monitoring (J. Royer, pers. comm). Similarities between *B. mutabilis* (May) and members of the *tryoni* complex have been noted in Drew (1989). In fact, similar to *B. humilis, B. mutabilis* also exhibits the key characters that define the complex, but is unresponsive to either cue-lure or methyl eugenol (Drew and Hooper, 1981).

7.1.4. Phylogenetics

Previous studies have found that the *B. tryoni* species complex *sensu stricto* is monophyletic however, species within the group are not easily resolved (Blacket et al., 2012, Krosch et al., 2020b). Based on mitochondrial cytochrome c oxidase subunit I (COI) genetic barcodes, Blacket et al. (2012) and Jiang et al. (2014) found that *B. curvipennis* was paraphyletic with the complex, however Asokan et al. (2011) found that species to be sister to the group. Subsequent multigene phylogenetic analyses also place *B. curvipennis* within the complex (Krosch et al., 2013, Smith et al., 2003). A phylogenomic approach, which incorporated 878 amplicons, was also unable to resolve the complex (Dupuis et al., 2018). The only identified genetic difference between members in this species complex exists between *B. tryoni* and *B. neohumeralis.*

Morrow et al. (2000) were able to differentiate *B. aquilonis* from other species in the complex based on fixed differences in mitochondrial loci. Additionally, Wang et al. (2003) provided microsatellite evidence that supported the possibility that *B. tryoni* and *B. aquilonis* have undergone hybridisation; while subsequent microsatellite and mitochondrial analysis has suggested that the two are conspecific (Cameron et al., 2010). A SNP analysis that included *B. aquilonis, B. tryoni* and *B. neohumeralis* was also able to separate the three species, however the study reported very low genetic distance between the three (Catullo et al., 2019). Another SNP analysis, focussed on *B. tryoni* and *B. aquilonis* found that there was gene flow between the two species,

but also identified two isolated populations in Broome and Alice Springs (Popa-Baez et al., 2020).

The literature has glossed over the species status of *B. melas* (it remains absent from identification keys) (Plant Health Australia, 2018a), often due to the difficulty of discerning this species from dark *B. tryoni* variants, or suspicion that it is a hybrid of *B. neohumeralis* and *B. tryoni* (Hancock et al., 2000, Doorenweerd et al., 2018). Despite the importance of this species as a declared agricultural pest (Clarke et al., 2011), there are no studies that have explicitly investigated the species status of *B. melas*. *Bactrocera melas* has been included in genetic studies which were unable to differentiate *B. melas* from other species in the complex (Blacket et al., 2012, Krosch et al., 2020b), but no further investigation has been undertaken.

7.1.5. Chapter aims and hypotheses

Three hypotheses were tested in this chapter: i) that *B. humilis* is not a true species; ii) that *B. melas* is not a true species; and iii) that there are cryptic populations of species in the northern distribution range of *B. neohumeralis.* In order to test these, the first aim of this chapter is to investigate the systematic boundaries of the *B. tryoni* species complex by expanding upon the sampling of Chapter 3, which identified the *B. tryoni* species complex to be larger than the traditional four members, with *B. ustulata, B. erubescentis, B. mutabilis* and *B. curvipennis* all falling within the complex and *B. neohumeralis* resolved as polyphyletic (Fig. 7.2). Expansion of sampling refers to an increase in taxon replicates and an increase in genetic data. The second aim of the chapter is to focus more specifically on applying molecular and morphological tools, as required, to systematic questions concerning *B. humilis, B. neohumeralis* and *B. melas:* specifically, with respect to seeking evidence for their species status (*B. melas* and *B. humilis*), or evidence for cryptic species lineages (*B. neohumeralis*). Having systematically redefined the complex, the final aim of the chapter is to undertake taxonomic revision and redescription to align the complex's systematics and taxonomy. A focus on the species status of *B. aquilonis,* while important, was considered beyond the logistic scope of this PhD project, especially as other were known to be working on this question (PBCRC, 2018, Popa-Baez et al., 2020). However, the status of *B. aquilonis* is addressed in the Discussion.

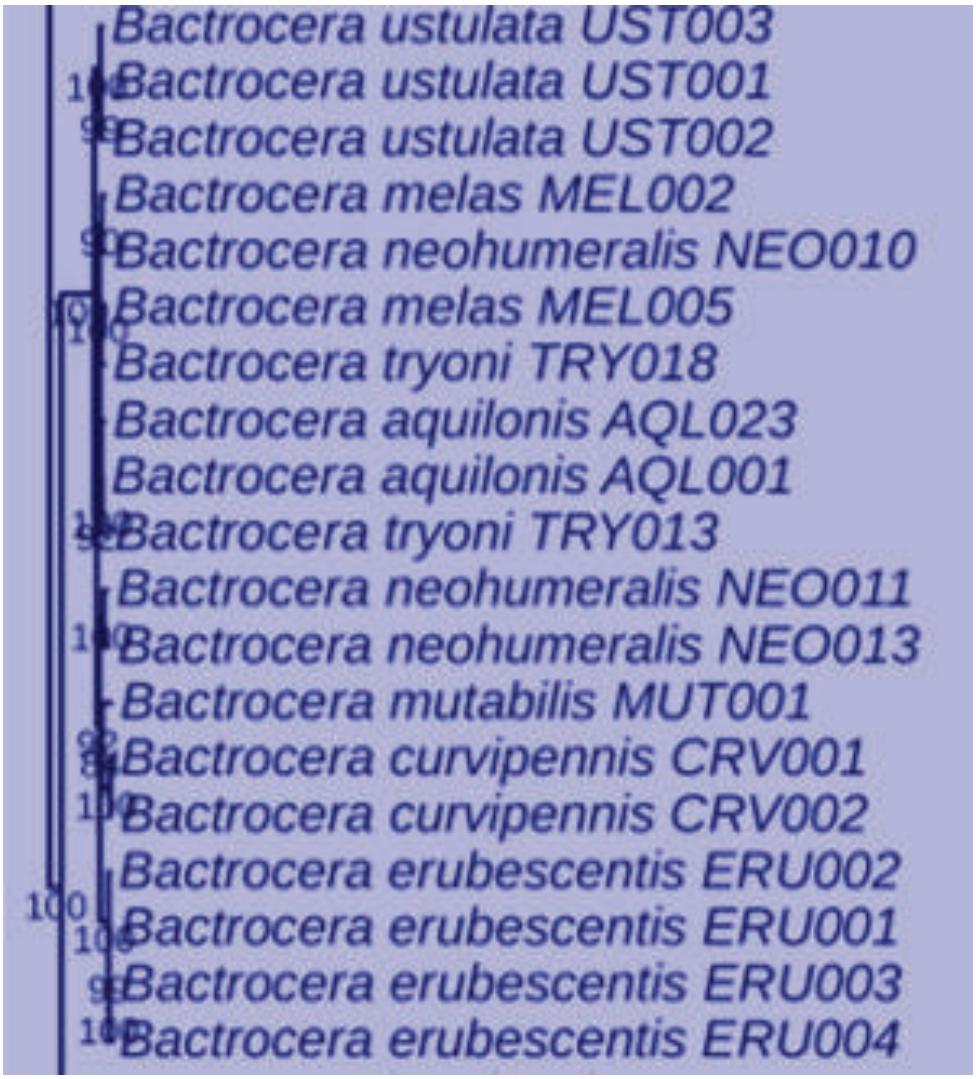


Figure 7.2: Extract from Chapter 3 phylogeny showing relationships resolved within the *Bactrocera tryoni* species complex. All nodes >90 bootstrap support.

7.2. Methodology

7.2.1. Molecular systematics

Fifty-eight individuals were used for molecular analysis. Individuals were chosen in order to expand the geographic sampling of species and to include more representatives of each species. Previously extracted genomic DNA was made available as the result of a concurrent diagnostics project led by Dr Mark Schutze (as discussed in Chapter 3). All individuals used for molecular analysis in this chapter are listed in Table 7.1.

This study was able to acquire SNP data using the method below, for a subset of 20 individuals (indicated Table 7.1) due to another concurrently running project; which funded the analysis. Inclusion of the SNP data was purely exploratory and used to support morphological and sanger sequence datasets gathered for *B. melas*.

Table 7.1: *Bactrocera tryoni* complex *sensu lato* species used for extraction and sequence analysis in this chapter as well as outgroups *A. fraterculus, A. serpentina* and two *B. quadrata* specimens. Collection information is provided here, and data already made available through the CRC project. Specimens selected for additional DArTseq SNP analysis are indicated by 'Y'. Specimens were determined by J. McMahon (QUT), J. Royer (QDAF) and M. Starkie (QUT).

Genus	Species	Code	Country	Location	Collected by	Trap date	SNP analysis
Anastrepha	*fraterculus*	AFR001	Brazil	Ex colony Pelatan Brazil Seibersdorf	M. Schutze	29.iii.2011	
Anastrepha	*serpentina*	ASR001	Panama	Lago	Y. Basset	8.iii.2013	
Bactrocera	*aquilonis*	AQL001	Australia	Department of Agriculture and Fisheries cultures, Western Australia	B. Woods	2.ii.2017	
Bactrocera	*aquilonis*	AQL010	Australia	Cable Beach, Broome, Western Australia	B. Woods	2.ii.2017	Y
Bactrocera	*aquilonis*	AQL015	Australia	Cable Beach, Broome, Western Australia	B. Woods	3.iii.2017	
Bactrocera	*aquilonis*	AQL023	Australia	Kununurra, Western Australia	B. Woods	15.iv.2016	
Bactrocera	*aquilonis*	AQL024	Australia	Black Point, Ranger Station Cobourg Peninsula, Northern Territory	F. Timaepatua	18.v.2019	
Bactrocera	*aquilonis*	AQL025	Australia	Black Point, Ranger Station Cobourg Peninsula, Northern Territory	F. Timaepatua	18.v.2019	Y

Genus	Species	Code	Country	Location	Collected by	Trap date	SNP analysis
Bactrocera	*curvipennis*	CRV001	New Caledonia	La Foa, South Province	J. Royer	13.x.2017	
Bactrocera	*curvipennis*	CRV002	New Caledonia	La Foa, South Province	J. Royer	10.xii.2017	Y
Bactrocera	*erubescentis*	ERU001	Australia	Cape York, Queensland	L. Bailey	28.xi.2015	
Bactrocera	*erubescentis*	ERU002	Australia	Lockhart River, Queensland	J. Pritchard	27.vii.2015	
Bactrocera	*erubescentis*	ERU003	Australia	Cape York, Queensland	L. Bailey	28.xi.2015	
Bactrocera	*erubescentis*	ERU004	Australia	Cape York, Queensland	L. Bailey	28.xi.2015	Y
Bactrocera	*humilis*	HUM001	Australia	Smithfield, Queensland	I. Schneider	13.vi.2013	
Bactrocera	*humilis*	HUM002	Australia	Umagico, Queensland	E. Cottis	9.v.2016	
Bactrocera	*humilis*	HUM003	Australia	Roma Flats, Queensland	J. Sailor	9.xi.2009	
Bactrocera	*humilis*	HUM004	Australia	Roma Flats, Queensland	J. Bond	21.v.2007	
Bactrocera	*humilis*	HUM005	Australia	Pormpurnaw, Queensland	PFFP	5.ii.1999	
Bactrocera	*melas*	BBR002	Australia	Brisbane, Queensland	BQ Trapper	17.ii.2015	Y
Bactrocera	*melas*	MEL002	Australia	Brisbane, Queensland	J. Royer	14.i.2015	
Bactrocera	*melas*	MEL005	Australia	Cairns, Queensland	J. Royer	7.vi.2016	
Bactrocera	*melas*	MEL006	Australia	Lockhart, Queensland	J. Pritchard	6.v.2019	Y

Genus	Species	Code	Country	Location	Collected by	Trap date	SNP analysis
Bactrocera	*melas*	MEL007	Australia	Lockhart, Queensland	J. Pritchard	6.v.2019	
Bactrocera	*melas*	MEL008	Australia	Gladstone, Queensland	J Royer	8.v.2014	Y
Bactrocera	*melas*	MEL009	Australia	Gladstone, Queensland	J Royer	6.i.2015	
Bactrocera	*melas*	MEL010	Australia	Cairns, Queensland	R. Allen	12.v.2015	
Bactrocera	*mutabilis*	MUT001	Australia	Foley's Road, Bundaberg, Queensland	L. Senior	25.xi.2016	
Bactrocera	*mutabilis*	MUT002	Australia	Bulburin National Park, Queensland	F. Strutt, M. Starkie	12.ii-3.iv.2019	Y
Bactrocera	*neohumeralis*	NEO010	Australia	Brisbane, Queensland	C. M.	10.xi.2015	Y
Bactrocera	*neohumeralis*	NEO011	Australia	Lockhart River dump, Queensland	J. Royer	15.ix.2014	Y
Bactrocera	*neohumeralis*	NEO013	PNG	PAU near Port Moresby, National Capital District	J. Royer	28.iii.2013	Y
Bactrocera	*neohumeralis*	NEO014	Australia	Mackay, Queensland	G. Green	14.v.2019	
Bactrocera	*neohumeralis*	NEO015	Australia	Mackay, Queensland	G. Green	14.v.2019	Y
Bactrocera	*neohumeralis*	NEO016	Australia	Lockhart, Queensland	J. Pritchard	6.v.2019	Y
Bactrocera	*neohumeralis*	NEO017	Australia	Lockhart, Queensland	J. Pritchard	11.iii.2019	
Bactrocera	*neohumeralis*	NEO018	Australia	Lockhart, Queensland	J. Pritchard	6.v.2019	Y
Bactrocera	*neohumeralis*	NEO019	Australia	Lockhart River dump, Queensland	J. Royer	13.x.2014	

Genus	Species	Code	Country	Location	Collected by	Trap date	SNP analysis
Bactrocera	*neohumeralis*	NEO1	Australia	Brisbane, Queensland	S. Collingwood	7.vii.2015	
Bactrocera	*neohumeralis*	NEO2	Australia	Cairns, Queensland	M. Berridge	6.vii.2015	
Bactrocera	*neohumeralis*	NEO3	Australia	Cairns, Queensland	M. Berridge	6.vii.2015	
Bactrocera	*neohumeralis*	TRY004	Australia	Brisbane, Queensland	S. Collingwood	7.vii.2015	Y
Bactrocera	*neohumeralis*	TRY006	Australia	Cairns, Queensland	M. Berridge	6.vii.2015	
Bactrocera	*neohumeralis*	TRY012	Australia	Buronga, New South Wales	N/A	24.ix.2015	Y
Bactrocera	*neohumeralis*	TRY013	Australia	Buronga, New South Wales	N/A	24.ix.2015	
Bactrocera	*quadrata*	QUD002	Australia	Brisbane, Queensland	C. Maneckshana	7.i.2016	
Bactrocera	*quadrata*	QUD003	Australia	Brisbane, Queensland	C. Maneckshana	23.xii.2015	
Bactrocera	*tryoni*	TRY018	New Caledonia	La Foa, South Province	J. Royer	9.x.2017	Y
Bactrocera	*tryoni*	TRY019	Australia	Mackay, Queensland	G. Green	14.v.2019	Y
Bactrocera	*tryoni*	TRY020	Australia	Mackay, Queensland	G. Green	14.v.2019	
Bactrocera	*tryoni*	TRY021	Australia	Coen, Queensland	J. Walker	28.v.2019	Y
Bactrocera	*tryoni*	TRY022	Australia	Coen, Queensland	J. Walker	28.v.2019	
Bactrocera	*tryoni*	TRY023	New Caledonia	Pocquereux, South Province	J. Royer	28.xi.2017	

Genus	Species	Code	Country	Location	Collected by	Trap date	SNP analysis
Bactrocera	*tryoni*	TRY024	New Caledonia	Pocquereux, South Province	J. Royer	28.xi.2017	
Bactrocera	*tryoni*	TRY1	Australia	Brisbane, Queensland	S. Collingwood	7.vii.2015	
Bactrocera	*tryoni*	TRY3	Australia	Cairns, Queensland	M. Berridge	6.vii.2015	
Bactrocera	*ustulata*	UST001	PNG	PASI agricultural station near Vanimo, Sanduan Province	S. Cowan	23-28.iv.2016	
Bactrocera	*ustulata*	UST002	PNG	PASI agricultural station near Vanimo, Sanduan Province	S. Cowan	23-28.iv.2016	Y
Bactrocera	*ustulata*	UST003	PNG	Baitabag, Madang Province	R. Opasa, F. Phillip	19-25.xi.2016	

7.2.1.1. Loci selection, amplification and sequencing

Protocols for extraction and DNA sequencing followed that of Chapter 3. Additional to the genes used in Chapter 3, one additional locus was amplified for each individual: POP4 (Ribonuclease P protein subunit p29) with primer information provided in Table 7.2. Specimens included in this chapter, their collection information and Genbank accession numbers can be found in Appendices 3 & 8. Sequencing was carried out on a 3500 Genetic Analyzer (Applied Biosystems, USA) at the Molecular Genetics Research Facility at QUT, with some additional specimens sequenced through Macrogen Inc. (Seoul, South Korea). DNA from a subset of individuals (20 in total) was also sent to Diversity Arrays Technologies (Canberra, Australia) where high density DArTseq (=SNP) analysis was undertaken. DArTseq utilises a combination of genome complexity reduction methods followed by next-generation sequencing (Cruz et al., 2013). This technology is optimized for each organism through the application of different restriction enzymes to choose the most appropriate complexity reduction method. The method of genome complexity reduction used here is proprietary information, but information on the technique can be found in Melville et al. (2017). Poor quality sequences were filtered, with more stringent selection criteria to barcode regions was applied during the first part of the pipeline. Using DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14), identical sequences were collated for use in the secondary pipeline. All library tags included in the DArTsoft14 analysis were clustered using DArT PL's C++ algorithm at the threshold distance of 3. Then, clusters were parsed into separate SNP loci.

Table 7.2: Loci, primers and annealing temperatures used for amplification of the POP4 gene.

Loci	Length	Primer name	Sequence (5'-3')	Tm (°C)	Reference
POP 4	520bp	POP4-f	ACATTACAATGTTGGAAGGGGG	55.0	Krosch et al. (2019b)
		POP4-r	CTTYAYCTTYTTGACGCTGCG	55.0	Krosch et al. (2019b)

7.2.2. Analysis of genetic data

7.2.2.1. Sanger sequence data

Sequence data was analysed using the same methods employed in Chapter 3, however, due to the focus on species boundaries, the dataset was split into mitochondrial and nuclear loci and each analysed separately first, before being concatenated. Alignments were first input into the IQ-Tree ModelFinder (Kalyaanamoorthy et al., 2017) to determine the appropriate evolutionary model for each partition (Table 7.3). COI and COII were partitioned into first, second and third codon positions. All trees were run on the IQ-Tree online server (Trifinopoulos et al., 2016) using the proportionally linked analysis as per Chapter 3.

Because of the rarity of specimens, *B. humilis* samples were only analysed for the cytochrome c oxidase subunit I (COI) barcode region. Specimens sampled for molecular analysis, represented all known individuals of this species (Table 7.1). This included specimens that had responded to methyl eugenol, as had the single holotype specimen, as well as specimens that had responded to cue-lure.

Table 7.3: Loci model selection and partitions used in the phylogenetic analyses of the *Bactrocera tryoni* species complex as determined through IQ-Tree ModelFinder (Kalyaanamoorthy et al., 2017).

Loci	Model selection
COI+COII first codon	TN+F+I
COI+COII second codon	TN+F+I
COI+COII third codon	TPM2u+F+G4
16S	K3Pu+F+I
RPA2	HKY+F+G4
DDOSTs2	HKY+F+I
EIF3L	K2P+I+G4
POP4	K2P+I

7.2.2.2. Single nucleotide polymorphism (SNP) data

SNP data was analysed in the RStudio (RStudio Team, 2019) package dartR (Gruber et al., 2019) for principal coordinate and phylogenetic analysis. Raw data was filtered to remove monomorphic loci, to a 95% threshold call rate. Populations were grouped based on species and a principal coordinate analyses (PCoA) were run with and without the four additional species included in the analysis. Additionally, prompted by the results of the PCoAs, the species status of *B. melas* was explored in greater detail with a final analysis with only *B. tryoni, B. melas* and *B. neohumeralis*. Scripts used here were taken from those available online at the Introduction to dartR CRAN: https://rdrr.io/cran/dartR/f/vignettes/IntroTutorial_dartR.Rmd (Gruber et al., 2019).

7.2.3. Morphological taxonomy

In addition to the specimens listed in Table 7.1, holotype specimens were examined where possible[2]. Morphological character states assessed were those used in the descriptions of the species, with special attention paid to purported diagnostic traits (Drew, 1989). If access to a type was impractical, images were provided by the holding institution for examination. In addition to examination of the types and the small number of specimens used for molecular analysis, all pinned holdings of the target taxa in the Queensland Department of Agriculture and Fisheries Insect Collection were examined. Further, I hold a casual position with Biosecurity Queensland where, I examine and identify thousands of Australian fruit flies daily; most of which are *B. tryoni* complex species. Through this position, and the morphological character examinations undertaken for Chapter 2, I have examined thousands of *B. tryoni* complex flies over the course of this study.

[2] COVID-19 interrupted Queensland Museum access

7.3. Results

7.3.1. Molecular systematics

7.3.1.1. Sanger sequence data

I estimated three phylogenetic trees: mitochondrial, nuclear and a combined tree. The mitochondrial tree was not able to resolve any species within the *B. tryoni* species complex *sensu stricto* as monophyletic, but the additional species *B. curvipennis, B. erubescentis, B. ustulata* and *B. mutabilis* were monophyletic (Fig. 7.3). *Bactrocera aquilonis* specimens were all resolved within one clade, which was polyphyletic with *B. tryoni.* The nuclear phylogeny (Fig. 7.4) had some poorly supported relationships and polytomies present. Some samples also had a proportion of missing data, including *A. fraterculus, A. serpentina,* QUD002 and TRY018, which resulted in long branch lengths and in the case of QUD003 and QUD002 no sequences in common; therefore they were not resolved monophyletically. Like the mitochondrial reconstruction, the nuclear phylogeny (Fig. 7.4) could only resolve *B. ustulata, B. erubescentis, B. mutabilis* and *B. curvipennis* clades as monophyletic. Again, these four species were nested within the original four members *B. tryoni* species complex. When the mitochondrial and nuclear partitions were combined (Fig. 7.5), the *B. ustulata, B. erubescentis, B. mutabilis* and *B. curvipennis* were still nested within the four original members of the *B. tryoni* species complex. Similar to the results of Chapter 3, in which some specimens of *B. neohumeralis* were falling out within a separate clade (with the proposed newer complex members), the mitochondrial tree also resolved additional *B. tryoni, B. melas* and *B. neohumeralis* specimens as polyphyletic from the primary *B. aquilonis-B. melas-B. neohumeralis-B. tryoni* clade.

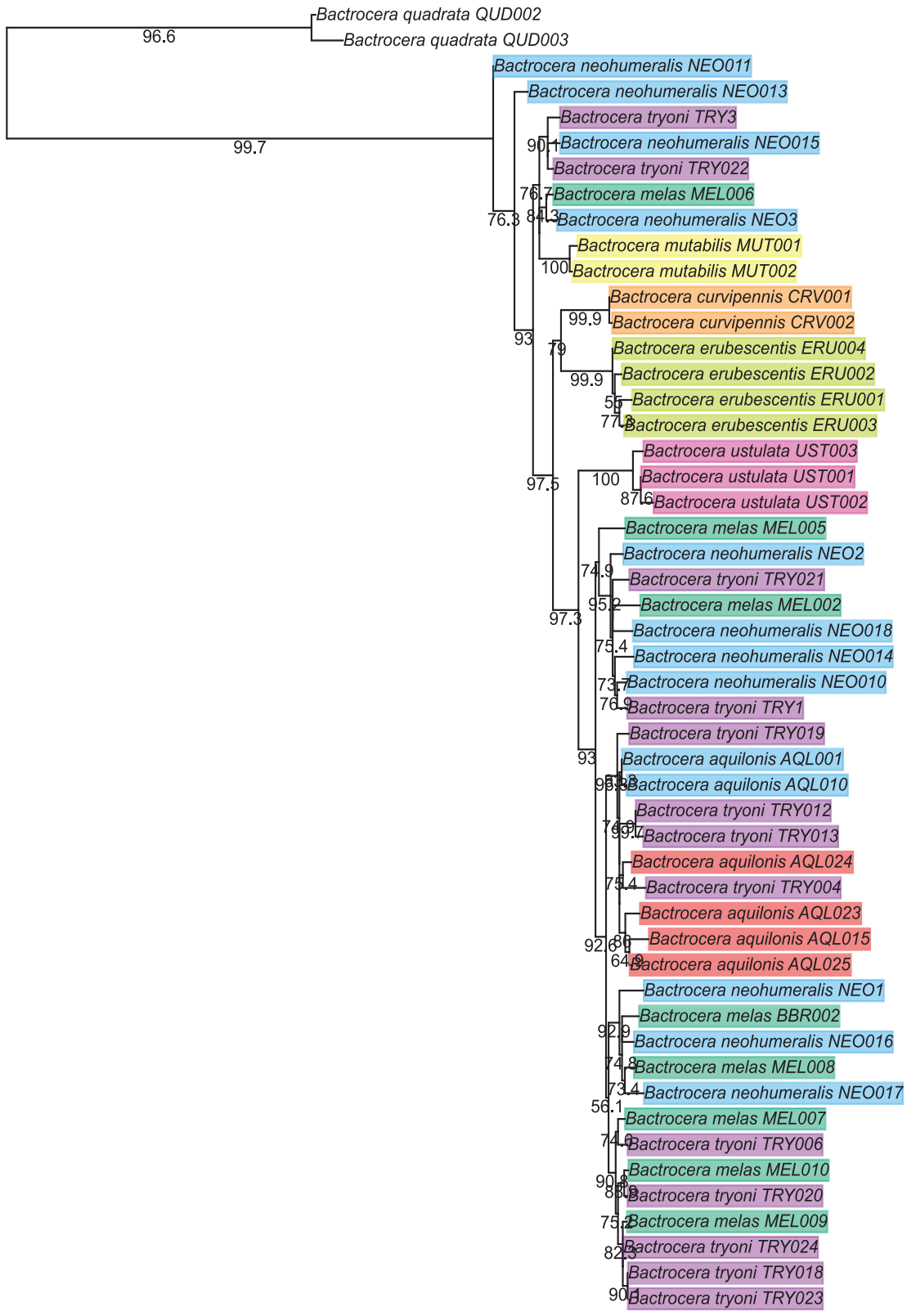


Figure 7.3: Proportionally linked Maximum Likelihood phylogeny of the *Bactrocera tryoni* species complex *sensu lato* based on three mitochondrial loci: COI, COII and 16S. Branch supports are SH-aLRT values.

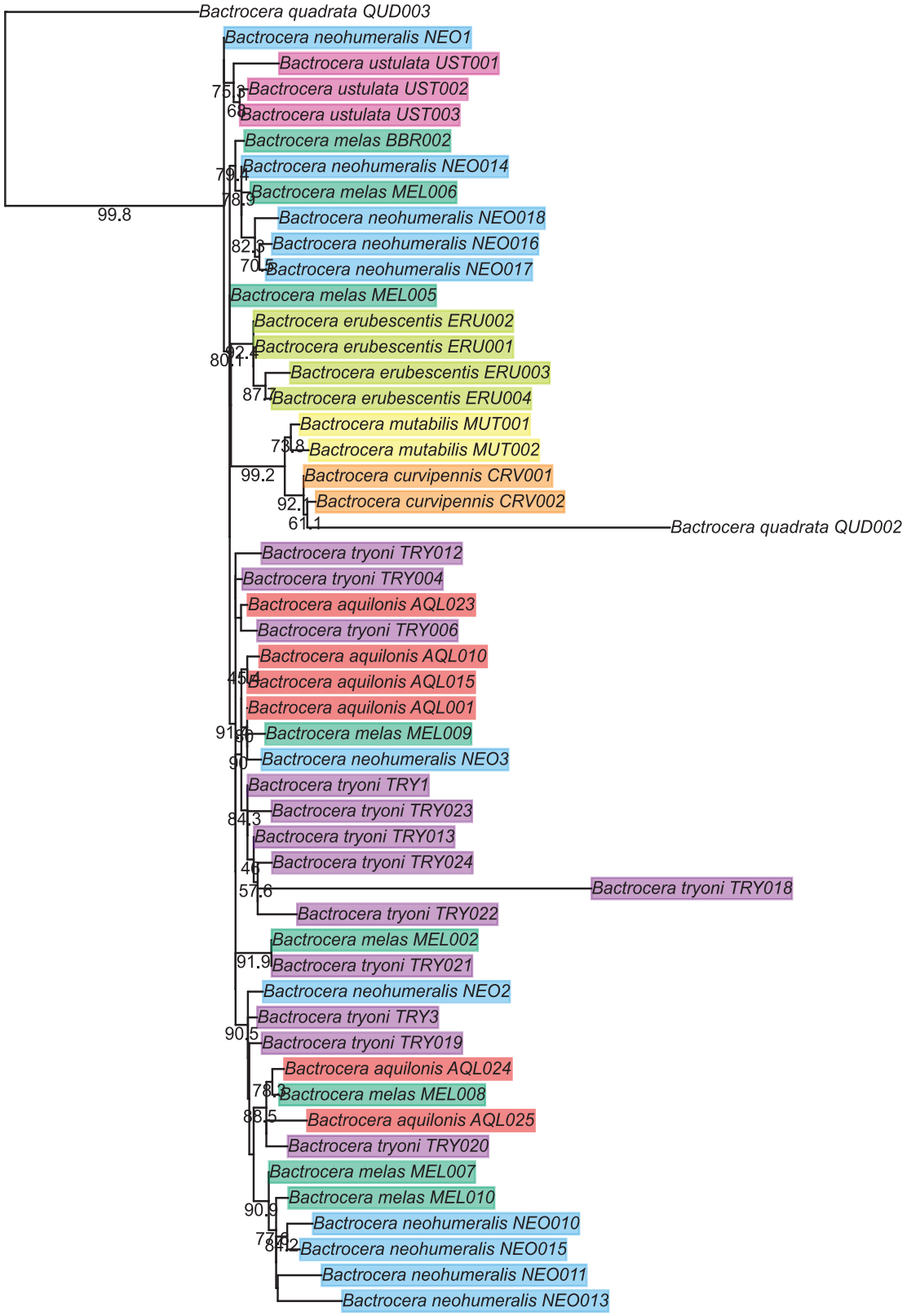


Figure 7.4: Proportionally linked Maximum Likelihood phylogeny of the *Bactrocera tryoni* species complex *sensu lato* based on four nuclear loci: RPA2, DDOSTs2, EIF3L and POP4. Branch supports are SH-aLRT values.

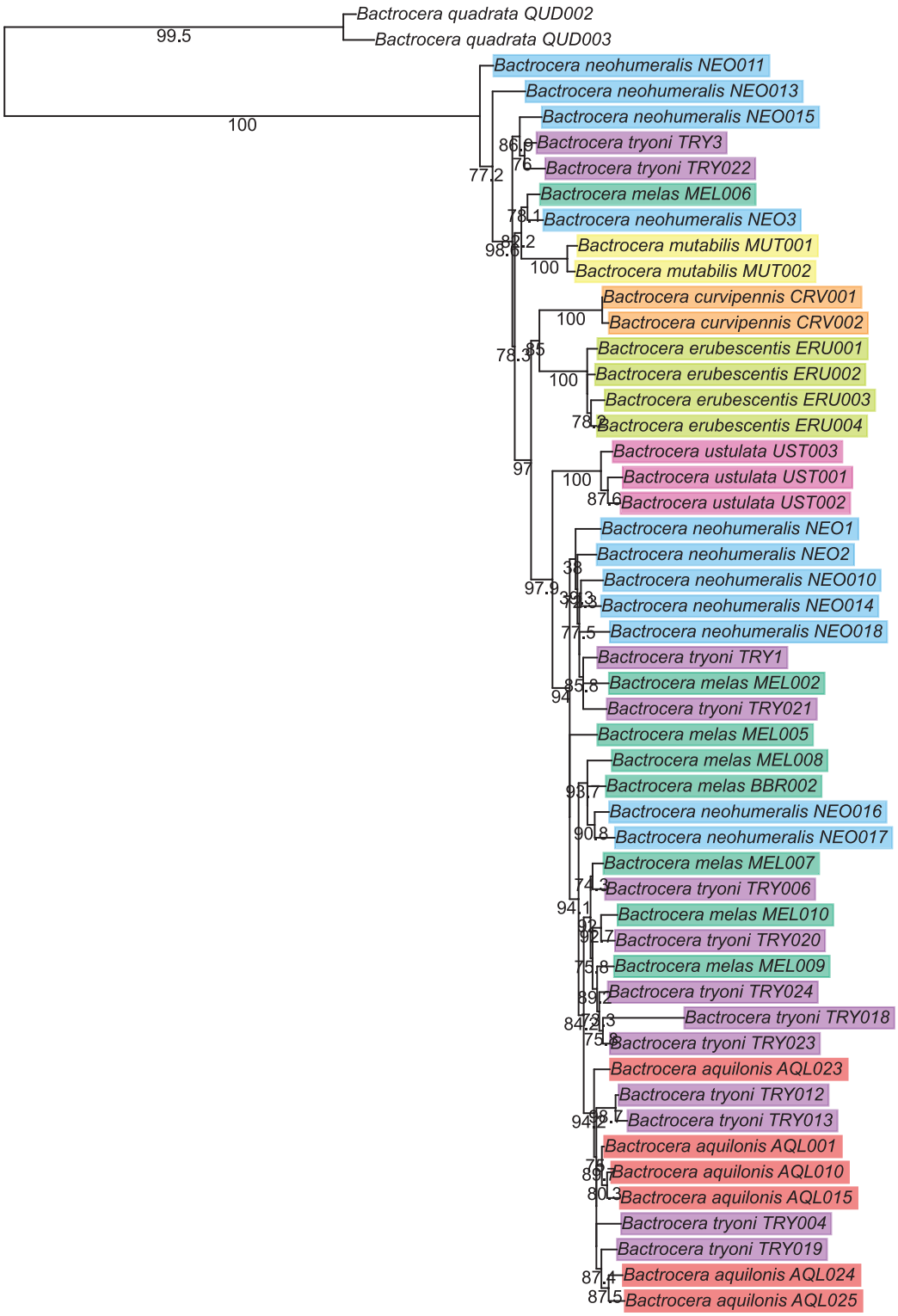


Figure 7.5: Proportionally linked Maximum Likelihood phylogeny of the *Bactrocera tryoni* species complex *sensu lato* based on three mitochondrial loci: COI, COII and 16S and four nuclear loci: RPA2, DDOSTs2, EIF3L and POP4. Branch supports are SH-aLRT values.

7.3.1.2. SNP data

Data was filtered from a total of 50126 binary SNPs for each of the three analyses. The PCoA analysis of all eight species showed that the four members of the *B. tryoni* species complex *sensu stricto* clustered together, while the four additional species of interest occupied distinctly different ordination space (Fig. 7.6). When the analysis was run with only the four traditional members of the complex, *B. melas* did not appear as a separate cluster, instead it always clustered with either *B. tryoni* or *B. neohumeralis* (Fig. 7.7). When the PCoA was run with only *B. tryoni, B. neohumeralis* and *B. melas, B. melas* again clustered with *B. tryoni* or *B. neohumeralis.* Some specimens of *B. tryoni* and *B. neohumeralis* fell almost midway between the two main species clusters (Fig. 7.8).

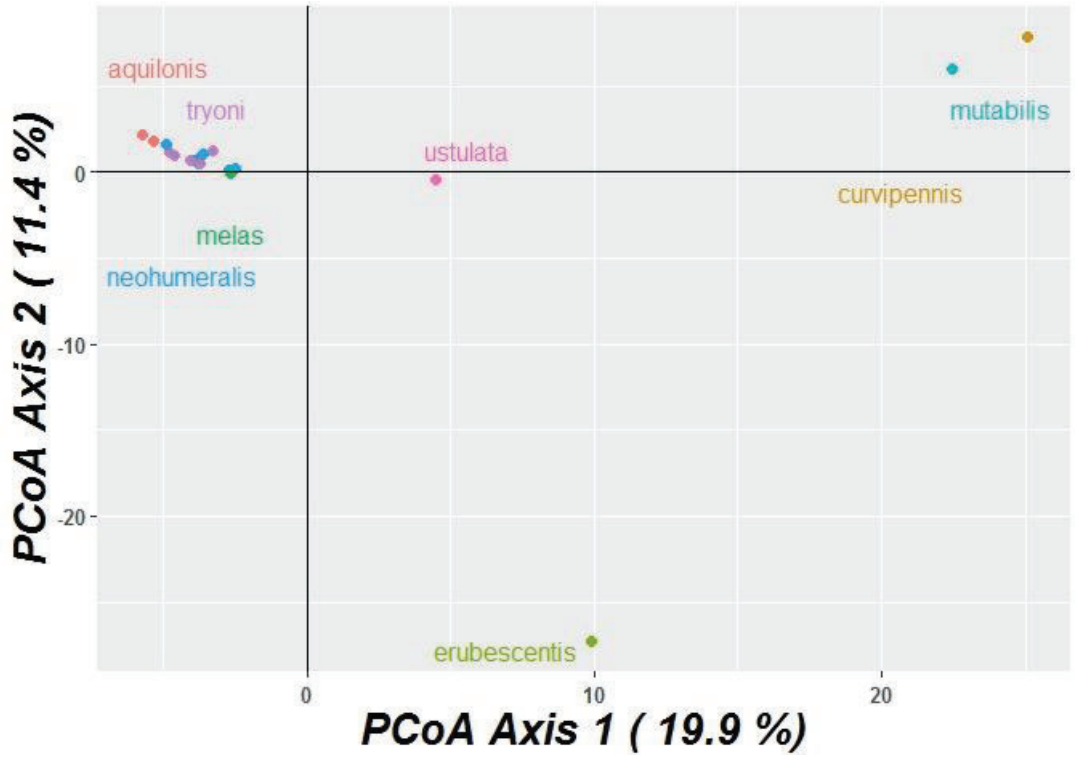


Figure 7.6: Principal coordinate analysis of 39283 SNPs from 20 individuals of the *Bactrocera tryoni* species complex *sensu lato.*

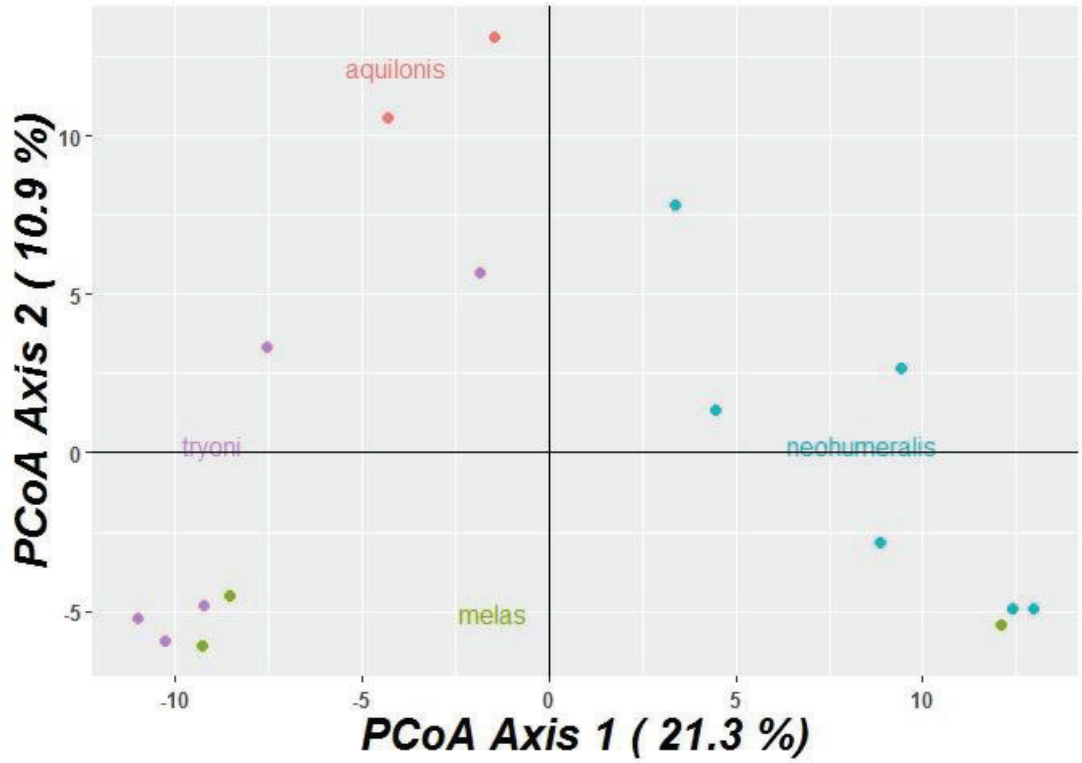


Figure 7.7: Principal coordinate analysis of 29785 SNPs from 16 individuals of the *Bactrocera tryoni* species complex *sensu stricto.*

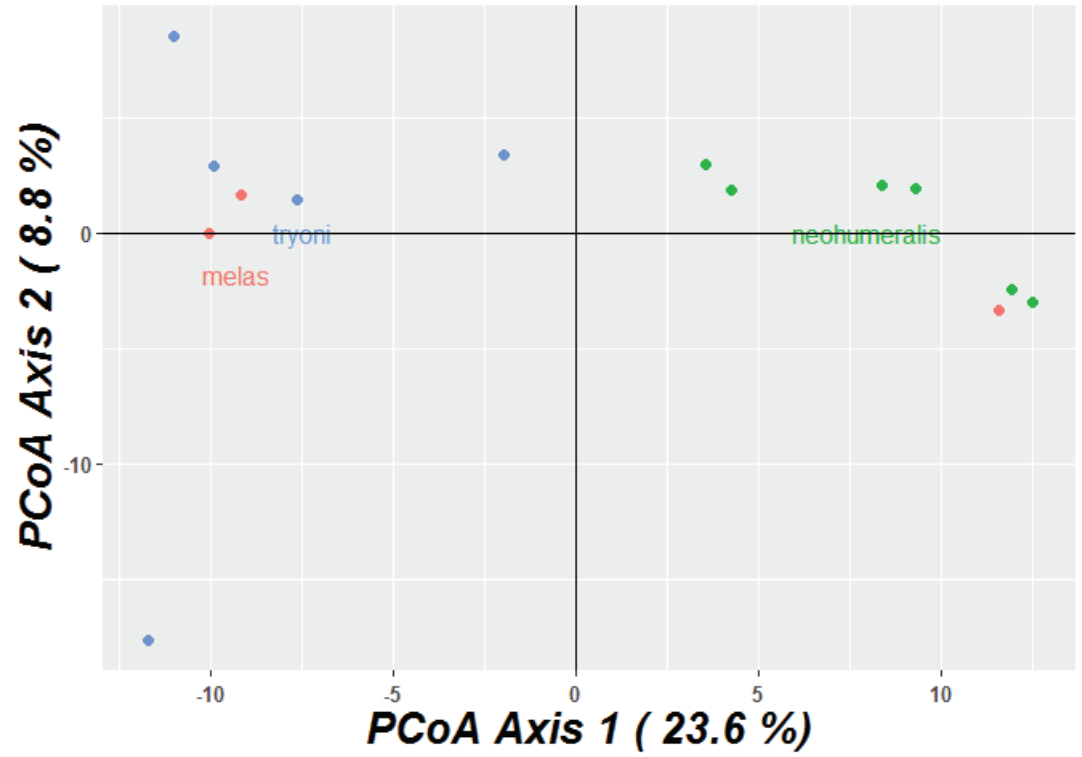


Figure 7.8: Principal coordinate analysis of 27817 SNPs data from 14 individuals from three species of the *Bactrocera tryoni* species complex; *B. tryoni, B. neohumeralis* and *B. melas.*

7.3.2. Species level systematics and taxonomy

7.3.2.1. Bactrocera humilis

Five *B. humilis* individuals were sequenced for COI in this chapter. Sequences were compared against those gathered for Chapter 3, and checked against the online database BLAST (date accessed: September 9, 2019) (Altschul et al., 1990). All *B. humilis* individuals were resolved within species clades, that were not their own (Table 7.4). Identification to species level was not achieved due to lack of sequence variation from other members of the *B. tryoni* species complex. The patterns observed seem to suggest that this is not a true species. The *B. humilis* holotype held at the Queensland Museum, Brisbane, exhibited all of the characteristics of typical *B. tryoni,* including microtrichia present in both costal cells (Fig. 7.9). There were no discernible features that could morphologically separate this species from *B. tryoni.*

Table 7.4: Results of the COI barcode sequencing of five *Bactrocera humilis* specimens and their respective genetic identification when matched against other sequences from the Australian Dacini database collated in Chapter 3 of this thesis.

D	Specimen	Country	Location	Lure	Trap date	Trapper	Identifier	BLAST result
s	HUM001	Australia	Smithfield, near Cairns, QLD, 16°49.031'S, 145°41.180E	ME	13.vi.2013	I. Schneider	I. Schneider	*B. breviaculeus/ peninsularis*
s	HUM002	Australia	Umagico, QLD, 10°53.300'S, 142°21.00'E	ME	9.v.2016	E. Cottis	I. Schneider	*B. breviaculeus/ peninsularis*
s	HUM003	Australia	Roma Flats, Cape York, QLD, 10 41.925'S 142 31.834'E	ME	9.xi.2009	J. Sailor	S. Cowan	*B. mayi/B. near quadrata/B. tenuifascia*
s	HUM004	Australia	Roma Flats, Cape York, QLD, 10 47'17"S 142 27'31"E	CUE	21.v.2007	J. Bond	D. Hancock	*B. breviaculeus/ peninsularis*
s	HUM005	Australia	Pormpurnaw	CUE	5.ii.1999	PFFP	D. Hancock	*B. tryoni/B. aquilonis*

Figure 7.9: *Bactrocera humilis* holotype. A: whole body dorsal view; and B: wing. Images provided by Geoff Thompson.

7.3.2.2. Bactrocera melas

The *B. melas* lectotype image was examined (Fig. 7.10) along with other specimens held at the Department of Agriculture and Fisheries insect collection. This examination revealed no identifying characters that could definitively separate *B. melas* from the variation that has been observed and documented within *B. tryoni.*

Figure 7.10: Image of the *Bactrocera melas* lectotype held at the Queensland Museum (Queensland Museum Network, 2020).

7.3.3. Taxonomic revision of the *Bactrocera tryoni* species group

Based on genetic and morphological analyses, I provide a new definition of this species group (Table 7.5) and a revision of the species within it. I will refer to this 'complex' from now on as the '*B. tryoni* species group' and will address the issues surrounding the term 'complex' in greater detail in the discussion chapter of this thesis. The group now encompasses seven species: *B. aquilonis, B. curvipennis, B. erubescentis, B. mutabilis, B. neohumeralis, B. tryoni* and *B. ustulata.* Based on morphological and molecular evidence, *B. humilis* and *B. melas* are synonymised with *B. tryoni* and amendments have been made to the description of *B. tryoni* to incorporate this phenotypic variation. I recognise that this new species group definition is broader than that provided previously and may require further revisions as more genetic data becomes available in the future (I identify additional species of interest in the discussion).

Table 7.5: Previous definition of the *Bactrocera tryoni* species complex compared to the revised definition of the *Bactrocera tryoni* species group. Key differences in the definitions are highlighted in grey.

Bactrocera tryoni species complex Drew (1989)	*Bactrocera tryoni* species group (this thesis)
"*Bactrocera species with clear wing membrane except for narrow costal band (not confluent with R4+5) and anal streak, costal cells fulvous or fuscous and generally covered with microtrichia, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow with narrow dark basal band, mesonotum red-brown (with or without dark colour patterns), abdominal terga generally red-brown with variable dark colour patterns, males attracted to cue lure".*	*Bactrocera* species with clear wing membrane except for some species with fuscous markings on r-m crossvein, costal band often wider than R2+3 with some species confluent with R4+5, anal streak, costal cells fulvous or fuscous with microtrichia covering one or both costal cells, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow with narrow dark basal band, mesonotum red-brown or black (with or without dark colour patterns), abdominal terga generally

Bactrocera tryoni species complex Drew (1989)	*Bactrocera tryoni* species group (this thesis)
	red-brown or orange-brown with variable colour patterns. Males attracted to cue-lure and isoeugenol.

Species included in the* Bactrocera tryoni *species group

Bactrocera (*Bactrocera*) *aquilonis* (May)

Bactrocera (*Bactrocera*) *curvipennis* (Froggatt)

Bactrocera (*Bactrocera*) *erubescentis* (Drew & Hancock)

Bactrocera (*Bactrocera*) *mutabilis* (May)

Bactrocera (*Bactrocera*) *neohumeralis* (Hardy)

Bactrocera (*Bactrocera*) *tryoni* (Froggatt)

Bactrocera (*Bactrocera*) *ustulata* Drew

***Bactrocera* (*Bactrocera*) *aquilonis* (May)**

Strumenta aquilonis May, 1965: 62-64

Dacus (*Bactrocera*) *aquilonis* Drew, 1982: 18-20

Bactrocera (*Bactrocera*) *aquilonis* Drew, 1989: 113-114

MATERIAL EXAMINED

Included (but not limited to) databased specimens held in QDAF collections: insecoll 109265-109458.

DIAGNOSIS

Fig. 7.11. Medium sized species: large black facial spots present; humeral and notopleural calli yellow; mesonotum pale red-brown with fuscous markings, mesopleural stripe reaching almost to anterior npl. bristle, lateral postsutural vittae

present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, costal cells fuscous, microtrichia covering second costal cell and most of first costal cell; abdominal terga III-V pale orange-brown with pale fuscous along anterior margin of tergum III and widening over lateral margins of that tergum, a medial longitudinal pale fuscous band on terga III and IV.

DESCRIPTION

Bactrocera aquilonis is adequately described in by Drew in Drew et al. (1982).

ATTRACTANT

Males attracted to Cue-lure.

DISTRIBUTION

Northern regions of the Northern Territory and Western Australia. Type locality Nightcliff, Darwin, Northern Territory.

Figure 7.11: *Bactrocera aquilonis* male lateral. Scale: 2mm. Image from Plant Health Australia (2018a).

***Bactrocera* (*Bactrocera*) *curvipennis* (Froggatt)**

Dacus curvipennis Froggatt 1909: 93

Strumenta curvipennis Perkins, 1939: 8-9

Dacus (*Strumenta*) *curvipennis* Drew, 1947: 30-32

Bactrocera (*Bactrocera*) *curvipennis* Drew, 1989: 128-129

MATERIAL EXAMINED

Included (but not limited to) databased specimen held in QDAF collections: insecoll 139293.

DIAGNOSIS

Fig. 7.12. Small species; very small pale fuscous facial spots present; humeral and notopleural calli yellow; mesonotum black, mesopleural stripe reaching midway between anterior margin of notopleural callus and anterior npl. bristle, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a broad fuscous costal band and anal streak, a broad fuscous band along r-m crossvein, costal cells pale fuscous, microtrichia covering second costal cell and outer corner of first costal cell; abdominal terga III-V orange-brown with a narrow transverse fuscous band along anterior margin of tergum III merging into broad lateral black margins and with anterolateral corners of terga IV and V fuscous.

DESCRIPTION

Bactrocera curvipennis is adequately described in Drew (1974).

ATTRACTANT

Males attracted to isoeugenol.

DISTRIBUTION

New Caledonia and Vanuatu. Type locality, New Caledonia.

Figure 7.12: *Bactrocera curvipennis* male dorsal. Scale: 2mm. Image from Plant Health Australia (2018a).

***Bactrocera* (*Bactrocera*) *erubescentis* (Drew & Hancock)**

Dacus (*Bactrocera*) *erubescentis* Drew and Hancock, 1981: 64-66

Bactrocera (*Bactrocera*) *erubescentis* Drew, 1989: 99

MATERIAL EXAMINED

Included (but not limited to) databased specimens held in QDAF collections: insecoll 107685-107707.

DIAGNOSIS

Fig. 7.13. Medium sized species; medium sized black facial spots present; humeral and notopleural calli yellow; mesonotum red-brown, mesopleural stripe ending midway between anterior margin of notopleural callus and anterior npl. bristle, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, costal cells fulvous with microtrichia in outer 1/3 of second costal cell only; abdominal terga III-V orange-brown with a narrow transverse dark band across anterior margin of tergum III, narrow dark lateral margins and a medial longitudinal dark band over all 3 terga.

DESCRIPTION

Bactrocera erubescentis is adequately described by Drew and Hancock in Drew et al. (1981).

ATTRACTANT

Males attracted to Cue-lure.

DISTRIBUTION

Known from Cape York Peninsula, the Torres Strait and Papua New Guinea. Type locality, Weipa, Queensland, Australia.

Figure 7.13: *Bactrocera erubescentis* male dorsal. Image from (Plant Health Australia, 2018b).

***Bactrocera* (*Bactrocera*) *mutabilis* (May)**

Strumenta mutabilis May 1951: 6-8

Dacus (*Bactrocera*) *mutabilis* Drew, 1982: 38-40

Bactrocera (*Bactrocera*) *mutabilis* Drew, 1989: 147

MATERIAL EXAMINED

Included (but not limited to) databased specimens held in QDAF collections: insecoll 117984-118045 (*B. mutabilis*).

DIAGNOSIS

Medium sized species; facial spots absent; humeral and notopleural calli yellow; mesonotum red-brown with oval black spots on anterior margin, mesopleural stripe reaching midway between anterior margin of notopleural callus and anterior npl. bristle, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, costal cells fuscous, microtrichia covering second costal cell and most of first costal cell; all abdominal terga entirely orange-brown. Images provided in Chapter 2 (Fig. 2.2A-F).

DESCRIPTION

Bactrocera mutabilis is adequately described by Drew in Drew et al. (1978).

ATTRACTANT

Males attracted to isoeugenol.

DISTRIBUTION

Known from eastern coast of Queensland south from Atherton; type locality, Toowoomba, Queensland, Australia.

***Bactrocera* (*Bactrocera*) *neohumeralis* (Hardy)**

Chaetodacus humeralis Perkins, 1934: 42-43

Strumenta melas Perkins and May, 1949: 12-14; May, 1963: 50

Dacus (*Strumenta*) *tryoni* var. *neohumeralis* Hardy, 1951: 169-170

Strumenta humeralis May, 1963: 46

Dacus (*Strumenta*) *neohumeralis* Drew, 1974: 67

Dacus (*Bactrocera*) *neohumeralis* Drew, 1982: 40-43

Bactrocera (*Bactrocera*) *neohumeralis* Drew, 1989: 114-115

MATERIAL EXAMINED

Included (but not limited to) databased specimens held in QDAF collections: insecoll 109500-111070.

DIAGNOSIS

Fig. 7.14. Medium sized species; medium sized black facial spots present; humeral calli dark brown to fuscous (see Fig. 7.15 for variation); notopleural calli yellow; mesonotum dark redbrown with dark fuscous to black markings, mesopleural stripe reaching midway between anterior margin of notopleural callus and anterior npl. bristle, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, costal cells fuscous, microtrichia covering second costal cell and outer 1/2 of first costal cell; abdominal terga III-V generally dark fuscous to dull black and tending red-brown medially.

DESCRIPTION

Bactrocera neohumeralis is adequately described by Drew in Drew et al. (1978).

ATTRACTANT

Males attracted to Cue-lure.

DISTRIBUTION

Eastern Queensland, northern New South Wales, Torres Strait islands and Papua New Guinea. More prevalent in wet tropical areas; Type locality, Cairns, Queensland, Australia.

COMMENTS

Phylogenetic analysis suggests that *B. neohumeralis* may consist of at least two sibling clades, one predominantly located in the southern and central portions of its geographic range, the other in the northern part of its range. The northern clade may also contain individuals which cannot be morphologically separated from *B. tryoni*. Morphological examination could find no consistent differences between specimens from each group. There is insufficient evidence at this point to justify splitting the species, but more research is warranted.

Figure 7.14: *Bactrocera neohumeralis* male dorsal. Scale: 2mm. Image from Plant Health Australia (2018a).

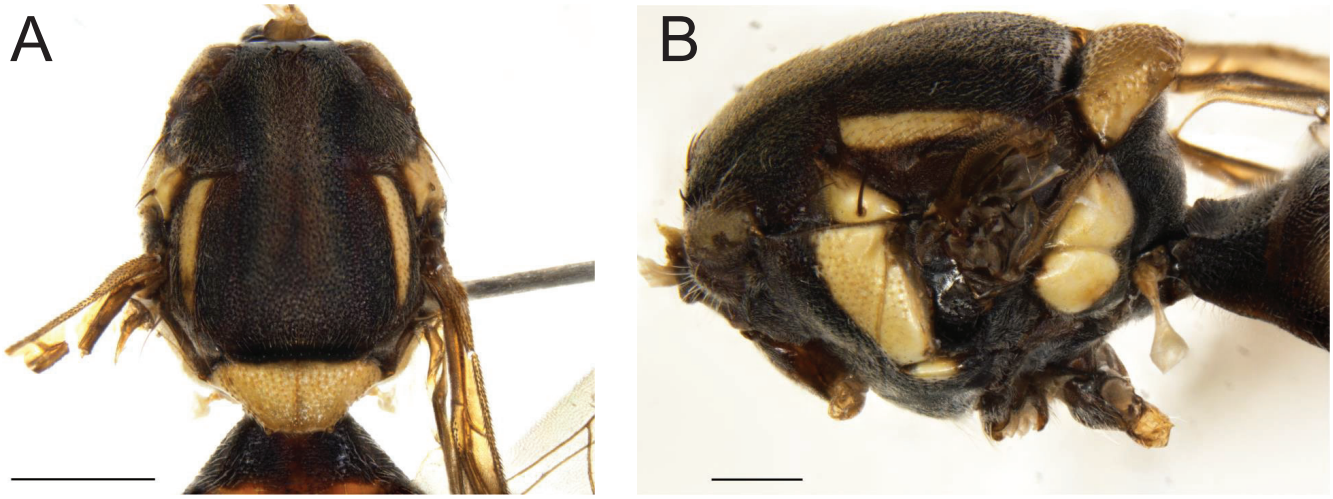


Figure 7.15: Variation in notopleural calli of *B. neohumeralis* (initially identified as an intermediate sp. and possible *B. melas* specimen (MEL006)). A: scutum dorsal (scale: 1mm); and B: scutum lateral (scale 0.5mm).

***Bactrocera* (*Bactrocera*) *tryoni* (Froggatt) new description**

Tephritis tryoni Froggatt, 1897: 410-412; Froggatt, 1899: 498

Dacus tryoni Froggatt, 1909: 79-80; 1910: 865; Malloch, 1931: 263

Chaetodacus tryoni Tryon, 1927: 181-183

Chaetodacus tryoni var. *juglandis* Tryon, 1927: 188

Chaetodacus tryoni var. *musa* Tryon, 1927: 187

Chaetodacus tryoni var. *sarcocephali* Tryon, 1927: 188

Strumenta melas Perkins and May, 1949: 12-14; May, 1963: 50

Dacus (*Strumenta*) *tryoni* Hardy, 1951: 167-168; Drew, 1974: 85-88

Dacus tryoni var. *melas* Hardy, 1951: 168-169

Strumenta tryoni May, 1963: 48

Dacus (*Bactrocera*) *humilis* Drew and Hancock, 1981: 68-70

Dacus (*Bactrocera*) *tryoni* Drew, 1982: 43-47

Dacus (*Bactrocera*) *melas* Drew, 1983: 34-35

Bactrocera (*Bactrocera*) *humilis* Drew, 1989: 138 **syn. n.**

Bactrocera (*Bactrocera*) *melas* Drew, 1989: 114 **syn. n.**

Bactrocera (*Bactrocera*) *tryoni* Drew, 1989: 115-116

MATERIAL EXAMINED

Included (but not limited to) databased specimens held in QDAF collections: insecoll 109500-111070. insecoll 111071-145692 and 109475-109559.

DIAGNOSIS

Medium sized species; medium sized black facial spots present; humeral and notopleural calli yellow; mesonotum red-brown with fuscous markings, mesopleural stripe reaching midway between anterior margin of notopleural callus and anterior npl. bristle, lateral postsutural vittae present, medial postsutural vitta absent,

scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, costal cells varying from fulvous to fuscous, microtrichia covering second costal cell and outer 1/2 of first costal cell; abdominal terga III-V generally red-brown with a medial and 2 broad lateral longitudinal fuscous bands over all 3 terga and joined along anterior margin of tergum III; paler forms of the abdomen are often present.

DESCRIPTION OF MALE

HEAD

Generally fulvous. Frons of even width; fulvous with fuscous around orbital bristles and on antero-medial hump; bristles fuscous: 1 s.or., 2 i.or.; lunule darkened. Ocellar triangle black. Vertex pale fuscous. Face fulvous with two pear shaped spots (tending oval) not quite reaching epistoma. Genae fulvous; sub-ocular spots pale; bristles dark fulvous. Occiput fulvous, yellow along eye margin; occipital rows with 6-8 strong fuscous bristles each side. Antennae fulvous with fuscous on apex and outer surface of third segment; arista fulvous proximally (remainder black).

THORAX

Generally rich red-brown to dark-brown (see Fig. 7.16A-C for variation). Pleura rich red-brown with black on most of sternopleuron, a spot above hind coxae and beneath wings; blotched fuscous to black along anterior and posterior edges of mesoplueral stripes. Mesonotum with a central tomentose band appearing greyish; fuscous markings as follows: two narrow longitudinal bands which run from anterior mesonotal suture; between humeral and notopleural calli; along inner posterior margins of post-sutural vittae; on posterior marginal area of mesonotum (may be absent in some specimens). Yellow markings as follows: humeral calli; notopleural calli; narrow mesopleural stripes ending midway between notopleural callus and anterior npl. bristle above, continuing onto sternopleuron below as a transverse spot; upper hypopleural callli (posterior apices red-brown); 5/8 lower hypopleural calli (remainder red-brown); two narrow, triangular, lateral post-sutural vittae ending before upper p.sa. bristles (level with lower p.sa. bristle). Postnotum fuscous laterally and red-brown centrally. Scutellum yellow with narrow black basal band. Bristles: sc. 2, prsc. 2, p.sa. 2, a.sa. 1, mpl. 1, npl. 2, scp. 4; all bristles well developed and fuscous. Legs: fulvous except for middle and hind coxae and hind tibiae fuscous;

middle tibiae each with apical black spur. Wings: costal cells fulvous to fuscous, microtrichia covering all of second costal cell and outer ½ of first costal cell; remainder of wings colourless except dark fulvous stigma, narrow fuscous costal band overlapping R2+3 and ending midway between extremities of R4+5 and M vein, broad fuscous anal strak ending at wing margin. A dense aggregation of microtrichia around CuA+1A. Supernumerary lobe of medium development in males and weak in females.

ABDOMEN

Oval; tergites free; pecten present on tergite III. Tergite I dark red-brown to fuscous; tergite II fulvous with posterior ½ tending whitish; chiefly in central areas of each tergite. Variation high in abdomen markings from a faintly visible thin medial longitudinal fuscous band on tergites III-V (not visible in some specimens) to darker and more prominent medial longitudinal fuscous band (see Fig. 7.17A-D for variation). A pair of shining spots on tergite V which tend towards yellow-brown. In some specimens tergites III-V are paler in the central areas, the fuscous pattern being confined to the anterior margin of tergite III and lateral margins of three tergites.

ATTRACTANT

Males attracted to Cue-lure.

DISTRIBUTION

French Polynesia, Pitcairn Islands, Papua New Guinea, New Caledonia, Torres Strait islands, Eastern coast of Australia from Cape York in Queensland to Gippsland, Victoria, Northern Territory. Type localities, Tenterfield and Penrith, New South Wales, Australia.

COMMENTS

Bactrocera humilis and *B. melas* are synonymised here after a combination of morphological and genetic data provided evidence that they are not biological species. Specifically, *B. humilis* could not be uniquely identified using COI barcode data, while the type specimen was morphologically identical to *B. tryoni. Bactrocera melas* specimens clustered with both *B. tryoni* and *B. neohumeralis* in SNP analysis, but is synonymised with *B, tryoni* based on comparison of the types which had no morphological differences. Phylogenetic analysis provides preliminary analysis of

the cryptic clade from northern Australia that includes specimens morphologically similar to both *B. tryoni* and *B. neohumeralis*.

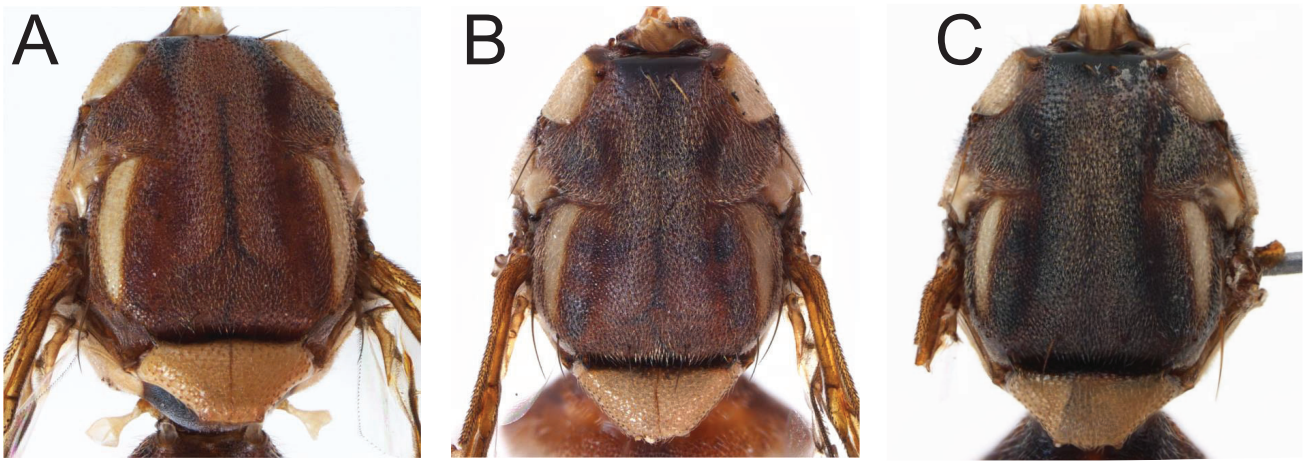


Figure 7.16: *Bactrocera tryoni* scutum variation dorsal. A: TRY021; B: TRY019; and C: BBR002.

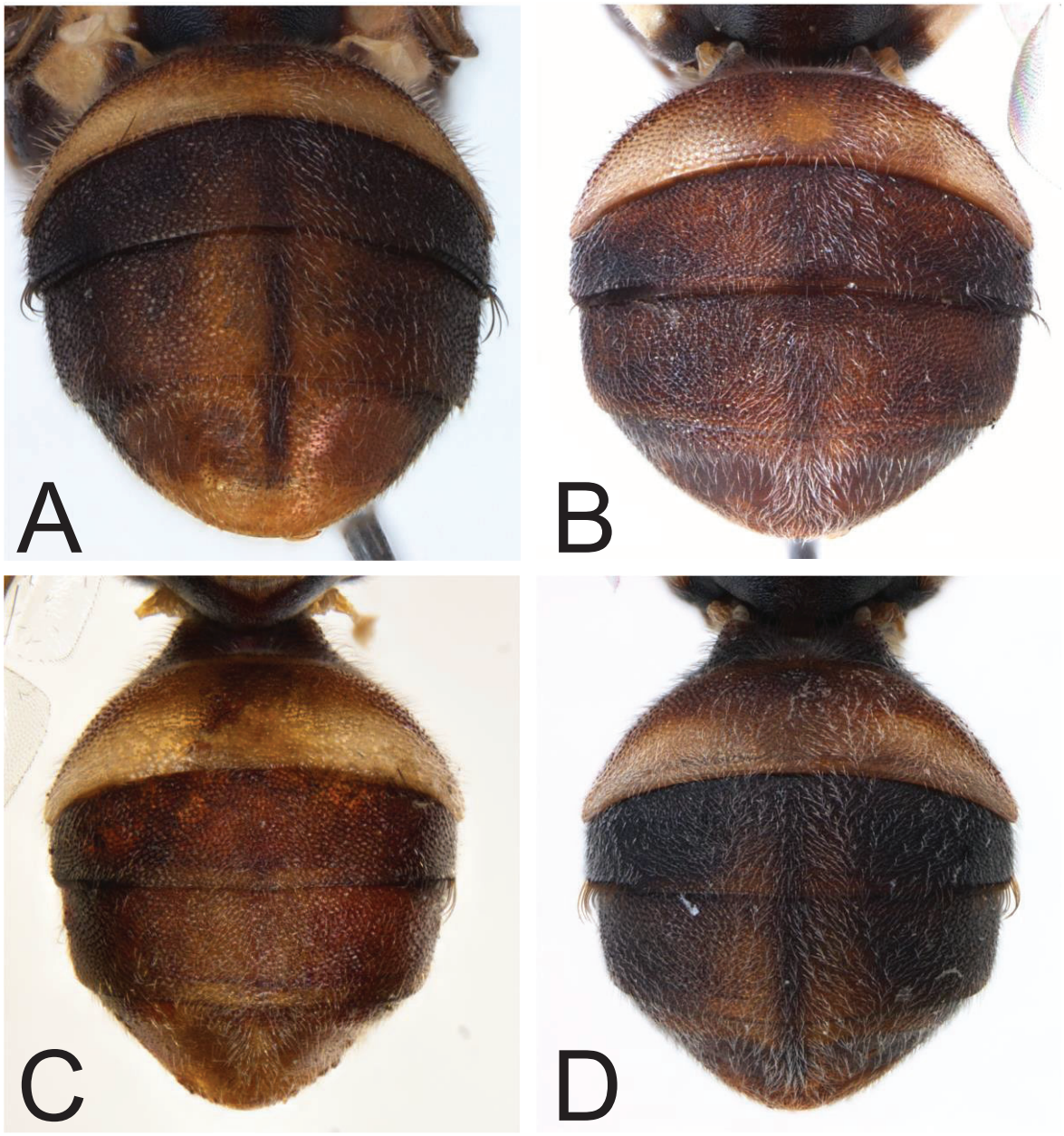


Figure 7.17: *Bactrocera tryoni* abdomen variation dorsal. A: TRY021; B: TRY019; C: uncoded specimen from QDAF collections; and D: BBR002.

***Bactrocera* (*Bactrocera*) *ustulata* Drew**

Bactrocera (*Bactrocera*) *ustulata* Drew, 1989: 86-87

MATERIAL EXAMINED

Included (but not limited to) databased specimen held in QDAF collections: insecoll 145692.

DIAGNOSIS

Fig. 7.18A-B. Medium sized species; facial spots medium sized and pear shaped; humeral and notopleural calli yellow; mesonotum pale fuscous with dark fuscous to black patterns, lateral postsutural vittae present, medial postsutural vitla absent, mesopleural stripe of medium width (reaching midway between anterior margin of notopleural callus and anterior npl. bristle), scutellum yellow; wing with a broad fuscous costal band and anal streak, costal cells fuscous, microtrichia covering both costal cells; abdominal terga Ill-V orange-brown except for a very narrow indefinite medial longitudinal pale fuscous band and 2 broad lateral longitudinal dark fuscous to black bands over all 3 terga.

DESCRIPTION

Bactrocera ustulata is adequately described Drew (1989).

ATTRACTANT

Males attracted to Cue-lure.

DISTRIBUTION

Morobe and Central Provinces, Papua New Guinea. Type locality, Morobe Province, Papua New Guinea.

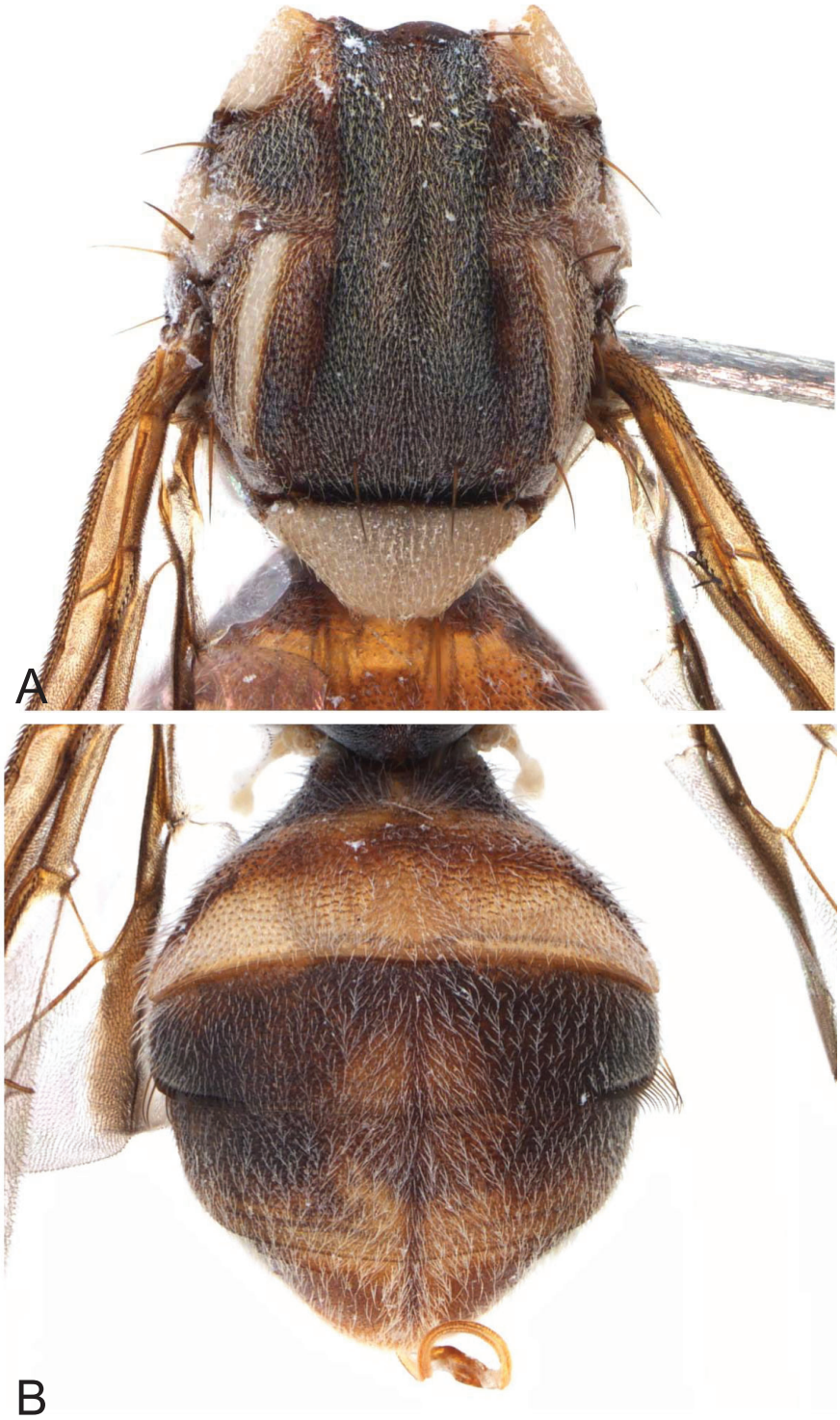


Figure 7.18: *Bactrocera ustulata* (UST002) male. A: scutum dorsal; and B: abdomen dorsal.

7.4. Discussion

I have revised the *B. tryoni* species group to consist of seven species that form a monphyletic clade. This includes synonymization of *B. humilis* and *B. melas* with *B. tryoni* Additionally, there is preliminary evidence that there may be a cryptic basal species (currently fitting the morphological descriptions of *B. neohumeralis* and *B. tryoni*) also present in Queensland and Papua New Guinea, which may have been influenced by historical biogeography. Here I will provide a general discussion on the results of this chapter and identify some issues that require further investigation.

7.4.1. Systematics and taxonomy

7.4.1.1. B. humilis

The *B. humilis* specimens included in this chapter represent the majority of specimens that have ever been identified as this species. Several that were initially identified as *B. humilis,* in part because they came from methyl eugenol (ME) traps, were genetically identified as cue-lure responsive species and so may represent "blow-ins" (very occasional captures of individuals of a species from the "wrong" trap), or a small level of cross-lure contamination in traps. Given the available evidence, the original single specimen of a "*B. tryoni*-like" individual caught in an ME trap, and so described as the new species of *B. humilis,* was probably a similar blow-in or the result of trap contamination. Of the specimens that were examined, only one possessed microtrichia in both costal cells as per the initial description of Drew et al. (1999). While there is an absence of physiological data for *B. humilis* in the literature, what is known, is that this species shares a geographic range with *B. tryoni* (Drew et al., 1999) and overlaps in morphology with documented variants of *B, tryoni* (Plant Health Australia, 2016). These lines of evidence provide support toward this species being conspecific with *B. tryoni.*

7.4.1.2. B. melas

The SNP data provides evidence that *B. melas* is not a morphologically or genetically distinct species. Additionally, it appears that this species is also not a hybrid of *B. tryoni* and *B. neohumeralis* (as suggested by Hancock (2013)) but is simply one or

the other. I note that the *B. melas* specimen (MEL006) that clusters with *B. neohumeralis* was included because morphologically, it had intermediately coloured postpronotal lobes (rusty orange instead of yellow as in *B. tryoni* and dark brown as in *B. neohumeralis*). It is extremely important for diagnosticians to note that it is entirely possible that intermediates could be *B. tryoni* or *B. neohumeralis*. *Bactrocera melas* overlaps in its geographic range, host range (Hancock et al., 2000), and (as shown here), morphological variation with *B. tryoni* and *B. neohumeralis*. Further work on this group could investigate the nuclear Internal Transcribed Spacer regions 2 (ITS2) locus, which has been shown to exhibit fixed differences between *B. tryoni* and *B. neohumeralis* (Morrow et al., 2000). This study did not include intermediates or any species identified as *B. melas,* but is definitely something that should be investigated further given that the evidence provided in this chapter suggest *B. melas* may not be a true species.

7.4.1.3. Other species for consideration

Sampling for Chapter 3 of this thesis failed to acquire genetic material for *B. notatagena* (May), but was able to amplify a small fragment of the COI barcode region from *B. nigrovittata*. Based on the diagnosis for both species, the new definition of the *B. tryoni* species group would encompass both species. *Bactrocera nigrovittata* was not resolved as close to the *B. tryoni* species complex. Because of this, I am hesitant to include other species in this group based solely on morphological evidence; as I know from Chapter 4 that shared morphological characters for the Dacini does not guarantee genetic affinity. Further work on this species group should prioritise the inclusion of fresh specimens of both of these species.

7.4.1.4. Comments on B. aquilonis

This chapter did not aim to investigate the species status of *B. aquilonis,* however, the results offer evidence that could be useful in making taxonomic changes in future. Previous studies have suggested this species is not genetically distinct from *B. tryoni* (Cameron et al., 2010), which is consistent with my Sanger sequencing results.

The SNP data suggests that the level of interspecific variation between *B. tryoni* and *B. aquilonis* is only slightly less than the level of variation that exists between *B. neohumeralis* and *B. tryoni.* There were some *B. tryoni* and *B. neohumeralis* individuals that clustered in between the *B. aquilonis* cluster and the clusters of *B. neohumeralis* and *B. tryoni* in the PCoA. This agreed with Popa-Baez et al. (2020) who found evidence for gene flow between *B. tryoni* in Queensland and *B. aquilonis* in the Northern Territory; suggesting there may be gene flow/hybridisation between these populations.

7.4.2. Biogeographic considerations and the presence of cryptic species in the group

Several individuals were resolved as sister to the rest of the *B. tryoni* species group and as a second sister clade to *B. mutabilis* respectively. These individuals are northern distributed specimens (Papua New Guinea, Coen, Cairns, Lockhart, Mackay). From a biogeographic perspective, this could suggest that some of these individuals represent basal taxa and potential cryptic taxa. There may have been multiple introductions of this group into Australia from Papua New Guinea. When sea levels were lower, a land bridge was present between Cape York and Papua New Guinea, which has been shown to have influenced speciation in mammals (Malekian et al., 2010, Macqueen et al., 2012) and other insect species (Beebe and Cooper, 2002). There is evidence to suggest that this sister group may encompass one or more cryptic species due to previous evidence of the presence of *B. neohumeralis* and *B. tryoni* in Papua New Guinea which are not considered to be pests (i.e. different host ranges) (Drew, 1989). Non-pestiferous species could also be have been sampled here, providing evidence toward them sharing geographic ranges with pestiferous species (the less divergent clade on the tree) in Australia.

The *B. neohumeralis* specimen from Papua New Guinea is resolved within this basal clade, along with a number of other specimens collected from north Queensland locations (no further south than Mackay); however, they do occur in sympatry with specimens that are within the larger *B. tryoni sensu stricto* clade. There is enough evidence provided here to suggest the presence of a cryptic species, but not enough

to determine how many species there may be. More comprehensive population-level sampling across these ranges would provide greater insight into these species.

7.4.3. Limitations

Because the SNP analysis was based on a small number of individuals, it is difficult to draw conclusions on ongoing issues such as the species status of *B. aquilonis* and the potential for hybridisation between *B. tryoni* and *B. neohumeralis.* Expanded population-level studies could be used to investigate the basal species that were identified here in greater detail.

Chapter 8: General Discussion

8.1. Summary

This thesis aimed to comprehensively sample the Australian Dacini taxa and produce a dated phylogeny in order to investigate evolutionary traits and pathways. Here I draw together the findings from each chapter and discuss the practical applications of these findings for investigating key species groups, as well as implications for pest management and diagnostics.

I produced a dated phylogeny for the Australian Dacini and closely related members from the Asia-Pacific region, an analysis that had previously not been undertaken. The dataset consisted of over 80% of the Australian Dacini taxa, with additional taxonomic and phylogenetically related species from the South-east Asian and Pacific region. For the majority of the Australian taxa, this was the first time that these species had ever been used for genetic analysis. I found that the Australian taxa were polyphyletic across the tree, with multiple radiations between Australia and Papua New Guinea emerging in the biogeographic analysis. The phylogeny found that many relationships that have been made based on taxonomy were not supported by phylogenetic analysis.

Node calibrations found that divergence time estimates (e.g. *Bactrocera* split from *Dacus* + *Zeugodacus* 33.61-46.11mya and *Dacus* and *Zeugodacus* split 32.7-45.23mya) were much younger (~30my) than the only previously dated Dacini phylogeny, but my dates were in agreement with higher level Dipteran analyses (Wiegmann et al., 2011, Han and Ro, 2016). Based on these dates, I investigated the divergence pathways and speciation of the Australian Dacini. Species were found to have radiated eastward into the Pacific, with evidence of species reaching New Caledonia from Papua New Guinea (through Melanesia) and Australia. There was no evidence of species lineages moving back into Australia or Papua New Guinea from the Pacific once they had colonised the region, suggesting unidirectional movement.

Evidence suggested that Australia was colonised by Dacini species only from Papua New Guinea, and that this occurred only via the land bridge that existed between Cape York and Papua New Guinea. There was no evidence to indicate species colonised Australia from Indonesia. Species then colonised other identified Australian biogeographic regions (west and south) from the source region identified

to be: Cape York and Atherton. Within Australia, there was no indication that biogeographic barriers that have restricted movement of other taxa, had any impact on the movement of Dacini species.

Ancestral trait analysis based on existing records found that the ancestral male lure response was cue-lure, and that response to other plant derived chemicals has evolved multiple times across the phylogeny. Lure response was found to have a high phylogenetic signal across the tree. Additionally, host diet breadth was also investigated and was found to follow the general trend of evolution from generalist to specialist; however, observations of the tree revealed *Bactrocera* consisted of a high number of generalists compared to *Dacus,* the majority of which were specialists. Phylogenetic signal for host diet breadth was statistically significant across the tree, but not as strong as for lure response. Analysis for dependency between the two traits found that there was no correlation between lure response and host diet breadth.

Morphological characters were tested here for the first time for phylogenetic utility. I found that colour patterns and structural characters were not able to produce a well resolved phylogeny when used alone. When combined with molecular data, morphology did not add any additional resolution. Therefore, I concluded that morphological characters within the Dacini should only serve diagnostic and descriptive purposes, rather than being used for determining evolutionary relationships via cladistic analyses. I utilised taxonomic characters in defining two new species groups, but use the term ‘group’ instead of ‘complex’ because of the evolutionary assumptions often applied to the term complex (discussed further in this chapter).

The first of the new species groups was the *B. aglaiae* species group. The *B. aglaiae* species group was resolved as a basal clade to all *Bactrocera* sampled in this dataset. A likelihood mapping analysis found that this placement was well supported and corroborated this relationship which had been previously hypothesised based only on morphological characters. In addition to this group being basal, I identified several species existing within the group that had not previously been identified as separate biological species. When examining existing species descriptions, I identified taxonomic discrepancies between the description of the holotype and the subsequent description of the paratypes. This led me to define the species group, provide a

description of the new species, and move away from the use of the term species complexes.

Additional members were also identified via the phylogenetic analysis to exist within the current interpretation of the *B. tryoni* species complex. Separate species clades were identified that further analysis revealed consisted of a number of basal species. Four additional members were also resolved within the species complex. Two species, *B. humilis* and *B. melas,* for which their species status has been debated in the literature were synonymised with *B. tryoni* based on morphological examinations and supporting molecular data. Given this information, I redefined this species complex as a species group based on additional sequencing to include the additional four species in the group.

This chapter will draw together key issues of this thesis in the context of the broader Dacini literature. This will include important implications for systematics and taxonomy of the group as well as outcomes and recommendations for pest management and diagnostics. Throughout this chapter I will provide specific examples of how the findings can be applied in a practical setting along with a case study which will demonstrate how the findings of this thesis are a good starting point for investigating difficult species groups within the Dacini.

8.2. Implications for the systematics and taxonomy of the Australian Dacini

Informal taxonomic groupings (subgeneric groups, subgenera, species complexes) are used extensively within the Dacini, but there are many examples of their use in plants (Brown et al., 1995, Muschner et al., 2006), birds (Daily et al., 1993), fungi (Nirenberg and O'Donnell, 1998)and other insects (De Meyer et al., 2015a). These taxonomic ranks, that do not hold status under the ICZN, exist to aid in better identification of species (Drew, 1972), and are commonly applied to infer systematic relationships (Clarke and Schutze, 2014). For a tribe as large as the Dacini, these groupings should be useful if they provide both an accurate representation of species relationships (i.e. systematics), whilst also aiding identification and taxonomy. This phylogeny adds to a larger dataset provided by those that came before it (Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018), given all of this information,

there is now extensive phylogenetic understanding of a large proportion of the tribe, which now enables the utility of these groups to be questioned.

8.2.1. Subgeneric groupings and subgenera

Chapter 3 resolved the three subgeneric groupings (i.e. the Bactrocera group, Melanodacus group and Zeugodacus group) as polyphyletic across the phylogeny (Table 3.6). Ignoring the issue of them being non-natural groupings, I still question the usefulness of these subgeneric groupings, as each contains hundreds of species. Additionally, these groups do not aid in taxonomic identification or classification as they have not been included in any dichotomous key, instead, all keys for the tribe begin at the subgeneric level. At the subgeneric group level, there are four groups for which very few characters are used to classify species. For example, the Melanodacus group of subgenera is only separated from the Zeugodacus group by the length of the posterior lobe of the male surstylus (Drew, 1989). I suggest that Dacini workers consider abandoning these groups.

The subgeneric classifications of species within *Bactrocera* and *Zeugodacus* remain the subject of constant scrutiny and ongoing revision. This was primarily prompted by the elevation of *Zeugodacus* to genus level which was not supported by all taxonomists (Drew and Romig, 2013), despite extensive phylogenetic evidence (Krosch et al., 2012, Virgilio et al., 2015, San Jose et al., 2018, Dupuis et al., 2018). There are seven characters that are used to discriminate among *Bactrocera* and *Zeugodacus* subgenera (presented in the context of the subgenera included in this thesis in Table 8.1). These include: the length of the posterior lobe of the male surstylus (short or long), the shape of the male abdominal sternum V (concave or deeply concave), the number of scutellar bristles present (1 or 2 pairs), and the presence or absence of: pecten on terga III, humeral bristles, prescutellar bristles and supra-alar bristles (Drew, 1989).

This strict classification system provides for only two states (e.g. present/absent) however, this should only hold if there is no variation in these states. However, it is clear from the table that some subgenera exhibit both states e.g., one or two pairs of scutellar bristles present in *B.* (*Neozeugodacus*). Drew (1989) mentions that there is intraspecific variability of prescutellar bristles within some species of genus

Bactrocera. Absence of prescutellar and supra-alar bristles has also been reported in *B. coccinae* (Premlata and Singh), despite this species being assigned to subgenus *Bactrocera* (which consists of species with these traits present). Similarly, this has also been observed in *Z. cucurbitae,* which showed variation in the presence and absence of all setae (White, 2000). Additionally, characters are sometimes physically dislodged from specimens which can create difficulties for diagnosticians (Fleming et al., 2000). Hardy (1969) did not agree with the use of a number of ambiguous bristles or, more importantly, use of male-based characters (e.g. male surstylus), which are only useful 50% of the time.

Additionally, there are also a number of examples across the phylogeny of subgeneric polyphyly, in particular within the subgenera of *Dacus* and *Zeugodacus*. As multi-entry keys (Plant Health Australia, 2018b) and molecular diagnostics become the norm (Plant Health Australia, 2020), the focus and utility of subgenera must be on aiding the classification of species. So, given the information provided in Chapter 3 of this thesis, it may better serve Dacini workers to i) undertake a taxonomic review of these subgenera so that morphological and molecular data reflect the same relationships; and ii) in doing so, reduce the number of subgenera for ease of reference (i.e. as some contain thousands of species while others only a handful) and better handling of the tribe.

Table 8.1: Eight subgenera included in this thesis and the seven morphological characters used for classification (Drew, 1989, Hancock and Drew, 2015, 2016, 2018a, 2018b). Key character differences are highlighted.

Genus	Subgenus	Abdominal sternite V of male	Posterior lobe of male surstylus	Pecten of cilia on abdominal tergite III	Postpronotal setae	Supra-alar setae	Prescutellar setae	Scutellar bristles
Bactrocera	*Apodacus*	deep posterior emargination	short	present	absent	present or absent	present or absent	one pair
Bactrocera	*Hemizeugodacus*	shallow posterior emargination	short	present	absent	present	present	two pairs
Bactrocera	*Neozeugodacus*	shallow posterior emargination	short	present	absent	present or absent	present	one or two pairs
Bactrocera	*Parazeugodacus*	shallow posterior emargination	short	present or absent	absent	present	present	two pairs
Zeugodacus	*Austrodacus*	shallow posterior emargination	long	absent	absent	present or absent	present or absent	two pairs
Zeugodacus	*Zeugodacus*	slightly concave on posterior margin	long	present	absent	generally present	generally present	two pairs
Zeugodacus	*Sinodacus*	slightly concave on posterior margin	long	present	absent	present	absent	one pair

Genus	Subgenus	Abdominal sternite V of male	Posterior lobe of male surstylus	Pecten of cilia on abdominal tergite III	Postpronotal setae	Supra-alar setae	Prescutellar setae	Scutellar bristles
Zeugodacus	*Parasinodacus*	shallow posterior emargination	long	present	absent	generally present	present or absent	one pair

8.2.2. Species complexes

There are numerous definitions for 'species complex' that exist for the Dacini (Schutze et al., 2017), but in addition to this, there are many other definitions and examples of species complexes that exist outside of the Dacini (Mayr, 1963, Mayr, 1940) (Table 8.2). Sigovini et al. (2016) defines a species complex as *".. a group of related species characterized by unclear boundaries.."*. However, many more definitions exist, and it is often not clear which definition is being applied.

Table 8.2: Various definitions and examples of how species complexes are used in taxonomy.

Name used	Definition applied	Reference
Cryptic species complex	Morphologically identical species	Campillo et al. (2005) and Walter (2005)
Sibling species complex	A group of monophyletic species	Walter (2005)
Species complex	Morphological similarity and overlapping distribution	Mateos (2008)
Species complex	Species assigned based on genetics	Weir et al. (2012)
Species complex	Morphological similarity and suspected introgression	Jones et al. (2013)
Taxonomic species complex	Species assigned based on morphology and convenience	Clarke and Schutze (2014)

8.2.2.1. Usefulness of complexes and groups

Using the *B. dorsalis* species complex as an example, which consists of over 85 species (Drew and Romig, 2013, Leblanc et al., 2015), the question must be asked: does this complex still serve a diagnostic and functional purpose? Membership within this group is largely based on the presence of a black scutum and clear costal cells on the wing (Drew and Romig, 2013). However, from the results of Chapters 3

and 4, it is clear these morphological characters are extremely common within the Dacini as the *B. dorsalis* complex was resolved as polyphyletic across the tree. Therefore, based on my results, I can conclude that this complex can no longer be considered a group that consists of separate closely related species, nor a group that exclusively shares morphological characters. On the other end of the scale, is the *B. quadrata* species complex which consists of a group of species that inhabit a similar geographic range (Hancock et al., 2000), but do not share close morphological or genetic affinity (Chapter 3). As such, in contrast to reliance on the definition of Sigovini et al. (2016) (above) these examples highlight the importance of having clearly defined boundaries for these complexes and groups, which will ensure they continue to serve a taxonomic purpose.

8.2.2.2. Recommendations

I recommend avoiding the term 'complex' and using the term 'species group' for the groups within the Dacini. When implementing the 'group' classification, I would advise that workers clearly define their lines of evidence as to why (or why not) species are placed in these groups (e.g. morphology, ecology, genetics) and provide a clear definition for the species group. Not only will this streamline taxonomy for this clade and their diagnosis, but this approach will also align with nomenclature used for other taxa, such as grasses (Dekker, 2003), amphibians (Macey et al., 2000), small mammals (Sullivan et al., 1997), other insects (Magowski and Moser, 2003, Kaminski et al., 2020) and sometimes other tephritids (Berlocher, 2000). However, I will continue to refer to existing species complexes here as 'complexes' until they have undergone taxonomic review.

8.2.3. Diagnostics

Species diagnostics has not been specifically addressed in this thesis however, given the wide range of genetic data gathered, and the analysis of the utility of morphological characters, it is appropriate to discuss the future of diagnostics for this group. I recommend that morphology be the first port of call for identification due to the ease and efficiency of this approach. However, this is not always possible or

feasible as taxonomic expertise may be limited, or adult specimens may not be available. In this instance, it would be necessary to rely on a database of molecular data, for which this thesis has contributed a large number of new sequences from the Australian and Pacific region.

8.2.3.1. Comments on diagnostic loci

I employed a number of different loci for the phylogenetic reconstruction that were developed specifically for diagnostics within this tribe (Krosch et al., 2019b, Plant Health Australia, 2020). I did not set out to formally test the utility of the chosen loci, but can make some comments on observations I had during sequence editing and tree building. Despite employing the purpose-built loci, I was still unable to resolve some difficult species complexes and groups. This was to be expected for some groups such as the *B. tryoni* species group and the *B. frauenfeldi* species complex, as large phylogenomic datasets have failed to discern species in these groups (Dupuis et al., 2018); however, other species groups also proved difficult. One example is the difficulty encountered with a group that consisted of *B. peninsularis, B. breviaculeus* and *B. rufofuscula* that all loci struggled to separate individually. If multiple molecular loci and morphological characters are unable to separate some of these species, perhaps the search for a 'barcode' is not the right approach. Perhaps the search is simply for enough SNPs or nucleotides of difference that are present in a wide variety of different loci. Using the results from Chapter 7 as an example; *B. tryoni* and *B. neohumeralis* are extremely difficult to separate using numerous loci, some of which had been developed specifically for the Dacini, and yet the SNP data was able to easily provide separation between the two.

8.2.4. Successful applications of morphology

It is important to recognise the utility of morphology for integrative taxonomic approaches. As I have identified, morphological characters were not capable of resolving species relationships when used for phylogenetic reconstruction. However, phylogenetic reconstructions incorporate many species and a large number of shared morphological characters. If instead, the aim of the study was to delimit species

boundaries or inform taxonomic assignments, morphology could be far more useful. Schutze et al. (2015b) incorporated morphological characters such as wing shape and size, width of vittae, scutum colouration and aedeagus length in conjunction with phylogenetic and haplotype analyses in an integrative taxonomic approach investigating the species boundaries of (then) *B. invadens* and *B. dorsalis*. This use of morphology provided a statistical comparison across populations and evidence towards synonymising the two species. This was a targeted approach that utilised morphological characters that were known to be diagnostic or species-specific and in this case, morphology was extremely useful.

8.2.4.1. Using other evidence to support morphological taxonomy

At the species level, morphological taxonomy was extremely useful for identification of species throughout this project. Where possible, two individuals were included of each species, and in most cases, species were monophyletic. The approach worked as a positive feedback loop, where morphology acted as the first identification; then genetic data confirmed or rejected the diagnosis; followed by further morphological examination; and then additional specimens sequenced if necessary. I think this worked well in confirming identifications of species across the phylogeny. An example of when other data such as genetic and physiological (whether in a formal analysis or not - in this case morphology was used for identification purposes only) is useful, is in the case of *B. bryoniae.* Individuals from two regions were identified as *B. bryoniae* however, after genetic analysis it was found that this species has genetically diverged, and while still forming a monophyletic clade, most likely consists of two separate species. In addition, of the two regions sampled (Australia and Papua New Guinea), *B. bryoniae* is not considered to be significant pest in Australia (Drew et al., 1978), but has been reared and confirmed to significantly impact Birdseye chilli crops in Papua New Guinea (Leblanc et al., 2001). The addition of other data here has helped to unravel a potential cryptic species.

8.2.5. Case study: *B. frauenfeldi* species complex

The findings of each chapter of this thesis provide a good starting point for investigating the relationships and evolution of species complexes. Here I use my results and apply it to a case study on the *B. frauenfeldi* species complex. The *B. frauenfeldi* species complex currently consists of five species; *B. frauenfeldi, B. caledoniensis, B. trilineola, B. albistrigata* and *B. parafrauenfeldi,* four of which were sampled within this thesis. I will utilise my results to infer possible information on the fifth species *B. parafrauenfeldi* which was unable to be sampled and for which there is little associated biological information due to the rare nature of this species.

8.2.5.1. Gathering evidence

All four members sampled in this thesis formed a monophyletic clade, with *B. frauenfeldi* and *B. caledoniensis* difficult to resolve (Fig. 8.1). In addition, the four members sampled are cue-lure responsive and are polyphagous species (Fig. 8.2A-C). The complex is widely distributed, with members scattered throughout South-east Asia, Papua New Guinea, and the Pacific, with *B. parafrauenfeldi* found only in the Northern Territory, Australia. Species in the group are estimated to have diverged in the last 5my. Morphologically, all five species are extremely similar, with the minor differences between *B. trilineola* and *B. parafrauenfeldi* considered to be the extent of wing and leg markings (Drew, 1989).

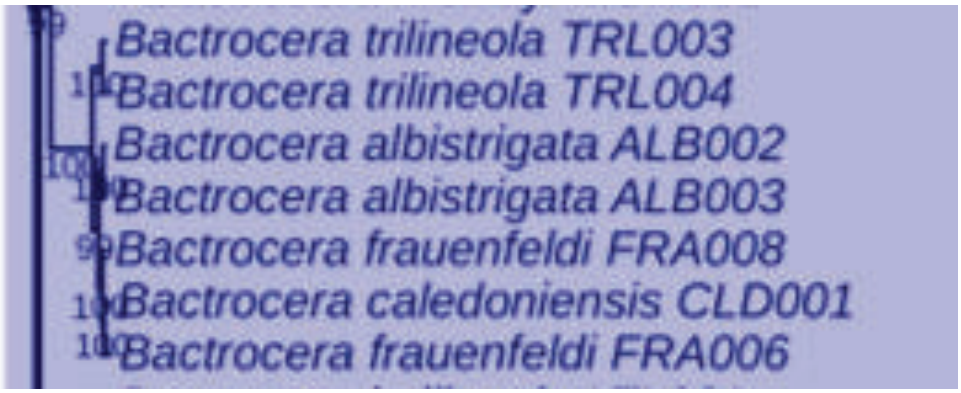


Figure 8.1: Extract of phylogenetic tree produced in Chapter 3 showing resolution of the four members of the *B. frauenfeldi* species complex that were sampled.

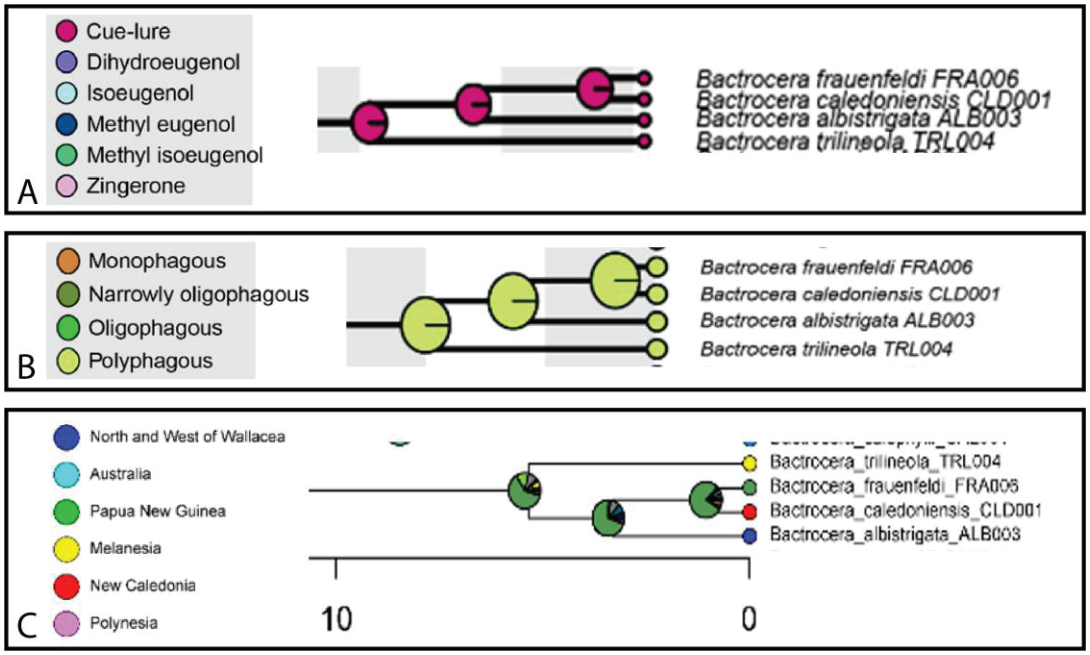


Figure 8.2: Extracts from three separate analyses in this thesis. A: Male lure response; B: larval diet breadth; and C: biogeographic distribution of the four species belonging to the *B. frauenfeldi* species complex.

8.2.6.2. Inferring evolutionary patterns

Based on the evidence above and the knowledge of other evolutionary patterns found within this thesis, we can deduce the following about *B. parafrauenfeldi:*

- It is either cue-lure (which is the lure it has responded to previously, although it is only occasionally encountered (J. Royer., pers comm.)) or isoeugenol responsive based on patterns observed in the ancestral trait reconstruction of male lure response of other species.
- Phylogenetic signal was weaker in the host breadth tree, therefore patterns are difficult to infer. Host plants have not been recorded for *B. parafrauenfeldi* (Hancock et al., 2000) and it is not a listed pest (Plant Health Australia, 2016). This may suggest that this fly is not polyphagous however, more investigation is needed.
- Based on the biogeographic analysis and pathways that were identified for other close species groups, the most likely divergence pathway for *B. parafrauenfeldi* was from *B. frauenfeldi,* a Papua New Guinea species. Following this the species made its way west into the Northern Territory (Fig. 8.3).

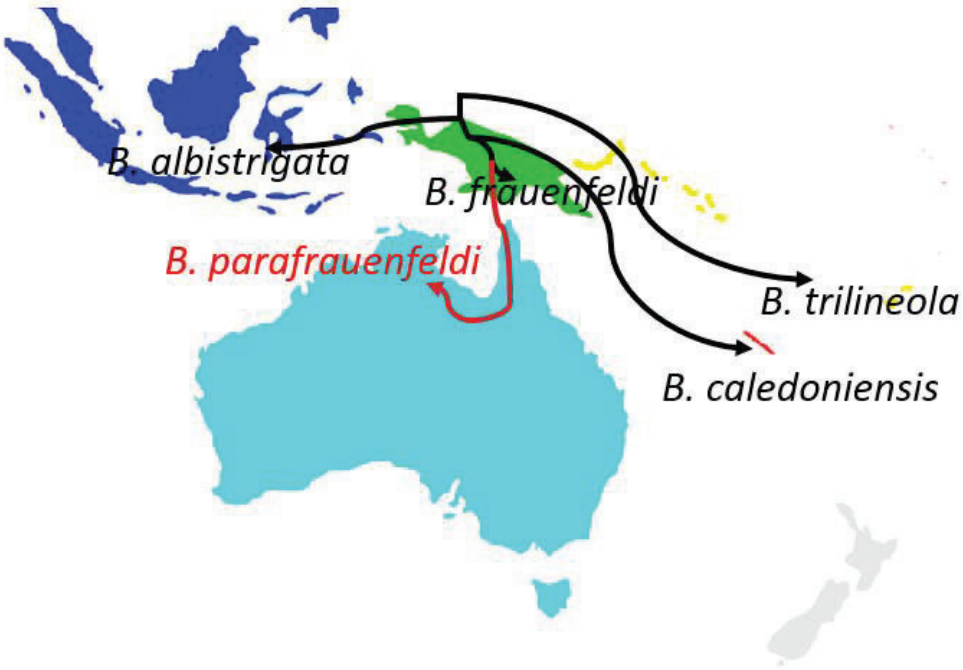


Figure 8.3: Proposed divergence pathways for five members of the *B. frauenfeldi* species complex. Red pathway of *B. parafrauenfeldi* is postulated based on previously identified divergence pathways for other species.

8.2.6.3. Further considerations

Based on the proposed divergence pathways for other species, and the patterns observed there (Chapter 5); groups of sister species are usually present across Papua New Guinea + North Queensland + Northern Territory. It is unusual that there is an absence in North Queensland of any species closely related to *B. parafrauenfeldi* (given that *B. frauenfeldi* is native to Papua New Guinea). When comparing morphology between *B. frauenfeldi* and *B. parafrauenfeldi* (Fig. 8.4), the only difference is the presence and absence of vittae. However, the variability of vittae length in *B. frauenfeldi* has been well documented (Plant Health Australia, 2016) (Fig. 8.5) and cannot be considered a diagnostic species character as it is well within the variation observed for *B. parafrauenfeldi*. Delving into recent history would reveal that the two species were described a year apart. First, *B. frauenfeldi* invaded and established in North Queensland in 1974 (Drew et al., 1978) and then in 1975 the first detection of *B. parafrauenfeldi* occurred in the Northern Territory, with the species officially described in 1989 (Drew, 1989). Further evidence is needed to confirm that these species are conspecific, but there is evidence to support the theory that after *B. frauenfeldi* invaded Queensland, it did not take long for humans to spread it west into the Northern Territory.

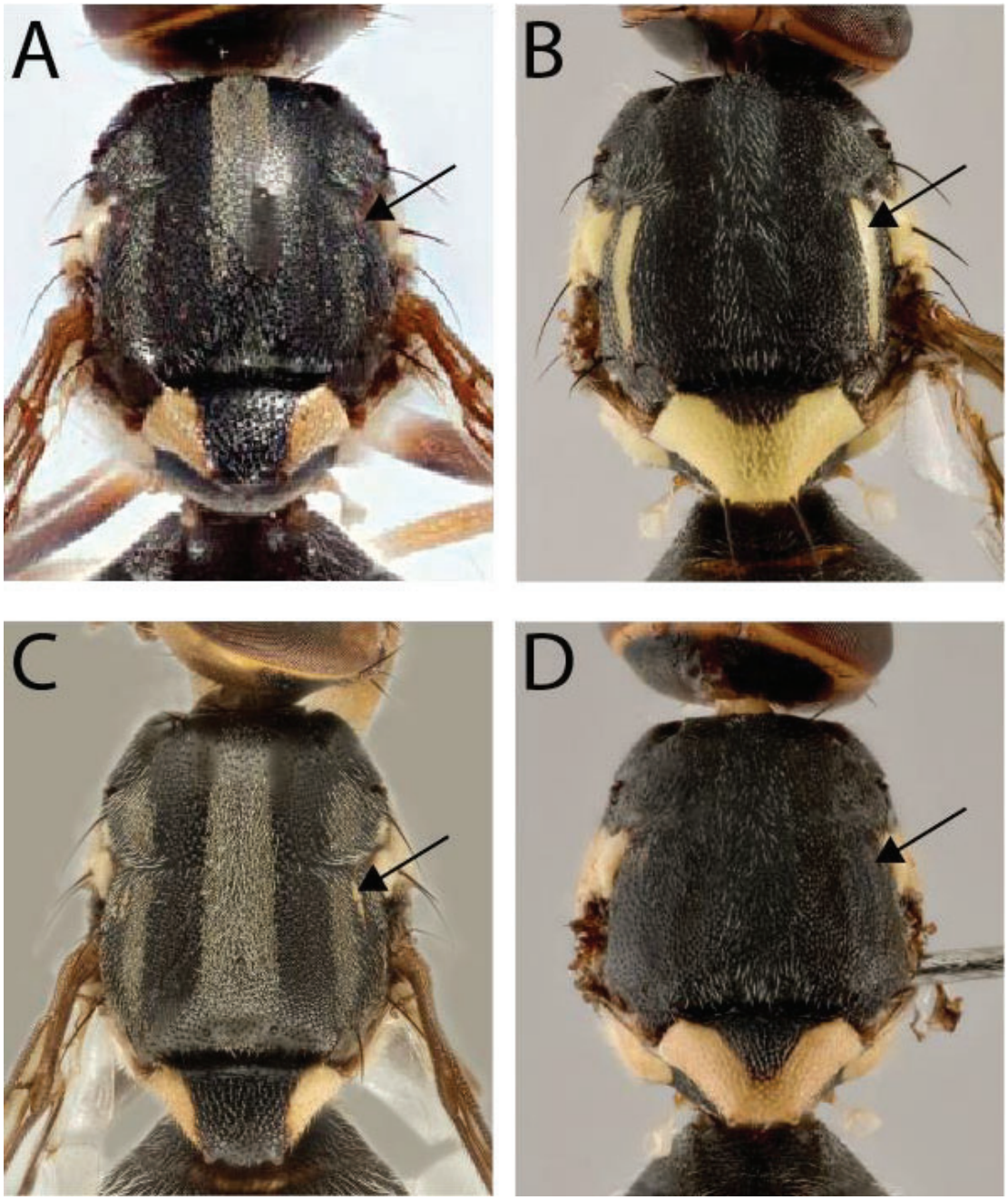


Figure 8.4: Variation in lateral post-sutural vittae length for *B. parafrauenfeldi* and *B. frauenfeldi.* A: *B. parafrauenfeldi;* B: *B. frauenfeldi;* C: variation of vittae length of *B. parafrauenfeldi;* and D: variation in vittae length of *B. frauenfeldi.* Images: Queensland Museum Network (2020), J. McMahon and Plant Health Australia (2016).

8.2.6.4. Concluding remarks on case study

To conclude, this thesis provides the basis for the beginnings of a taxonomic revision for the *B. frauenfeldi* species complex. Further investigation of the morphological variation within this group is important, but further investigation into the population genetics of this group could shed light on the geographic origins of *B. parafrauenfeldi* in the Northern Territory.

8.3. Conclusions and further research

I will outline some further research directions that could build upon the findings here. Firstly, I want to highlight the many applications of this phylogeny: ancestral trait reconstruction, likelihood mapping, biogeographic analyses of speciation, testing against morphological characters, and contributions towards taxonomic revisions. While these were my uses for this dataset, I want to acknowledge that this dataset will become available for others to use for their own questions. For example, I have only mapped two evolutionary traits, but there may other traits such as host plant family that could be investigated for this tribe.

8.3.1. Taxonomy

I used my findings to inform a taxonomic revision of the *B. tryoni* species group. Further work on this group could expand to better understand the paraphyly of *B. neohumeralis* and *B. tryoni*. This might include expanded sequencing of the internal transcribed spacer region 2, a locus that has previously been identified as having fixed differences in the sequence and can pull apart *B. neohumeralis* and *B. tryoni* (Morrow et al., 2000). This locus could be effective at determining whether these paraphyletic individuals represent cryptic species within this group.

Between this study, and other large phylogenies on the group (Krosch et al., 2012, Dupuis et al., 2018, San Jose et al., 2018), there is enough evidence available for informative taxonomic revision of the tribe. In particular, a revision of subgenera within *Dacus* and *Bactrocera.* This would reconcile the issues that continue to be identified between taxonomy and phylogenetics, and would be extremely helpful for all that work on this tribe.

8.3.2. Systematics and phylogenetics

Further phylogenetic work could look at combining datasets with other existing phylogenetic studies in order to expand our understanding of the origins of the tribe. This would add further evidence toward the two proposed origin hypotheses from Chapter 5 of this thesis. In addition to this, further taxon sampling of the *B.* (*Hemizeugodacus*) subgenus would add to the existing knowledge provided in this thesis surrounding the basal clade of all *Bactrocera.* Determining the basal lineage, could shed light on the origins of this genus.

A further step in developing better uses for morphology could include mapping morphological traits onto the nodes of the morphological and molecular phylogenies. This might allow for a better understanding of the evolution of character traits, and if there are any characters that are capable of discerning species clades.

Going forward, a greater understanding of the drivers of Dacini speciation would be invaluable. In particular, investigating the relationship between host plant families and large radiations across the phylogeny, could provide insights into how these species have evolved and why there are so many young clades of *Bactrocera.*

Bibliography

ACTON, G. D. 1999. Apparent polar wander of India since the Cretaceous with implications for regional tectonics and true polar wander. *Memoirs of the Geological Society of India,* 44**,** 129-175.

AITCHISON, J. C., ALI, J. R. & DAVIS, A. M. 2007. When and where did India and Asia collide? *Journal of Geophysical Research,* 12**,** B05423.

ALBERCTSEN, B. R. 2000. Flowering phenology and seed predation by a tephritid fly: escape of seeds in time and space. *Ecoscience,* 7**,** 433-438.

ALI, J. R. & AITCHISON, J. C. 2008. Gondwana to Asia: plate tectonics, palaeogeography and the biological connectivity of the Indian sub-continent from the middle Jurassic through latest Eocene (166-35 Ma). *Earth-Science Reviews,* 88**,** 145-166.

ALLWOOD, A. J. & ANGELES, T. S. 1979. Host records of fruit flies (Family Tephritidae) in the Northern Territory. *Queensland Journal of Agricultural and Animal Sciences,* 36**,** 105-113.

ALLWOOD, A. J., CHINAJARIYAWONG, A., KRITSANEEPAIBOON, S., DREW, R. A. I., HAMACEK, E. L., HANCOCK, D. L., HENGSAWAD, C., JIPANIN, J. C., JIRASURAT, M., KRONG, C. K., LEONG, C. T. S. & VIJAYSEGARAN, S. 1999. Host plant records for fruit flies (Diptera: Tephritidae) in Southest Asia. *Raffles Bulletin of Zoology,* 47**,** 1-92.

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. & LIPMAN, D. J. 1990. Basic local alignment search tool. *Journal of Molecular Biology,* 215**,** 403-410.

AMICE, R. & SALES, F. 1997. Fruit fly fauna in New Caledonia. *In:* ALLWOOD, A. J. & DREW, R. A. I. (eds.) *Management of fruit flies in the Pacific.* Nadi, Fiji: ACIAR.

ARCHIE, J. W. 1996. Measures of homoplasy. *In:* SANDERSON, M. J. & HUFFORD, L. (eds.) *Homoplasy: the recurrence of similarity in evolution.* San Diego, California: Academic Press.

ARMSTRONG, K. F. & BALL, S. L. 2005. DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 360**,** 1813-1823.

ARZUFFI, R., ROBLEDO, N. & VALDEZ, J. 2008. Antennal sensilla of *Toxotrypana curvicauda* (Diptera: Tephritidae). *The Florida Entomologist,* 91**,** 669-673.

ASOKAN, R., REBIJITH, K. B., SINGH, S. K., SIDHU, A. S., SIDDHARTHAN, S., KARANTH, P. K., ELLANGO, R. & RAMAMURTHY, V. V. 2011. Molecular identification and phylogeny of *Bactrocera* species (Diptera: Tephritidae). *Florida Entomologist,* 94**,** 1026-1035.

ATHAR, M. 2005. Infestation of olive fruit fly, *Bactrocera oleae,* in California and taxonomy of its host trees. *Agriculturae Conspectus Scientificus,* 70**,** 135-138.

ATLAS OF LIVING AUSTRALIA. 2019. Alangium villosum *subsp.* tomentosum *(F.Muell.) Bloemb.* [Online]. Available: https://biocache.ala.org.au/occurrences/search?q=lsid:http://id.biodiversity.org.au/node/apni/2916803#tab_recordsView [Accessed November 2 2018].

AUDLEY-CHARLES, M. G. 1987. Dispersal of Gondwanaland: relevance to evolution of the Angiosperms. *In:* WHITMORE, T. C. (ed.) *Biogeographical evolution of the Malay Archipelago.* Oxford: Clarendon Press.

AUSTWICK, E. 1961. *Annual Report 1960-1961*, Agricultural Branch, Northern Territory Administration.
AXELROD, D. I. & RAVEN, P. H. 1982. Paleobiogeography and origin of the New Guinea flora. *In:* GRESSIT, J. L. (ed.) *Biogeography and ecology of New Guinea.* Dordrecht: Springer.
BAI, X., ZENG, T., NI, X., SU, J., YE, G., LU, Y. & QI, Y. 2019. CRISPR/Cas9-mediated knockout of the eye pigmentation gene *white* leads to alterations in color of head spots in the oriental fruit fly, *Bactrocera dorsalis*. *Insect Molecular Biology*.
BAUER, A. M. & SADLIER, R. A. 1993. Systematics, biogeography and conservation of the lizards of New Caledonia. *Biodiversity Letters,* 1**,** 107-122.
BECK, J., KITCHING, I. J. & LINSENMAIR, K. E. 2006. Wallace's line revisited: has vicariance or dispersal shaped the distribution of Malesian hawkmoths (Lepidoptera: Sphingidae)? *Biological Journal of the Linnean Society,* 89**,** 455-468.
BEEBE, N. W. & COOPER, R. D. 2002. Distribution and evolution of the *Anopheles punctulatus* group (Diptera: Culicidae) in Australia and Papua New Guinea. *International Journal for Parasitology,* 32**,** 563-574.
BEER, D. & BEER, A. 2019. phylotate: phylogenies with annotations. Available from: https://CRAN.R-project.org/package=phylotate.
BELL, K. L., MORITZ, C., MOUSSALLI, A. & YEATES, D. K. 2007. Comparative phylogeography and speciation of dung beatles from the Australian Wet Tropics rainforest. *Molecular Ecology,* 16**,** 4984-4998.
BELL, M. A. & LLOYD, G. T. 2014. strap: stratigraphic tree analysis for palaeontology. Available from: https://CRAN.R-project.org/package=strap.
BELL, R. C., PARRA, J. L., TONIONE, M., HOSKIN, C. J., MACKENZIE, J. B., WILLIAMS, S. E. & MORITZ, C. 2010. Patterns of persistence and isolation indivate resilience to climate change in montance rainforest lizards. *Molecular Ecology,* 19**,** 2531-2544.
BELT, E. S., HARTMAN, J. H., DIEMER, J. A., DROEGER, T. J., TIBERT, N. E. & CURRAN, H. A. 2004. Unconformities and age relationships, Tongue River and older members of the Fort Union Formation (Paleocene), western Williston Basin, U.S.A. *Rocky Mountain Geology,* 39**,** 113-140.
BERLOCHER, S. H. 2000. Radiation and divergence in the *Rhagoletis pomonella* species group: inferences from allozymes. *Evolution,* 54**,** 543-557.
BERNAYS, E. A. & MINKENBERG, P. 1997. Insect herbivores: different reasons for being a generalist. *Ecology,* 78**,** 1157-1169.
BEZZI, M. 1915. On the Ethiopian fruit-flies of the genus *Dacus*. *Bulletin of Entomological Research,* 6**,** 85-101.
BEZZI, M. 1916. On the fruit-flies of the genus *Dacus* (s. 1) occurring in India, Burma, and Ceylon. *Bulletin of Entomological Research,* 7**,** 99-121.
BLACKET, M. J., SEMERARO, L. & MALIPATIL, M. B. 2012. Barcoding Queensland fruit flies (*Bactrocera tryoni*): impediments and improvements. *Molecular Ecology Resources,* 12**,** 428-436.
BOONTOP, Y., KUMARAN, N., SCHUTZE, M. K., CLARKE, A. R., CAMERON, S. L. & KROSCH, M. N. 2017. Population structure in *Zeugodacus cucurbitae* (Diptera: Tephritidae) across Thailand and the Thai-Malay peninsula: natural barriers to a great disperser. *Biological Journal of the Linnean Society,* 121**,** 540-555.

BOUGEARD, S. & DRAY, S. 2018. Supervised multiblock analysis in R with the ade4 package. *Journal of Statistical Software,* 86**,** 1-17.
BOYKIN, L. M., SCHUTZE, M. K., KROSCH, M. N., CHOMIC, A., CHAPMAN, T. A., ENGLEZOU, A., ARMSTRONG, K. F., CLARKE, A. R., HAILSTONES, D. & CAMERON, S. L. 2013. Multi-gene phylogenetic analysis of south-east Asian pest members of the *Bactrocera dorsalis* species complex (Diptera:Tephritidae) does not support current taxonomy. *Journal of Applied Entomology,* 138**,** 235-253.
BROOKS, D. R., O'GRADY, R. T. & GLEN, D. R. 1986. A measure of the information content of phylogenetic trees, and its use as an optimality criterion. *Systematic Zoology,* 35**,** 571-581.
BROWN, J. K., FROHLICH, D. R. & ROSELL, R. C. 1995. The sweetpoatao or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? *Annual Review of Entomology,* 40**,** 511-534.
BRYANT, L. M. & FULLER, S. J. 2014. Pleistocene climate fluctuations influence phylogeographical patterns in *Melomys cervinipes* across the mesic forests of eastern Australia. *Journal of Biogeography,* 41**,** 1923-1935.
BRYANT, L. M. & KROSCH, M. N. 2016. Lines in the land: a review of evidence for eastern Australia's major biogeographical barriers to closed forest taxa. *Biological Journal of the Linnean Society,* 119**,** 238-264.
BURBIDGE, N. 1960. The phytogeography of Australia. *Australian Journal of Botany,* 8**,** 75-209.
BURRETT, C., DUHIG, N., BERRY, R. & VARNE, R. 1991. Asian and South-western Pacific continental terranes derived from Gondwana, and their biogeographic significance. *Australian Systematic Botany,* 4**,** 13-24.
CAMERON, E. C., SVED, J. A. & GILCHRIST, A. S. 2010. Pest fruit fly (Diptera: Tephritidae) in northwestern Australia: one species or two? *Bulletin of Entomological Research,* 100**,** 197-206.
CAMPILLO, S., GARCIA-ROGER, E. M., MARTINEZ-TORRES, D. & SERRA, M. 2005. Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. *Hydrobiologia,* 546**,** 181-187.
CARNEY, J. N. & MACFARLANE, A. 1982. Geological evidence bearing on the Miocene to Recent structural evolution of the New Hebrides arc. *Tectonophysics,* 87**,** 147-175.
CATULLO, R. A., YEAP, H. L., LEE, S. F., BRAGG, J. G., CHEESMAN, J., DE FAVERI, S. G., EDWARDS, O., HEE, A. K. W., POPA, A. D., SCHIFFER, M. & OAKESHOTT, J. G. 2019. A genome-wide approach for uncovering evolutionary relationships of Australian *Bactrocera* species complexes (Diptera: Tephritidae). *Invertebrate Systematics,* 33**,** 618-627.
CHATTERJEE, S., GOSWAMI, A. & SCOTESE, C. 2013. The longest voyage: tectonic, magmatic, and paleoclimatic evolution of the Indian plate during its northward flight from Gondwana to Asia. *Gondwana Research,* 23**,** 238-267.
CHATTERJEE, S. & SCOTESE, C. 1999. The breakup of Gondwana and the evolution and biogeography of the Indian plate. *PINSA,* 65**,** 397-425.
CHATTERJEE, S. & SCOTESE, C. 2010. The wandering Indian plate and its changing biogeography during the late Cretaceous-early Tertiary period. *In:* BANDYOPADHYAY, S. (ed.) *New aspects of Mesozoic biodiversity. Lecture notes in earth sciences.* Springer: Berlin, Hiedelberg.
CHESSEL, D., DUFOUR, A. & THIOULOUSE, J. 2004. The ade4 package - I: one-table methods. *R News,* 4**,** 5-10.

CHRISTENSON, L. D. & FOOTE, R. H. 1960. Biology of fruit flies. *Annual Review of Entomology,* 5**,** 171-192.
CLARKE, A. R. 2017. Why so many polyphagous fruit flies (Diptera: Tephritidae)? A further contribution to the 'generalism' debate. *Biological Journal of the Linnean Society,* 120**,** 245-257.
CLARKE, A. R. 2019. *Biology and management of* Bactrocera *and related fruit flies,* Boston, MA, CAB International.
CLARKE, A. R., ALLWOOD, A. J., CHINAJARIYAWONG, A., DREW, R. A. I., HENGSAWAD, C., JIRASURAT, M., KONG KRONG, C., KRITSANEEPAIBOON, S. & VIJAYSEGARAN, S. 2001. Seasonal abundance and host use patterns of seven *Bactrocera* Macquart species (Diptera: Tephritidae) in Thailand and Peninsular Malaysia. *The Raffles Bulletin of Zoology,* 49**,** 207-220.
CLARKE, A. R., ARMSTRONG, K. F., CARMICHAEL, A. E., MILNE, J. R., RAGHU, S., RODERICK, G. K. & YEATES, D. K. 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology,* 50**,** 293-319.
CLARKE, A. R., BALAGAWI, S., CLIFFORD, B., DREW, R. A. I., LEBLANC, L., MARARUAI, A., MCGUIRE, D., PUTULAN, D., ROMIG, T., SAR, S. & TENAKANAI, D. 2004. Distribution and biogeography of *Bactrocera* and *Dacus* species (Diptera: Tephritidae) in Papua New Guinea. *Austral Entomology,* 43**,** 148-156.
CLARKE, A. R., LI, Z. H., QIN, Y., ZHAO, Z., LIU, L. & SCHUTZE, M. K. 2019. *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is not invasive through Asia: it's been there all along *Journal of Applied Entomology,* 143**,** 797-801.
CLARKE, A. R., POWELL, K. S., WELDON, C. W. & TAYLOR, P. W. 2011. The ecology of *Bactrocera tryoni* (Diptera: Tephritidae): what do we know to assist pest management. *Annals of Applied Biology,* 158**,** 26-54.
CLARKE, A. R. & SCHUTZE, M. K. 2014. The complexities of knowing what it is you are trapping. *In:* SHELLY, T. E. (ed.) *Trapping and the detection, control, and regulation of tephritid fruit flies.* Dordrecht: Springer.
COLEMAN, P. J. & PACKHAM, G. H. 1976. The Melanesian Borderlands and India - Pacific plates' boundary. *Earth-Science Reviews,* 12**,** 197-233.
CONDAMINE, F. L., LESLIE, A. B. & ANTONELLI, A. 2017. Ancient islands acted as refugia and pumps for conifer diversity. *Cladistics,* 33**,** 69-92.
COOPER, W. & COOPER, W. T. 2013. *Australian rainforest fruits: a field guide,* Collingwood, Victoria, CSIRO Publishing.
COX, C. B. & MOORE, P. D. 2005. *Biogeography: an ecological and evolutionary approach,* Carlton, Victoria, Blackwell Publishing.
CRUZ, V. M., KILIAN, A., MCKAY, J. & DIERIG, D. Molecular genetic characterization of *Lasquerella* new industrial crop using DArTseq markers. Plant and Animal Genome XXI Conference, 2013 San Diego, CA, USA. Plant and Animal Genome.
DAHL, D. B., SCOTT, D., ROOSEN, C., MAGNUSSON, A., SWINTON, J., SHAH, A., HENNINGSEN, A., PUETZ, B., PFAFF, B., AGOSTINELLI, C., LOEHNERT, C., MITCHELL, D., WHITING, D., DA ROSA, F., GAY, G., SCHULZ, G., FELLOWS, I., LAAKE, J., WALKER, J., YAN, J., ANDRONIC, L., LOECHER, M., GUBRI, M., STIGLER, M., CASTELO, R., FALCON, S., EDWARDS, S., GARBADE, S. & LIGGES, U. 2019.

xtable: export tables to LaTEX or HTML. Available from: https://CRAN.R-project.org/package=xtable.
DAILY, G. C., EHRLICH, P. R. & HADDAD, N. M. 1993. Double keystone bird in a keystone species complex. *Proceedings of the National Academy of Sciences of the United States of America,* 90**,** 592-594.
DAY, E. H., HUA, X. & BROMHAM, L. 2016. Is specialization an evolutionary dead-end? Testing for differences in speciation, extinction and trait transition rates across diverse phylogenies of specialists and generalists. *Journal of Evolutionary Biology,* 29**,** 1257-1267.
DE MEYER, M. 2000. Systematic revision of the subgenus *Ceratitis* MacLeay *s.s.* (Diptera, Tephritidae). *Zoological Journal of the Linnean Society,* 128**,** 439-467.
DE MEYER, M., DELATTE, H., EKESI, S., JORDAENS, K., KALINOVA, B., MANRAKHAN, A., MWATAWALA, M., STECK, G., J VAN CANN, VANICKOVA, L., BRIZOVA, R. & VIRGILIO, M. 2015a. An integrative approach to unravel the *Ceratitis* FAR (Diptera, Tephritidae) cryptic species complex: a review. *ZooKeys,* 540**,** 405-427.
DE MEYER, M., DELATTE, H., MWATAWALA, M., QUILICI, S., VAYSSIERES, J. & VIRGILIO, M. 2015b. A review of the current knowledge on *Zeugodacus cucurbitae* (Coquillett) (Diptera, Tephritidae) in Africa, with a list of species included in *Zeugodacus*. *Zookeys,* 540**,** 539-557.
DE QUEIROZ, K. 2007. Species concepts and species delimitation. *Systematic Biology,* 56**,** 879-886.
DEKKER, J. 2003. The foxtail (*Setaria*) species-group. *Weed Science,* 51**,** 641-656.
DHILLON, M. K., SINGH, R., NARESH, J. S. & SHARMA, H. C. 2005. The melon fly, *Bactrocera cucurbitae:* a review of its biology and management. *Journal of Insect Science,* 5**,** 1-16.
DIAMOND, J. 1984. Biogeographic mosaics in the Pacific. *In:* RADOVSKY, F. J., RAVEN, P. H. & SOHMER, S. H. (eds.) *Biogeography of the tropical Pacific.* Honolulu, USA.
DICKENS, J. C., HART, W. G., LIGHT, D. M. & JANG, E. B. 1988. Tephritid olfaction: morphology of the antennae of four tropical species of economic importance (Diptera: Tephritidae). *Annals of the Entomological Society of America,* 81**,** 325-331.
DOBSON, H. E. M. 2006. Relationship between floral fragrance composition and type of pollinator. *In:* DUDAREVA, N. & PICHERSKY, E. (eds.) *Biology of floral scent.* CRC Press.
DODSON, G. 1978. Morphology of the reproductive system in *Anastrepha suspensa* (Loew) and notes on related species. *The Florida Entomologist,* 61**,** 231-239.
DODSON, G. N. 2000. Behaviour of the Phytalmiinae and the evolution of antlers in tephritid fruit flies. *In:* ALUJA, M. & NORRBOM, A. (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior.* Boca Raton, Florida: CRC Press.
DOMINIAK, B. C. & MAPSON, R. 2017. Revised distributions of *Bactrocera tryoni* in eastern Australia and effect on possible incursions of Meditteranean fruit fly: development of Australia's eastern trading block. *Horticultural Entomology,* 110**,** 2459-2465.
DOORENWEERD, C., LEBLANC, L., NORRBOM, A. L., SAN JOSE, M. & RUBINOFF, D. 2018. A global checklist of the 932 fruit fly species in the tribe Dacini (Diptera, Tephritidae). *Zookeys,* 730**,** 19-56.

DOUTCH, H. F. 1972. The Paleogeography of Northern Australia and New Guinea, and its relevance to the Torres Strait area. *In:* WALKER, D. (ed.) *Bridge and Barrier: the natural and cultural history of Torres Strait.* Canberra: Department of Biogeography and Geomorphology, Research School of Pacific Studies, Australian National University.
DOYEN, J. T. & TSCHINKEL, W. R. 1982. Phenetic and cladistic relationships among tenebrionid beetles (*Coleoptera*). *Systematic Entomology,* 7**,** 127-183.
DRAY, S. & DUFOUR, A. 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software,* 22**,** 1-20.
DRAY, S., DUFOUR, A. & CHESSEL, D. 2007. The ade4 package - II: two-table and K-table methods. *R News,* 7**,** 47-52.
DREW, R. A. I. 1969. Morphology of the reproductive system of *Strumenta tryoni* (Froggatt) (Diptera: Tephritidae) with a method of distinguishing sexually mature adult males. *Journal of the Australian Entomological Society,* 8**,** 21-32.
DREW, R. A. I. 1972. The generic and subgeneric classification of Dacini (Diptera: Tephritidae) from the South Pacific area. *Australian Journal of Entomology,* 11**,** 1-22.
DREW, R. A. I. 1974. Revised descriptions of species of Dacini (Diptera: Tephritidae) from the south Pacific area. II. The *Strumenta* group of subgenera of genus *Dacus*. *Queensland Department of Primary Industries, Division of Plant Industy Bulletin,* 653**,** 1-111.
DREW, R. A. I. 1989. *The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions,* Brisbane, Queensland, Queensland Museum.
DREW, R. A. I. 2004. Biogeography and speciation in the Dacini (Diptera: Tephritidae: Dacinae). *Bishop Museum Bulletin in Entomology,* 12**,** 165-178.
DREW, R. A. I. & HANCOCK, D. L. 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research***,** 1-68.
DREW, R. A. I. & HANCOCK, D. L. 1999. Phylogeny of the tribe Dacini (Dacinae) based on morphological, distributional, and biological data. *In:* ALUJA, M. & NORRBOM, A. L. (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior.* Boca Raton, Florida: CRC Press.
DREW, R. A. I. & HANCOCK, D. L. 2016. A review of the subgenus *Bulladacus* Drew & Hancock of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae), with description of two new species from Papua New Guinea. *Australian Entomologist,* 43**,** 189-210.
DREW, R. A. I., HANCOCK, D. L. & ROMIG, M. C. 1981. Australian Dacinae (Diptera: Tephritidae): new species from Cape York Peninsula, A discussion of species complexes and key to species. *Australian Journal of Zoology,* 29**,** 49-91.
DREW, R. A. I., HANCOCK, D. L. & ROMIG, M. C. 1999. New species and records of fruit flies (Diptera: Tephritidae: Dacinae) from North Queensland. *Australian Entomologist,* 26**,** 1-12.
DREW, R. A. I. & HOOPER, G. H. S. 1981. The responses of fruit fly species (Diptera: Tephritidae) in Australia to various attractants. *Journal of the Australian Entomological Society,* 20**,** 201-205.
DREW, R. A. I., HOOPER, G. H. S. & BATEMAN, M. A. 1978. Economic fruit flies of the South Pacific region. Brisbane, Australia: Watson Ferguson & Co.

DREW, R. A. I., HOOPER, G. H. S. & BATEMAN, M. A. 1982. *Economic fruit flies of the south Pacific region,* Brisbane, Australia, Watson Ferguson & Co.
DREW, R. A. I. & LAMBERT, D. M. 1986. On the specific status of *Dacus (Bactrocera) aquilonis* and *D. (Bactrocera) tryoni* (Diptera: Tephritidae). *Annals of the Entomological Society of America,* 79**,** 870-878.
DREW, R. A. I., MA, J., SMITH, S. & HUGHES, J. M. 2011. The taxonomy and phylogenetic relationships of species in the *Bactrocera musae* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Papua New Guinea. *The Raffles Bulletin of Zoology,* 59**,** 145-162.
DREW, R. A. I., RAGHU, S. & HALCOOP, P. 2008. Bridging the morphological and biological species concepts: studies on the *Bactrocera dosalis* (Hendel) complex (Diptera: Tephritidae: Dacinae) in South-east Asia. *Biological Journal of the Linnean Society,* 93**,** 217-226.
DREW, R. A. I. & ROMIG, M. C. 2001. The fruit fly fauna (Diptera: Tephritidae:Dacinae) of Bougainville, the Solomon Islands and Vanuatu. *Australian Journal of Entomology,* 40**,** 113-150.
DREW, R. A. I. & ROMIG, M. C. 2013. *Tropical fruit flies of South-east Asia,* Wallingford, CAB International.
DREW, R. A. I. & ROMIG, M. C. 2016. *Keys to the tropical fruit flies (Tephritidae: Dacinae) of South-East Asia: Indomalaya to North-West Australasia,* Wallingford, CABI.
DREW, R. A. I., TSURUTA, K. & WHITE, I. M. 2005. A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomology,* 13**,** 149-154.
DUAN, J. J. & MESSING, R. H. 1997. Biological control of fruit flies in Hawaii: factors affecting non-target risk analysis. *Agriculture and Human Values,* 14**,** 227-236.
DUCHENE, D. A., TONG, K. J., FOSTER, C. S. P., DUCHENE, S., LANFEAR, R. & HO, S. Y. W. 2020. Linking branch lengths across sets of loci provides the highest statistical support for phylogenetic inference. *Molecular Biology and Evolution,* msz291.
DUNNUM, J. L. & SALAZAR-BRAVO, J. 2010. Molecular systematics, taxonomy and biogeography of the genus *Cavia* (Rodentia: Caviidae). *Journal of Zoological Systematics and Evolutionary Research,* 48**,** 376-388.
DUPUIS, J. R., BREMER, F. T., KAUWE, A., SAN JOSE, M., LEBLANC, L. & RUBINOFF, D. 2018. HiMAP: Robust phylogenomics from highly multiplexed amplicon sequencing. *bioRxiv*.
EBACH, M. C., MURPHY, D. J., GONZALEZ-OROZCO, C. E. & MILLER, J. T. 2015. A revised area taxonomy of phytogeographical regions within the Australian Bioregionalisation Atlas. *Phytotaxa,* 208**,** 261-277.
EBERHARD, W. G. 2010. Rapid divergent evolution of genitalia: theory and data updated. *In:* LEONARD, J. & CORDOBA-AGUILAR, A. (eds.) *The evolution of primary sexual characters in animals.* Oxford: Oxford University Press.
EDDELBUETTEL, D. & BALAMUTA, J. J. 2018. Extending R with C++: a brief introduction to Rcpp. *The American Statistician,* 72**,** 28-36.
EDDELBUETTEL, D. & SANDERSON, C. 2014. RcppArmadillo: accelerating R with high-performance C++ linear algebra. *Computational Statistics & Data Analysis,* 71**,** 1054-1063.

EDGAR, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research,* 32**,** 1792-1797.
ELFEKIH, S., CHEN, C., HSU, J., BELCAID, M. & HAYMER, D. 2016. Identification and preliminary characterization of chemosensory perception-associated proteins in the melon fly *Bactrocera cucurbitae* using RNA-seq. *Scientific Reports,* 6**,** 1-10.
EWERS, R. M., DIDHAM, R. K., PEARSE, W. D., LEFEBVRE, V., ROSA, I. M. D., CARREIRAS, J. M. B., LUCAS, R. M. & REUMAN, D. C. 2013. Using landscape history to predict biodiversity patterns in fragmented landscapes. *Ecology Letters,* 16**,** 1221-1233.
FAY, H. A. C. 2010. Exploring structure-activity relationships in the phenylpropanoids *Proceedings of the 8th International Symposium on Fruit Flies of Economic Importance, 26 September - 1 October 2010.* Valencia, Spain.
FAY, H. A. C. 2012. A highly effective and selective male lure for *Bactrocera jarvisi* (Diptera: Tephritidae). *Australian Journal of Entomology,* 51**,** 189-197.
FEGAN, M. & PRIOR, P. 2005. How complex is the *Ralstonia solanacearum* species complex? *In:* ALLEN, C., PRIOR, P. & HAYWARD, A. C. (eds.) *Bacterial wilt disease and the Ralstonia solanacearum species complex.* St. Paul, MN: APS Press.
FLEMING, C. C., RAO, J., MORELAND, B., CRAIG, D. & TURNER, S. J. 2000. Diagnostics of cyst nematodes and tephritid fruit flies using mitochondrial and ribosomal DNA. *Bulletin OEPP,* 30**,** 585-590.
FLETCHER, B. S. 1998. Dacine fruit flies collected during the dry season in the lowland rainforest of Madang Province, Papua New Guinea (Diptera: Tephritidae). *Australian Journal of Entomology,* 37**,** 315-318.
FOLMER, O., BLACK, M., HOEH, W. & LUTZ, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3**,** 294-299.
FOOTE, R. H., BLANC, F. L. & NORRBOM, A. L. 1994. *Handbook of the fruit flies (Diptera: Tephritidae) of America north of Mexico,* Ithaca, Comstock Publishing Associates.
FRANKHAM, G. J., HANDASYDE, K. A. & ELDRIDGE, M. D. B. 2015. Evolutionary and comtemporary responses to habitat fragmentation detected in mesic zone marsupial, the long-nosed potoroo (*Potorous tridactylus*) in south-eastern Australia. *Journal of Biogeography,* 43**,** 653-665.
FRIAS, D. 2008. Morphology of immature stages in the neotropical nonfrugivorous Tephritinae fruit fly species *Rachptera limbata* Bigot (Diptera: Tephritidae) on *Baccharis linearis* (R. et Pav.) (Asteraceae). *Neotropical Entomology,* 37**,** 536-545.
FRITZ, S. A. & PURVIS, A. 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology,* 24**,** 1042-1051.
FROGGATT, W. W. 1897. The fruit-maggot fly, (*Tephritis tryoni* n. sp.). *Agricultural Gazette of New South Wales,* 8**,** 410-412.
GADAGKAR, S., ROSENBERG, M. S. & KUMAR, S. 2005. Inferring species phylogenies from multiple genes: concatenated sequence tree versus

consensus gene tree. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution,* 304**,** 64-74.
GAINA, C., MULLER, R. D., BROWN, B., ISHIHARA, T. & IVANOV, S. 2007. Breakup and early seafloor spreading between India and Antarctica. *Geophysical Journal International,* 170**,** 151-169.
GALLOWAY, D. J. 2007. *Flora of New Zealand: Lichens,* Lincoln, New Zealand, Manaaki Whenua Press.
GATESY, J., DESALLE, R. & WAHLBERG, N. 2007. How many genes should a systematist sample? Conflicting insights from a phylogenomic matrix characterized by replicated incongruence. *Systematic Biology,* 56**,** 355-363.
GENTILINI, G., KORNEYEV, V. A. & KAMENEVA, E. P. 2006. Fossil tephritoid flies (Diptera: Pallopteridae, Ulidiidae, Tephritidae) from the Upper Miocene of Monte Castellaro, Italy, and a review of fossil European tephritoids. *Instrumenta Biodiversitatis,* 7**,** 1-20.
GERBER, S. 2019. Use and misuse of discrete character data for morphospace and disparity analyses. *Palaeontology,* 62**,** 305-319.
GLOR, R. E. & WARREN, D. 2010. Testing ecological explanations for biogeographic boundaries. *Evolution,* 65**,** 673-683.
GOVAERTS, R., FRODIN, D. G. & PENNINGTON, T. D. 2001. *World checklist and bibliography of Sapotaceae,* Kew, United Kingdom, Royal Botanic Gardens.
GRAND, A., CORVEZ, A., VELEZ, L. M. D. & LAURIN, M. 2013. Phylogenetic inference using discrete characters: performance of ordered and unordered parsimony and three-item statements. *Biological Journal of the Linnean Society,* 110**,** 914-930.
GRISMER, J. L., SHULTE, J. A., ALEXANDER, A., WAGNER, P., TRAVERS, S. L., BUEHLER, M. D., WELTON, L. J. & BROWN, R. M. 2016. The Eurasian invasion: phylogenomic data reveal multiple Southeast Asian origins for Indian Dragon Lizards. *BMC Evolutionary Biology,* 16**,** 43.
GRUBER, B., GEORGES, A., UNMACK, P. J., CLARK, L. V. & BERRY, O. 2019. dartR: importing and analysing SNP and Silicodart data generated by genome-wide restriction fragment analysis. Available from: https://CRAN.R-project.org/package=dartR.
GUINDON, S., DUFAYARD, J., LEFORT, V., ANISIMOVA, M., HORDIJK, W. & GASCUEL, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology,* 59**,** 307-321.
HACKATHON, R., BOLKER, B., BUTLER, M., COWAN, P., DE VIENNE, D., EDDELBUETTEL, D., HOLDER, M., JOMBART, T., KEMBEL, S., MICHONNEAU, F., ORME, D., O'MEARA, B., PARADIS, E., REGETZ, J. & ZWICKL, D. 2020. phylobase: base package for phylogenetic structures and comparative data. Available from: https://CRAN.R-project.org/package=phylobase.
HAGEDORN, G., RAMBOLD, G. & MARTELLOS, S. 2012. Types of identification keys. *In:* NIMIS, P. L. & VIGNES LEBBE, R. (eds.) *Tools for identifying biodiversity: progress and problems, September 20-22, 2010.* Paris: Edizioni Università di Trieste.
HAJIBABAEI, M., SINGER, G. A. C., HEBERT, P. D. N. & HICKEY, D. A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics,* 23**,** 167-172.

HALL, R. 2001. Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. *In:* METCALFE, I. (ed.) *Faunal and floral migrations and evolution in SE Asia and Australia.* Rotterdam, Netherlands: Balkema.
HALL, R. 2009. Southeast Asia's changing palaeogrography. *Blumea,* 54**,** 148-161.
HALL, T. 2011. BioEdit: an important software for molecular biology. *GERF Bulletin of Biosciences,* 2**,** 60-61.
HAN, H.-Y. & RO, K.-E. 2016. Molecular phylogeny of the superfamily Tephritoidea (Insecta: Diptera) reanalysed based on expanded taxon sampling and sequence data. *Journal of Zoological Systematics and Evolutionary Research,* 54**,** 276-288.
HANCOCK, D. L. 1986. Classification of the Trypetinae (Diptera: Tephritidae), with a discussion of the Afrotropical fauna. *Journal of the Entomological Society of Southern Africa,* 49**,** 275-305.
HANCOCK, D. L. 2013. A revised checklist of Australian fruit flies (Diptera: Tephritidae). *Australian Entomologist,* 40**,** 219-236.
HANCOCK, D. L. 2015. A new subgenus for six Indo-Australian species of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae) and subgeneric transfer of four other species. *Australian Entomologist,* 42**,** 39-44.
HANCOCK, D. L. & DREW, R. A. I. 2006. A revised classification of subgenera and species groups in Dacus Fabricius (Diptera: Tephritidae). *Instrumenta Biodiversitatis,* VII**,** 167-205.
HANCOCK, D. L. & DREW, R. A. I. 2015. A review of the Indo-Australian subgenus *Parazeugodacus* Shiraki of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae). *Australian Entomologist,* 42**,** 91-104.
HANCOCK, D. L. & DREW, R. A. I. 2016. A review of the subgenus *Austrodacus* Perkins of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae). *Australian Entomologist,* 43**,** 75-82.
HANCOCK, D. L. & DREW, R. A. I. 2017a. A review of the Indo-Australian subgenera *Heminotodacus* Drew, *Paradacus* Perkins and *Perkinsidacus* subgen. n. of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae). *Australian Entomologist,* 44**,** 137-146.
HANCOCK, D. L. & DREW, R. A. I. 2017b. A review of the Pacific islands subgenus *Notodacus* Perkins of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae). *Australian Entomologist,* 44**,** 113-120.
HANCOCK, D. L. & DREW, R. A. I. 2017c. A review of the subgenus *Javadacus* Hardy of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae). *Australian Entomologist,* 44**,** 105-112.
HANCOCK, D. L. & DREW, R. A. I. 2018a. A review of the subgenera '*Apodacus*' Perkins, '*Hemizeugodacus*' Hardy, '*Neozeugodacus*' May, stat. rev., '*Semicallantra*' Drew and '*Tetradacus*' Miyake of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae) *Australian Entomologist,* 45**,** 105-132.
HANCOCK, D. L. & DREW, R. A. I. 2018b. A review of the subgenera *Asiadacus* Perkins, *Diplodacus* May, *Hemigymnodacus* Hardy, *Niuginidacus* Drew, *Papuodacus* Drew and *Sinodacus* Zia of *Bactrocera* Macquart (Diptera: Tephritidae: Dacinae). *Australian Entomologist,* 45**,** 181-208.
HANCOCK, D. L. & DREW, R. A. I. 2018c. A review of the subgenus '*Zeugodacus*' Hendel of *'Bactrocera'* Macquart (Diptera: Tephritidae: Dacinae): an integrative approach. *Australian Entomologist,* 45**,** 251-272.

HANCOCK, D. L., HAMACEK, E. L., LLOYD, A. C. & ELSON-HARRIS, M. M. 2000. *The Distribution and host plants of fruit flies (Diptera: Tephritidae) in Australia,* Brisbane, Queensland, Department of Primary Industries.
HARDY, D. E. 1951. The Krauss collection of Australian fruit flies (Tephritdae-Diptera). *Pacific Science,* 5**,** 115-189.
HARDY, D. E. 1969. Taxonomy and distribution of the oriental fruit fly and related species (Tephritidae-Diptera). *Proceedings of the Hawaiian Entomological Society,* 10**,** 395-428.
HARDY, D. E. 1973. The fruit flies (Tephritidae-Diptera) of Thailand and bordering countries. *Pacific Insects Monograph,* 31**,** 1-353.
HARDY, N. B. & OTTO, S. P. 2014. Specialization and generalization in the diversification of phytophagous insects: tests of the musical chairs and oscillation hypotheses. *Proceedings of the Royal Society B: Biological Sciences,* 281**,** 20132960.
HARVEY, M. S., BERRY, O., EDWARD, K. L. & HUMPHREYS, G. 2008. Molecular and morphological systematics of hypogean schizomids (Schizomida: Hubbardiidae) in semiarid Australia. *Invertebrate Systematics,* 22**,** 167-194.
HAUSER, D. L. & PRESCH, W. 1991. The effect of ordered characters on phylogenetic reconstruction. *Cladistics,* 7**,** 243-265.
HEADS, M. 2001. Regional patterns of biodiversity in New Guinea plants. *Botanical Journal of the Linnean Society,* 136**,** 67-73.
HEADS, M. 2002a. Birds of paradise, vicariance biogeography and terrane tectonics in New Guinea. *Journal of Biogeography,* 29**,** 261-283.
HEADS, M. 2002b. Regional patterns of biodiversity in New Guinea animals. *Journal of Biogeography,* 29**,** 285-294.
HEADS, M. 2010. Biogeographical affinities of the New Caledonian biota: a puzzle with 24 pieces. *Journal of Biogeography,* 37**,** 1179-1201.
HEATH, T. A., HEDTKE, S. M. & HILLIS, D. M. 2008. Taxon sampling and the accuracy of phylogenetic analyses. *Journal of Systematics and Evolution,* 46**,** 239-257.
HEIBL, C. 2008. PHYLOCH: R language tree plotting tools and interfaces to diverse phylogentic software packages. Available from: http://www.christophheibl.de/Rpackages.html.
HOLDER, M. & LEWIS, P. O. 2003. Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics,* 4**,** 275-284.
HOLLINGSWORTH, R. G., DREW, R. A. I., ALLWOOD, A. J., ROMIG, M. C., VAGALO, M. & TSATSIA, F. 2003. Host plants and relative abundance of fruit fly (Diptera: Tephritidae) species in the Solomon Islands. *Australian Journal of Entomology,* 42**,** 95-108.
HU, F., ZHANG, G., JIA, F., DOU, W. & WANG, J. 2010. Morphological characterization and distribution of antennal sensilla of six fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America,* 103**,** 661-670.
HUELSENBECK, J. P., BULL, J. J. & CUNNINGHAM, C. W. 1996. Combining data in phylogenetic analysis. *Trends in Ecology & Evolution,* 11**,** 152-158.
HUELSENBECK, J. P. & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics,* 17**,** 754-755.
HUXHAM, K. A., FAY, H. A. C. & HANCOCK, D. L. 2006. Two new species and a new Australian record of *Bactrocera* Macquart (Diptera: Tephritidae:

Dacinae) from northern Queensland, Torres Strait and Papua New Guinea. *Australian Journal of Entomology,* 45**,** 34-37.
HUXHAM, K. A. & HANCOCK, D. L. 2002. New records of Dacinae (Diptera: Tephritidae) from northern Queensland and Torres Strait, Australia. *Australian Entomologist,* 29**,** 123-125.
ITO, S. 1983. *Die japanischen Bohrfliegen,* Osaka, Maruzen Co., Ltd.
IWAHASHI, O. 1999a. Distinguishing between the two sympatric species *Bactrocera carambolae* and *B. papayae* (Diptera: Tephritidae) based on aedeagal length. *Annals of the Entomological Society of America,* 92**,** 639-643.
IWAHASHI, O. 1999b. Distinguishing between two sympatric species, *Bactrocera occipitalis* and *B. philippinensis* (Diptera: Tephritidae), based on aedeagal length. *Annals of the Entomological Society of America,* 92**,** 182-187.
IWAHASHI, O. & ROUTHIER, W. 2001. Aedeagal length and its variation of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), which recently invaded Egypt. *Applied Entomology and Zoology,* 36**,** 13-17.
IWAIZUMI, R., KANEDA, M. & IWAHASHI, O. 1997. Correlation of length of terminalia of males and females among nine species of *Bactrocera* (Diptera: Tephritidae) and differences among sympatric species of *B. dorsalis* complex. *Annals of the Entomological Society of America,* 90**,** 664-666.
JEONG, S., REBEIZ, M., ANDOLFATTO, P., WERNER, T., TRUE, J. & CARROLL, S. B. 2008. The evolution of gene regulation underlies a morphological difference between two *Drosophila* sister species. *Cell,* 132**,** 783-793.
JIANG, F., JIN, Q., LIANG, L., ZHANG, A. B. & LI, Z. H. 2014. Existence of species complex largely reduced barcoding success for invasive species of Tephritidae: a case study in *Bactrocera* spp. . *Molecular Ecology Resources,* 14**,** 1114-1128.
JOMBART, T., DRAY, S. & BILGRAU, A. E. 2017. adephylo: exploratory analysis for the phylogenetic comparative method. Available from: https://CRAN.R-project.org/package=adephylo.
JONES, R. C., STEANE, D. A., LAVERY, M., VAILLANCOURT, R. E. & POTTS, B. M. 2013. Multiple evolutionary processes drive the patterns of genetic differentiation in a forest tree species complex. *Ecology and Evolution,* 3**,** 1-17.
KALYAANAMOORTHY, S., MINH, B. Q., WONG, T. K. F., A, V. H. & JERMIIN, L. S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods,* 14**,** 587.
KAMINSKI, M. J., GEARNER, O. M., KANDA, K., SWICHTENBERG, K., PURCHART, L. & SMITH, A. D. 2020. First insights into the phylogeny of tok-tokkie beetles (Tenebrionidae: Molurina, Phanerotomeina) and examination of the status of the *Psammodes vialis* species-group. *Zoological Journal of the Linnean Society***,** 1-19.
KARIG, D. E. 1974. Evolution of arc systems in the western Pacific. *Annual Review of Earth and Planetary Sciences,* 2**,** 51-75.
KARORI SANCTUARY TRUST. 2016. *The Continent of Zealandia* [Online]. Available: https://www.visitzealandia.com/About/History/The-Continent-of-Zealandia [Accessed 6th February, 2018].
KEMBEL, S. W., COWAN, P. D., HELMUS, M. R., CORNWELL, W. K., MORLON, H., ACKERLY, D. D., BLOMBERG, S. P. & WEBB, C. O.

2010. picante: R tools for integrating phylogenies and ecology. *Bioinformatics,* 26**,** 1463-1464.
KERSHAW, A. P. 1994. Pleistocene vegetation of the humid tropics of northeastern Queensland, Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 109**,** 399-412.
KITCHING, R. L., EASTWOOD, R. & HURLEY, K. 2001. Butterflies and Wallace's line: faunistic patterns and explanatory hypotheses within the south-east Asian butterflies. *In:* METCALFE, I., SMITH, J. M. B., MORWOOD, M. & DAVIDSON, I. (eds.) *Faunal and floral migration and evolution in SE Asia-Australasia.* Lisse, Netherlands: Swets Zeitlinger.
KITTO, G. B. 1983. An immunological approach to the phylogeny of the Tephritidae. *In:* CAVALLORO, R. (ed.) *Fruit flies of economic importance.* Rotterdam, Netherlands: A. A. Balkema.
KLAUS, S., SCHUBART, C. D., STREIT, B. & PFENNINGER, M. 2010. When Indian crabs were not yet Asian - biogeographic evidence for Eocene proximity of India and Southeast Asia. *BMC Evolutionary Biology,* 10**,** 287.
KLUGE, A. G. 1991. Boine snake phylogeny and research cycles. *Miscellaneous Publications Museum of Zoology, University of Michigan,* 178**,** 1-58.
KOENKER, R. & NG, P. 2019. SparseM: sparse linear algebra. Available from: https://CRAN.R-project.org/package=SparseM.
KOVAC, D. 2015. Reproductive behaviour and basic biology of the oriental bamboo-inhabiting *Anoplomus rufipes* and a comparison with frugivorous Dacinae fruit flies. *Insects,* 6**,** 869-896.
KOVAC, D., FREIDBERG, A. & STECK, G. J. 2013. Biology and description of the third instar larva and puparium of *Ichneumonopsis burmensis* Hardy (Diptera: Tephritidae: Dacinae: Gastrozonini), a bamboo-breeding fruit fly from the oriental region. *The Raffles Bulletin of Zoology,* 61**,** 117-132.
KROSCH, M. N. 2011. Phylogeography of *Echinocladius martini* Cranston (Diptera: Chironimidae) in closed forest streams of eastern Australia. *Australian Journal of Entomology,* 50**,** 258-268.
KROSCH, M. N., HEROLD, N., THORNHILL, A. H. & CRANSTON, P. S. 2020a. How 'Gondwanan' is *Riethia?* Molecular phylogenetics elucidates the mode and tempo of diversification in Austro-Pacific Chironominae (Diptera). *Invertebrate Systematics,* 34**,** 328-341.
KROSCH, M. N., SCHUTZE, M. K., ARMSTRONG, K. F., BOONTOP, Y., BOYKIN, L. M., CHAPMAN, T. A., ENGLEZOU, A., CAMERON, S. L. & CLARKE, A. R. 2013. Piecing together an integrative taxonomic puzzle: microsatellite, wing shape and aedeagus length analyses of *Bactrocera dorsalis s.l.* (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. *Systematic Entomology,* 38**,** 2-13.
KROSCH, M. N., SCHUTZE, M. K., ARMSTRONG, K. F., GRAHAM, G. C., YEATES, D. K. & CLARKE, A. R. 2012. A molecular phylogeny for the tribe Dacini (Diptera: Tephritidae): systematic and biogeographic implications. *Molecular Phylogenetics and Evolution,* 64**,** 513-523.
KROSCH, M. N., SCHUTZE, M. K., NEWMAN, J., STRUTT, F., BRYANT, L. M., MCMAHON, J. & CLARKE, A. R. 2019a. In the footsteps of Wallace: population structure in the breadfruit fruit fly, *Bactrocera umbrosa* (F.) (Diptera: Tephritidae), suggests disjunction across the Indo-Australian archipelago. *Austral Entomology,* 58**,** 602-613.

KROSCH, M. N., SCHUTZE, M. K., STRUTT, F., CLARKE, A. R. & CAMERON, S. L. 2019b. A transcriptome-based analytical workflow for identifying loci for species diagnosis: a case study with *Bactrocera* fruit flies (Diptera: Tephritidae). *Austral Entomology,* 58**,** 395-408.

KROSCH, M. N., STRUTT, F., BLACKET, M. J., BATOVSKA, J., STARKIE, M., CLARKE, A. R., CAMERON, S. L. & SCHUTZE, M. K. 2020b. Development of internal COI primers to improve and extend barcoding of fruit flies (Diptera: Tephritidae: Dacini). *Insect Science,* 27**,** 143-158.

KRUCKEBERG, A. R. 1969. The implications of ecology for plant systematics. *Taxon,* 18**,** 92-120.

KUBOTA, U., LOYOLA, R. D., ALMEIDA, A. M., CARVALHO, D. A. & LEWINSOHN, T. M. 2007. Body size and host range co-determine the altitudinal distribution of Neotropical tephritid flies. *Global Ecology and Biogeography,* 16**,** 632-639.

KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution,* 33**,** 1870-1874.

KUMARAN, N., BALAGAWI, S., SCHUTZE, M. K. & CLARKE, A. R. 2013. Evolution of lure response in tephritid fruit flies: phytochemicals as drivers of sexual selection. *Animal Behaviour,* 85**,** 781-789.

KUMARAN, N. & CLARKE, A. R. 2014. Indirect effects of phytochemicals on offspring performance of Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *Journal of Applied Entomology,* 138**,** 361-367.

LABEDA, D. P., DUNLAP, C. A., RONG, X., HUANG, Y., DOROGHAZI, J. R., JU, K.-S. & METCALF, W. W. 2017. Phylogenetic relationships in the family *Streptomycetaceae* using multi-locus sequence analysis. *Journal of Microbiology,* 110**,** 563-583.

LAWSON, A. E., MCGUIRE, D. J., YEATES, D. K., DREW, R. A. I. & CLARKE, A. R. 2003. Dorsalis: an interactive indentification tool to fruit flies of the *Bactrocera dorsalis* complex. Brisbane, Australia: Griffith University.

LEBLANC, L., BALAGAWI, S., MARARUAI, A., PUTULAN, D. & CLARKE, A. R. 2001. Fruit flies in Papua New Guinea. *Pest Advisory Leaflet no. 37.* Suva, Fiji: Secretariat of the Pacific Community-Plant Protection Service.

LEBLANC, L., HOSSAIN, M. A., KHAN, S. A., SAN JOSE, M. & RUBINOFF, D. 2013. A preliminary survey of the fruit flies (Diptera: Tephritidae: Dacinae) of Bangladesh. *Proceedings of the Hawaiian Entomological Society,* 45**,** 51-58.

LEBLANC, L., HOSSAIN, M. A., KHAN, S. A., SAN JOSE, M. & RUBINOFF, D. 2014. Additions to the fruit fly fauna (Diptera: Tephritidae: Dacinae) of Bangladesh, with a key to the species. *Proceedings of the Hawaiian Entomological Society,* 46**,** 31-40.

LEBLANC, L., SAN JOSE, M., BARR, N. & RUBINOFF, D. 2015. A phylogenetic assessment of the polyphyletic nature and intraspecific colour polymorphism in the *Bactrocera dorsalis* complex (Diptera: Tephritidae). *Zookeys,* 540**,** 339-367.

LEBLANC, L., VUET, E. T., DREW, R. A. I. & ALLWOOD, A. J. 2012. Host plant records for fruit flies (Diptera: Tephritidae: Dacini) in the Pacific Islands. *Proceedings of the Hawaiian Entomological Society,* 44**,** 11-53.

LECOINTRE, G., PHILIPPE, H., LE, H. L. V. & LE GUYADER, H. 1993. Species sampling has a major impact on phylogenetic inference. *Molecular Phylogenetics and Evolution,* 2**,** 205-224.
LEMON, J. 2006. plotrix: a package in the red light district of R. *R-News,* 6**,** 8-12.
LEWIS, P. O. 2001. A likelihood appraoch to estimating phylogeny from discrete morphological character data. *Systematic Biology,* 50**,** 913-925.
LI, J., LI, Y., KLAUS, S., RAO, D., HILLIS, D. M. & ZHANG, Y. 2013. Diversification of rhacophorid frogs provides evidence for accelerate faunal exchange between India and Eurasia during the Oligocene. *PNAS,* 110**,** 3441-3446.
LI, L., MA, H., NIU, L., HAN, D., ZHANG, F., CHEN, J. & FU, Y. 2017. Evaluation of chromatic cues for trapping *Bactrocera tau*. *Pest Management Science,* 73**,** 217-222.
LI, Z. X. & POWELL, C. M. 2001. An outline of the palaeogeographic evolution of the Australasian region since the beginning of the Neoproterozoic. *Earth-Science Reviews,* 53**,** 237-277.
LIANG, G.-Q., HANCOCK, D. L., XU, W. & LIANG, F. 1993. Notes on the Dacinae of southern China (Diptera: Tephritidae. *Journal of the Australian Entomological Society,* 32**,** 137-140.
LIDICKER, W. Z. 1973. A phenetic analysis of some New Guinea rodents. *Systematic Zoology,* 22**,** 36-45.
LINDA, L., WAITJAKSONO, W. & SUPUTA, S. 2018. Species composition of fruit flies (Diptera: Tephritidae) in Sorong and Raja Ampat, West Papua. *Jurnal Perlindungan Tanaman Indonesia,* 22**,** 193-200.
LINDAHL, T. 1993. Instability and decay of the primary structure of DNA. *Nature,* 362**,** 709.
LIU, B., LI, B., ZHAN, G., ZHA, T., WANG, Y. & MA, C. 2017. Forced hot-air treatment against *Bactrocera papayae* (Diptera: Tephritidae) in papaya. *Applied Entomology and Zoology,* 52**,** 531-541.
LOXDALE, H. D. & BALOG, A. 2018. Aphid specialism as an example of ecological-evolutionary divergence. *Biological Reviews,* 93**,** 642-657.
LOXDALE, H. D., BALOG, A. & HARVEY, J. A. 2019. Generalism in nature... the great misnomer: aphids and wasp parasitoids as examples. *Insects,* 10**,** 314.
LOXDALE, H. D. & HARVEY, J. A. 2016. The 'generalism' debate: misinterpreting the term in the empirical literature focussing on dietary breadth in insects. *Biological Journal of the Linnean Society,* 119**,** 265-282.
LOXDALE, H. D., LUSHAI, G. & HARVEY, J. A. 2011. The evolutionary improbability of 'generalism' in nature, with special reference to insects. *Biological Journal of the Linnean Society,* 103**,** 1-18.
LUCKY, A. 2011. Molecular phylogeny and biogeography of the spider ants, genus *Leptomyrmex* Mayr (Hymenoptera: Formicidae. *Molecular Phylogenetics and Evolution,* 59**,** 281-292.
LUTERBACHER, J., DIETRICH, D., XOPLAKI, E., GROSJEAN, M. & WANNER, J. 2004. European seasonal and annual temperature variability, trends, and extremes since 1500. *Science,* 303**,** 1499-1503.
MACEY, J. R., STRASBURG, J. L., BRISSON, J. A., VREDENBURG, V. T., JENNINGS, M. & LARSON, A. 2000. Molecular phylogenetics of western North American frogs of the *Rana boylii* species group. *Molecular Phylogenetics and Evolution,* 19**,** 131-143.

MACFARLANE, J. R., EAST, R. W., DREW, R. A. I. & BETLINSKI, G. A. 1987. Dispersal of irradiated Queensland fruit flies *Dacus tryoni* (Froggatt) (Diptera: Tephritidae), in south-eastern Australia. *Australian Journal of Zoology,* 35**,** 275-281.
MACQUEEN, P., SEDDON, J. M. & GOLDIZEN, A. W. 2012. Effects of historical forest contraction on the phylogeographic structure of Australo-Papuan populations of the red-legged pademelon (Macropodidae: *Thylogale stigmatica*). *Austral Ecology,* 37**,** 479-490.
MAGOWSKI, W. L. & MOSER, J. C. 2003. Redescription of *Tarsonemus minimax* and definition of its species-group in the genus *Tarsonemus* (Acari: Tarsonemidae) with descriptions of two new species. *Annals of the Entomological Society of America,* 96**,** 345-368.
MALEKIAN, M., COOPER, S. J. B., NORMAN, J. A., CHRISTIDIS, L. & CARTHEW, S. M. 2010. Molecular systematics and evolutionary origins of the genus *Petaurus* (Marsupialia: Petauridae) in Australia and New Guinea. *Molecular Phylogenetics and Evolution,* 54**,** 122-135.
MANGUILLA, E., ESCUDERO, M., WATERWAY, M. J., HIPP, A. L. & LUCENO, M. 2015. Phylogeny, systematics, and trait evolution of *Carex* section *Glareosae*. *American Journal of Botany,* 102**,** 1128-1144.
MATEOS, E. 2008. Definition of *Lepidocyrtus lusitanicus* Gama, 1964 species-complex (Collembola, Entomobryidae), with description of a new species and color forms from the Iberian Peninsula. *Zootaxa,* 1917**,** 38-54.
MATZKE, N. J. 2013a. BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R scripts. Available from: http://CRAN.R-project.org/package=BioGeoBEARS.
MATZKE, N. J. 2013b. cladoRcpp: C++ implementations of phylogenetic calculations. Available from: http://CRAN.R-project.org/package=cladoRcpp.
MATZKE, N. J. & SIDJE, R. B. 2013. rexpokit: R wrappers for EXPOKIT. Available from: http://CRAN.R-project.org/package=rexpokit.
MAY, A. W. S. 1953. Queensland host records for the Dacinae (*fam.* Trypetidae). *Queensland Journal of Agricultural Science,* 10**,** 36-79.
MAY, A. W. S. 1957. Queensland host records for the Dacinae (fam. Trypetidae). First supplementary lists. *Queensland Journal of Agricultural Science,* 14**,** 29-39.
MAY, A. W. S. 1960. Queensland host records for the Dacinae (fam. Trypetidae). Second supplementary lists. *Queensland Journal of Agricultural Science,* 17**,** 195-200.
MAY, A. W. S. 1962. Additions to the species of Dacinae (Trypetidae: Diptera) from Queensland and New Guinea. *Queensland Journal of Agricultural and Animal Sciences,* 19**,** 63-76.
MAY, A. W. S. 1963. An investigation of fruit flies (Trypetidae: Diptera) in Queensland. 1. Introduction, species, pest status and distribution. *Queensland Journal of Agricultural Science,* 20**,** 1-82.
MAY, A. W. S. 1965. New species and records of dacinae (Diptera: Trypetidae) from northern Australia. *Australian Journal of Entomology,* 4**,** 58-66.
MAYR, E. 1940. Speciation phenomena in birds. *American Naturalist,* 74**,** 249-278.
MAYR, E. 1963. *Animal species and evolution,* Cambridge, MA, USA, Harvard University Press.

MCGUIRE, J. A., WITT, C. C., ALTSHULER, D. L. & REMSEN, J. V. 2007. Phylogenetic systematics and biogeography of hummingbirds: Bayesian and Maximum Likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. *Systematic Biology,* 56**,** 837-856.
MCLOUGHLIN, S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany,* 49**,** 271-300.
MEADE, A. & PAGEL, M. 2016. BayesTraits V3 manual. Reading, UK: University of Reading.
MEATS, A. 2006. Attributes pertinent to overwintering potential do not explain why *Bactrocera neohumeralis* (Hardy) (Diptera: Tephritidae) does not spread further south within the geographical range of *B. tryoni* (Froggatt). *Australian Journal of Entomology,* 45**,** 20-25.
MELVILLE, J., HAINES, M. L., BOYSEN, K., HODKINSON, L., KILIAN, A., KL SMITH DATE, POTVIN, D. A. & PARRIS, K. M. 2017. Identifying hybridization and admixture using SNPs: application of the DArTseq platform in phylogeographic research on vertebrates *Royal Society of Open Science,* 4**,** 161061.
METCALF, R. L. 1990. Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America,* 83**,** 1017-1030.
METCALFE, I. 1998. Paleozoic and Mesozoic geological evolution of the SE Asian region: multidisciplinary constraints and implications for biogeography. *In:* HALL, R. & HOLLOWAY, D. (eds.) *Biogeography and geological evolution of SE Asia.* Leiden, Netherlands: Backhuys.
MICHAUX, B. 1996. The origin of southwest Sulawesi and other Indonesian terranes: a biological view. *Paleogeography, Paleoclimatology, Paleoecology,* 122**,** 167-183.
MICHAUX, B. & WHITE, I. M. 1999. Systematics and biogeography of southwest Pacific *Bactrocera* (Diptera: Tephritidae: Dacini). *Palaeogeography, Palaeoclimatology, Palaeoecology,* 153**,** 337-351.
MILLER, M. A., PFEIFFER, W. & SCHWARTZ, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments workshop (GCE), November 14, 2010.* New Orleans, LA.
MINH, B. G., NGUYEN, M. A. T. & HAESELER, A. V. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution,* 30**,** 1188-1195.
MINH, B. Q., TRIFINOPOULOS, J., SCHREMPF, D. & SCHMIDT, H. A. 2019. *IQ-TREE version 2.0: tutorials and manual* [Online]. Available: http://www.iqtree.org/doc/iqtree-doc.pdf [Accessed January 22 2020].
MIRAMS, A. G. K., TREML, E. A., SHIELDS, J. L., LIGGINS, L. & RIGINOS, C. 2011. Vicariance and dispersal across an intermittent barrier: population genetic structure of marine animals across the Torres Strait land bridge. *Coral Reefs,* 30**,** 937-949.
MISHLER, B., BREMER, K., HUMPHRIES, C. & CHURCHILL, S. 1988. The use of nucleic acid sequence data in phylogenetic reconstruction. *Taxon,* 37**,** 391-395.
MORLEY, R. J. 2000. *Origin and evolution of tropical rainforests,* Chichester, UK, Wiley & Sons.
MORROW, J., SCOTT, L., CONGDON, B., YEATES, D. K., FROMMER, M. & SVED, J. A. 2000. Close genetic similarity between two sympatric species of

Tephritid fruit fly reproductively isolated by mating time. *Evolution,* 54**,** 899-910.
MORTIMER, N., CAMPBELL, H. J., TULLOCH, A. J., KING, P. R., STAGPOOLE, V. M., WOOD, R. A., RATTENBURY, M. S., SUTHERLAND, R., ADAMS, C. J., COLLOT, J. & SETON, M. 2017. Zealandia: Earth's hidden continent. *GSA Today,* 27**,** 27-35.
MURAJI, M. & NAKAHARA, S. 2001. Phylogenetic relationships among fruit flies, *Bactrocera* (Diptera, Tephritidae), based on the mitochondrial rDNA sequences. *Insect Molecular Biology,* 10**,** 549-559.
MURIENNE, J., GRANDCOLAS, P., PUILACHS, M. D., BELLES, X., D'HAESE, C., LEGENDRE, F., PELLENS, R. & GUILBERT, E. 2005. Evolution on a shaky piece of Gondwana: is local endemism recent in New Caledonia? *Cladistics,* 21**,** 2-7.
MUSCHNER, V. C., LORENZ-LEMKE, A. P., VECCHIA, M., BONATTO, S. L., SALZANO, F. M. & FREITAS, L. B. 2006. Differential organellar inheritance in *Passiflora'*s (Passifloraceae) subgenera. *Genetica,* 128**,** 449-453.
NAIR, N., THANGJAM, B. C., BHATTACHARJEE, T. & DEBNATH, M. R. 2017. Species composition of Dacine fruit flies (Diptera: Tephritidae: Dacinae: Dacini) associated with cucurbits in Tripura, a north easteran state of India. *Journal of Entomology and Zoology Studies,* 5**,** 330-335.
NAKAHARA, S. & MURAJI, M. 2008. Phylogenetic analyses of *Bactrocera* fruit flies (Diptera: Tephritidae) based on nucleotide sequences of the mitochondrial COI and COII genes. *Research Bulletin of Plant Protection Japan,* 44**,** 1-12.
NASH, J. C. & VARADHAN, R. 2011. Unifying optimization algorithms to aid software system users: optimx for R. *Journal of Statistical Software,* 43**,** 1-14.
NEI, M., XU, P. & GLAZKO, G. 2001. Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. *PNAS,* 98**,** 2497-2502.
NICHOLLS, J. A. & AUSTIN, J. J. 2005. Phylogeography of an east Australian wet-forest bird, the satin bowerbird (*Ptilonorhynchus violaceus)*, derived from mtDNA, and its relationship to morphology. *Molecular Ecology,* 14**,** 1485-1496.
NIRENBERG, H. I. & O'DONNELL, K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species comples. *Mycologia,* 90**,** 434-458.
NORRBOM, A. 2004. Updates to biosystematic database of world Diptera for Tephritidae through 1999. *Diptera Data Dissemination Disk.* Washington DC: US Department of Agriculture.
NORRBOM, A. & CONDON, M. A. 2000. Phylogeny of the subfamily Blepharoneurinae. *In:* ALUJA, M. & NORRBOM, A. (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior.* Boca Raton, Florida: CRC Press.
NOVOTNY, V., BASSET, Y., MILLER, S. E., WEIBLEN, G. D., BREMER, B., CIZEK, L. & DROZD, P. 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature,* 416**,** 841-844.
NOVOTNY, V., CLARKE, A. R., DREW, R. A. I., BALAGAWI, S. & CLIFFORD, B. 2005. Host specialization and species richness of fruit flies (Diptera:

Tephritidae) in a New Guinea rainforest. *Journal of Tropical Ecology,* 21**,** 67-77.
OKSANEN, J., BLANCHET, F. G., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P. R., O'HARA, R. B., SIMPSON, G. L., SOLYMOS, P., HENRY, M., STEVENS, H., SZOECS, E. & WAGNER, H. 2019. vegan: community ecology package. Available from: https://CRAN.R-project.org/package=vegan.
ORME, D., FRECKLETON, R., THOMAS, G., PETZOLDT, T., FRITZ, S., ISAAC, N. & PEARSE, W. 2018. caper: comparative analyses of phylogenetics and evolution in R. Available from: https://CRAN.R-project.org/package=caper.
OSBORNE, D. V. 1963. Some aspects of the theory of dichotomous keys. *New Phytologist,* 62**,** 144-160.
OSBORNE, R., MEATS, A., FROMMER, M., SVED, J., DREW, R. A. I. & ROBSON, M. K. 1997. Australian distribution of 17 species of fruit flies (Diptera: Tephritidae) caught in cue lure traps in February 1994. *Australian Journal of Entomology,* 36.
PAGEL, M. 1994. Detecting correlated evolution on phylogenies: a general methods for the comparative analysis of discrete characters. *Proceedings of the Royal Society B: Biological Sciences,* 255**,** 27-45.
PAGEL, M. & MEADE, A. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *The American Naturalist,* 167**,** 808-825.
PALUMBI, S. R. 1996. Nucleic acids II: the polymerase chain reaction. *In:* HILLIS, D. M., MORITZ, C. & MABLE, B. K. (eds.) *Molecular systematics.* 2nd ed. Sunderland, Massachusetts: Sinauer Associates, Inc.
PAPUA NEW GUINEA DEPARTMENT OF CONSERVATION 2017. Papua New Guinea's fifth national report to the Convention on Biological Diversity. Port Moresby.
PARADIS, E. & SCHLIEP, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics,* 35**,** 526-528.
PARSONS, M. J. 1999. *The butterflies of Papua New Guinea: their systematics and biology,* London, UK, Academic Press.
PBCRC. 2018. *Next generation national fruit fly diagnostics and handbook* [Online]. CRC Plant Biosecurity. Available: http://www.pbcrc.com.au/research/project/2147/ [Accessed January 6, 2018].
PENNELL, M., EASTMAN, J., SLATER, G., BROWN, J., UYEDA, J., FITZJOHN, R., ALFARO, M. & HARMON, L. 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics,* 30**,** 2216-2218.
PERKINS, F. A. & MAY, A. W. S. 1949. Studies in Australian and Oriental Trypetidae Part IV. New Species of Dacinae from Queensland. *University of Queensland Papers Department of Biology,* 2**,** 1-21.
PLANT HEALTH AUSTRALIA 2016. The Australian Handbook for the identification of fruit flies. Canberra, ACT: Plant Health Australia.
PLANT HEALTH AUSTRALIA. 2018a. *Fruit Fly ID Australia* [Online]. Available: http://fruitflyidentification.org.au/ [Accessed June 12 2018].
PLANT HEALTH AUSTRALIA. 2018b. *Fruit fly ID Key 2018 - LUCID Web Player* [Online]. Available: https://fruitflyidentification.org.au/identify/ [Accessed January 5, 2019].

PLANT HEALTH AUSTRALIA. 2020. *DNA barcoding* [Online]. Available: https://fruitflyidentification.org.au/diagnostic-methods/molecular-identification/dna-barcoding/ [Accessed October 16 2018].
PLETSCH, T., ERBACHER, J., HOLBOURN, A. E. L., KUHNT, W., MOULLADE, M., OBOH-IKUENOBEDE, F. E., SODING, E. & WAGNER, T. 2001. Cretaceous separation of Africa and South America: the view from the West African margin (ODP Leg 159). *Journal of South American Earth Sciences,* 14**,** 147-174.
POLHEMUS, D. A. & POLHEMUS, J. T. 1998. Assembling New Guinea: 40 million years of island arc accretion as indicated by the distributions of aquatic Heteroptera (Insecta). *In:* HALL, R. & HOLLOWAY, D. (eds.) *Biogeography and geological evolution of SE Asia.* Leiden, Netherlands: Backhuys.
POLLOCK, D. D., ZWICKL, D., MCGUIRE, J. A. & HILLIS, D. M. 2002. Increased taxon sampling is advantageous for phylogenetic inference. *Systematic Biology,* 51**,** 664-671.
POPA-BAEZ, A., CATULLO, R., LEE, S. F., YEAP, H. L., MOURANT, R. G., FROMMER, M., SVED, J., CAMERON, E. C., EDWARDS, O. R., TAYLOR, P. W. & OAKESHOTT, J. G. 2020. Genome-wide patterns of differentiation over space and time in the Queensland fruit fly. *Scientific Reports,* 10**,** 10788.
POWELL, A. J. 1992. Dinoflagellate cysts of the Tertiary system. *In:* POWELL, A. J. (ed.) *A stratigraphic index of dinoflagellate cysts.* London: Chapman and Hall.
PUTTICK, M. & TITLE, P. 2019. MCMCtreeR: prepare MCMCtree analyses and plot bayesian divergence time analyses estimates on trees. Available from: https://CRAN.R-project.org/package=MCMCtreeR
QUEENSLAND MUSEUM NETWORK. 2020. *Bactrocera (Bactrocera) melas* [Online]. The State of Queensland (Queensland Museum). Available: https://collections.qm.qld.gov.au/objects/IN50702/bactrocera-bactrocera-melas#&gid=1&pid=1 [Accessed July 1, 2020].
R CORE TEAM 2003. R: A language and environment for statistical computing. Vienna, Austria: R foundation for statistical computing.
RAGANATH, H. R. & VEENAKUMARI, K. 1999. Notes on the Dacine fruit flies (Diptera: Tephritidae) of Andaman and Nicobar Islands - II. *The Raffles Bulletin of Zoology,* 47**,** 221-224.
RAGHU, S. 2004. Functional significance of phytochemical lures to dacine fruit flies (Diptera: Tephritidae): an ecological and evolutionary synthesis. *Bulletin of Entomological Research,* 94**,** 385-399.
RAGHU, S., CLARKE, A. R., DREW, R. A. I. & HULSMAN, K. 2000. Impact of habitat modification on the distribution and abundance of fruit flies (Diptera: Tephritidae) in Southeast Queensland. *Population Ecology,* 42**,** 153-160.
RAMBAUT, A., DRUMMOND, A. J., XIE, D., BAELE, G. & SUCHARD, M. A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology,* 67**,** 901-904.
RANNALA, B. & YANG, Z. 2007. Inferring speciation times under an episodic molecular clock. *Systematic Biology,* 56**,** 453-466.
RAVAL, U. & VEERASWAMY, K. 2003. India-Madagascar separation: breakup along a pre-existing mobile belt and chipping of the Craton. *Gondwana Research,* 6**,** 467-485.

RAVEN, P. H. & AXELROD, D. I. 1972. Plate tectonics and Australasian paleobiogeography. *Science,* 176**,** 1379-1386.
RAVEN, P. H. & AXELROD, D. I. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden,* 61**,** 539-673.
REEVES, J. M., CHIVAS, A. R., GARCIA, A., HOLT, S., COUAPEL, M. J. J., JONES, B. G., CENDON, D. I. & FINK, D. 2008. The sedimentary record of palaeoenvironments and sea-level change in the Gulf of Carpentaria, Australia, through the last glacial cycle. *Quarternary International,* 183**,** 3-22.
REMUND, U., BOLLER, E. F. & ECONOMOPOULOS, A. P. 1976. Flight performance of *Dacus oleae* reared on olives and artifical diet. *Zeitschrift fur Angewandte Entomologie,* 81**,** 330-339.
REVELL, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution,* 3**,** 217-223.
ROKAS, A., WILLIAMS, B. L., KING, N. & CARROLL, S. B. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature,* 425**,** 798-804.
ROMIGUIER, J., RANWEZ, V., DELSUC, F., GALTIER, N. & DOUZERY, E. J. P. 2013. Less is more in mammalian phylogenomics: AT-rich genes minimize tree conflicts and unravel the root of placental mammals. *Molecular Biology and Evolution,* 30**,** 2134-2144.
RONQUIST, F. & HUELSENBECK, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* 19**,** 1572-1574.
ROONEY, A. P., PRICE, N. P. J., EHRHARDT, C., SWEZEY, J. L. & BANNAN, J. D. 2009. Phylogeny and molecular taxonomy of the *Bacillus subtilis* species complex and description of *Bacillus subtilis* subsp. *inaquosorum* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 59**,** 2429-2436.
ROYER, J. E. 2015. Responses of fruit flies (Tephritidae: Dacinae) to novel male attractants in north Queensland, Australia, and improved lures for some pest species. *Austral Entomology,* 54**,** 411-426.
ROYER, J. E., AGOVAUA, S., BOKOSOU, J., KURIKA, K., MARARUAI, A., MAYER, D. G. & NIANGU, B. 2018. Responses of fruit flies (Diptera: Tephritidae) to new attractants in Papua New Guinea. *Austral Entomology,* 57**,** 40-49.
ROYER, J. E., DE FAVERI, S. G., LOWE, G. E. & WRIGHT, C. L. 2014. Cucumber volatile blend, a promising female-biased lure for *Bactrocera cucumis* (French 1907) (Diptera: Tephritidae: Dacinae), a pest fruit fly that does not respond to male attractants. *Austral Entomology,* 53**,** 347-352.
ROYER, J. E. & HANCOCK, D. L. 2012. New distribution and lure records of Dacinae (Diptera: Tephritidae) from Queensland, Australia, and description of a new species of *Dacus* Fabricius. *Austral Entomology,* 51**,** 239-247.
ROYER, J. E., TEAKLE, G. E., AHOAFI, E. & MAYER, D. G. 2019. Methyl-isoeugenol, a significantly more attractive male lure for the methyl eugenol-responsive Pacific fruit fly, *Bactrocera xanthodes* (Diptera: Tephritidae). *Austral Entomology,* 58**,** 800-804.
ROYER, J. E., WRIGHT, C. L. & HANCOCK, D. L. 2016. *Bactrocera frauenfeldi* (Diptera: Tephritidae), an invasive fruit fly in Australia that may have

reached the extent of its spread due to environmental variables. *Austral Entomology,* 55**,** 100-111.
RSTUDIO TEAM 2019. RStudio: integrated development for R. Boston, MA: RStudio Inc.
SAN JOSE, M., DOORENWEERD, C., LEBLANC, L., BARR, N., GEIB, S. & RUBINOFF, D. 2018. Incongruence between molecules and morphology: a seven-gene phylogeny of Dacini fruit flies paves the way for reclassification. *Molecular Phylogenetics and Evolution,* 121**,** 139-149.
SAN JOSE, M., LEBLANC, L., GEIB, S. M. & RUBINOFF, D. 2013. An evaluation of the species status of *Bactrocera invadens* and the systematics of the *Bactrocera dorsalis* (Diptera: Tephritidae) complex. *Annals of the Entomological Society of America,* 106**,** 684-694.
SANDERSON, C. & DONOGHUE, M. J. 1989. Patterns of variation in levels of homoplasy. *Evolution,* 43**,** 1781-1795.
SAR, S., BALAGAWI, S., MARARUAI, A. & PUTULAN, D. Fruit fly research and development in PNG. *In:* BOURKE, R. M., ALLEN, M. G. & SALISBURY, J. G., eds. Food Security for Papua New Guinea. Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference, 2000 PNG University of Technology, Lae. ACIAR Proceedings, 571-576.
SCHLICK-STEINER, B. C., STEINER, F. M., SEIFERT, B., STAUFFER, C., CHRISTIAN, E. & CROZIER, R. H. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology,* 55**,** 421-438.
SCHUTZE, M. K., AKETARAWONG, N., AMORNSAK, W., ARMSTRONG, K. F., AUGUSTINOS, A. A., BARR, N., BO, W., BOURTZIS, K., BOYKIN, L. M., CACERES, C., CAMERON, S. L., CHAPMAN, T. A., CHINVINIJKUL, S., CHOMIC, A., DE MEYER, M., DROSOPOULOU, E., ENGLEZOU, A., EKESI, S., GARIOU-PAPALEXIOU, A., GEIB, S. M., HAILSTONES, D., HASANUZZAMAN, M., HAYMER, D., HEE, A. K. W., HENDRICHS, J., JESSUP, A., JI, Q., KHAMIS, F. M., KROSCH, M. N., LEBLANC, L., MAHMOOD, K., MALACRIDA, A. R., MAVRAGANI-TSIPIDOU, P., MWATAWALA, M., NISHIDA, R., ONO, H., REYES, J., RUBINOFF, D., SAN JOSE, M., SHELLY, T. E., SRIKACHAR, S., TAN, K. H., THANAPHUM, S., HAQ, I., VIJAYSEGARAN, S., WEE, S. L., YESMIN, F., ZACHAROPOULOU, A. & CLARKE, A. R. 2015a. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. *Systematic Entomology,* 40**,** 456-471.
SCHUTZE, M. K., MAHMOOD, K., PAVASOVIC, A., BO, W., NEWMAN, J., CLARKE, A. R., KROSCH, M. N. & CAMERON, S. L. 2015b. One and the same: integrative taxonomic evidence that *Bactrocera invadens* (Diptera: Tephritidae) is the same species as the Oriental fruit fly *Bactrocera dorsalis*. *Systematic Entomology,* 40**,** 472-486.
SCHUTZE, M. K., VIRGILIO, M., NORRBOM, A. & CLARKE, A. R. 2017. Tephritid integrative taxonomy: where we are now, with a focus on the resolution of three tropical fruit fly species complexes. *Annual Review of Entomology,* 62**,** 147-164.

SCOTLAND, R. W., OLMSTEAD, R. G. & BENNETT, J. R. 2003. Phylogeny reconstruction: the role of morphology. *Society of Systematic Biologists,* 52**,** 539-548.
SEGURA, M. D., CALLEJAS, C., FERNANDEZ, M. P. & OCHANDO, M. D. 2006. New contributions towards the understanding of the phylogenetic relationships among economically important fruit flies (Diptera: Tephritidae). *Bulletin of Entomological Research,* 96**,** 279-288.
SHELLY, T. E. 2017. Zingerone and the mating success and field attraction of male melon flies (Diptera: Tephritidae). *Journal of Asia-Pacific Entomology,* 20**,** 175-178.
SHIMODAIRA, H. 2002. An approximately unbiased test of phylogenetic tree selection. *Systematic Biology,* 51**,** 492-508.
SIGOVINI, M., KEPPEL, E. & TAGLIAGPIETRA, D. 2016. Open Nomenclature in the biodiversity era. *Methods in Ecology and Evolution,* 7**,** 1217-1225.
SIMMONS, N. B. 2005. *Pteropus*. *In:* WILSON, D. E. & REEDER, D. A. M. (eds.) *Mammal species of the world: a taxonomic and geographic reference.* 3rd ed. Baltimore, MD: Johns Hopkins University Press.
SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LIU, H. & FLOOK, P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America,* 87**,** 651-701.
SMITH, A. B. 1994. *Systematics and the fossil record: documenting evolutionary patterns,* Oxford, Blackwell Scientific Publications.
SMITH, B. T., MCCORMACK, J. E., CUERVO, A. M., HICKERSON, M. J., ALEIXO, A., CADENA, C. D., PEREZ-EMAN, J., BURNEY, C. W., XIE, X., HARVEY, M. G., FAIRCLOTH, B. C., GLENN, T. C., DERRYBERRY, E. P., PREJEAN, J., FIELDS, S. & BRUMFIELD, R. T. 2014. The drivers of tropical speciation. *Nature,* 515**,** 406-409.
SMITH, D. R. & POINAR, G. O. 1992. Sawflies (Hymenoptera: Argidae) from Dominican amber. *Entomological News,* 103**,** 117-124.
SMITH, E. S. C., CHIN, D., ALLWOOD, A. J. & COLLINS, S. G. 1988. A revised host list of fruit flies (Diptera: Tephritidae) from the Northern Territory of Australia. *Queensland Journal of Agricultural and Animal Sciences,* 45**,** 19-28.
SMITH, P. T., ARMSTRONG, K. F. & KAMBHAMPATI, S. 2005. Phylogenetic relationships and character evolution among selected species of *Bactrocera* (Diptera: Tephritidae) based on multiple mitochondrial genes. *Insect Systematics & Evolution,* 36**,** 343-359.
SMITH, P. T., KAMBHAMPATI, S. & ARMSTRONG, K. F. 2003. Phylogenetic relationships among *Bactrocera* species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution,* 26**,** 8-17.
SMITH, P. T., MCPHERON, B. A. & KAMBHAMPATI, S. 2002. Phylogenetic analysis of mitochondrial DNA supports the monophyly of Dacini fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America,* 95**,** 658-664.
SOARES, A. E. R. & SCHRAGO, C. G. 2015. The influence of taxon sampling on Bayesian divergence time inference under scenarios of rate heterogeneity among lineages. *Journal of Theoretical Biology,* 364**,** 31-39.

SOKAL, R. R. 1986. Phenetic taxonomy: theory and methods. *Annual Review of Ecology and Systematics,* 17**,** 423-442.
SULLIVAN, J., MARKERT, J. A. & KILPATRICK, C. W. 1997. Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. *Systematic Biology,* 46**,** 426-440.
SWENSON, U., MUNZINGER, J. & BARTISH, I. V. 2007. Molecular phylogeny of *Planchonella* (Sapotaceae) and eight new species from New Caledonia. *Taxon,* 56**,** 329-354.
SWOFFORD, D. L. 1998. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods),* Sunderland, Massachusetts, Sinauer Associates.
TAN, K., NISHIDA, R. & TOONG, Y. 2002. Floral synomone of a wild orchid, *Bulbophyllum cheiri,* lures *Bactrocera* fruit flies for pollination. *Journal of Chemical Ecology,* 28**,** 1161-1172.
TAN, K. H. & NISHIDA, R. 2000. Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens,* and *Bactrocera* fruit flies via a floral synonome. *Journal of Chemical Ecology,* 26**,** 533-546.
TIERNEY, L., ROSSINI, A. J., LI, N. & SEVCIKOVA, H. 2018. snow: simple network of workstations. Available from: https://CRAN.R-project.org/package=snow.
TOON, A., HUGHES, J. M. & JOSEPH, L. 2010. Multilocus analysis of honeyeaters (Aves: Meliphagidae) highlights spatio-temporal heterogeneity in the influence of biogeographic barriers in the Australian monsoonal zone. *Molecular Ecology,* 19**,** 2980-2994.
TRIFINOPOULOS, J., NGUYEN, L. T., VON HAESELER, A. & MINH, B. Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research,* 44**,** W232-W235.
TRIPATHY-LANG, A., HODGES, K. V., VAN SOEST, M. C. & AHMAD, T. 2013. Evidence of pre-Oligocene emergence of the Indian passive margin and the timing of collision initiation between India and Eurasia. *Lithosphere,* 5**,** 501-506.
TRYON, H. 1889. Report on insect and fungus pests. Brisbane, Queensland: Department of Agriculture.
TRYON, H. 1927. Queensland fruit flies (Trypetidae), Series 1. *Proceedings of the Royal Society of Queensland,* 38**,** 176-224.
TURNER, H., HOVENKAMP, P. H. & VAN WELZEN, P. C. 2001. Biogeography of Southeast Asia and the West Pacific. *Journal of Biogeography,* 28**,** 217-230.
USDA. 2016. *Bactrocera tau host list 2016* [Online]. Available: https://www.aphis.usda.gov/plant_health/plant_pest_info/fruit_flies/downloads/host-lists/bactrocera-tau-host-list.pdf [Accessed July 7, 2019].
USDA. 2019. *White striped fruit fly, Bactrocera albistrigata, host list* [Online]. Available: https://www.aphis.usda.gov/plant_health/plant_pest_info/fruit_flies/downloads/host-lists/white-striped-ff-host-list.pdf [Accessed July 7, 2019].
VAN BALGOOY, M. M. J. 1971. Plant-geography of the Pacific. *Blumea,* 6**,** 1-222.
VAN HINSBERGEN, D. J. J., LIPPERT, P. C., DUPONT-NIVET, G., MCQUARRIE, N., DOUBROVINE, P. V., SPAKMAN, W. & TORSVIK, T. H. 2012. Greater India basin hypothesis and a two-stage Cenozoic collision between India and Asia. *PNAS,* 109**,** 7659-7664.

VAN WELZEN, P. C., PARNELL, J. A. N. & SLIK, J. W. F. 2011. Wallace's Line and plant distributions: two of three phytogeographical areas and where to group Java? *Biological Journal of the Linnean Society,* 103**,** 531-545.
VAN WELZEN, P. C., SLIK, J. W. F. & ALAHUHTA, J. 2005. Plant distribution patterns and plate tectonics in Malesia. *Kongelige Danske Videnskabernes Selskab Biologiske Skrifter,* 55**,** 199-217.
VAN WELZEN, P. C., TURNER, H. & HOVENKAMP, P. H. 2003. Historical biogeography of Southeast Asia and the West Pacific, or the generality of unrooted area networks as historical biogeographic hypotheses. *Journal of Biogeography,* 30**,** 181-192.
VANICKOVA, L., BRIZOVA, R., MENDOCA, A. L., POMPEIANO, A. & NASCIMENTO, R. R. D. 2015. Intraspecific variation of cuticular hydrocarbon profiles in the *Anastrepha fraterculus* (Diptera: Tephritidae) species complex. *Journal of Applied Entomology,* 139**,** 679-689.
VARGAS, R. I., MAU, R. F. L., JANG, E. B., FAUST, R. M. & WONG, L. 2008. The Hawaii fruit fly areawide pest management programme. *In:* KOUL, O. & CUPERUS, G. (eds.) *Areawide pest management: theory and implementation.* London, UK: CABI Books.
VARGAS, R. I., PINERO, J. C. & LEBLANC, L. 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the pacific region. *Insects,* 6**,** 297-318.
VIRGILIO, M., DE MEYER, M., WHITE, I. M. & BACKELJAU, T. 2009. African *Dacus* (Diptera: Tephritidae: molecular data and host plant associations do not corroborate morphology based classifications. *Molecular Phylogenetics and Evolution,* 51**,** 531-539.
VIRGILIO, M., JORDAENS, K., VERWIMP, C., WHITE, I. M. & DE MEYER, M. 2015. Higher phylogeny of frugivorous flies (Diptera, Tephritidae, Dacini): Localised partition conflicts and a novel generic classification. *Molecular Phylogenetics and Evolution,* 85**,** 171-179.
VORIS, H. K. 2000. Maps of Pleistocene sea levels in southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography,* 27**,** 1153-1167.
WALTER, G. H. 2005. *Insect pest management and ecological research,* New York, Cambridge University Press.
WANG, Y., YU, H., RAPHAEL, K. & GILCHRIST, A. S. 2003. Genetic delineation of sibling species of the pest fruit fly *Bactrocera* (Diptera: Tephritidae) using microsatellites. *Bulletin of Entomological Research,* 93**,** 351-360.
WARNES, G. R., BOLKER, B., GORJANC, G., GROTHENDIECK, G., KOROSEC, A., LUMLEY, T., MACQUEEN, D., MAGNUSSON, A. & ROGERS, J. 2017. gdata: various R programming tools for data manipulation. Available from: https://CRAN.R-project.org/package=gdata.
WARNES, G. R., BOLKER, B. & LUMLEY, T. 2020. gtools: various R programming tools. Available from: https://CRAN.R-project.org/package=gtools.
WEBER, L. C., VANDERWAL, J., SCHMIDT, S., MCDONALD, W. J. F. & SHOO, L. P. 2014. Patterns of rain forest plant endemism in subtropical Australia relate to stable mesic refugia and species dispersal limitations. *Journal of Biogeography,* 41**,** 222-238.
WEIR, B. S., JOHNSTON, P. R. & DAMM, U. 2012. The *Colletotrichum gloesporioides* species complex. *Studies in Mycology,* 73**,** 115-180.

WHITE, I. M. 2000. Morphological features of the tribe Dacini (Dacinae): their significance to behaviour and classification. *In:* ALUJA, M. & NORRBOM, A. L. (eds.) *Fruit flies (Tephritidae): phylogeny and evolution of behavior.* Boca Raton, Florida: CRC Press.
WHITE, I. M. 2006. *Taxonomy of the Dacina (Diptera: Tephritidae) of Africa and the Middle East,* Heriotdale, Johannesburg, Ultra Litho.
WHITE, I. M. & ELSON-HARRIS, M. M. 1992. *Fruit flies of economic significance: their identification and bionomics,* Wallingford, UK, CABI International.
WHITE, I. M. & ELSON-HARRIS, M. M. 1994. *Fruit flies of economic significance: their identification and bionomics,* Wallingford, UK, CAB International.
WHITE, I. M. & EVENHUIS, N. L. 1999. New species and records of Indo-Australasian Dacini (Diptera: Tephritidae). *The Raffles Bulletin of Zoology,* 47**,** 487-540.
WHITE, I. M. & HANCOCK, D. L. 1997. CABIKEY to the Indo-Australasian Dacini Fruit flies. Wallingford: CAB International
WHITMORE, T. C. 1982. Wallace's line: a result of plate tectonics. *Annals of the Missouri Botanical Garden,* 69**,** 668-675.
WICKHAM, H., HESTER, J. & CHANG, W. 2020. devtools: tools to make developing R packages easier. Available from: https://CRAN.R-project.org/package=devtools.
WIEGMANN, B. M., TRAUTWEIN, M. D., WINKLER, I. S., BARR, N. B., KIM, J.-W., LAMBKIN, C., BERTONE, M. A., CASSEL, B. K., BAYLESS, K. M., HEIMBERG, A. M., WHEELER, B. M., PETERSON, K. J., PAPE, T., SINCLAIR, B. J., SKEVINGTON, J. H., BLAGODEROV, V., CARAVAS, J., KUTTY, S. N., SCHMIDT-OTT, U., KAMPMEIER, G. E., THOMPSON, F. C., GRIMALDI, D. A., BECKENBACH, A. T., COURTNEY, G. W., FRIEDRICH, M., MEIER, R. & YEATES, D. K. 2011. Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences of the United States of America,* 108**,** 5690-5695.
WIKLUND, C. 1982. Generalist versus specialist utilization of host plants among butterflies. *In:* VISSER, J. H. & MINKS, A. K. (eds.) *Proceedings of the 5th international symposium on insect-plant relationships, March 1-4, 1982.* Sweden: Centre for Agricultural Publishing and Documentation.
WILLIAMS, S. E. & PEARSON, R. G. 1997. Historical rainforest contractions, localized extinctions and patterns of vertebrate endemism in the rainforests of Australia's wet tropics. *Proceedings of the Royal Society B: Biological Sciences,* 264**,** 709-716.
WINKLER, I. S., LABANDEIRA, C. C., WAPPLER, T. & WILF, P. 2010. Distinguishing Agromyzidae (Diptera) leaf mines in the fossil record: new taxa from the paleogene of north America and Germany and their evolutionary implications. *Journal of Paleontology,* 84**,** 935-954.
WITTKOPP, P. J., VACCARO, K. & CARROLL, S. B. 2002. Evolution of *yellow* gene regulation and pigmentation in *Drosophila*. *Current Biology,* 12**,** 1547-1556.
YANG, Z. 2007. PAML 4: a program package for phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution,* 24**,** 1586-1591.

YANG, Z. & RANNALA, B. 2006. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. *Molecular Biology and Evolution,* 23**,** 212-226.
YANG, Z. & RANNALA, B. 2012. Molecular phylogenetics: principles and practice. *Nature Reviews Genetics,* 13**,** 303-314.
YU, G., LAM, T. T. Y., ZHU, H. & GUAN, Y. 2018. Two methods for mapping and visualizing associated data on phylogeny using ggtree. *Molecular Biology and Evolution,* 35**,** 3041-3043.
YU, G., SMITH, D. K., ZHU, H., GUAN, Y. & LAM, T. T. Y. 2017. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution,* 8**,** 28-36.
YU, H., FROMMER, M., ROBSON, M. K., MEATS, A. W., SHEARMAN, D. C. A. & SVED, J. 2001. Microsatellite analysis of the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae) indicates spatial structuring: implications for population control. *Bulletin of Entomological Research,* 91**,** 139-147.
ZEREGA, N. J. C., RAGONE, D. & MOTLEY, T. J. 2004. Complex origins of breadfruit (*Artocarpus altilis,* Moraceae): implications for human migrations in Oceania. *American Journal of Botany,* 91**,** 760-766.
ZHANG, B., LIU, Y. H., WU, W. X. & WANG, Z. L. 2010. Molecular phylogeny of *Bactrocera* species (Dipera: Tephritidae: Dacini) inferred from mitochondrial sequences of 16S rDNA and COI sequences. *Florida Entomologist,* 93**,** 369-377.
ZWICKL, D. J. & HILLIS, D. M. 2002. Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology,* 51**,** 588-598.
ZWOLFER, H. 1982. Fruit flies of economic importance. *In:* CAVALLORO, R. (ed.) *Fruit flies of economic importance. Proceedings of the CEC/IOBC International Symposium.* Athens, Greece: A. A. Balkema, Rotterdam, Netherlands.

Appendices

Appendix 1: Records for new fruit fly collections made during this thesis

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD		Isoeugenol	-17.272806S, 145.466E	*B. barringtoniae*	1	15.i.2018-20.i.2018
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD		Zingerone	-17.272806S, 145.466E	*B. aurea*	1	15.i.2018-20.i.2018
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD		Zingerone	-17.272806S, 145.466E	*B. silvicola*	1	15.i.2018-20.i.2018
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD		Zingerone	-17.272806S, 145.466E	*D. absonifacies*	1	15.i.2018-20.i.2018
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD		Zingerone	-17.272806S, 145.466E	*B. aglaiae*	5	15.i.2018-20.i.2018
Baldy Mountain Creek/Rifle Range Road near Atherton, QLD		Zingerone	-17.272806S, 145.466E	*B. jarvisi*	50	15.i.2018-20.i.2018
Barrington Tops National Park, NSW	1	Cue-lure	-32.062S, 151.683E	*D. aequalis*	15	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	1	Cue-lure	-32.062S, 151.683E	*D. absonifacies*	40	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	1	Isoeugenol	-32.062S, 151.683E	*B. halfordiae*	15	3.vi.2019-16.iv.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Barrington Tops National Park, NSW	1	Methyl Eugenol	-32.062S, 151.683E	*B. cacuminata*	1	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	1	Zingerone	-32.062S, 151.683E	*B. aurea*	1	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	1	Zingerone	-32.062S, 151.683E	*D. aequalis*	3	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	1	Zingerone	-32.062S, 151.683E	*D. absonifacies*	50	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Biogel	-32.051S, 151.637E	*D. absonifacies*	1	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Cue-lure	-32.051S, 151.637E	*B. tryoni*	1	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Cue-lure	-32.051S, 151.637E	*D. aequalis*	6	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Cue-lure	-32.051S, 151.637E	*D. absonifacies*	40	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Isoeugenol	-32.051S, 151.637E	*B. halfordiae*	20	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Methyl Eugenol	-32.051S, 151.637E	*D. aequalis*	1	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Methyl Eugenol	-32.051S, 151.637E	*D. absonifacies*	2	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Zingerone	-32.051S, 151.637E	*D. aequalis*	2	3.vi.2019-16.iv.2019
Barrington Tops National Park, NSW	2	Zingerone	-32.051S, 151.637E	*D. absonifacies*	50	3.vi.2019-16.iv.2019
Border Ranges National Park, NSW	1	Cue-lure	-28.408S, 153.034E	*D. absonifacies*	2	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Dihydroeugenol	-28.408S, 153.034E	*B. halfordiae*	30	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Isoeugenol	-28.408S, 153.034E	*B. halfordiae*	200	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Methyl Eugenol	-28.408S, 153.034E	*B. bancroftii*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Methyl Eugenol	-28.408S, 153.034E	*B. halfordiae*	1	30.i.2019-12.iii.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Border Ranges National Park, NSW	1	Methyl Eugenol	-28.408S, 153.034E	*B. endiandrae*	4	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Methyl Eugenol	-28.408S, 153.034E	*B. cacuminata*	200	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Methyl Isoeugenol	-28.408S, 153.034E	*B. halfordiae*	4	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Methyl Isoeugenol	-28.408S, 153.034E	*B. cacuminata*	15	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Zingerone	-28.408S, 153.034E	*D. aequalis*	15	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Zingerone	-28.408S, 153.034E	*B. aurea*	20	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	1	Zingerone	-28.408S, 153.034E	*D. absonifacies*	150	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Cue-lure	-28.388S, 153.064E	*B. quadrata*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Cue-lure	-28.388S, 153.064E	*D. absonifacies*	2	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Isoeugenol	-28.388S, 153.064E	*B. halfordiae*	30	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Methyl Eugenol	-28.388S, 153.064E	*B. cacuminata*	10	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Zingerone	-28.388S, 153.064E	*B. jarvisi*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Zingerone	-28.388S, 153.064E	*B. species A*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Zingerone	-28.388S, 153.064E	*D. aequalis*	2	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Zingerone	-28.388S, 153.064E	*B. aurea*	20	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	2	Zingerone	-28.388S, 153.064E	*D. absonifacies*	150	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Cue-lure	-28.368S, 153.072E	*B. tryoni*	2	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Cue-lure	-28.368S, 153.072E	*D. aequalis*	3	30.i.2019-12.iii.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Border Ranges National Park, NSW	3	Cue-lure	-28.368S, 153.072E	*D. absonifacies*	50	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Isoeugenol	-28.368S, 153.072E	*B. halfordiae*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Methyl Eugenol	-28.368S, 153.072E	*B. endiandrae*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Methyl Eugenol	-28.368S, 153.072E	*B. batemani*	10	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Methyl Eugenol	-28.368S, 153.072E	*B. cacuminata*	200	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Zingerone	-28.368S, 153.072E	*D. aequalis*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Zingerone	-28.368S, 153.072E	*B. aurea*	50	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	3	Zingerone	-28.368S, 153.072E	*D. absonifacies*	100	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Cue-lure	-28.345S, 152.968E	*B. bryoniae*	4	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Cue-lure	-28.345S, 152.968E	*B. tryoni*	4	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Cue-lure	-28.345S, 152.968E	*D. aequalis*	4	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Cue-lure	-28.345S, 152.968E	*D. absonifacies*	20	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Cue-lure	-28.345S, 152.968E	*B. quadrata*	30	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Methyl Eugenol	-28.345S, 152.968E	*B. cacuminata*	300	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Methyl Isoeugenol	-28.345S, 152.968E	*B. cacuminata*	150	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Zingerone	-28.345S, 152.968E	*B. cacuminata*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Zingerone	-28.345S, 152.968E	*D. aequalis*	1	30.i.2019-12.iii.2019
Border Ranges National Park, NSW	4	Zingerone	-28.345S, 152.968E	*B. aurea*	4	30.i.2019-12.iii.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Border Ranges National Park, NSW	4	Zingerone	-28.345S, 152.968E	*D. absonifacies*	200	30.i.2019-12.iii.2019
Bulburin National Park, QLD	1	Biogel	-24.505010S, 151.449969E	*B. jarvisi*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Biogel	-24.505010S, 151.449969E	*B. mutabilis*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Biogel	-24.505010S, 151.449969E	*B. tryoni*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Biogel	-24.505010S, 151.449969E	*B. brunnea*	2	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*Z. choristus*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*B. silvicola?*	3	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*D. absonifacies*	4	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*B. neohumeralis*	10	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*B. bryoniae*	20	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*B. tryoni*	40	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*D. aequalis*	70	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Cue-lure	-24.505010S, 151.449969E	*B. quadrata*	100	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Isoeugenol	-24.505010S, 151.449969E	*B. halfordiae*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Isoeugenol	-24.505010S, 151.449969E	*B. mutabilis*	2	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Methyl Eugenol	-24.505010S, 151.449969E	*B. endiandrae*	4	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Methyl Eugenol	-24.505010S, 151.449969E	*B. batemani*	7	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Methyl Eugenol	-24.505010S, 151.449969E	*B. cacuminata*	60	13.ii.2019-2.iv.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Bulburin National Park, QLD	1	Methyl Isoeugenol	-24.505010S, 151.449969E	*B. cacuminata*	2	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Zingerone	-24.505010S, 151.449969E	*D. absonifacies*	6	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Zingerone	-24.505010S, 151.449969E	*B. aurea*	30	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Zingerone	-24.505010S, 151.449969E	*D. aequalis*	60	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	1	Zingerone	-24.505010S, 151.449969E	*B. jarvisi*	100	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Biogel	-24.511468S, 151.461447E	*B. bancroftii*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Biogel	-24.511468S, 151.461447E	*B. neohumeralis*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*B. silvicola*	10	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*Z. choristus*	10	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*D. aequalis*	50	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*B. neohumeralis*	80	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*B. bryoniae*	200	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*B. tryoni*	200	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Cue-lure	-24.511468S, 151.461447E	*B. quadrata*	300	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Methyl Eugenol	-24.511468S, 151.461447E	*B. endiandrae*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Methyl Eugenol	-24.511468S, 151.461447E	*B. mayi*	5	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Methyl Eugenol	-24.511468S, 151.461447E	*B. batemani*	60	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Methyl Eugenol	-24.511468S, 151.461447E	*B. cacuminata*	100	13.ii.2019-2.iv.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Bulburin National Park, QLD	2	Methyl Isoeugenol	-24.511468S, 151.461447E	*B. cacuminata*	1	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Zingerone	-24.511468S, 151.461447E	*D. absonifacies*	2	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Zingerone	-24.511468S, 151.461447E	*B. tryoni*	5	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Zingerone	-24.511468S, 151.461447E	*B. aurea*	15	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Zingerone	-24.511468S, 151.461447E	*D. aequalis*	50	13.ii.2019-2.iv.2019
Bulburin National Park, QLD	2	Zingerone	-24.511468S, 151.461447E	*B. jarvisi*	200	13.ii.2019-2.iv.2019
Cairns Cemetary, QLD		Dry Protein	-17.244127S, 145.480586E	*B. frauenfeldi*	3	15.i.2018-20.i.2018
Daintree Rainforest Observatory, Cape Tribulation, QLD		Cue-lure	-16.103983S, 145.449177E	*B. breviaculeus*	3	1.iv.2017
Daintree Rainforest Observatory, Cape Tribulation, QLD		Methyl Eugenol	-16.103983S, 145.449177E	*B. visenda*	1	1.iv.2017
Dorrigo National Park, NSW	1	Cue-lure	-30.357142S, 152.774391E	*D. absonifacies*	2	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	1	Cue-lure	-30.357142S, 152.774391E	*D. aequalis*	2	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	1	Methyl Isoeugenol	-30.357142S, 152.774391E	*B. cacuminata*	4	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	1	Zingerone	-30.357142S, 152.774391E	*B. aurea*	2	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	1	Zingerone	-30.357142S, 152.774391E	*D. aequalis*	2	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	1	Zingerone	-30.357142S, 152.774391E	*D. aequalis*	3	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	1	Zingerone	-30.357142S, 152.774391E	*D. absonifacies*	4	6.iii.2019-17.iv.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Dorrigo National Park, NSW	1	Zingerone	-30.357142S, 152.774391E	*D. absonifacies*	5	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	2	Cue-lure	-30.357142S, 152.774391E	*D. absonifacies*	2	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	2	Cue-lure	-30.357142S, 152.774391E	*D. aequalis*	4	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	2	Isoeugenol	-30.357142S, 152.774391E	*B. halfordiae*	2	6.iii.2019-17.iv.2019
Dorrigo National Park, NSW	2	Methyl Eugenol	-30.357142S, 152.774391E	*B. cacuminata*	6	6.iii.2019-17.iv.2019
Lake Morris Road, Cairns, QLD		Dihydroeugenol	-16.924971S, 145.718430E	*B. decurtans?*	1	15.i.2018-20.i.2018
Lake Morris Road, Cairns, QLD		found on trapping gear	-16.924971S, 145.718430E	*B. neohumeralis*	1	15.i.2018-20.i.2018
Lake Morris Road, Cairns, QLD		Isoeugenol	-16.93S, 145.72E	*B. kraussi*	1	15.i.2018-20.i.2018
Lake Morris Road, Cairns, QLD		Isoeugenol	-16.924971S, 145.718430E	*B. murrayi*	2	15.i.2018-20.i.2018
Lake Morris Road, Cairns, QLD		Zingerone	-16.93S, 145.72E	*B. aglaiae* complex	15	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Ceratrap	-16.860310S, 145.758040E	*B. breviaculeus*	2	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Isoeugenol	-16.860310S, 145.758040E	*B. barringtoniae*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Isoeugenol	-16.860310S, 145.758040E	*B. perkinsi*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Isoeugenol	-16.860310S, 145.758040E	*unsure*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Isoeugenol	-16.860310S, 145.758040E	*B. decurtans*	3	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Isoeugenol	-16.860310S, 145.758040E	*B. barringtoniae*	6	15.i.2018-20.i.2018

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Machan's Beach, Cairns, QLD		Methyl Isoeugenol	-16.860310S, 145.758040E	*B. barringtoniae*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Methyl Isoeugenol	-16.860310S, 145.758040E	*B. opiliae*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Zingerone	-16.860310S, 145.758040E	*B. breviaculeus*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Zingerone	-16.860310S, 145.758040E	*B. frauenfeldi*	1	15.i.2018-20.i.2018
Machan's Beach, Cairns, QLD		Zingerone	-16.860310S, 145.758040E	*B. jarvisi*	60	15.i.2018-20.i.2018
Nambour, QLD		-	-26.636525S, 152.965024E	*monitoring only*		vi.2018-present
Noosa National Park, QLD	1	Cue-lure	-26.383129S, 153.100324E	*B. quadrata*	2	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Cue-lure	-26.383129S, 153.100324E	*D. aequalis*	2	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Cue-lure	-26.383129S, 153.100324E	*B. bryoniae*	3	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Cue-lure	-26.383129S, 153.100324E	*B. neohumeralis*	20	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Cue-lure	-26.383129S, 153.100324E	*B. tryoni*	30	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Isoeugenol	-26.383129S, 153.100324E	*B. halfordiae*	2	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Methyl Eugenol	-26.383129S, 153.100324E	*B. mayi*	2	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Methyl Eugenol	-26.383129S, 153.100324E	*B. cacuminata*	30	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Zingerone	-26.383129S, 153.100324E	*B. jarvisi*	4	12.ii.2019-2.iv.2019
Noosa National Park, QLD	1	Zingerone	-26.383129S, 153.100324E	*B. aurea*	10	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Biogel	-26.383129S, 153.100324E	*B. cacuminata*	1	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Cue-lure	-26.383129S, 153.100324E	*B. quadrata*	1	12.ii.2019-2.iv.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Noosa National Park, QLD	2	Cue-lure	-26.383129S, 153.100324E	*B. bryoniae*	3	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Cue-lure	-26.383129S, 153.100324E	*B. neohumeralis*	10	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Cue-lure	-26.383129S, 153.100324E	*B. tryoni*	40	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Methyl Eugenol	-26.383129S, 153.100324E	*B. mayi*	2	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Methyl Eugenol	-26.383129S, 153.100324E	*B. cacuminata*	30	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Methyl Isoeugenol	-26.383129S, 153.100324E	*B. cacuminata*	1	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Zingerone	-26.383129S, 153.100324E	*D. absonifacies*	1	12.ii.2019-2.iv.2019
Noosa National Park, QLD	2	Zingerone	-26.383129S, 153.100324E	*B. jarvisi*	40	12.ii.2019-2.iv.2019
Sherwood arboretum, Brisbane, QLD		Ceratrap	-27.532151S, 152.974444E	*B. bancroftii*	1	xii.2017-vii.2018
Sherwood arboretum, Brisbane, QLD		Ceratrap	-27.532151S, 152.974444E	*B. tryoni*	4	xii.2017-vii.2018
Sherwood arboretum, Brisbane, QLD		Dihydroeugenol	-27.532151S, 152.974444E	*B. cacuminata*	1	xii.2017-vii.2018
Tolga Scrub, near Atherton, QLD		Cue-lure	-17.244127S, 145.480586E	*B. tryoni*	200	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Dihydroeugenol	-17.244127S, 145.480586E	*B. neohumeralis*	1	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		ex. *Syzygium sp.*	-17.244127S, 145.480586E	*B. aglaiae* complex	1	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Isoeugenol	-17.244127S, 145.480586E	*B. phaleriae*	1	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Isoeugenol	-17.244127S, 145.480586E	*B. fagraea complex*	7	15.i.2018-20.i.2018

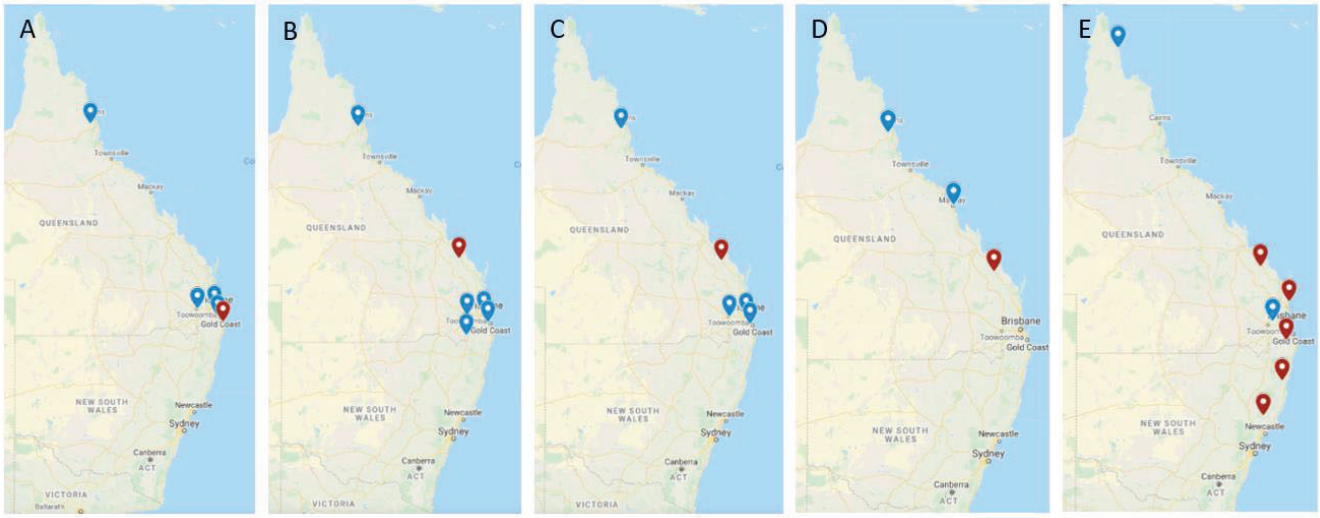
Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Tolga Scrub, near Atherton, QLD		Methyl Eugenol	-17.244127S, 145.480586E	*B. endiandrae*	30	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Methyl Eugenol	-17.244127S, 145.480586E	*B. cacuminata*	200	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Zingerone	-17.244127S, 145.480586E	*B. aglaiae* complex	10	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Zingerone	-17.244127S, 145.480586E	*B. aglaiae* complex	10	15.i.2018-20.i.2018
Tolga Scrub, near Atherton, QLD		Zingerone	-17.244127S, 145.480586E	*B. jarvisi*	10	15.i.2018-20.i.2018
Tropical Fruit World, Duranbah, NSW		Biogel	-28.284946S, 153.525139E	*Z. cucumis*	1	15.xi.2018-18.vi.2019
Tropical Fruit World, Duranbah, NSW		Biogel	-28.284946S, 153.525139E	*B. bancroftii*	5	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Biogel	-28.284946S, 153.525139E	*B. cacuminata*	5	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Biogel	-28.284946S, 153.525139E	*B. cacuminata*	60	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*Z. choristus*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*D. aequalis*	3	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. bryoniae*	10	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. bryoniae*	10	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. bryoniae*	16	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. neohumeralis*	20	23.v.2018-19.ix.2018

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. neohumeralis*	30	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. neohumeralis*	40	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. tryoni*	200	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. tryoni/B. neohumeralis*	200	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Cue-lure	-28.284946S, 153.525139E	*B. tryoni*	500	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Dihydroeugenol	-28.284946S, 153.525139E	*B. cacuminata*	5	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Dry Protein	-28.284946S, 153.525139E	*B. cacuminata*	2	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. aberrans*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. cacuminata*	1	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. halfordiae*	3	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. cacuminata*	10	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. halfordiae*	12	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. aberrans*	15	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Isoeugenol	-28.284946S, 153.525139E	*B. aberrans*	60	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. aurea*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. batemani*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	4	23.v.2018-19.ix.2018

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	20	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. endiandrae*	20	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. endiandrae*	20	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	30	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	200	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	200	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	200	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	not identified	200	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	400	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Methyl Eugenol	-28.284946S, 153.525139E	*B. cacuminata*	1000	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Methyl Isoeugenol	-28.284946S, 153.525139E	*B. cacuminata*	7	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Methyl Isoeugenol	-28.284946S, 153.525139E	*B. cacuminata*	15	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. cacuminata*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. jarvisi*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. jarvisi*	1	15.xi.2018-16.i.2019
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. neohumeralis*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*D. aequalis*	1	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*D. aequalis*	1	15.xi.2018-16.i.2019

Location	Site	Lure or fruit species	Latitude and Longitude	Species	Count	Date
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*D. aequalis*	2	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. bryoniae*	3	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. tryoni*	6	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. aurea*	7	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. aurea*	20	23.v.2018-19.ix.2018
Tropical Fruit World, Duranbah, NSW		Zingerone	-28.284946S, 153.525139E	*B. tryoni*	30	23.v.2018-19.ix.2018
Woodford, QLD		Biogel	-26.963027S, 152.784398E	*B. bancroftii*	1	ii.2018-x.2018
Woodford, QLD		Biogel	-26.963027S, 152.784398E	*B. neohumeralis*	1	ii.2018-x.2018
Woodford, QLD		Biogel	-26.963027S, 152.784398E	*Z. cucumis*	2	ii.2018-x.2018
Woodford, QLD		Biogel	-26.963027S, 152.784398E	*Z. cucumis*	2	ii.2018-x.2018
Woodford, QLD		Biogel	-26.963027S, 152.784398E	*B. cacuminata*	7	ii.2018-x.2018
Woodford, QLD		Biogel	-26.963027S, 152.784398E	*B. tryoni*	7	ii.2018-x.2018

Appendix 2: New distributions of species collected in Chapter 2. A: *B. aberrans;* B: *B. brunnea;* C: *B. muatbilis;* D: *B. silvicola;* and E: *B. aurea.* Blue indicates previous records, red indicates records from this thesis. Note: not all are new geographic ranges, some represent new elevations or climatic zones.



Appendix 3: Collection information for species included in this thesis.

Genus	Species	Specimen	Country	Location	Lure	Trapped by:	Trap date
Anastrepha	*fraterculus*	AFR001	Brazil	Ex colony Pelatan Brazil Seibersdorf	Culture	M. Schutze	29.iii.2011
Anastrepha	*serpentina*	ASR001	Panama	Lago	Reared from *Chrysophyllum argenteum*	Y. Basset	8.iii.2013
Bactrocera	*abdonigella*	ABD004	Papua New Guinea	Wanang, Madang Province	Cue-lure	M. Schutze	19-23.x.2014
Bactrocera	*abdonigella*	ABD005	Papua New Guinea	Baitabag, Madang Province	Cue-lure	M. Schutze, E. Bris	19-23.x.2015
Bactrocera	*aberrans*	ABE001	Australia	180 Coes Creek Road, Coes Creek, Nambour, Queensland	Dry protein trap	T. Wheatland	28.vi.2016
Bactrocera	*aberrans*	ABE002	Australia	181 Coes Creek Road, Coes Creek, Nambour, Queensland	Dry protein trap	T. Wheatland	7.vi.2016
Bactrocera	*abscondita*	ABC001	Australia	Cairns, Queensland	Cue-lure	M. Berridge	2.viii.2016
Bactrocera	*abscondita*	BRV002	Australia	Iron Range, Queensland	Cue-lure	S. Cameron	7.iii.2015
Bactrocera	*absidata*	ASD001	Papua New Guinea	Swire station, Wanang, Madang Province	Cue-lure	M. Schutze	19-23.x.2014
Bactrocera	*absidata*	ASD002	Papua New Guinea	Swire station, Wanang, Madang Province	Cue-lure	M. Schutze	19-23.x.2014
Bactrocera	*absidata*	ASD003	Papua New Guinea	Swire station, Wanang, Madang Province	Cue-lure	M. Schutze	19-23.x.2014
Bactrocera	*aeroginosa*	ARG001	Australia	Cairns, Queensland	Cue-lure	BQ Trapper	24.xi.2015
Bactrocera	*aeroginosa*	ARG002	Australia	Cairns, Queensland	Cue-lure	BQ Trapper	19.i.2016
Bactrocera	*aglaiae*	AGL001	Australia	Chambers Wildlife Lodge, Eacham Close, Lake Eacham, Queensland	Zingerone	M. Krosch	8-11.x.2016

Bactrocera	*aglaiae*	AGL003	Australia	Atherton Tablelands, Winfield Park, Queensland	Zingerone	J. Royer	3.iv.2013
Bactrocera	*aglaiae*	AGL006	Australia	Lake Eacham, Queensland	Zingerone	8-11.x.2016	M. Krosch
Bactrocera	*aglaiae*	AGL007	Australia	Lake Eacham, Queensland	Zingerone	8-11.x.2016	M. Krosch
Bactrocera	*aglaiae*	AGL009	Australia	Julatten, Queensland	Zingerone	5-7.x.2016	M. Krosch
Bactrocera	*aglaiae*	AGL011	Australia	Cow Bay, Queensland	Zingerone	1-3.x.2016	M. Krosch
Bactrocera	*aglaiae* complex	AGL002	Australia	Lockhart River dump, Queensland	Zingerone	J. Royer	13.x.2014
Bactrocera	*aglaiae* complex	AGL004	Australia	Tolga Scrub, Queensland	Reared from *Szygium* sp.	M. Starkie, J. Royer	emerged ii.2018
Bactrocera	*aglaiae* complex	AGL005	Papua New Guinea	Baitabag, Madang Province	Zingerone	R. Opasa, F. Phillip	5-11.xi.2016
Bactrocera	*aglaiae* complex	AGL010	Australia	Cow Bay, Queensland	Zingerone	1-3.x.2016	M. Krosch
Bactrocera	*aglaiae* complex	AGL012	Australia	Cow Bay, Queensland	Zingerone	1-3.x.2016	M. Krosch
Bactrocera	*aglaiae* complex	AGL013	Australia	Lockhart River dump, Queensland	Zingerone	29.i.2019	J. Royer
Bactrocera	*aglaiae* complex	MAC001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*aglaiae complex*	MAC004	Papua New Guinea	Baitabag, Madang Province	Zingerone	R. Opasa, F. Phillip	29.x-4.xi.2016
Bactrocera	*albistrigata*	ALB002	Australia	Christmas Island	No lure details	B. Woods	08.xi.2012
Bactrocera	*albistrigata*	ALB003	Malaysia	Serdang, Selangor	Cue-lure	Mohd. Hannifah Yahaya	28.i.2016
Bactrocera	*allwoodi*	ALL001	Australia	Alyangula Golf Park, Groote Aylandt, Northern Territory	Cue-lure	Anindilyakwa rangers	23.ix.2008
Bactrocera	*alyxiae*	ALX001	Australia	Iron Range, Queensland	Cue-lure	S. Cameron	4.iii.2015-7.iii.2015
Bactrocera	*alyxiae*	ALX003	Papua New Guinea	Wanang, III Swires Station Road, Madang Province	Cue-lure	M. Schutze	23.x.2014
Bactrocera	*amplexiseta*	AMP002	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.2015-1.iii.2015

Bactrocera	*amplexiseta*	AMP003	Australia	Mourilyan Harbour, Queensland	Cue-lure	K. Leutton	8.vi.2016
Bactrocera	*antigone*	ANT001	Australia	Iron Range, Queensland	Cue-lure	S. Cameron	4.iii.2015-7.iii.2015
Bactrocera	*antigone*	ANT002	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	7.xii.2015
Bactrocera	*aquilonis*	AQL001	Australia	Department of Agriculture and Fisheries cultures, Western Australia	Ex lab colony	B. Woods	15.iv.2016
Bactrocera	*aquilonis*	AQL010	Australia	Cable Beach, Broome, Western Australia	emerged from Gubinge (Terminalia ferdinandiana)	B. Woods	2.ii.2017
Bactrocera	*aquilonis*	AQL015	Australia	Cable Beach, Broome, Western Australia	emerged from Red Gubinge Hybrid (*Terminalia ferdinandiana* x *petiolaris*)	B. Woods	3.iii.2017
Bactrocera	*aquilonis*	AQL023	Australia	Kununurra, Western Australia	Ex lab colony	B. Woods	15.iv.2016
Bactrocera	*aquilonis*	AQL024	Australia	Black Point, Ranger Station Cobourg Peninsula, Northern Territory	Cue-lure	F. Timaepatua	18.v.2019
Bactrocera	*aquilonis*	AQL025	Australia	Black Point, Ranger Station Cobourg Peninsula, Northern Territory	Cue-lure	F. Timaepatua	18.v.2019
Bactrocera	*atramentata*	ATM001	Papua New Guinea	Keravat Golf, East New Britain Province	Cue-lure	J. Royer	18.iv.2013
Bactrocera	*aurantiaca*	AUR001	Malaysia	Bentong, Selangor	Cue-lure	Mohd. Hannifah Yahaya	31.i.2016
Bactrocera	*aurantiaca*	AUR002	Australia	Cape York, Queensland	Cue-lure	L. Bailey	20.ii.2016
Bactrocera	*aurea*	AEA002	Australia	Lockhart River dump, Queensland	Zingerone	J. Royer	15.ix.2014
Bactrocera	*aurea*	AEA001	Australia	Mt Mee, Queensland	Zingerone	J. Royer	22.x.2014

Bactrocera	*bancroftii*	BAN002	Australia	Bundaberg, Queensland	Methyl Eugenol	BQ Trapper	13.i.2016
Bactrocera	*bancroftii*	BAN003	Australia	Brisbane, Queensland	Methyl Eugenol	C. Manechkshana	13.ii.2016
Bactrocera	*barringtoniae*	BAR001	Australia	Atherton CSIRO, Queensland	Methyl Isoeugenol	J. Royer	14.ii.2013
Bactrocera	*barringtoniae*	BAR002	Australia	Atherton CSIRO, Queensland	Methyl Isoeugenol	J. Royer	14.ii.2013
Bactrocera	*batemani*	BAT001	Australia	Brisbane, Queensland	Cue-lure	C. Manechkshana	14.iv.2015
Bactrocera	*bidentata*	BID001	Australia	Lockhart River dump, Queensland	Methyl Isoeugenol	J. Royer	4.viii.2014
Bactrocera	*bidentata*	BID002	Australia	Lockhart River dump, Queensland	Methyl Isoeugenol	J. Royer	4.viii.2014
Bactrocera	*breviaculeus*	BRV004	Australia	Malanda, Queensland	Cue-lure	S. Cameron	26.ii.2015
Bactrocera	*breviaculeus*	BRV007	Australia	Horn Island, Torres Strait	Cue-lure	V. Kirk	22.v.2017
Bactrocera	*brunnea*	BRU001	Australia	Bulburin National Park, Queensland	Biogel	F. Strutt, M. Starkie	12.ii-3.iv.2019
Bactrocera	*brunnea*	BRU001	Australia	Bulburin National Park, Queensland	Biogel	12.ii-3.iv.2019	F. Strutt, M. Starkie
Bactrocera	*bryoniae*	BRY001	Papua New Guinea	Mt Hagen, Western Highlands Province	Cue-lure	S. Cowan	21.iv.2016
Bactrocera	*bryoniae*	BRY005	Australia	Bundaberg, Queensland	Cue-lure	L. Senior	30.iv.2016
Bactrocera	*cacuminata*	CAC004	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.15-1.iii.2015
Bactrocera	*cacuminata*	CAC006	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.15-1.iii.2015
Bactrocera	*cacuminata*	CAC007	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.15-1.iii.2015

Bactrocera	*cacuminata*	CAC008	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.15-1.iii.2015
Bactrocera	*cacuminata*	CAC010	Australia	Cairns, Queensland	Methyl Eugenol	M. Berridge	6.vii.2015
Bactrocera	*cacuminata*	CAC011	Australia	Brisbane, Queensland	Methyl Eugenol	S. Colingwood	7.vii.2015
Bactrocera	*cacuminata*	CAC014	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii-1.iii.2015
Bactrocera	*cacuminata*	CAC015	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii-1.iii.2015
Bactrocera	*caledoniensis*	CLD001	New Caledonia	La Foa, South Province	Cue-lure	J. Royer	13.x.2017
Bactrocera	*calophylli*	CAL001	Australia	McLeod Street, Cairns, Queensland	Ceratrap	J. Royer	16.iv.2012
Bactrocera	*cheesmanae*	CHE001	Papua New Guinea	Madang Province	Methyl Eugenol	M. Schutze	21.x.2014
Bactrocera	*cheesmanae*	CHE003	Papua New Guinea	Wanang, III Swires Station Road, Madang Province	Methyl Eugenol	M. Schutze	17-21.x.2014
Bactrocera	*consectorata*	CON001	Papua New Guinea	Madang Province	CUE	M. Schutze	21.x.2014
Bactrocera	*curreyi*	CUR001	Papua New Guinea	Madang Province	CUE	M. Schutze	23.x.2014
Bactrocera	*curvifera*	CVF001	Papua New Guinea	BRC Nagada Harbour, Madang Province	Methyl Eugenol	M. Schutze	14-15.x.2014
Bactrocera	*curvipennis*	CRV001	New Caledonia	La Foa, South Province	Isoeugenol	J. Royer	13.x.2017
Bactrocera	*curvipennis*	CRV002	New Caledonia	La Foa, South Province	Isoeugenol	J. Royer	10.xii.2017
Bactrocera	*decurtans*	DEC001	Australia	Cairns, Queensland	Methyl Eugenol	M. Berridge	24.xi.2015
Bactrocera	*decurtans*	DEC002	Australia	Badu island, Torres Strait	Methyl Eugenol	D. Nona	6.x.2017
Bactrocera	*distincta*	DIS001	Tonga	Nuku'alofa, Tongatapu	Cue-lure	J. Royer	26.ix.2017
Bactrocera	*dyscrita*	DYS001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	23.x.2014
Bactrocera	*ebenea*	EBE001	New Caledonia	La Foa, South Province	Methyl Eugenol	J. Royer	28.xi.2017
Bactrocera	*endiandre*	END002	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.2015
Bactrocera	*endiandre*	END005	Australia	Cow Bay, Queensland	Methyl Eugenol	M. Krosch	1-3.x.2016
Bactrocera	*endiandre*	END006	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.2015

Bactrocera	*endiandre*	END007	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.2015
Bactrocera	*endiandre*	END008	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	26.ii.2015
Bactrocera	*endiandre*	END010	Papua New Guinea	Swire station, Wanang, Madang Province	Methyl Eugenol	M. Schutze	19-23.x.2014
Bactrocera	*erubescentis*	ERU001	Australia	Cape York, Queensland	Cue-lure	L. Bailey	28.xi.2015
Bactrocera	*erubescentis*	ERU002	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	27.vii.2015
Bactrocera	*erubescentis*	ERU003	Australia	Cape York, Queensland	Cue-lure	L. Bailey	28.xi.2015
Bactrocera	*erubescentis*	ERU004	Australia	Cape York, Queensland	Cue-lure	L. Bailey	28.xi.2015
Bactrocera	*facialis*	FAC006	Tonga	Nuku'alofa, Tongatapu	Cue-lure	J. Royer	26.ix.2017
Bactrocera	*facialis*	FAC007	Tonga	Nuku'alofa, Tongatapu	Cue-lure	J. Royer	26.ix.2017
Bactrocera	*fagraea*	FAG001	Australia	Mourilyan Harbour, Queensland	Cue-lure	A. Russell	17.viii.2016
Bactrocera	*fagraea*	FAG002	Australia	Mourilyan Harbour, Queensland	Cue-lure	A. Russell	18.viii.2016
Bactrocera	*frauenfeldi*	FRA006	Solomon Islands	Solomon Islands	Cue-lure	S. Cowan	4.viii.2015
Bactrocera	*frauenfeldi*	FRA008	Australia	Cairns, Queensland	Cue-lure	M. Berridge	6.vii.2015
Bactrocera	*fulvicauda*	BLH003	Papua New Guinea	Kiunga, Western Province	Methyl eugenol	L. Halling	24.v.2015
Bactrocera	*fulvicauda*	FUL002	Papua New Guinea	Wanang, Madang Province	Methyl Eugenol	M. Schutze	19-23.x.2014
Bactrocera	*fulvifacies*	FLF001	New Caledonia	La Foa, South Province	Zingerone	J. Royer	9-13.x.2017
Bactrocera	*furvilineata*	FUR001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	23.x.2014
Bactrocera	*furvilineata*	FUR003	Papua New Guinea	Baitabag, Madang Province	Cue-lure	M. Schutze	17-21.x.2014
Bactrocera	*halfordiae*	HAL001	Australia	180 Coes Creek Road, Coes Creek, Nambour, Queensland	Dry protein trap	T. Wheatland	7.vi.2016
Bactrocera	*halfordiae*	HAL002	Australia	180 Coes Creek Road, Coes Creek, Nambour, Queensland	Dry protein trap	T. Wheatland	17.v.2016
Bactrocera	*halfordiae*	HAL003	Australia	Tropical Fruit World, New South Wales	Isoeugenol	M. Starkie	23.5-18.ix.2018

Bactrocera	*humilis*	HUM001	Australia	Smithfield, Queensland	Methyl Eugenol	I. Schneider	13.vi.2013
Bactrocera	*humilis*	HUM002	Australia	Umagico, Queensland	Methyl Eugenol	E. Cottis	9.v.2016
Bactrocera	*humilis*	HUM003	Australia	Roma Flats, Queensland	Methyl Eugenol	J. Sailor	9.xi.2009
Bactrocera	*humilis*	HUM004	Australia	Roma Flats, Queensland	Cue-lure	J. Bond	21.v.2007
Bactrocera	*humilis*	HUM005	Australia	Pormpurnaw, Queensland	Cue-lure	PFFP	5.ii.1999
Bactrocera	*jarvisi*	JAR007	Australia	Ex colony Cairns QDAF, Queensland	ex colony	T. Peek	11.i.2016
Bactrocera	*jarvisi*	JAR008	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	21.xii.2015
Bactrocera	*kraussi*	KRA001	Australia	Malanda, Queensland	No lure details	S. Cameron	24.ii.2015
Bactrocera	*kraussi*	KRA003	Australia	Cairns, Queensland	Ex lab colony	Cairns DAF	1.v.2016
Bactrocera	*lampabilis*	LAM001	Papua New Guinea	Keravat Golf, East New Britain Province	Methyl Eugenol	J. Royer	2.v.2013
Bactrocera	*laticaudus*	LCD003	Australia	Mackay, Queensland	Cue-lure	C. Kemp	14.xii.2015
Bactrocera	*laticaudus*	LCD004	Australia	Cow Bay, Queensland	Cue-lure	M. Krosch	1-3.x.2016
Bactrocera	*laticaudus*	LCD005	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	4-7.iii.2015
Bactrocera	*latilineola*	LTL001	Malaysia	Selangor	Zingerone	Mohd. Hannifah Yahaya	31.i-7.ii.2016
Bactrocera	*lineata*	LIN002	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*lineata*	LIN003	Papua New Guinea	Baitabag, Madang Province	Cue-lure	M. Schutze	7-21.x.2014
Bactrocera	*manskii*	MAN001	Australia	Iron Range, Queensland	Cue-lure	S. Cameron	7.iii.2015
Bactrocera	*manskii*	MAN003	Australia	Cape York, Queensland	Cue-lure	J. Pritchard	4.i.2015
Bactrocera	*mayi*	MAY002	Australia	Cairns, Queensland	Methyl Eugenol	M. Berridge	6.vii.2015
Bactrocera	*mayi*	MAY003	Australia	Cairns, Queensland	Methyl Eugenol	M. Berridge	6.vii.2015
Bactrocera	*mayi*	MAY004	Australia	Cairns, Queensland	Methyl Eugenol	M. Berridge	6.vii.2015
Bactrocera	*mayi*	MAY005	Australia	Cairns, Queensland	Methyl Eugenol	M. Berridge	6.vii.2015
Bactrocera	*melanothoracica*	MTH002	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	7.iii.2015
Bactrocera	*melanothoracica*	UNI003	Australia	Coen, Queensland	Methyl Eugenol	S. Templeton	22.ii.2016

Bactrocera	*melas*	BBR002	Australia	Brisbane, Queensland	Cue-lure	BQ Trapper	17.ii.2015
Bactrocera	*melas*	MEL002	Australia	Brisbane, Queensland	Cue-lure	J. Royer	14.i.2015
Bactrocera	*melas*	MEL005	Australia	Cairns, Queensland	Cue-lure	J. Royer	7.vi.2016
Bactrocera	*melas*	MEL006	Australia	Lockhart, Queensland	Cue-lure	J. Pritchard	6.v.2019
Bactrocera	*melas*	MEL007	Australia	Lockhart, Queensland	Cue-lure	J. Pritchard	6.v.2019
Bactrocera	*melas*	MEL008	Australia	Gladstone, Queensland	Cue-lure	J Royer	8.v.2014
Bactrocera	*melas*	MEL009	Australia	Gladstone, Queensland	Cue-lure	J Royer	6.i.2015
Bactrocera	*melas*	MEL010	Australia	Cairns, Queensland	Cue-lure	R. Allen	12.v.2015
Bactrocera	*melastomatos*	MLS001	Malaysia	Selangor, Malaysia	Cue-lure	W. Rattanapun	17-24.i.2016
Bactrocera	*mendosa*	MND001	Australia	Mataranka, Northern Territory	bred from *Pouteria sericea*	T. Angles	26.iii.1976
Bactrocera	*minax*	MIN001	China	Yichang, Hubei Province	ex citrus fruit	N. Changying	1.xii.2017
Bactrocera	*moluccensis*	BLH004	Papua New Guinea	Kiunga, Western Province	Cue-lure	L. Halling	3.vi.2015
Bactrocera	*moluccensis*	MOL002	Australia	Boigu, Torres Strait	Cue-lure	N. Gorton	29.v.2017
Bactrocera	*morobiensis*	MOR001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*mucronis*	MUC001	New Caledonia	La Foa, South Province	Cue-lure	J. Royer	10.x.2017
Bactrocera	*murrayi*	MUR001	Australia	Ugar island, Torres Strait	Methyl Eugenol	H. Newman	10.x.2017
Bactrocera	*murrayi*	MUR002	Australia	Mer island, Torres Strait	Methyl Eugenol	B. Kaigay	5.ix.2017
Bactrocera	*musae*	MUS002	Papua New Guinea	Madang Province	Methyl eugenol	M. Schutze	21.x.2014
Bactrocera	*musae*	MUS030	Papua New Guinea	Baitabag, Madang Province	Methyl Eugenol	R. Opasa	5-12.x.2016
Bactrocera	*mutabilis*	MUT001	Australia	Foley's Road, Bundaberg, Queensland	Biotrap	L. Senior	25.xi.2016
Bactrocera	*mutabilis*	MUT002	Australia	Bulburin National Park, Queensland	Isoeugenol	F. Strutt, M. Starkie	12.ii-3.iv.2019
Bactrocera	*near aglaiae*	AGL008	Australia	Julatten, Queensland	Zingerone	5-7.x.2016	M. Krosch
Bactrocera	*near musae*	END011	Papua New Guinea	Madang Province	Methyl eugenol	M. Schutze	23.x.2014
Bactrocera	*near quadrata*	NQD001	Australia	Iron Range, Queensland	ME	S. Cameron	7.iii.2015

Bactrocera	*near quadrata*	NQD002	Australia	Coen, Queensland	Cue-lure	J. Walker	15.xi.2016
Bactrocera	*neocheesmanae*	NCH002	Papua New Guinea	Madang Province	ME	M. Schutze	15.x.2014
Bactrocera	*neohumeralis*	NEO010	Australia	Brisbane, Queensland	Cue-lure	C. Maneckshana	10.xi.2015
Bactrocera	*neohumeralis*	NEO011	Australia	Lockhart River dump, Queensland	Zingerone	J. Royer	15.ix.2014
Bactrocera	*neohumeralis*	NEO013	PNG	PAU near Port Moresby, National Capital District	ME/CL	J. Royer	28.iii.2013
Bactrocera	*neohumeralis*	NEO014	Australia	Mackay, Queensland	Cue-lure	G. Green	14.v.2019
Bactrocera	*neohumeralis*	NEO015	Australia	Mackay, Queensland	Cue-lure	G. Green	14.v.2019
Bactrocera	*neohumeralis*	NEO016	Australia	Lockhart, Queensland	Cue-lure	J. Pritchard	6.v.2019
Bactrocera	*neohumeralis*	NEO017	Australia	Lockhart, Queensland	Cue-lure	J. Pritchard	11.iii.2019
Bactrocera	*neohumeralis*	NEO018	Australia	Lockhart, Queensland	Cue-lure	J. Pritchard	6.v.2019
Bactrocera	*neohumeralis*	NEO019	Australia	Lockhart River dump, Queensland	CL analogue HAL1	J. Royer	13.x.2014
Bactrocera	*neohumeralis*	NEO1	Australia	Brisbane, Queensland	Cue-lure	S. Collingwood	7.vii.2015
Bactrocera	*neohumeralis*	NEO2	Australia	Cairns, Queensland	Cue-lure	M. Berridge	6.vii.2015
Bactrocera	*neohumeralis*	NEO3	Australia	Cairns, Queensland	Cue-lure	M. Berridge	6.vii.2015
Bactrocera	*neohumeralis*	NEO013	Papua New Guinea	PAU near Port Moresby, National Capital District	Methyl Eugenol/Cue-lure	J. Royer	28.iii.2013
Bactrocera	*nigra*	NIG001	Australia	Foley's Road, Bundaberg, Queensland	Biotrap	L. Lowe	13.iii.2017
Bactrocera	*nigrescentis*	NGS001	Papua New Guinea	Keravat, East New Britain Province	Cue-lure	J. Royer	11.iv.2013
Bactrocera	*nigrovitatta*	NGV001	PNG	Bulolo	Ex *Solanum* sp.		14.vii.1980
Bactrocera	*oleae*	OLE003	Austria	Ex colony Vienna IAEA Seibersdorf	ex colony	S. Ahmad	20.iv.2016
Bactrocera	*oleae*	OLE004	Austria	Ex colony Vienna IAEA Seibersdorf	ex colony	S. Ahmad	20.iv.2016
Bactrocera	*opiliae*	CAC012	Australia	Malanda, Queensland	Methyl eugenol	S. Cameron	1.iii.2015
Bactrocera	*opiliae*	OPL001	Australia	Noonamah, Northern Territory	Methyl Eugenol	M. Finlay-Doney	24.xii.2009

Bactrocera	*opiliae*	OPL002	Australia	Noonamah, Northern Territory	Methyl Eugenol	M. Finlay-Doney	24.xii.2009
Bactrocera	*opiliae*	OPL003	Australia	Noonamah, Northern Territory	Methyl Eugenol	M. Finlay-Doney	24.xii.2009
Bactrocera	*opiliae*	OPL004	Australia	Noonamah, Northern Territory	Methyl Eugenol	M. Finlay-Doney	24.xii.2009
Bactrocera	*opiliae*	OPL005	Australia	Noonamah, Northern Territory	Methyl Eugenol	M. Finlay-Doney	24.xii.2009
Bactrocera	*opiliae*	OPL007	Australia	Noonamah, Northern Territory	Methyl Eugenol	M. Finlay-Doney	24.xii.2009
Bactrocera	*opiliae*	PAL005	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	4-7.iii.2015
Bactrocera	*pallida*	PAL003	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	4-7.iii.2015
Bactrocera	*pallida*	PAL006	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	4-7.iii.2015
Bactrocera	*pallida*	PAL007	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	4-7.iii.2015
Bactrocera	*pallida*	PAL008	Australia	Mackay, Queensland	Methyl Eugenol	C. Kemp	5.xii.2013
Bactrocera	*parabarringtoniae*	PRB001	Australia	Thursday Island, Torres Strait	Methyl Eugenol	H. Matthew	24.v.2017
Bactrocera	*parabarringtoniae*	PRB002	Australia	Ugar island, Torres Strait	Methyl Eugenol	H. Newman	10.x.2017
Bactrocera	*paramusae*	PAR001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	15.x.2014
Bactrocera	*paraxanthodes*	PAX001	New Caledonia	Pocquereux	Eug 3	J. Royer	14.xi.2017
Bactrocera	*passiflorae*	PAS002	Fiji	Koronivia Research Station, Nausori	CUE	A. Caucau	N/A
Bactrocera	*pendleburyi*	PBY001	Malaysia	Selangor	Zingerone	Mohd. Hannifah Yahaya	24-31.i.2016
Bactrocera	*peneobscura*	PNC001	Vanuatu	Havannah, Efate	Cue-lure	J. Royer	21.x.2017
Bactrocera	*peninsularis*	PEN001	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	27.i.2016
Bactrocera	*peninsularis*	PEN002	Australia	Cairns, Queensland	Cue-lure	L. Baker	17.v.2013
Bactrocera	*peninsularis*	PEN003	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	27.i.2015
Bactrocera	*peninsularis*	PEN004	Australia	Thursday Island, Torres Strait	Cue-lure	H. Matthew	25.v.2017

Bactrocera	*pepsialae*	BSA001	Solomon Islands	Solomon Islands	Methyl eugenol	S. Cowan	12.viii.2016
Bactrocera	*perkinsi*	PRK001	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	15.ii.2016
Bactrocera	*perkinsi*	PRK002	Australia	Cape York, Queensland	Cue-lure	T. Lifu	26.v.2017
Bactrocera	*phaleriae*	PHA001	Australia	Tolga Scrub, Queensland	Isoeugenol	J. Royer, M. Starkie	17-19.i.2018
Bactrocera	*propinqua*	PRO001	Thailand	Surat Thani	Cue-lure	W. Rattanapun	14-21.i.2016
Bactrocera	*quadrata*	QUD002	Australia	Brisbane, Queensland	Cue-lure	C. Maneckshana	7.i.2016
Bactrocera	*quadrata*	QUD003	Australia	Brisbane, Queensland	Cue-lure	C. Maneckshana	23.xii.2015
Bactrocera	*quadrata*	QUD004	Australia	Moreton, Queensland	Cue-lure	L. Bailey	26.xii.2015
Bactrocera	*recurrens*	REC003	Papua New Guinea	Wanang, III Swires Station Road, Madang Province	Cue-lure	M. Schutze	23-27.x.2014
Bactrocera	*recurrens*	REC001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*redunca*	ANF003	Papua New Guinea	Boigu, Torres Strait	Cue-lure	G. Banu	6.ix.2017
Bactrocera	*repanda*	RPD001	Papua New Guinea	Madang Province	Methyl Eugenol	M. Schutze	21.x.2014
Bactrocera	*repanda*	RPD002	Papua New Guinea	Madang Province	Cue-lure lure	R. Opasa, F. Phillip	29.x-4.xi.2016
Bactrocera	*resima*	ANF001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*resima*	RES002	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*resima*	RES003	Papua New Guinea	Baitabag, Madang Province	Cue-lure	M. Schutze	17-21.x.2014
Bactrocera	*romigae*	ROM001	Australia	Malanda, Queensland	Methyl Eugenol	S. Cameron	1.iii.2015
Bactrocera	*romigae*	ROM002	Australia	Lockhart River, Queensland	Methyl Eugenol	J. Pritchard	23.v.2016
Bactrocera	*rufescens*	RFN001	Australia	Mourilyan Harbour, Queensland	Cue-lure	A. Russell	18.viii.2016
Bactrocera	*rufofuscula*	RUF002	Australia	Lockhart River, Queensland	Cue-lure	J. Pritchard	21.xii.2015
Bactrocera	*russeola*	RSS001	Australia	Mourilyan Harbour, Queensland	Cue-lure	A. Russell	18.viii.2016
Bactrocera	*seguyi*	SEG001	Papua New Guinea	Madang Province	Methyl Eugenol	R. Opasa	5-12.x.2016

Bactrocera	*silvicola*	RUF003	Australia	Lake Eacham, Queensland	Zingerone	M. Krosch	8.xi.2016
Bactrocera	*silvicola*	SIL006	Australia	Mackay, Queensland	Cue-lure	C. Kemp	30.ix.2016
Bactrocera	*species*	DSP001	Papua New Guinea	Madang Province	Zingerone	R. Opasa, F. Philip	5-11.xi.2016
Bactrocera	*speculifera*	BLH002	Papua New Guinea	Kiunga, Western Province	Methyl eugenol	L. Halling	24.v.2015
Bactrocera	*speewahensis*	SPE001	Australia	Speewah, Queensland	Zingerone	C. Weymouth	27.x.2014
Bactrocera	*speewahensis*	SPE002	Australia	Portland Roads, Cape Weymouth, Queensland	Zingerone	J. Pritchard	22.vii.2013
Bactrocera	*strigata*	STR001	Australia	Gympie	Methyl Eugenol		7.x.1997
Bactrocera	*tapahensis*	TAP001	Malaysia	Selangor	Methyl Eugenol	Mohd. Hannifah Yahaya	10-17.i.2016
Bactrocera	*tenuifascia*	PAL004	Australia	Iron Range, Queensland	Methyl Eugenol	S. Cameron	26.iii.2015
Bactrocera	*tenuifascia*	TNF001	Australia	Melville Island, Tiwi Islands, Northern Territory	Methyl Eugenol	L. Halling	2.vi.2017
Bactrocera	*tenuifascia*	TNF002	Australia	Melville Island, Tiwi Islands, Northern Territory	Methyl Eugenol	L. Halling	2.vi.2017
Bactrocera	*terminaliae*	TER001	Papua New Guinea	Madang Province	Zingerone	R. Opasa, F. Philip	29.x-4.xi.2016
Bactrocera	*tigrina*	TIG001	Australia	Cairns, Queensland	Cue-lure	BQ Trapper	17.ii.2015
Bactrocera	*tinomiscii*	TIN001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*trilineola*	TRL003	Vanuatu	Havannah, Efate	Cue-lure	J. Royer	20.x.2017
Bactrocera	*trilineola*	TRL004	Vanuatu	Malafau, Efate	Cue-lure	J. Royer	12.x.2017
Bactrocera	*trivialis*	TRV002	Papua New Guinea	PAU near Port Moresby, National Capital District	Cue-lure	J. Royer	28.iii.2013
Bactrocera	*trivialis*	TRV003	Papua New Guinea	Baitabag, Madang Province	Cue-lure	M. Schutze	17-21.x.2014
Bactrocera	*tryoni*	TRY004	Australia	Brisbane, Queensland	Cue-lure	S. Collingwood	7.vii.2015

Bactrocera	*tryoni*	TRY006	Australia	Cairns, Queensland	Cue-lure	M. Berridge	6.vii.2015
Bactrocera	*tryoni*	TRY012	Australia	Buronga, New South Wales	Colony	N/A	24.ix.2015
Bactrocera	*tryoni*	TRY013	Australia	Buronga, New South Wales	Colony	N/A	24.ix.2015
Bactrocera	*tryoni*	TRY013	Australia	Buronga, New South Wales	ex colony		24.ix.2015
Bactrocera	*tryoni*	TRY018	New Caledonia	La Foa, South Province	Cue-lure	J. Royer	9.x.2017
Bactrocera	*tryoni*	TRY019	Australia	Mackay, Queensland	Cue-lure	G. Green	14.v.2019
Bactrocera	*tryoni*	TRY020	Australia	Mackay, Queensland	Cue-lure	G. Green	14.v.2019
Bactrocera	*tryoni*	TRY021	Australia	Coen, Queensland	Cue-lure	J. Walker	28.v.2019
Bactrocera	*tryoni*	TRY022	Australia	Coen, Queensland	Cue-lure	J. Walker	28.v.2019
Bactrocera	*tryoni*	TRY023	New Caledonia	Pocquereux	Cue-lure	J. Royer	28.xi.2017
Bactrocera	*tryoni*	TRY024	New Caledonia	Pocquereux	Cue-lure	J. Royer	28.xi.2017
Bactrocera	*tryoni*	TRY1	Australia	Brisbane, Queensland	Cue-lure	S. Collingwood	7.vii.2015
Bactrocera	*tryoni*	TRY3	Australia	Cairns, Queensland	Cue-lure	M. Berridge	6.vii.2015
Bactrocera	*tsuneonis*	TSU001	China	Yichang Hubei Province	Reared	N. Changying	1.vi.2017
Bactrocera	*tsuneonis*	TSU002	China	Yichang Hubei Province	Reared	N. Changying	1.vi.2017
Bactrocera	*umbrosa*	UMB002	Papua New Guinea	Madang Province	Methyl eugenol	M. Schutze	21.x.2015
Bactrocera	*umbrosa*	UMB006	East Timor	Dili	Methyl eugenol	G. Bellis	26.x.2015
Bactrocera	*umbrosa*	UMB011	Thailand	Phuket	Methyl eugenol	Y. Boontop	N/A
Bactrocera	*undescribed*	VFL001	Australia	Couchy Creek Nature Reserve, New South Wales	Zingerone	24-27.i.2018	V. Varghese
Bactrocera	*undescribed with medial spot*	BMS001	Malaysia	Labuan island	Zingerone	J. Royer	3.iv.2018
Bactrocera	*unitaeneola*	UNF001	Vanuatu	Havannah, Efate	Cue-lure	J. Royer	21.x.2017
Bactrocera	*unitaeneola*	UNF002	Vanuatu	Havannah, Efate	Cue-lure	J. Royer	21.x.2017
Bactrocera	*ustulata*	UST001	Papua New Guinea	PASI agricultural station near Vanimo, Sanduan Province	Melolure	S. Cowan	23-28.iv.2016

Bactrocera	*ustulata*	UST002	Papua New Guinea	PASI agricultural station near Vanimo, Sanduan Province	Melolure	S. Cowan	23-28.iv.2016
Bactrocera	*ustulata*	UST003	Papua New Guinea	Baitabag, Madang Province	Cue-lure	R. Opasa, F. Phillip	19-25.xi.2016
Bactrocera	*visenda*	Bvis1	Australia	Malanda, Queensland	Methyl eugenol	S. Cameron	1.iii.2015
Bactrocera	*visenda*	VIS002	Australia	Iron Range, Queensland	Methyl eugenol	S. Cameron	7.iii.2015
Bactrocera	*vulgaris*	VUL001	Papua New Guinea	Madang Province	Cue-lure	M. Schutze	21.x.2014
Bactrocera	*vulgaris*	VUL003	Papua New Guinea	Wanang, Madang Province	Cue-lure	M. Schutze	19-23.x.2014
Bactrocera	*xanthodes*	XAN001	Fiji	Koronivia Research Station, Nausori	ME	A. Caucau	20.ix.2016
Bactrocera	*xanthodes*	XAN002	Fiji	Koronivia Research Station, Nausori	ME	A. Caucau	20.ix.2016
Bactrocera	*yorkensis*	YOR002	Australia	Lockhart River dump, Queensland	Methyl Isoeugenol	J. Pritchard	28.iv.2014
Bactrocera	*yorkensis*	YOR003	Australia	Wangetti Beach, Queensland	Methyl Isoeugenol	L. Baker, E. Edwards	23.i.2013
Dacus	*absonifacies*	ABS001	Australia	Mt Hypipamee National Park, Queensland	Zingerone	L. Baker	23.iv.2013
Dacus	*aequalis*	AEQ001	Australia	Bundaberg, Queensland	Cue-lure	L. Senior	13.vi.2016
Dacus	*aequalis*	AEQ002	Australia	Brisbane, Queensland	Cue-lure	C. Maneckshana	7.i.2016
Dacus	*aneuvittatus*	ANE001	New Caledonia	La Foa, South Province	Zingerone	J. Royer	19-13.x.2017
Dacus	*axanus*	AXN001	Papua New Guinea	Vanimo Beach Hotel, Vanimo, Sandaun Province	Cue-lure	S. Cowan	29.iv.2016
Dacus	*axanus*	AXN002	Papua New Guinea	Vanimo Beach Hotel, Vanimo, Sandaun Province	Melolure	S. Cowan	28-29.iv.2016
Dacus	*bellulus*	BEL001	Australia	Mackay, Queensland	Cue-lure	C. Kemp	14.xii.2015
Dacus	*bellulus*	BEL002	Australia	Coen, Queensland	Cue-lure	BQ Trapper	21.ii.2016
Dacus	*hardyi*	HAR001	Australia	Cairns, Queensland	Cue-lure	R. Allen	1.iv.2015

Dacus	*impar*	IMP001	Papua New Guinea	Madang Province	Zingerone	R. Opasa, F. Phillip	5-11.xi.2016
Dacus	*longicornis*	LON002	Bangladesh	Dhaka	Zingerone	J. Royer	17.viii.2017
Dacus	*mayi*	DMY001	Papua New Guinea	Madang Province	Methyl eugenol	M. Schutze	23.x.2014
Dacus	*near pusillus*	PUS003	Australia	Lockhart River dump, Queensland	Zingerone	J. Royer	8.vii.2013
Dacus	*newmani*	NEW001	Australia	Carnarvon, Western Australia	CUE	B. Woods	10.iii.2016
Dacus	*newmani*	NEW002	Australia	Carnarvon, Western Australia	CUE	B. Woods	10.iii.2016
Dacus	*palmerensis*	PLM001	Australia	Cairns, Queensland	Cue-lure	R. Allen	12.v.2015
Dacus	*pusilis*	PUS005	Australia	Lockhart River, Queensland	Methyl Eugenol	J. Pritchard	15.ii.2016
Dacus	*pusillus*	PUS001	Australia	Coen, Queensland	Methyl eugenol	S. Templeton	20.i.2016
Dacus	*salamander*	SAL001	Australia	Coen, Queensland	Cue-lure	S. Templeton	20.i.2016
Dacus	*secamonae*	SEC001	Australia	Portland Roads, Cape Weymouth, Queensland	Zingerone	J. Pritchard	8.vii.2013
Dacus	*signatifrons*	SIG001	Australia	Brisbane, Queensland	Cue-lure	C. Maneckshana	26.i.2016
Dacus	*species*	DSP002	Papua New Guinea	Madang Province	Zingerone	R. Opasa	28.ix-4.x.2017
Dacus	*near pusillus*	PUS004	Australia	Lockhart River dump, Queensland	Zingerone	J. Royer	8.vii.2013
Zeugodacus	*atrifacies*	ATR001	Malaysia	Bentong, Selangor	Cue-lure	Mohd. Hannifah Yahaya	17-24-Jan-2016
Zeugodacus	*choristus*	CHO002	Australia	Julatten, Queensland	Cue-lure	M. Krosch	5-7.x.2016
Zeugodacus	*choristus*	CHO003	Australia	Julatten, Queensland	Cue-lure	M. Krosch	5-7.x.2016
Zeugodacus	*choristus*	CHO004	Australia	Julatten, Queensland	Cue-lure	M. Krosch	5-7.x.2016
Zeugodacus	*cilifer*	CIL001	Thailand	Surat Thani	Cue-lure	W. Rattanapun	14-21.i.2016
Zeugodacus	*cucumis*	CUM001	Australia	Ex colony Cairns QDAF, Queensland	Colony	T. Peek	11.i.2014
Zeugodacus	*cucumis*	CUM004	Australia	Ex colony Cairns QDAF, Queensland	Colony	T. Peek	11.i.2014

Zeugodacus	*cucurbitae*	CUC002	Malaysia	Shah Alam, Selangor	Cue-lure	Mohd. Hannifah Yahaya	18.ii.2016
Zeugodacus	*cucurbitae*	CUC004	Malaysia	Shah Alam, Selangor	CUE	Mohd. Hannifah Yahaya	18.ii.2016
Zeugodacus	*depressus*	DEP001	Korea	Gyeonsangbuk-do Bukhu-myeon, Daehyeon-ri	No lure details	K. M. Kwon	12.ix.2016
Zeugodacus	*depressus*	DEP002	Korea	Gyeonsangbuk-do Bukhu-myeon, Daehyeon-ri	No lure details	K. M. Kwon	12.ix.2016
Zeugodacus	*diversus*	DIV001	Bangladesh	Dhaka	Methyl Isoeugenol	J. Royer	22.iii.2017
Zeugodacus	*fallacis*	FAL002	Australia	Iron Range, Queensland	Cue-lure	S. Cameron	30.v.2015
Zeugodacus	*fallacis*	FAL004	Australia	Cairns, Queensland	Cue-lure	M. Berridge	19.i.2016
Zeugodacus	*hochii*	HOC002	Malaysia	Selangor	Cue-lure/Zingerone	Mohd. Hannifah Yahaya	28.i.2016
Zeugodacus	*hululangitae*	HUL001	Malaysia	Selangor	Cue-lure	Mohd. Hannifah Yahaya	24-31.i.2016
Zeugodacus	*incisus*	INC001	Thailand	Surat Thani	Cue-lure	W. Rattanapun	14-21.i.2017
Zeugodacus	*macrovittatus*	MAC003	Papua New Guinea	Wanang, Madang Province	Cue-lure	M. Schutze	19-23.x.2014
Zeugodacus	*neopallescentis*	NPL001	Papua New Guinea	Madang Province	Zingerone	R. Opasa, F. Phillip	17-21-Oct-2014
Zeugodacus	*platamus*	TAU001	Malaysia	Bentong, Selangor	CUE	Mohd. Hannifah Yahaya	6.iii.2016
Zeugodacus	*platamus*	TAU004	Malaysia	Bentong, Selangor	CUE	Mohd. Hannifah Yahaya	6.iii.2016
Zeugodacus	*reflexus*	REF001	Papua New Guinea	Keravat, East New Britain Province	Cue-lure	J. Royer	2.v.2013
Zeugodacus	*sandaracinus*	SAN001	Papua New Guinea	Madang Province	CUE	M. Schutze	21.x.2014

Zeugodacus	*scutellatus*	SCT001	China	Yunnan Province	From culture	Prof. Zhihong	19.iv.2016
Zeugodacus	*strigifinis*	BLH001	Papua New Guinea	Daru Island	Cue-lure	L. Halling	21.v.2015
Zeugodacus	*strigifinis*	STG002	Australia	Iron Range, Queensland	Cue-lure	S. Cameron	4-7.iii.2015
Zeugodacus	*tau*	TAU002	Malaysia	Bentong, Selangor	Cue-lure	Mohd. Hannifah Yahaya	6.iii.2016
Zeugodacus	*tau*	TAU003	Malaysia	Bentong, Selangor	Cue-lure	Mohd. Hannifah Yahaya	6.iii.2016
Zeugodacus	*triangularis*	TAG001	Papua New Guinea	Keravat, East New Britain Province	Cue-lure	J. Royer	28.iii.2013
Zeugodacus	*vinnulus*	VIN001	Malaysia	Selangor	Cue-lure	Mohd. Hannifah Yahaya	17.i.2016

Appendix 4: PCR mastermix recipes for amplification of the COI barcode, COI, COII, 16S, DDOSTs2, RPA2, EIF3L and POP4 loci. For difficult to amplify specimens, recipes were altered with increased $MgCl_2$, BSA and gDNA to a total reaction volume of 25µL. The sequencing PCR mastermix was the same for all specimens, with BigDye and gDNA volumes increased for difficult species to a total reaction volume of 20µL.

PCR mastermix		Sequencing PCR mastermix	
Reagent	Volume (µL)	Reagent	Volume (µL)
dH_20	7-7.5	dH_20	12.5
OneTaq hot start quick-load 2X master mix with standard buffer (New England BioLabs, USA)	12.5	BigDye Terminator v3.1 ready reaction mix (Applied Biosystems)	1
Primers	0.5	5X Sequencing buffer (Applied Biosystems)	3.5
$MgCl_2$ (50mM)	1-1.5	Primer (10pmol)	1
BSA (Bovine Serum Albumin) (10%)	1-2	gDNA	2-5
gDNA	2		

Appendix 5: Thermocycler protocols for multiple COI barcode primers, COI, COII, 16S, DDOSTs2, RPA2 and EIF3L loci. PCR protocols were altered for pinned specimens with an increase in the number of cycles. *Upon development of the nested primers (during the data collection phase of this project), the COI barcode primer pair and FFCOI primer pair were replaced with the forward of LCO1490-mod and the reverse HCO2198-mod (where a nested PCR was not necessary). #The remainder of the COI region (non-barcode region) was amplified using a second pair of primers.

COI barcode	FFCOI	LCO1490-mod*	Dac-COI-f	COI#	COII	16S	RPA2 and DDOSTs2	EIF3L	POP4
2 min @ 94ºC	2 min @ 94ºC	1 min @ 94ºC	1 min @ 94ºC	2 min @ 94ºC	1 min @ 94ºC	1 min @ 94ºC	1 min @ 94ºC	2 min @ 94ºC	2 min @ 94ºc
30 sec @ 94ºC	30 sec @ 94ºC	30 sec @ 94ºC	30 sec @ 94ºC	30 sec @ 96ºC	30 sec @ 94ºC	30 sec @ 94ºC	30 sec @ 94ºC	30 sec @ 94ºC	30 sec @ 94 ºC
30 sec @ 52ºC × 35	30 sec @ 52ºC × 33	30 sec @ 53ºC × 35	30 sec @ 51ºC × 35	30 sec @ 51ºC × 35	30 sec @ 52ºC × 32	30 sec @ 53ºC × 32	30 sec @ 53ºC × 35	30 sec @ 50ºC × 30	30 sec @ 50 ºC × 35
45 sec @ 68ºC	40 sec @ 68ºC	30 sec @ 68ºc	30 sec @ 68ºc	1 min @ 68ºC	45 sec @ 68ºC	45 sec @ 68ºC	30 sec @ 68ºC	45 sec @ 68ºC	1 min @ 68 ºC
2 min @ 68ºC	2 min @ 68ºC	5 min @ 68ºC	5 min @ 68ºC	5 min @ 68ºC	5 min @ 68ºC	5 min @ 68ºC	5 min @ 68ºC	5 min @ 68ºC	5 min @ 68 ºC

Appendix 6: Standard thermocycler protocol for BigDye sequencing reactions.

Thermocycler BigDye sequencing reaction protocol for all loci	
1 min @ 96ºC	
10 sec @ 96ºC	×30
5 sec @ 50ºC	
4 min @ 60ºC	

Appendix 7: Relevant publications that data from this thesis contributed to.

Insect science

Insect Science (2020) 27, 143–158, DOI 10.1111/1744-7917.12612

ORIGINAL ARTICLE

Development of internal COI primers to improve and extend barcoding of fruit flies (Diptera: Tephritidae: Dacini)

Matt N. Krosch[1,2,*], Francesca Strutt[1,2,*], Mark J. Blacket[3], Jana Batovska[3], Melissa Starkie[1], Anthony R. Clarke[1], Stephen L. Cameron[1,2,4] and Mark K. Schutze[1,2,5]

[1]*School of Earth, Environmental & Biological Sciences, Queensland University of Technology, Brisbane, Queensland, Australia;* [2]*Plant Biosecurity Co-operative Research Centre, Bruce, Australian Capital Territory, Australia;* [3]*Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia;* [4]*Department of Entomology, Purdue University, West Lafayette, Indiana, USA and* [5]*Department of Agriculture & Fisheries, Queensland Primary Industries Insect Collection (QDPC), Dutton Park, Queensland, Australia*

Abstract Accurate species-level identifications underpin many aspects of basic and applied biology; however, identifications can be hampered by a lack of discriminating morphological characters, taxonomic expertise or time. Molecular approaches, such as DNA "barcoding" of the *cytochrome c oxidase* (*COI*) gene, are argued to overcome these issues. However, nuclear encoding of mitochondrial genes (numts) and poor amplification success of suboptimally preserved specimens can lead to erroneous identifications. One insect group for which these molecular and morphological problems are significant are the dacine fruit flies (Diptera: Tephritidae: Dacini). We addressed these issues associated with COI barcoding in the dacines by first assessing several "universal" COI primers against public mitochondrial genome and numt sequences for dacine taxa. We then modified a set of four primers that more closely matched true dacine COI sequence and amplified two overlapping portions of the COI barcode region. Our new primers were tested alongside universal primers on a selection of dacine species, including both fresh preserved and decades-old dry specimens. Additionally, *Bactrocera tryoni* mitochondrial and nuclear genomes were compared to identify putative numts. Four numt clades were identified, three of which were amplified using existing universal primers. In contrast, our new primers preferentially amplified the "true" mitochondrial COI barcode in all dacine species tested. The new primers also successfully amplified partial barcodes from dry specimens for which full length barcodes were unobtainable. Thus we recommend these new primers be incorporated into the suites of primers used by diagnosticians and quarantine labs for the accurate identification of dacine species.

Key words *Bactrocera*; biosecurity; *Dacus*; minibarcodes; pseudogene; *Zeugodacus*

Introduction

Accurate identification of insect species is fundamental to both basic biological research and applied fields such as quarantine, surveillance and biosecurity. It is widely recognized that traditional morphological approaches are not always sufficient in all taxa or in all life stages, and a multitude of approaches that harness the discriminating power of molecular data have been developed to overcome these issues (Krosch *et al.*, 2017). The burgeoning field of DNA barcoding, in particular the use of the mitochondrial *cytochrome c oxidase subunit 1* (*COI*) gene as a standard molecular "barcode," has seen rapid cataloguing of molecular variation alongside

Correspondence: Matt N. Krosch, School of Earth, Environmental & Biological Sciences, Queensland University of Technology, PO Box 2434, Brisbane, Queensland 4001, Australia. Email: m.krosch@qut.edu.au

*These authors contributed equally to this work.

Appendix 8: Species included in this thesis, with molecular voucher codes provided and GenBank accession numbers of species where appropriate. Sequences that were not generated as a part of this project are indicated with a '*', sequences generated in this project are indicated by a '^', '-' indicates no sequence was used or generated for this project.

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Aedes aegypti	-	AY056597*	AY056597*	KC913582*	AF034475*	-	-	XM 001653812*	-
Aedes albopictus	-	MK575475*	MK575475*	MK57547*	MK575475*	-	-	XM 019670370*	-
Anastrepha fraterculus	AFR001	MF970718*	NC 034912*	DQ116549*	^	MF970594*	*	-	-
Anastrepha serpentina	ASR001	MF970719*	HQ677069*	AY573141*	^	^	*	-	-
Bactrocera abdonigella	ABD004	^	^	^	^	^	^	^	-
Bactrocera abdonigella	ABD005	^	^	^	^	^	^	^	-
Bactrocera aberrans	ABE001	^	^	^	^	^	^	^	-
Bactrocera aberrans	ABE002	^	^	^	^	^	^	^	-
Bactrocera abscondita	ABC001	MF970722^	^	^	^	MF970591^	MF970959^	MH135058^	-
Bactrocera abscondita	BRV002	MF970757^	^	^	^	MF970606^	MF970971^	MH135059^	-
Bactrocera absidata	ASD001	^	^	^	^	-	-	^	-
Bactrocera absidata	ASD002	^	^	^	^	-	-	^	-
Bactrocera absidata	ASD003	^	^	^	^	-	-	^	-
Bactrocera aeroginosa	ARG001	*	^	*	^	^	^	^	-
Bactrocera aeroginosa	ARG002	^	^	^	^	^	^	^	-
Bactrocera aglaiae	AGL001	^	^	^	^	^	^	^	-
Bactrocera aglaiae	AGL002	^	^	^	^	^	^	^	-
Bactrocera aglaiae	AGL003	^	^	^	^	^	^	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera aglaiae	AGL003	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL004	^	^	^	^	^	^	^	-
Bactrocera aglaiae	AGL005	^	^	^	^	-	-	^	-
Bactrocera aglaiae	AGL006	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL007	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL008	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL009	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL010	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL011	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL012	^	-	-	-	-	-	-	-
Bactrocera aglaiae	AGL013	^	-	-	-	-	-	-	-
Bactrocera aglaiae	MAC001	*	^	*	^	*	*	^	-
Bactrocera aglaiae	MAC004	^	^	^	^	-	-	^	-
Bactrocera albistrigata	ALB002	MF970728*	*	^	^	^	^	^	-
Bactrocera albistrigata	ALB003	MF970729*	^	*	^	MH134968^	^	-	-
Bactrocera allwoodi	ALL001	^	-	^	^	^	^	-	-
Bactrocera alyxiae	ALX001	*	*	*	^	*	^	^	-
Bactrocera alyxiae	ALX003	MF970746^	^	^	^	MF970598^	MF970964^	MH135062^	-
Bactrocera amplexiseta	AMP002	^	^	^	^	^	^	^	-
Bactrocera amplexiseta	AMP003	^	^	^	^	^	^	^	-
Bactrocera antigone	ANT001	*	*	*	^	^	^	^	-
Bactrocera antigone	ANT002	^	^	^	^	^	^	^	-
Bactrocera aquilonis	AQL001	^	^	*	^	^	^	^	^

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera aquilonis	AQL010	MH125301*	^	^	^	^	^	^	MH134918*
Bactrocera aquilonis	AQL015	MH125305*	-	^	^	^	^	MH135066*	MH134920*
Bactrocera aquilonis	AQL023	^	^	^	^	^	^	^	^
Bactrocera aquilonis	AQL024	^	^	^	^	^	^	^	^
Bactrocera aquilonis	AQL025	^	^	^	^	^	^	^	^
Bactrocera atramentata	ATM001	^	^	^	^	^	^	^	-
Bactrocera aurantiaca	AUR001	*	^	*	^	-	^	^	-
Bactrocera aurantiaca	AUR002	^	^	-	^	^	^	^	-
Bactrocera aurea	AEA001	^	^	^	^	-	^	^	-
Bactrocera aurea	AEA002	^	^	^	^	^	^	^	-
Bactrocera bancroftii	BAN002	MF970754^	^	^	^	MF970604^	MF970969^	MH135068*	-
Bactrocera bancroftii	BAN003	MF970755^	^	^	^	MF970605^	MF970970^	MH135069*	-
Bactrocera barringtoniae	BAR001	^	^	^	^	^	^	^	-
Bactrocera barringtoniae	BAR002	^	^	^	^	^	^	^	-
Bactrocera batemani	BAT001	*	*	*	^	^	^	-	-
Bactrocera bidentata	BID001	^	^	^	^	^	^	^	-
Bactrocera bidentata	BID002	^	^	^	-	^	^	^	-
Bactrocera breviaculeus	BRV004	MF970758^	^	^	^	MF970607^	MF970972^	MH135070*	-
Bactrocera breviaculeus	BRV007	^	^	^	^	^	^	^	-
Bactrocera brunnea	BRU001	^	^	^	^	^	^	^	-
Bactrocera bryoniae	BRY001	MF970760*	^	*	^	MF970609*	MF970974*	MH135071*	-
Bactrocera bryoniae	BRY005	MF970764*	^	^	^	^	^	^	-
Bactrocera cacuminata	CAC004	MF970768^	^	^	^	MF970612^	MF970977^	MH135074*	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera cacuminata	CAC006	MF970769^	^	^	^	MF970613^	MF970978^	MH135075*	-
Bactrocera cacuminata	CAC007	MF970770^	^	^	^	MF970614^	MF970979^	^	-
Bactrocera cacuminata	CAC008	MF970771^	^	^	^	MF970615^	MF970980^	^	-
Bactrocera cacuminata	CAC010	MF970772^	^	^	^	MF970616^	MF970981^	^	-
Bactrocera cacuminata	CAC011	MF970773^	^	^	^	MF970617^	MF970982^	^	-
Bactrocera cacuminata	CAC014	MF970774^	^	^	^	MF970618^	MF970983^	MH135076*	-
Bactrocera cacuminata	CAC015	MF970775^	^	^	^	MF970619^	MF970984^	^	-
Bactrocera caledoniensis	CLD001	MH125330*	^	^	^	MH134971*	MH135017*	MH135077*	-
Bactrocera calophylli	CAL001	^	^	^	^	^	^	-	-
Bactrocera cheesmanae	CHE001	-	*	*	^	-	^	^	-
Bactrocera cheesmanae	CHE003	^	^	^	^	-	^	^	-
Bactrocera consectorata	CON001	*	*	*	^	^	^	^	-
Bactrocera curreyi	CUR001	MH125335*	*	*	^	MH134972^	MH135019^	^	-
Bactrocera curvifera	CVF001	^	^	^	^	^	^	^	-
Bactrocera curvipennis	CRV001	MH125332*	^	^	^	MH134973*	MH135020*	MH135086*	^
Bactrocera curvipennis	CRV002	MH125333*	^	^	^	MH134974*	MH135021*	MH135087*	^
Bactrocera decurtans	DEC001	*	^	*	^	^	^	^	-
Bactrocera decurtans	DEC002	^	^	^	^	^	^	^	-
Bactrocera distincta	DIS001	^	^	^	^	^	^	^	-
Bactrocera dyscrita	DYS001	*	*	*	^	^	^	^	-
Bactrocera ebenea	EBE001	^	^	^	^	^	^	^	-
Bactrocera endiandrae	END002	MF970830^	^	^	^	MF970652^	MF9710156	MH135096*	^
Bactrocera endiandrae	END005	MF970833^	^	^	^	^	MF971016^	MH135097*	^

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera endiandrae	END006	MF970834^	^	^	^	MF970654^	MF971017^	^	^
Bactrocera endiandrae	END007	MF970835^	^	^	^	MF970655^	MF971018^	^	^
Bactrocera endiandrae	END008	MF970836^	^	^	^	^	MF971019^	^	-
Bactrocera endiandrae	END010	MF970837^	^	^	^	MF970657^	MF971020^	MH135098*	^
Bactrocera erubescentis	ERU001	^	^	*	^	^	^	^	-
Bactrocera erubescentis	ERU002	^	^	*	^	^	^	*	-
Bactrocera erubescentis	ERU003	^	^	*	^	^	^	*	-
Bactrocera erubescentis	ERU004	^	^	^	^	^	^	-	-
Bactrocera facialis	FAC006	^	^	^	^	^	^	^	-
Bactrocera facialis	FAC007	^	^	^	^	^	^	^	-
Bactrocera fagraea	FAG001	^	^	^	^	^	^	^	-
Bactrocera fagraea	FAG002	^	^	^	^	^	^	^	-
Bactrocera frauenfeldi	FRA006	MF970840*	*	^	^	MF970658*	MF971021*	MH135105*	-
Bactrocera frauenfeldi	FRA008	MF970842*	^	^	^	MF970660*	MF971023*	MH135107*	-
Bactrocera fulvicauda	BLH003	*	^	^	^	^	^	^	-
Bactrocera fulvicauda	FUL002	^	^	^	^	^	^	^	-
Bactrocera fulvifacies	FLF001	^	^	^	^	^	^	^	-
Bactrocera furvilineata	FUR001	*	^	*	^	^	^	^	-
Bactrocera furvilineata	FUR003	*	^	^	^	^	^	^	-
Bactrocera halfordiae	HAL001	^	^	^	^	^	^	^	-
Bactrocera halfordiae	HAL002	^	^	^	^	^	^	-	-
Bactrocera halfordiae	HAL003	^	^	^	^	-	-	-	-
Bactrocera humilis	HUM001	^	-	-	-	-	-	-	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera humilis	HUM002	^	-	-	-	-	-	-	-
Bactrocera humilis	HUM003	^	-	-	-	-	-	-	-
Bactrocera humilis	HUM004	^	-	-	-	-	-	-	-
Bactrocera humilis	HUM005	^	-	-	-	-	-	-	-
Bactrocera jarvisi	JAR007	MF970853*	^	^	^	MF970663*	MF971026*	MH135114*	-
Bactrocera jarvisi	JAR008	MF970854^	^	^	^	MF970664^	MF971027^	^	-
Bactrocera kraussi	KRA001	MF970858*	*	*	^	^	^	^	-
Bactrocera kraussi	KRA003	MF970860^	^	^	^	MF970668^	MF971031^	-	-
Bactrocera lampabilis	LAM001	^	^	^	^	-	^	-	-
Bactrocera laticaudus	LCD003	MF970862^	^	^	^	MF970669^	MF971032^	MH135119*	-
Bactrocera laticaudus	LCD004	MF970863^	^	^	^	MF970670^	MF971033^	-	-
Bactrocera laticaudus	LCD005	MF970864^	^	^	^	MF970671^	MF971034^	MH135120*	-
Bactrocera latilineola	LTL001	^	^	^	^	^	^	-	-
Bactrocera lineata	LIN002	*	*	*	^	^	^	^	-
Bactrocera lineata	LIN003	^	^	^	^	^	^	^	-
Bactrocera manskii	MAN001	MF970865*	^	*	^	MF970672*	MF971035*	MH135123*	-
Bactrocera manskii	MAN003	^	^	^	^	^	^	^	-
Bactrocera mayi	MAY002	MF970867*	-	^	-	-	-	-	-
Bactrocera mayi	MAY003	MH125356*	-	-	^	MH134979*	MH135026*	-	-
Bactrocera mayi	MAY004	^	^	^	^	^	^	^	-
Bactrocera mayi	MAY005	^	^	^	^	^	^	^	-
Bactrocera melanothoracica	MTH002	*	^	*	^	^	^	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera melanothoracica	UNI003	^	^	^	^	^	^	^	-
Bactrocera melas	BBR002	MH125316*	^	*	^	^	^	^	MH134940*
Bactrocera melas	MEL002	MH125357*	^	^	^	MH134980*	MH135027*	*	*
Bactrocera melas	MEL005	MH125360^	^	^	^	^	^	^	^
Bactrocera melas	MEL006	^	^	-	^	^	-	^	^
Bactrocera melas	MEL007	^	^	^	^	^	-	^	-
Bactrocera melas	MEL008	^	^	^	^	^	^	^	^
Bactrocera melas	MEL009	^	-	^	^	^	^	^	^
Bactrocera melas	MEL010	^	-	^	^	^	^	^	^
Bactrocera melastomatos	MLS001	*	^	^	^	-	-	-	-
Bactrocera minax	MIN001	MH125361*	^	^	^	MH134982*	MH135029*	MH135128*	-
Bactrocera moluccensis	BLH004	*	*	*	*	^	^	^	-
Bactrocera moluccensis	MOL002	^	^	^	^	^	^	^	-
Bactrocera morobiensis	MOR001	*	*	*	^	-	^	^	-
Bactrocera mucronis	MUC001	*	^	^	^	^	^	^	-
Bactrocera murrayi	MUR001	^	^	^	^	^	^	^	-
Bactrocera murrayi	MUR002	^	^	^	^	^	^	^	-
Bactrocera musae	MUS002	MF970868*	*	*	^	MF970674*	MF971037*	MH135129^	-
Bactrocera musae	MUS030	^	^	^	^	^	^	^	-
Bactrocera mutabilis	MUT001	^	^	^	^	^	^	^	-
Bactrocera mutabilis	MUT002	^	^	^	^	^	^	^	^

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera neocheesmanae	NCH002	*	*	*	^	^	^	^	-
Bactrocera neohumeralis	NEO010	^	^	^	^	^	^	^	^
Bactrocera neohumeralis	NEO011	^	^	^	^	^	^	^	^
Bactrocera neohumeralis	NEO013	^	^	^	^	^	^	^	^
Bactrocera neohumeralis	NEO014	^	^	^	^	^	^	^	^
Bactrocera neohumeralis	NEO015	^	-	^	^	^	^	^	^
Bactrocera neohumeralis	NEO016	^	^	^	^	^	^	^	^
Bactrocera neohumeralis	NEO017	^	^	^	^	^	^	^	^
Bactrocera neohumeralis	NEO018	^	-	^	^	^	^	^	^
Bactrocera neohumeralis	NEO019	^	-	-	-	-	-	-	-
Bactrocera neohumeralis	NEO1	MH125385*	*	*	^	*	MF971043*	^	MF970559*
Bactrocera neohumeralis	NEO2	MH125386*	*	^	^	*	MG252661*	^	MF970560*
Bactrocera neohumeralis	NEO3	MH125387*	*	^	^	*	MG252662*	^	MF970561*
Bactrocera nigra	NIG001	^	^	^	^	^	^	^	-
Bactrocera nigrescentis	NGS001	*	^	-	^	^	^	^	-
Bactrocera oleae	OLE003	MF970906*	^	*	-	MF970688*	MF971048*	MH135146*	-
Bactrocera oleae	OLE004	MF970907*	^	^	^	MF970689*	MF971049*	MH135147*	-
Bactrocera opiliae	CAC012	MH125327^	^	^	^	MH134983^	MH135030^	MH135148*	-
Bactrocera opiliae	OPL001	MF970910^	^	^	^	MF970690^	MF971050^	MH135150*	-
Bactrocera opiliae	OPL002	MF970911^	^	^	^	MF970691^	MF971051^	MH135151*	-
Bactrocera opiliae	OPL003	MF970912^	^	^	^	MF970692^	MH135033^	^	-
Bactrocera opiliae	OPL004	MF970913^	^	^	^	MF970693^	MF971052^	MH135152*	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera opiliae	OPL005	MF970914^	^	^	^	MF970694^	MF971053^	MH135153*	-
Bactrocera opiliae	OPL007	MF970915^	^	^	^	MF970695^	MF971054^	MH135154*	-
Bactrocera opiliae	PAL005	MF970917^	^	^	^	MF970697^	MF971056^	MH135155*	-
Bactrocera pallida	PAL003	MF970916^	^	^	^	MF970696^	MF971055^	MH135156*	-
Bactrocera pallida	PAL006	^	^	^	^	^	^	-	-
Bactrocera pallida	PAL007	^	^	^	^	^	^	^	-
Bactrocera pallida	PAL008	^	^	^	^	^	^	^	-
Bactrocera parabarringtoniae	PRB001	^	^	^	^	^	^	^	-
Bactrocera parabarringtoniae	PRB002	^	^	^	^	^	^	^	-
Bactrocera paramusae	PAR001	*	*	*	^	^	-	*	-
Bactrocera paraxanthodes	PAX001	^	^	^	^	-	^	^	-
Bactrocera passiflorae	PAS002	MH125395*	^	*	^	MH134987*	MH135035*	MH135158*	-
Bactrocera pendleburyi	PBY001	^	^	^	^	^	^	^	-
Bactrocera peneobscura	PNC001	^	^	^	^	^	^	^	-
Bactrocera peninsularis	PEN001	^	^	^	^	^	^	^	-
Bactrocera peninsularis	PEN002	^	^	^	^	^	^	^	-
Bactrocera peninsularis	PEN003	^	^	^	^	^	^	^	-
Bactrocera peninsularis	PEN004	^	^	^	^	^	^	^	-
Bactrocera pepsialae	BSA001	*	*	*	*	^	^	^	-
Bactrocera perkinsi	PRK001	*	*	*	^	^	^	^	-
Bactrocera perkinsi	PRK002	^	^	^	^	^	^	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera phaleriae	PHA001	*	-	-	-	-	-	^	-
Bactrocera propinqua	PRO001	^	^	^	^	^	^	^	-
Bactrocera quadrata	QUD002	MF970920*	-	^	-	-	-	-	-
Bactrocera quadrata	QUD003	MF970921^	^	^	^	MF970699^	MF971058^	MH135163*	-
Bactrocera quadrata	QUD004	^	^	^	^	^	MH135038^	MH135164*	-
Bactrocera recurrens	REC001	*	^	*	^	-	-	^	-
Bactrocera recurrens	REC003	^	^	^	^	-	^	^	-
Bactrocera redunca	ANF003	^	^	^	^	^	^	^	-
Bactrocera repanda	RPD001	*	*	*	^	*	*	^	-
Bactrocera repanda	RPD002	^	^	^	^	-	^	^	-
Bactrocera resima	ANF001	*	*	*	^	^	^	^	-
Bactrocera resima	RES002	*	*	*	^	*	*	^	-
Bactrocera resima	RES003	^	^	^	^	^	^	^	-
Bactrocera romigae	ROM001	*	*	*	^	-	^	^	-
Bactrocera romigae	ROM002	^	^	^	^	^	^	^	-
Bactrocera rufescens	RFN001	^	-	^	^	^	-	^	-
Bactrocera rufofuscula	RUF002	MF970923^	^	^	^	MF970701^	MF971060^	MH135167*	-
Bactrocera russeola	RSS001	^	^	^	^	^	^	^	-
Bactrocera seguyi	SEG001	^	-	^	^	^	^	^	-
Bactrocera silvicola	RUF003	MF970924^	^	^	^	MF970702^	MF971061^	MH135168*	-
Bactrocera silvicola	SIL006	^	-	^	^	^	^	^	-
Bactrocera sp. near *musae*	END011	^	^	^	^	^	^	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera sp. near *quadrata*	NQD001	*	*	*	^	^	^	^	-
Bactrocera sp. near *quadrata*	NQD002	^	^	^	^	^	^	^	-
Bactrocera sp. A	VFL001	^	-	-	-	-	-	-	-
Bactrocera sp. with medial spot	BMS001	^	^	^	^	^	^	^	-
Bactrocera sp.	DSP001	^	^	^	^	-	^	^	-
Bactrocera speculifera	BLH002	-	-	^	-	-	-	-	-
Bactrocera speewahensis	SPE001	^	^	^	^	^	^	^	-
Bactrocera speewahensis	SPE002	^	^	^	^	^	^	^	-
Bactrocera tapahensis	TAP001	*	^	^	^	^	-	^	-
Bactrocera tenuifascia	PAL004	MH125391^	^	^	^	MH134993^	MH135041^	MH135172*	-
Bactrocera tenuifascia	TNF001	^	^	^	^	^	^	^	-
Bactrocera tenuifascia	TNF002	^	^	^	^	^	^	^	-
Bactrocera terminaliae	TER001	^	^	^	^	^	^	^	-
Bactrocera tigrina	TIG001	^	^	^	^	-	^	^	-
Bactrocera tinomiscii	TIN001	*	*	^	-	-	-	^	-
Bactrocera trilineola	TRL003	MH125423*	^	^	^	MH134996*	MH135044*	MH135173*	-
Bactrocera trilineola	TRL004	MH125424*	^	^	^	MH134997*	MH135045*	MH135174*	-
Bactrocera trivialis	TRV002	MF970930^	^	^	^	^	^	^	-
Bactrocera trivialis	TRV003	^	^	^	^	^	^	^	-
Bactrocera tryoni	TRY004	MH125426*	^	^	^	MF970707*	MF971064*	MH135175*	MF970571*
Bactrocera tryoni	TRY006	MH125428*	^	^	^	^	-	^	MF970573*

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera tryoni	TRY012	MH125434*	^	^	^	MF970708*	MF971065*	MH135176*	^
Bactrocera tryoni	TRY013	MH125435*	^	^	^	MF970709*	MF971066*	MH135177*	-
Bactrocera tryoni	TRY018	^	^	^	^	^	^	^	-
Bactrocera tryoni	TRY019	^	^	^	^	^	^	^	^
Bactrocera tryoni	TRY020	^	^	^	^	^	-	^	^
Bactrocera tryoni	TRY021	^	^	^	^	^	^	^	^
Bactrocera tryoni	TRY022	^	^	^	^	^	^	^	-
Bactrocera tryoni	TRY023	^	-	^	^	^	^	^	^
Bactrocera tryoni	TRY024	^	-	^	^	^	^	^	^
Bactrocera tryoni	TRY1	MH125436*	*	^	^	*	MF971067*	^	MF970576*
Bactrocera tryoni	TRY3	MH125440*	*	*	^	MF970710*	MF971068*	-	MF970578*
Bactrocera tsuneonis	TSU001	MH125441*	*	^	^	MH134998*	^	MH135178*	-
Bactrocera tsuneonis	TSU002	MH125442^	^	^	^	MH134999*	^	MH135179*	-
Bactrocera umbrosa	UMB002	MF970943*	^	^	^	MF970711*	MF971069*	MH135182*	-
Bactrocera umbrosa	UMB006	MF970945*	^	^	^	MF970712*	MF971070*	MH135183*	-
Bactrocera umbrosa	UMB011	MF970949*	^	^	^	MF970713*	MF971071*	MH135184*	-
Bactrocera unitaeneola	UNF001	MH125445	-	-	-	MH135002	MH135048	MH135185	
Bactrocera unitaeneola	UNF002	MH125446	-	-	-	MH135003	MH135049	MH135186	
Bactrocera ustulata	UST001	^	^	^	^	^	^	^	-
Bactrocera ustulata	UST002	^	^	^	^	^	^	^	-
Bactrocera ustulata	UST003	*	^	^	^	-	-	^	-
Bactrocera visenda	Bvis1	MH125326*	^	^	^	-	MH135051*	^	-
Bactrocera visenda	VIS002	MF970950*	^	^	^	MF970714*	MH135050*	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Bactrocera vulgaris	VUL001	*	*	*	^	^	^	^	-
Bactrocera vulgaris	VUL003	^	^	^	^	^	^	^	-
Bactrocera xanthodes	XAN001	MF970951*	*	*	-	-	MF971072*	MH135187*	-
Bactrocera xanthodes	XAN002	MF970952*	^	^	^	MH135005*	MF971073*	MH135188*	-
Bactrocera yorkensis	YOR002	^	^	-	^	-	-	-	-
Bactrocera yorkensis	YOR003	^	^	^	^	-	-	^	-
Ceratitis capitata		HQ677177*	HQ677177*	DQ011889*	KM023501*	XM 004526055*	-	XM 004519009*	-
Ceratitis rosa	-	-	EU276697*	EU926795*	EU926927*	-	-	-	-
Dacus absonifacies	ABS001	MF970723*	^	^	^	^	^	MH135191*	-
Dacus aequalis	AEQ001	MF970724*	^	^	^	MF970592*	-	MH135192*	-
Dacus aequalis	AEQ002	MF970725*	^	*	^	MF970593*	-	MH135193*	-
Dacus aneuvittatus	ANE001	*	^	^	^	^	^	^	-
Dacus axanus	ANX001	MH125295*	*	*	^	^	^	^	-
Dacus axanus	ANX002	MF970748^	^	^	^	MF970600^	MF970966^	MH135195*	-
Dacus bellulus	BEL001	MH125322*	*	*	^	^	-	^	-
Dacus bellulus	BEL002	MH125323*	^	^	^	MH135007^	MH135053*	MH135196*	-
Dacus hardyi	HAR001	^	^	^	^	^	^	^	-
Dacus impar	IMP001	^	^	^	^	^	^	^	-
Dacus longicornis	LON002	^	^	^	^	-	^	^	-
Dacus mayi	DMY001	*	*	*	^	^	^	^	-
Dacus newmani	NEW001	MF970893*	^	^	^	MF970683*	MF971044*	MH135198*	-
Dacus newmani	NEW002	MF970894*	^	*	^	MF970684*	^	^	-
Dacus palmerensis	PLM001	^	^	^	^	^	^	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Dacus pusillus	PUS001	*	^	*	-	-	^	*	-
Dacus pusillus	PUS005	MH125402^	^	^	^	MH135010^	MH135052^	^	-
Dacus salamander	SAL001	*	*	*	^	^	^	^	-
Dacus secamoneae	SEC001	^	^	^	^	-	-	^	-
Dacus signatifrons	SIG001	MH125415*	^	*	^	^	-	^	-
Dacus sp.	DSP002	^	^	^	^	-	^	^	-
Dacus sp. near *pusillus*	PUS003	MH125401*	^	^	^	MH135009^	MH135055*	MH135199*	-
Dacus sp. near *pusillus*	PUS004	^	^	^	^	^	-	^	-
Drosophila melanogaster	-	GQ229519*	GQ229519*	EU493757*	MK106020*	-	-	NM 140296*	-
Drosophila suzukii	-	AB824771*	AB824771*	LN867083*	KU588141*	-	-	XM 017078959*	-
Musca autumnalis	-	KF919023*	KF919023*	JQ821710*	FJ025457*	-	-	-	-
Musca domestica	-	KY001857*	KY001857*	FJ153278*	AY123346*	XM 005179270*	-	XM 005191615*	-
Rhagoletis pomonella	-	-	-	EU109161*	AF177127*	-	-	-	-
Rhagoletis zephyria	-	MH998965*	-	EU109172*	U39440*	-	-	XM 017624350*	-
Toxonevra saltuum	-	KR262683*	KR262683*	KR262711*	KR262612*	-	-	-	-
Toxonevra superba	-	MG110882*	-	AY573181*	AY573138*	-	-	-	-
Zeugodacus atrifacies	ATR001	*	*	^	^	^	^	^	-
Zeugodacus choristus	CHO002	MF970788^	^	^	^	MF970628^	MF970993^	^	-
Zeugodacus choristus	CHO003	MF970789^	^	^	^	MF970629^	MF970994^	MH135200*	-
Zeugodacus choristus	CHO004	^	^	^	^	^	^	*	-
Zeugodacus cilifer	CIL001	^	^	^	^	-	^	^	-
Zeugodacus cucumis	CUM001	MF970807*	^	*	^	MF970638*	MF971002*	MH135202*	-
Zeugodacus cucumis	CUM004	MF970809*	^	^	^	MF970640*	MF971004*	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Zeugodacus cucurbitae	CUC002	MF970800*	^	*	^	MF970635*	MF970999*	MH135204*	-
Zeugodacus cucurbitae	CUC004	MF970802*	^	^	^	MF970636*	MF971000*	^	-
Zeugodacus depressus	DEP001	MF970810*	^	*	^	MF970641*	MF971005*	MH135206*	-
Zeugodacus depressus	DEP002	MF970811*	^	^	^	MH135012*	^	MH135207*	-
Zeugodacus diversus	DIV001	^	^	^	^	-	^	^	-
Zeugodacus fallacis	FAL002	MH125344*	*	*	^	^	^	^	-
Zeugodacus fallacis	FAL004	MH125345*	^	^	^	^	^	^	-
Zeugodacus hochii	HOC002	*	*	*	^	^	^	^	-
Zeugodacus hululangitae	HUL001	^	^	^	^	^	^	^	-
Zeugodacus incisus	INC001	^	^	^	^	-	-	^	-
Zeugodacus macrovittatus	MAC003	^	^	^	^	-	-	^	-
Zeugodacus neopallescentis	NPL001	-	^	^	^	-	-	^	-
Zeugodacus platumus	TAU001	MH125419*	^	*	^	MH135013*	MH135056*	^	-
Zeugodacus platumus	TAU004	MH125420*	^	^	^	^	-	MH135208*	-
Zeugodacus reflexus	REF001	*	^	^	^	^	^	^	-
Zeugodacus sandaracinus	SAN001	*	*	*	^	-	^	^	-
Zeugodacus scutellatus	SCT001	*	^	*	^	-	^	^	-
Zeugodacus strigifinis	BLH001	*	*	*	*	-	-	^	-
Zeugodacus strigifinis	STG002	MF970926^	^	^	^	MH135014*	MH135057*	MH135209*	-
Zeugodacus tau	TAU002	MF970927*	^	^	^	MF970704*	MF971062*	MH135210*	-
Zeugodacus tau	TAU003	MF970928*	^	^	^	MF970705*	MF971063*	^	-

Species	Code	Genbank accessions							
		COI	SCOI	COII	16S	RPA2	DDOSTs2	EIF3L	POP4
Zeugodacus triangularis	TAG001	^	^	^	^	-	^	^	-
Zeugodacus vinnulus	VIN001	*	*	*	^	-	^	^	-

Appendix 9: Raw data used in the lure response and host diet breadth ancestral state reconstructions. Relevant publications provided.

Species	Host breadth and reference	Lure and reference
Bactrocera abdonigella	No known record (Drew and Romig, 2013)	Cue-lure
Bactrocera aberrans	Oligophagous	Isoeugenol (Royer, 2015) (unpubl. trapping data)
Bactrocera abscondita	No known record (Royer and Hancock, 2012)	Cue Lure (Drew, 1989)
Bactrocera absidata	No known record	Cue Lure (M. Schutze unpubl. trapping data, 2014)
Bactrocera aeroginosa	Specialist (Hancock et al., 2000)	Cue Lure (Drew, 1989)
Bactrocera aglaiae	Monophagous (Hancock et al., 2000)	Zingerone (Fay, 2012)
Bactrocera albistrigata	Polyphagous (Allwood et al., 1999, USDA, 2019)	Cue Lure (Drew and Romig, 2013)
Bactrocera allwoodi	None recorded (Hancock et al 2000)	Cue Lure (Drew, 1989)
Bactrocera alyxiae	Specialist (Hancock et al., 2000)	Cue Lure (Drew, 1989)
Bactrocera amplexiseta	None recorded (Hancock et al 2000)	Methyl eugenol (Drew, 1989)
Bactrocera antigone	None recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Bactrocera aquilonis	Polyphagous (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera atramentata	Specialist (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera aurantiaca	None recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Bactrocera aurea	Specialist (Hancock et al., 2000)	Zingerone (Hancock and Drew, 2015)
Bactrocera bancroftii	Polyphagous (Drew and Romig, 2001, Novotny et al., 2005)	Methyl eugenol (Drew, 1989)
Bactrocera barringtoniae	Specialist (Leblanc et al., 2012)	Methyl Isoeugenol (Royer, 2015)
Bactrocera batemani	None recorded (Hancock et al 2000)	Methyl eugenol (Drew, 1989)
Bactrocera bidentata	Specialist (Hancock et al., 2000)	Isoeugenol (Royer et al., 2019)
Bactrocera breviaculeus	Monophagous (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera bryoniae	Polyphagous (Drew, 1989, Hancock et al., 2000, Leblanc et al., 2012)	Cue-lure (Drew, 1989)

Species	Host breadth and reference	Lure and reference
Bactrocera brunnea	None recorded (Hancock et al 2000)	None
Bactrocera cacuminata	Specialist (Hancock et al., 2000)	Methyl eugenol (Hancock et al 2000)
Bactrocera caledoniensis	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera calophylli	Monophagous (Drew and Romig, 2013, Leblanc et al., 2012)	None
Bactrocera cheesmanae	Specialist (Novotny et al., 2005, Leblanc et al., 2012)	Methyl eugenol (Hancock et al 2000)
Bactrocera consectorata	None recorded	Cue-lure (Drew, 1989)
Bactrocera curreyi	None recorded	Cue-lure (Drew, 1989)
Bactrocera curvifera	Specialist (Leblanc et al., 2012)	Methyl eugenol (Drew, 1989)
Bactrocera curvipennis	Polyphagous (Amice and Sales 1997, Leblanc et al., 2012)	Isoeugenol (Royer, 2019)
Bactrocera decurtans	Specialist (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera distincta	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera dyscrita	No known record	Cue-lure (Drew, 1989)
Bactrocera ebenea	No known record	Methyl eugenol
Bactrocera endiandrae	Polyphagous (Hancock et al., 2000)	Methyl eugenol
Bactrocera erubescentis	None recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Bactrocera facialis	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera fagraea	Monophagous (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera frauenfeldi	Polyphagous (Hancock et al., 2000, Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera fulvicauda	None recorded	Methyl eugenol
Bactrocera fulvifacies	Specialist (Leblanc et al., 2012)	Zingerone (Royer, 2019)
Bactrocera furvilineata	None recorded	Cue-lure (Huxham and Hancock, 2002)
Bactrocera halfordiae	Polyphagous (Hancock et al., 2000)	Isoeugenol (unpubl. trapping data, Royer 2015)
Bactrocera jarvisi	Polyphagous (Hancock et al., 2000, Leblanc et al., 2012)	Zingerone (Plant Health Australia, 2018)
Bactrocera kraussi	Polyphagous (Hancock et al., 2000)	Isoeugenol (Royer 2015)

Species	Host breadth and reference	Lure and reference
Bactrocera lampabilis	None recorded	Methyl eugenol (Royer et al., 2018)
Bactrocera laticaudus	Monophagous (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera latilineola	None recorded	Methyl eugenol (Drew, 1989)
Bactrocera lineata	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera manskii	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera mayi	Polyphagous (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera melanothoracica	None recorded	Methyl eugenol (Drew, 1989)
Bactrocera melas	Polyphagous	Cue-lure (Drew, 1989)
Bactrocera melastomatos	Monophagous (Allwood et al., 1999)	Cue-lure (Drew and Romig, 2013)
Bactrocera minax	Monophagous (White and Elson-Harris, 1992)	None
Bactrocera moluccensis	Specialist (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera morobiensis	None recorded	Cue-lure (Drew, 1989)
Bactrocera mucronis	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera murrayi	Polyphagous (Hancock et al., 2000, Leblanc et al., 2012)	Methyl isoeugenol (Royer 2015)
Bactrocera musae	Polyphagous (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera mutabilis	Specialist (Hancock et al., 2000)	Isoeugenol (unpubl. trapping data)
Bactrocera neocheesmanae	Polyphagous (Leblanc et al., 2012)	Methyl eugenol (Drew, 1989)
Bactrocera neohumeralis	Polyphagous (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera nigra	Oligophagous (Hancock et al., 2000)	None
Bactrocera nigrescentis	None recorded	Cue-lure (Drew, 1989)
Bactrocera oleae	Polyphagous (Athar, 2005)	None
Bactrocera opiliae	Polyphagous (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera pallida	Specialist (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera parabarringtoniae	Specialist (Hancock et al., 2000)	Methyl eugenol (Royer and Hancock, 2012)

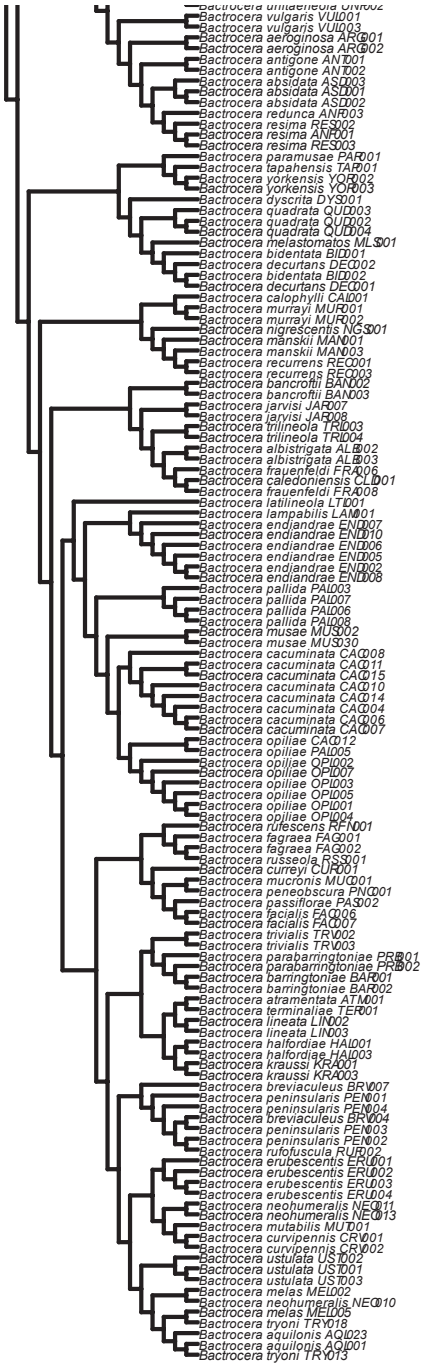
Species	Host breadth and reference	Lure and reference
Bactrocera paramusae	Oligophagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera paraxanthodes	Polyphagous (Amice and Sales, 1997, Leblanc et al., 2012)	Methyl eugenol (Amice and Sales, 1997)
Bactrocera passiflorae	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera pendleburyi	Polyphagous (Allwood et al., 1999)	Zingerone (QUT trapping data)
Bactrocera peneobscura	no known record (Drew and Romig, 2001)	Cue (Drew and Romig, 2001)
Bactrocera peninsularis	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera pepsialae	no known record (Drew and Romig, 2001)	Methyl eugenol (Drew, 1989)
Bactrocera perkinsi	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera phaleriae	Specialist (Hancock et al., 2000)	Isoeguenol (unpubl. trapping data)
Bactrocera propinqua	Monophagous (Drew and Hancock, 1994, Allwood et al., 1999)	Cue-lure (Drew, 1989)
Bactrocera quadrata	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera recurrens	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera redunca	Specialist (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera repanda	None recorded	Cue Lure (Huxham and Hancock, 2002)
Bactrocera resima	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera romigae	None recorded (Hancock et al., 2000)	Methyl eugenol (Drew and Hooper, 1981)
Bactrocera rufescens	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera rufofuscula	Polyphagous (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera russeola	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera seguyi	None recorded	Methyl eugenol (Drew, 1989)
Bactrocera silvicola	None recorded (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Bactrocera speculifera	None recorded	Methyl eugenol (Drew and Romig, 2013)
Bactrocera speewahensis	None recorded	Zingerone (Fay, 2012)
Bactrocera tapahensis	No known record (Drew and Romig 2013)	Methyl eugenol (Drew and Romig, 2013)

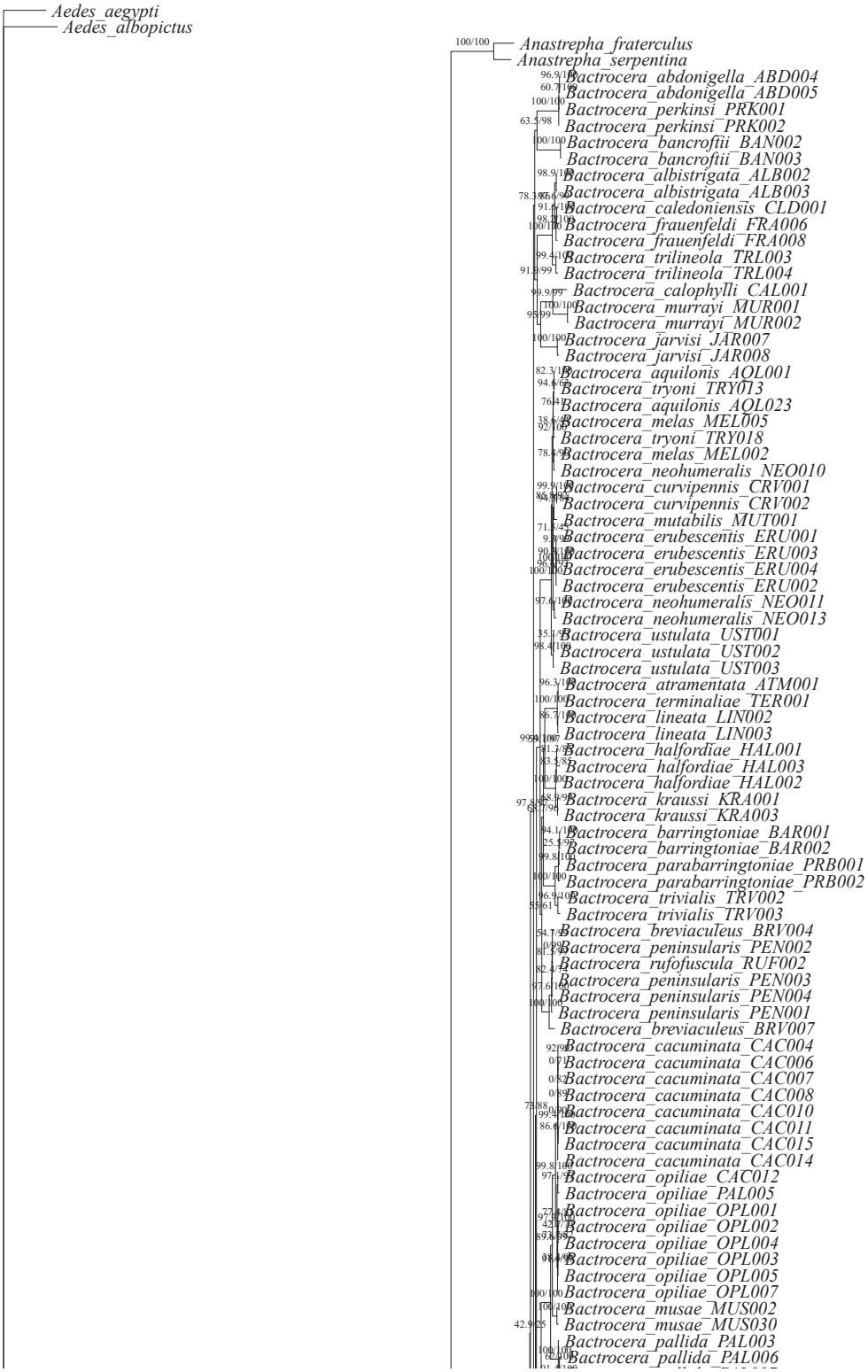
Species	Host breadth and reference	Lure and reference
Bactrocera tenuifascia	Monophagous (Hancock et al., 2000)	Methyl eugenol (Drew, 1989)
Bactrocera terminaliae	Polyphagous (Leblanc et al., 2012)	Zingerone (M. Schutze unpubl. trapping data)
Bactrocera tigrina	Monophaous (Hancock et al., 2000)	None
Bactrocera tinomiscii	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera trilineola	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera trivialis	Polyphagous (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera tryoni	Polyphagous (Hancock et al., 2000, Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Bactrocera tsuneonis	Monophagous (White and Elson-Harris, 1992)	None
Bactrocera umbrosa	Monophagous (Allwood et al., 1999, Leblanc et al., 2012)	Methyl eugenol (Drew, 1989)
Bactrocera unitaeneola	No known record (Drew and Romig 2001)	Cue-lure (Drew and Romig, 2001)
Bactrocera ustulata	None	Cue-lure (Drew, 1989)
Bactrocera visenda	Monophagous (Hancock et al., 2000)	Dihydroeugenol (Royer, 2016)
Bactrocera vulgaris	None	Cue-lure (Huxham and Hancock, 2002)
Bactrocera xanthodes	Polyphagous (Leblanc et al., 2012)	Methyl isoeugenol (Royer, 2019)
Bactrocera yorkensis	None recorded (Hancock et al., 2000)	Methyl Isoeugenol (Royer, 2015)
Dacus absonifacies	Specialist (Hancock et al., 2000)	Zingerone (Drew, 1989)
Dacus aequalis	Specialist (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Dacus aneuvittatus	Specialist (Leblanc et al., 2012)	Zingerone (Royer, 2019)
Dacus axanus	Oligophagous (Hancock et al., 2000, Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Dacus bellulus	None	Cue-lure (Drew, 1989)
Dacus hardyi	Specialist (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Dacus impar	None	Zingerone (QUT unpubl. collections)
Dacus longicornis	Oligophagous (Hardy, 1973)	Cue-lure (Drew, 1989)
Dacus mayi	None	Cue-lure (Drew, 1989)

Species	Host breadth and reference	Lure and reference
Dacus newmani	No host recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Dacus palmerensis	No host recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Dacus pusillus	No host recorded (Hancock et al 2000)	Methyl eugenol (Drew, 1989)
Dacus salamander	No host recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Dacus secamoneae	Specialist (Hancock et al., 2000)	Zingerone (Royer, 2015)
Dacus signatifrons	No host recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Zeugodacus atrifacies	no known record (Drew et al 2007)	Cue-lure (Drew and Romig, 2013)
Zeugodacus choristus	Specialist (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Zeugodacus cilifer	Specialist (Drew and Romig, 2013)	Cue-lure (Drew and Romig, 2013)
Zeugodacus cucumis	Polyphagous (Hancock et al., 2000)	Cue-lure (Drew, 1989)
Zeugodacus cucurbitae	Polyphagous (Allwood et al., 1999, Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Zeugodacus depressus	Oligophagous (Allwood et al., 1999, Drew and Romig, 2013)	Cue-lure (Drew, 1989)
Zeugodacus diversus	Oligophagous (White and Elson-Harris, 1992, Allwood et al., 1999)	Weakly attracted to Methyl eugenol
Zeugodacus fallacis	No host recorded (Hancock et al 2000)	Cue-lure (Drew, 1989)
Zeugodacus hochii	Oligophagous (Allwood et al., 1999, Vargas et al., 2015)	Cue-lure (Drew and Romig, 2013)
Zeugodacus hululangitae	No known record (Drew and Romig, 2013)	Cue-lure (Drew and Romig, 2013)
Zeugodacus incisus	No known host plant (Hancock and Drew, 2017)	Cue-lure (Drew, 1989)
Zeugodacus macrovittatus	None recorded	Cue-lure (Drew, 1989)
Zeugodacus neopallescentis	None recorded (Hancock and Drew, 2018)	Cue-lure (Drew, 1989)
Zeugodacus platamus	No known record (Drew and Romig, 2013)	Cue-lure (Drew and Romig, 2013)
Zeugodacus reflexus	None recorded	Cue-lure (Drew, 1989)
Zeugodacus sandaracinus	None recorded	No known record
Zeugodacus scutellatus	Oligophagous (Ito, 1983, Allwood et al., 1999)	Cue-lure (Drew and Romig, 2013)
Zeugodacus strigifinis	Specialist (Leblanc et al., 2012)	Cue-lure (Drew, 1989)

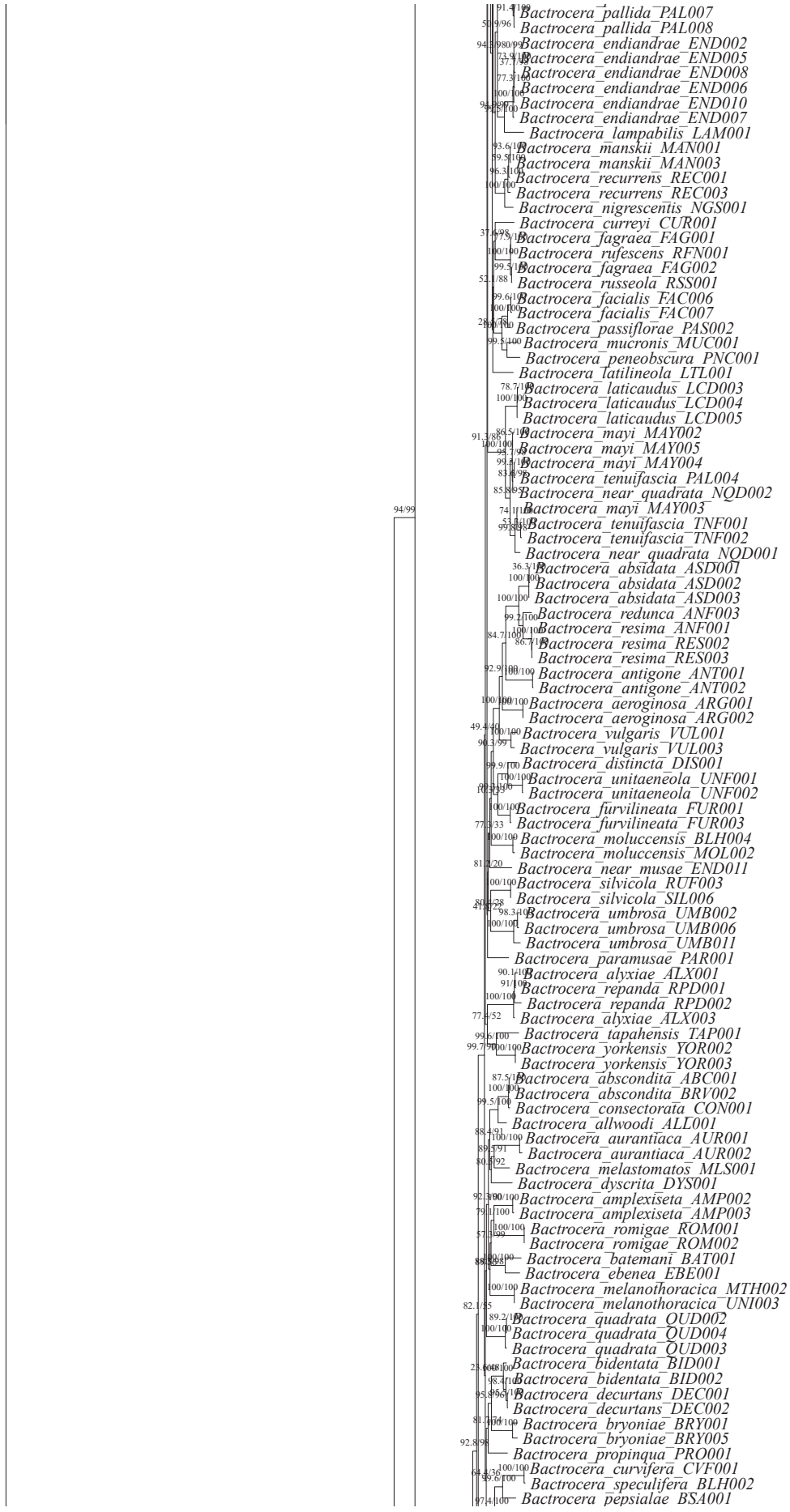
Species	Host breadth and reference	Lure and reference
Zeugodacus tau	Polyphagous (USDA, 2016)	Cue-lure (Drew and Romig, 2013)
Zeugodacus triangularis	Specialist (Leblanc et al., 2012)	Cue-lure (Drew, 1989)
Zeugodacus vinnulus	No known record (Hancock and Drew, 2017)	Cue-lure (Drew and Romig, 2013)

Appendix 10 (continued next page): Neighbour-Joining tree of the Dacini based on minimum evolution methods, reconstructed from six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L.


Bactrocera vulgaris VUL001
Bactrocera vulgaris VUL003
Bactrocera aeroginosa ARG001
Bactrocera aeroginosa ARG002
Bactrocera antigone ANT001
Bactrocera antigone ANT002
Bactrocera absidata ASD003
Bactrocera absidata ASD001
Bactrocera absidata ASD002
Bactrocera reduncа ANR003
Bactrocera resima RES002
Bactrocera resima ANR001
Bactrocera resima RES003
Bactrocera paramusae PAR001
Bactrocera tapahensis TAR001
Bactrocera yorkensis YOR002
Bactrocera yorkensis YOR003
Bactrocera dyscrita DYS001
Bactrocera quadrata QUD003
Bactrocera quadrata QUD002
Bactrocera quadrata QUD004
Bactrocera melastomatos MLS001
Bactrocera bidentata BID001
Bactrocera decurtans DEC002
Bactrocera bidentata BID002
Bactrocera decurtans DEC001
Bactrocera calophylli CAL001
Bactrocera murrayi MUR001
Bactrocera murrayi MUR002
Bactrocera nigrescentis NGS001
Bactrocera manskii MAN001
Bactrocera manskii MAN003
Bactrocera recurrens REC001
Bactrocera recurrens REC003
Bactrocera bancroftii BAN002
Bactrocera bancroftii BAN003
Bactrocera jarvisi JAR007
Bactrocera jarvisi JAR008
Bactrocera trilineola TRI003
Bactrocera trilineola TRI004
Bactrocera albistrigata ALB002
Bactrocera albistrigata ALB003
Bactrocera frauenfeldi FRA006
Bactrocera caledoniensis CLD001
Bactrocera frauenfeldi FRA008
Bactrocera latilineola LTI001
Bactrocera lampabilis LAM001
Bactrocera endiandrae END007
Bactrocera endiandrae END010
Bactrocera endiandrae END006
Bactrocera endiandrae END005
Bactrocera endiandrae END002
Bactrocera endiandrae END008
Bactrocera pallida PAL003
Bactrocera pallida PAL007
Bactrocera pallida PAL006
Bactrocera pallida PAL008
Bactrocera musae MUS002
Bactrocera musae MUS030
Bactrocera cacuminata CAC008
Bactrocera cacuminata CAC011
Bactrocera cacuminata CAC015
Bactrocera cacuminata CAC010
Bactrocera cacuminata CAC014
Bactrocera cacuminata CAC004
Bactrocera cacuminata CAC006
Bactrocera cacuminata CAC007
Bactrocera opiliae CAC012
Bactrocera opiliae PAL005
Bactrocera opiliae OPI002
Bactrocera opiliae OPI007
Bactrocera opiliae OPI003
Bactrocera opiliae OPI005
Bactrocera opiliae OPI001
Bactrocera opiliae OPI004
Bactrocera rufescens RFN001
Bactrocera fagraea FAG001
Bactrocera fagraea FAG002
Bactrocera russeola RSS001
Bactrocera curreyi CUR001
Bactrocera mucronis MUC001
Bactrocera peneobscura PNC001
Bactrocera passiflorae PAS002
Bactrocera facialis FAC006
Bactrocera facialis FAC007
Bactrocera trivialis TRV002
Bactrocera trivialis TRV003
Bactrocera parabarringtoniae PRB001
Bactrocera parabarringtoniae PRB002
Bactrocera barringtoniae BAR001
Bactrocera barringtoniae BAR002
Bactrocera atramentata ATM001
Bactrocera terminaliae TER001
Bactrocera lineata LIN002
Bactrocera lineata LIN003
Bactrocera halfordiae HAI001
Bactrocera halfordiae HAI003
Bactrocera kraussi KRA001
Bactrocera kraussi KRA003
Bactrocera breviaculeus BRV007
Bactrocera peninsularis PEN001
Bactrocera peninsularis PEN004
Bactrocera breviaculeus BRV004
Bactrocera peninsularis PEN003
Bactrocera peninsularis PEN002
Bactrocera rufofuscula RUF002
Bactrocera erubescentis ERU001
Bactrocera erubescentis ERU002
Bactrocera erubescentis ERU003
Bactrocera erubescentis ERU004
Bactrocera neohumeralis NEO011
Bactrocera neohumeralis NEO013
Bactrocera mutabilis MUT001
Bactrocera curvipennis CRV001
Bactrocera curvipennis CRV002
Bactrocera ustulata UST002
Bactrocera ustulata UST001
Bactrocera ustulata UST003
Bactrocera melas MEL002
Bactrocera neohumeralis NEO010
Bactrocera melas MEL005
Bactrocera tryoni TRY018
Bactrocera aquilonis AQI023
Bactrocera aquilonis AQI001
Bactrocera tryoni TRY013



Appendix 11 (continued next page): Proportionally linked ML phylogenetic tree of the Dacini reconstructed from seven partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L. Maximum likelihood SH-aLRT method was used to calculate branch supports with ultra-fast bootstrap values shown at the nodes; SH-aLRT/UFBoot.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Bactrocera_pallida_PAL008
Bactrocera_endiandrae_END002
Bactrocera_endiandrae_END005
Bactrocera_endiandrae_END008
Bactrocera_endiandrae_END006
Bactrocera_endiandrae_END010
Bactrocera_endiandrae_END007
Bactrocera_lampabilis_LAM001
Bactrocera_manskii_MAN001
Bactrocera_manskii_MAN003
Bactrocera_recurrens_REC001
Bactrocera_recurrens_REC003
Bactrocera_nigrescentis_NGS001
Bactrocera_curreyi_CUR001
Bactrocera_fagraea_FAG001
Bactrocera_rufescens_RFN001
Bactrocera_fagraea_FAG002
Bactrocera_russeola_RSS001
Bactrocera_facialis_FAC006
Bactrocera_facialis_FAC007
Bactrocera_passiflorae_PAS002
Bactrocera_mucronis_MUC001
Bactrocera_peneobscura_PNC001
Bactrocera_latilineola_LTL001
Bactrocera_laticaudus_LCD003
Bactrocera_laticaudus_LCD004
Bactrocera_laticaudus_LCD005
Bactrocera_mayi_MAY002
Bactrocera_mayi_MAY005
Bactrocera_mayi_MAY004
Bactrocera_tenuifascia_PAL004
Bactrocera_near_quadrata_NQD002
Bactrocera_mayi_MAY003
Bactrocera_tenuifascia_TNF001
Bactrocera_tenuifascia_TNF002
Bactrocera_near_quadrata_NQD001
Bactrocera_absidata_ASD001
Bactrocera_absidata_ASD002
Bactrocera_absidata_ASD003
Bactrocera_redunca_ANF003
Bactrocera_resima_ANF001
Bactrocera_resima_RES002
Bactrocera_resima_RES003
Bactrocera_antigone_ANT001
Bactrocera_antigone_ANT002
Bactrocera_aeroginosa_ARG001
Bactrocera_aeroginosa_ARG002
Bactrocera_vulgaris_VUL001
Bactrocera_vulgaris_VUL003
Bactrocera_distincta_DIS001
Bactrocera_unitaeneola_UNF001
Bactrocera_unitaeneola_UNF002
Bactrocera_furvilineata_FUR001
Bactrocera_furvilineata_FUR003
Bactrocera_moluccensis_BLH004
Bactrocera_moluccensis_MOL002
Bactrocera_near_musae_END011
Bactrocera_silvicola_RUF003
Bactrocera_silvicola_SIL006
Bactrocera_umbrosa_UMB002
Bactrocera_umbrosa_UMB006
Bactrocera_umbrosa_UMB011
Bactrocera_paramusae_PAR001
Bactrocera_alyxiae_ALX001
Bactrocera_repanda_RPD001
Bactrocera_repanda_RPD002
Bactrocera_alyxiae_ALX003
Bactrocera_tapahensis_TAP001
Bactrocera_yorkensis_YOR002
Bactrocera_yorkensis_YOR003
Bactrocera_abscondita_ABC001
Bactrocera_abscondita_BRV002
Bactrocera_consectorata_CON001
Bactrocera_allwoodi_ALL001
Bactrocera_aurantiaca_AUR001
Bactrocera_aurantiaca_AUR002
Bactrocera_melastomatos_MLS001
Bactrocera_dyscrita_DYS001
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Bactrocera_romigae_ROM001
Bactrocera_romigae_ROM002
Bactrocera_batemani_BAT001
Bactrocera_ebenea_EBE001
Bactrocera_melanothoracica_MTH002
Bactrocera_melanothoracica_UNI003
Bactrocera_quadrata_QUD002
Bactrocera_quadrata_QUD004
Bactrocera_quadrata_QUD003
Bactrocera_bidentata_BID001
Bactrocera_bidentata_BID002
Bactrocera_decurtans_DEC001
Bactrocera_decurtans_DEC002
Bactrocera_bryoniae_BRY001
Bactrocera_bryoniae_BRY005
Bactrocera_propinqua_PRO001
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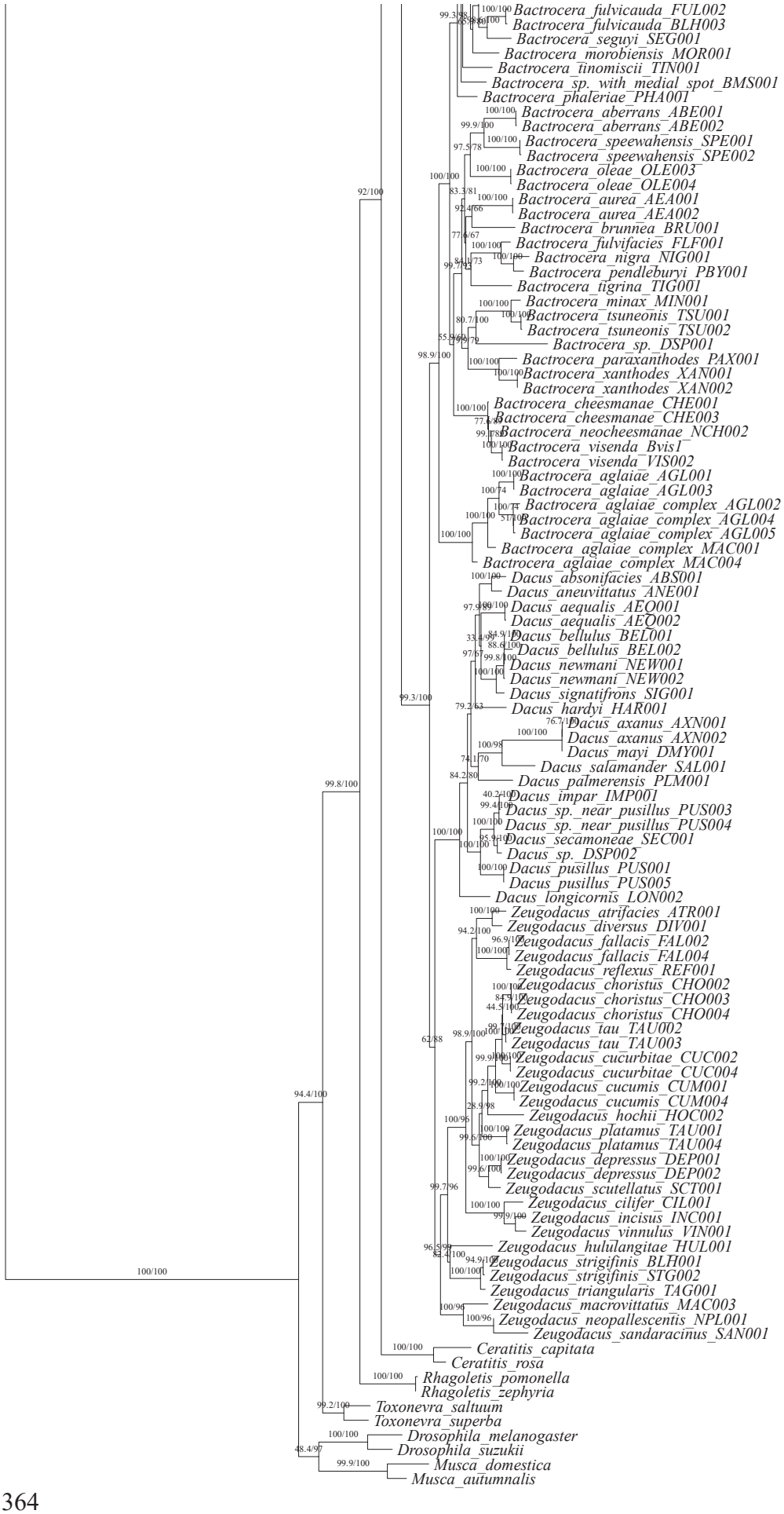
Bactrocera_speculifera_BLH002
Bactrocera_pepsialae_BSA001
94/99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Bactrocera_fulvicauda_BLH003
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Bactrocera_morobiensis_MOR001
Bactrocera_tinomiscii_TIN001
Bactrocera_sp._with_medial_spot_BMS001
Bactrocera_phaleriae_PHA001
Bactrocera_aberrans_ABE001
Bactrocera_aberrans_ABE002
Bactrocera_speewahensis_SPE001
Bactrocera_speewahensis_SPE002
Bactrocera_oleae_OLE003
Bactrocera_oleae_OLE004
Bactrocera_aurea_AEA001
Bactrocera_aurea_AEA002
Bactrocera_brunnea_BRU001
Bactrocera_fulvifacies_FLF001
Bactrocera_nigra_NIG001
Bactrocera_pendleburyi_PBY001
Bactrocera_tigrina_TIG001
Bactrocera_minax_MIN001
Bactrocera_tsuneonis_TSU001
Bactrocera_tsuneonis_TSU002
Bactrocera_sp._DSP001
Bactrocera_paraxanthodes_PAX001
Bactrocera_xanthodes_XAN001
Bactrocera_xanthodes_XAN002
Bactrocera_cheesmanae_CHE001
Bactrocera_cheesmanae_CHE003
Bactrocera_neocheesmanae_NCH002
Bactrocera_visenda_Bvis1
Bactrocera_visenda_VIS002
Bactrocera_aglaiae_AGL001
Bactrocera_aglaiae_AGL003
Bactrocera_aglaiae_complex_AGL002
Bactrocera_aglaiae_complex_AGL004
Bactrocera_aglaiae_complex_AGL005
Bactrocera_aglaiae_complex_MAC001
Bactrocera_aglaiae_complex_MAC004
Dacus_absonifacies_ABS001
Dacus_aneuvittatus_ANE001
Dacus_aequalis_AEQ001
Dacus_aequalis_AEQ002
Dacus_bellulus_BEL001
Dacus_bellulus_BEL002
Dacus_newmani_NEW001
Dacus_newmani_NEW002
Dacus_signatifrons_SIG001
Dacus_hardyi_HAR001
Dacus_axanus_AXN001
Dacus_axanus_AXN002
Dacus_mayi_DMY001
Dacus_salamander_SAL001
Dacus_palmerensis_PLM001
Dacus_impar_IMP001
Dacus_sp._near_pusillus_PUS003
Dacus_sp._near_pusillus_PUS004
Dacus_secamoneae_SEC001
Dacus_sp._DSP002
Dacus_pusillus_PUS001
Dacus_pusillus_PUS005
Dacus_longicornis_LON002
Zeugodacus_atrifacies_ATR001
Zeugodacus_diversus_DIV001
Zeugodacus_fallacis_FAL002
Zeugodacus_fallacis_FAL004
Zeugodacus_reflexus_REF001
Zeugodacus_choristus_CHO002
Zeugodacus_choristus_CHO003
Zeugodacus_choristus_CHO004
Zeugodacus_tau_TAU002
Zeugodacus_tau_TAU003
Zeugodacus_cucurbitae_CUC002
Zeugodacus_cucurbitae_CUC004
Zeugodacus_cucumis_CUM001
Zeugodacus_cucumis_CUM004
Zeugodacus_hochii_HOC002
Zeugodacus_platamus_TAU001
Zeugodacus_platamus_TAU004
Zeugodacus_depressus_DEP001
Zeugodacus_depressus_DEP002
Zeugodacus_scutellatus_SCT001
Zeugodacus_cilifer_CIL001
Zeugodacus_incisus_INC001
Zeugodacus_vinnulus_VIN001
Zeugodacus_hululangitae_HUL001
Zeugodacus_strigifinis_BLH001
Zeugodacus_strigifinis_STG002
Zeugodacus_triangularis_TAG001
Zeugodacus_macrovittatus_MAC003
Zeugodacus_neopallescentis_NPL001
Zeugodacus_sandaracinus_SAN001
Ceratitis_capitata
Ceratitis_rosa
Rhagoletis_pomonella
Rhagoletis_zephyria
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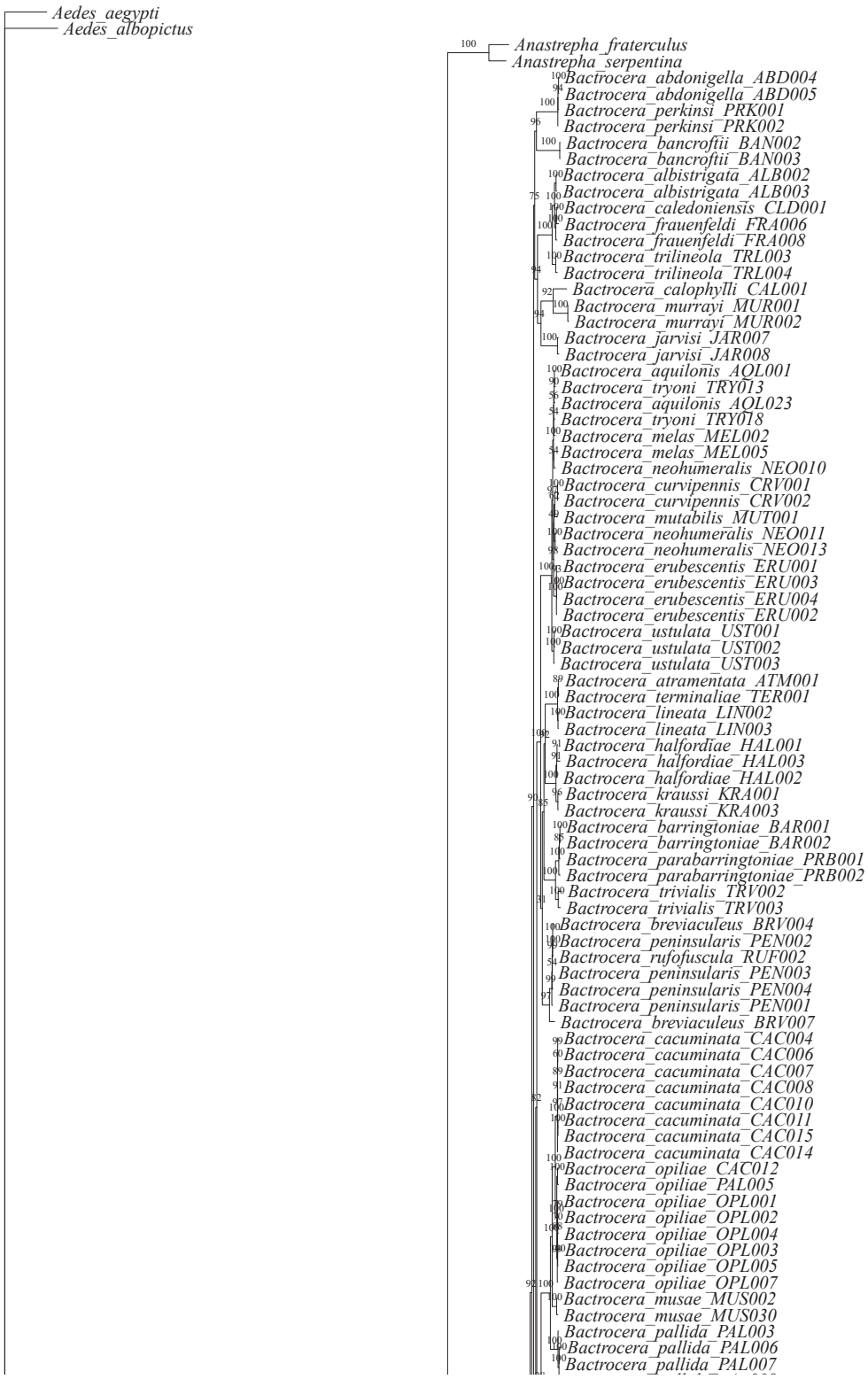
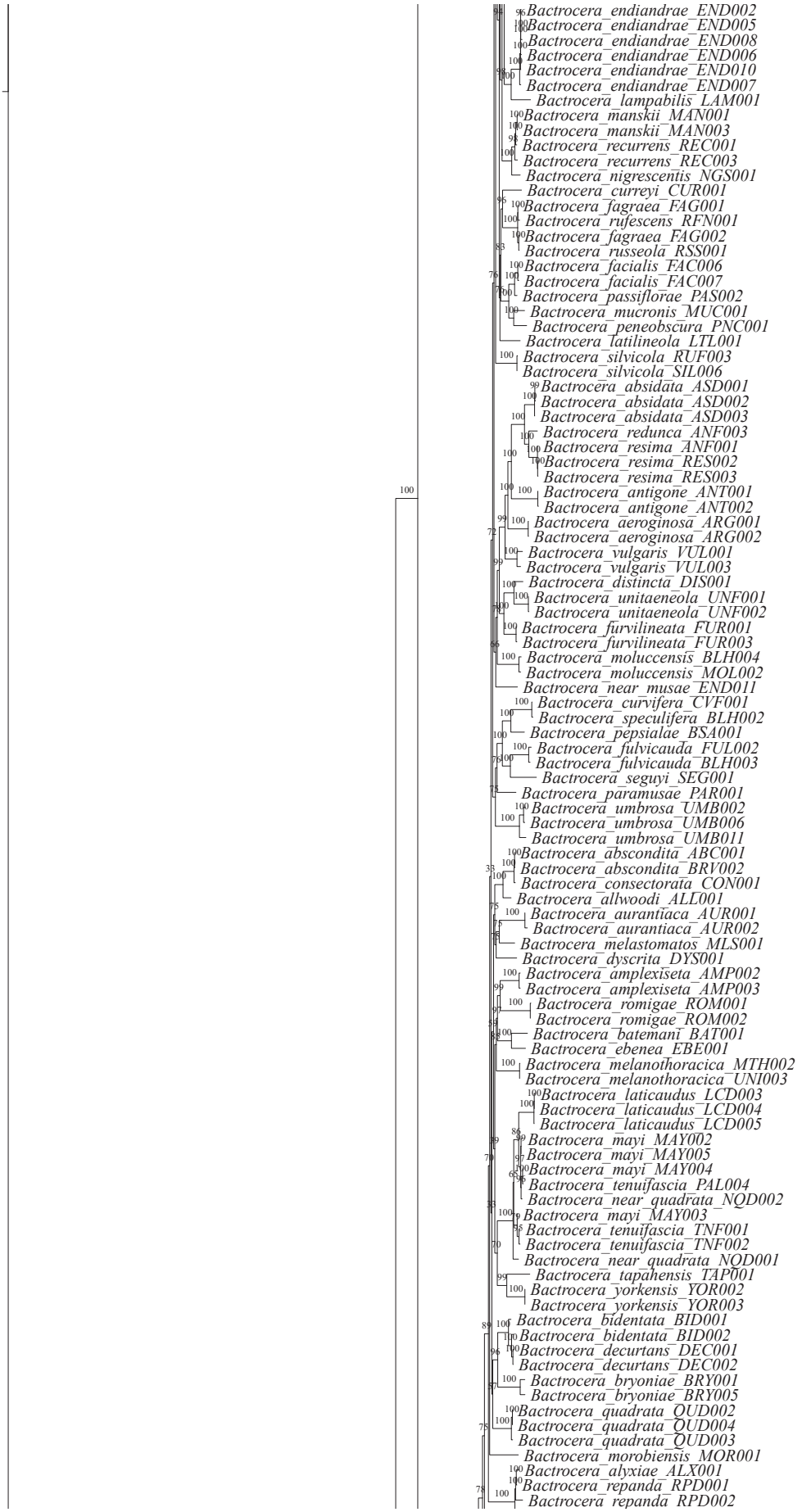
Drosophila_melanogaster
Drosophila_suzukii
Musca_domestica
Musca_autumnalis



Appendix 12 (continued next page): Proportionally linked phylogenetic tree of the Dacini reconstructed using the ML site resampling method from seven partitions of six loci: mitochondrial COI and COII; rRNA 16S; and nuclear DDOSTs2, RPA2 and EIF3L. Bootstrap values shown at the nodes.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Bactrocera_endiandrae_END006
Bactrocera_endiandrae_END010
Bactrocera_endiandrae_END007
Bactrocera_lampabilis_LAM001
Bactrocera_manskii_MAN001
Bactrocera_manskii_MAN003
Bactrocera_recurrens_REC001
Bactrocera_recurrens_REC003
Bactrocera_nigrescentis_NGS001
Bactrocera_curreyi_CUR001
Bactrocera_fagraea_FAG001
Bactrocera_rufescens_RFN001
Bactrocera_fagraea_FAG002
Bactrocera_russeola_RSS001
Bactrocera_facialis_FAC006
Bactrocera_facialis_FAC007
Bactrocera_passiflorae_PAS002
Bactrocera_mucronis_MUC001
Bactrocera_peneobscura_PNC001
Bactrocera_latilineola_LTL001
Bactrocera_silvicola_RUF003
Bactrocera_silvicola_SIL006
Bactrocera_absidata_ASD001
Bactrocera_absidata_ASD002
Bactrocera_absidata_ASD003
Bactrocera_redunca_ANF003
Bactrocera_resima_ANF001
Bactrocera_resima_RES002
Bactrocera_resima_RES003
Bactrocera_antigone_ANT001
Bactrocera_antigone_ANT002
Bactrocera_aeroginosa_ARG001
Bactrocera_aeroginosa_ARG002
Bactrocera_vulgaris_VUL001
Bactrocera_vulgaris_VUL003
Bactrocera_distincta_DIS001
Bactrocera_unitaeneola_UNF001
Bactrocera_unitaeneola_UNF002
Bactrocera_furvilineata_FUR001
Bactrocera_furvilineata_FUR003
Bactrocera_moluccensis_BLH004
Bactrocera_moluccensis_MOL002
Bactrocera_near_musae_END011
Bactrocera_curvifera_CVF001
Bactrocera_speculifera_BLH002
Bactrocera_pepsialae_BSA001
Bactrocera_fulvicauda_FUL002
Bactrocera_fulvicauda_BLH003
Bactrocera_seguyi_SEG001
Bactrocera_paramusae_PAR001
Bactrocera_umbrosa_UMB002
Bactrocera_umbrosa_UMB006
Bactrocera_umbrosa_UMB011
Bactrocera_abscondita_ABC001
Bactrocera_abscondita_BRV002
Bactrocera_consectorata_CON001
Bactrocera_allwoodi_ALL001
Bactrocera_aurantiaca_AUR001
Bactrocera_aurantiaca_AUR002
Bactrocera_melastomatos_MLS001
Bactrocera_dyscrita_DYS001
Bactrocera_amplexiseta_AMP002
Bactrocera_amplexiseta_AMP003
Bactrocera_romigae_ROM001
Bactrocera_romigae_ROM002
Bactrocera_batemani_BAT001
Bactrocera_ebenea_EBE001
Bactrocera_melanothoracica_MTH002
Bactrocera_melanothoracica_UNI003
Bactrocera_laticaudus_LCD003
Bactrocera_laticaudus_LCD004
Bactrocera_laticaudus_LCD005
Bactrocera_mayi_MAY002
Bactrocera_mayi_MAY005
Bactrocera_mayi_MAY004
Bactrocera_tenuifascia_PAL004
Bactrocera_near_quadrata_NQD002
Bactrocera_mayi_MAY003
Bactrocera_tenuifascia_TNF001
Bactrocera_tenuifascia_TNF002
Bactrocera_near_quadrata_NQD001
Bactrocera_tapahensis_TAP001
Bactrocera_yorkensis_YOR002
Bactrocera_yorkensis_YOR003
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Bactrocera_decurtans_DEC002
Bactrocera_bryoniae_BRY001
Bactrocera_bryoniae_BRY005
Bactrocera_quadrata_QUD002
Bactrocera_quadrata_QUD004
Bactrocera_quadrata_QUD003
Bactrocera_morobiensis_MOR001
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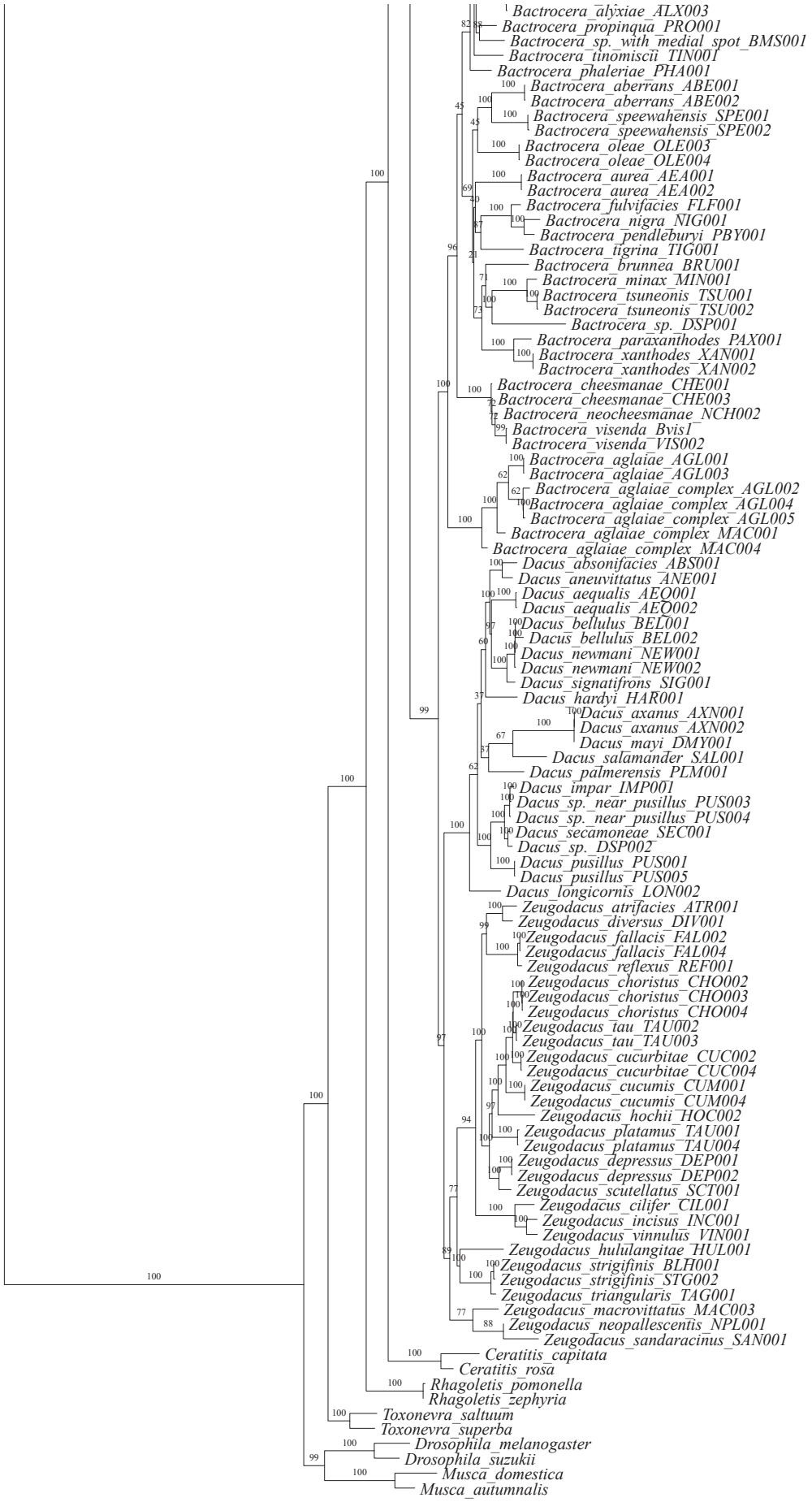
Bactrocera_repanda_RPD002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Bactrocera_aberrans_ABE001
Bactrocera_aberrans_ABE002
Bactrocera_speewahensis_SPE001
Bactrocera_speewahensis_SPE002
Bactrocera_oleae_OLE003
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Bactrocera_aurea_AEA002
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Bactrocera_pendleburyi_PBY001
Bactrocera_tigrina_TIG001
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Bactrocera_tsuneonis_TSU001
Bactrocera_tsuneonis_TSU002
Bactrocera_sp._DSP001
Bactrocera_paraxanthodes_PAX001
Bactrocera_xanthodes_XAN001
Bactrocera_xanthodes_XAN002
Bactrocera_cheesmanae_CHE001
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Bactrocera_neocheesmanae_NCH002
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Bactrocera_visenda_VIS002
Bactrocera_aglaiae_AGL001
Bactrocera_aglaiae_AGL003
Bactrocera_aglaiae_complex_AGL002
Bactrocera_aglaiae_complex_AGL004
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Dacus_hardyi_HAR001
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Dacus_sp._DSP002
Dacus_pusillus_PUS001
Dacus_pusillus_PUS005
Dacus_longicornis_LON002
Zeugodacus_atrifacies_ATR001
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Ceratitis_rosa
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Rhagoletis_zephyria
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Toxonevra_superba
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Musca_domestica
Musca_autumnalis