Cock et al.
CABI Agriculture and Bioscience (2025) 6:1, 0010
https://doi.org/10.1079/ab.2025.0010

CABI Agriculture and Bioscience

RESEARCH

OPEN ACCESS

Publicly-shared DNA barcodes and citizen science images provide new evidence on the establishment and spread of a lantana biological control agent, *Orphanostigma haemorrhoidalis* (Lepidoptera, Crambidae)

Matthew J.W. Cock[1,*], Michael D. Day[2], M. Alma Solis[3] and Rachel L. Winston[4]

Abstract

Orphanostigma haemorrhoidalis (Guenée) (Lepidoptera, Crambidae), indigenous to the Americas, was widely used in the Old World for the biological control of *Lantana camara* L. (Verbenaceae) from the 1950s to the 1980s. DNA barcodes from the Barcode of Life Data System (BOLD) and citizen scientist images from the iNaturalist and Afromoths websites were used to detect the establishment and spread of *O. haemorrhoidalis* in countries where it has not previously been reported. Analysis of DNA barcodes showed that there are two genetically distinguishable populations of *O. haemorrhoidalis* in the Americas, one in the south-eastern USA and the other widespread in the rest of the Neotropics. The two populations were introduced into different parts of the World and subsequently spread. We used DNA barcodes from BOLD to clarify that a population from Florida is established in Hawai'i, Australia and Fiji, while a population from Trinidad is established in parts of mainland Africa (including new records for Cameroon, Nigeria and Ghana), Madagascar, Mauritius and La Réunion. New country records for *O. haemorrhoidalis* were established from iNaturalist images from Eswatini, Kenya and Mozambique, and from Afromoths for Tanzania.

Keywords: Africa, BOLD, identification, iNaturalist, *Lantana camara*; monitoring, South Africa

Introduction

For over 100 years, biological control programmes have been conducted against target insect pests and weeds, resulting in the introduction and release of thousands of species of biological control agents around the world (Winston *et al*., 2014, 2024; Cock *et al*., 2016a). Although spectacular successes have been documented (e.g. Cock *et al*., 2016b), many of these programmes historically had insufficient resources to perform rigorous evaluations, and the status of many released organisms remains unknown (Cock *et al*., 2016a; Winston *et al*., 2024).

In recent years, new tools and resources have been developed that have applications for follow-up surveys of released biological control agents. DNA barcoding (Hebert *et al*., 2003) provides one such tool that can be used to help clarify the status of different populations of taxa (e.g. Zahiri *et al*., 2014). Citizen science platforms are an additional resource with applications for biological control agent surveys. Publicly shared and identified images (e.g. iNaturalist, 2024) are increasingly being used to monitor the establishment and spread of weed biological control agents (Cock *et al*., 2023; McClay *et al*., 2023).

The Neotropical flowering shrub, lantana (*Lantana camara* L.; Verbenaceae) was introduced as a garden and ornamental plant throughout the tropics and subtropics. It is now pervasive throughout the Old World (Holm *et al*., 1991), invading woodlands, forestry, orchards, grasslands and disturbed areas, where it

Affiliations: [1]CABI, Bakeham Lane, Egham, Surrey TW20 9TY, UK; [2] Department of Agriculture and Fisheries, GPO Box 267, Brisbane, Qld 4001, Australia; [3] Systematic Entomology Laboratory, Beltsville Agriculture Research Center, Agricultural Research Service, U.S. Department of Agriculture, c/o National Museum Natural History, MRC 168, 20013-7012, Smithsonian Institution, P.O. Box 37012, Washington, District of Columbia, USA; [4]MIA Consulting , Ogden, UT 84404, USA

*Corresponding Author: Matthew J.W. Cock. Email: m.cock@cabi.org

Submitted: 14 October 2024. Accepted: 24 December 2024. Published: 07 February 2025

displaces useful and indigenous plants. It has been the target for classical biological weed control since 1902. Although numerous biological control agents have been studied and released against lantana, the status of many releases is unknown, including some of the lantana leafroller, *Orphanostigma haemorrhoidalis* (Guenée) (Lepidoptera, Crambidae, Spilomelinae) (Winston *et al*., 2014, 2024). *O. haemorrhoidalis* was taken from Florida and Cuba to Hawai'i, and from there to Australia, South Africa and several Pacific territories. In a second initiative, it was taken from Trinidad to East Africa, South Africa, Mauritius and India. A detailed history of the use of this species, which is needed for the interpretation of our findings, is included as Supplementary Material 1.

In this article, we use, for the first time, a combination of publicly available DNA barcodes and citizen science images to assess the establishment and spread of an introduced weed biological control agent, *O. haemorrhoidalis*, as well as confirm historical distribution and establishment records reported in the literature.

Methods

DNA BARCODES

BOLD, the Barcode of Life Data System (BOLD, 2024) was searched to find DNA barcodes identified as *Orphanostigma* spp. Within BOLD, unique Barcode Index Numbers (BINs) are assigned to clusters of similar DNA barcodes (haplotypes) (Ratnasingham and Hebert, 2013). The BINs associated with *Orphanostigma* spp. were then examined to locate other samples that were not identified to genus. Those relating to *O. haemorrhoidalis* were compiled into a BOLD dataset (DS:ORPHAE). To these, we added a sequence from Hawai'i (O'ahu) (K. Austin, pers. comm. 2024) that has yet to be uploaded to BOLD. For our analysis, we excluded sequences of less than 400bp (base pairs).

Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). The evolutionary history was inferred using the maximum likelihood method and Tamura-Nei model (Tamura and Nei, 1993), using default parameters. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) (Felsenstein, 1985) were calculated. For illustrative purposes, a subset of 33 sequences was selected to include the diversity of countries and haplotypes, a Maximum-Likelihood tree calculated and figured. All sequences were grouped based on the allocation of BINs by BOLD, and the within and between groups variation was calculated using the standard function in MEGA X.

TAXONOMY

To interpret the results from the DNA barcoding assessment, male and female genitalia slides of *O. haemorrhoidalis* from selected populations (from Costa Rica, Cuba, Dominica, Dominican Republic, Fiji, Mexico, USA (Florida)) were prepared and examined. Abdomens were soaked in 10% potassium hydroxide and washed in water, stained with Chlorazol black, and slide mounted in Euparal (Robinson, 1976). Morphological structures were photographed using the Visionary Digital® imaging system at the National Museum of Natural History, Washington (NMNH). The genitalia from the two BINs were compared with the genitalia of *O. abruptalis* figured by Shaffer and Munroe (2007) and Bippus (2019) and a photograph of a preparation of *O. haemorrhoidalis* (BOLD: ACE4975) from Australia from the adult, as illustrated in Fig. 7a.

DIAGNOSTIC FEATURES

Orphanostigma abruptalis (Walker) was described from Sri Lanka and is reported from tropical Africa, most of the Indian Ocean Islands, South and South-east Asia to Australia (Shaffer and Munroe, 2007). The adults of *O. haemorrhoidalis* and *O. abruptalis*, are similar in habitus, although the genitalia are clearly different (Shaffer and Munroe, 2007; Landry, 2016; Bippus, 2019; Solis *et al*., 2024). In order to accurately assess the identification of citizen science images shared on iNaturalist, we had to formulate effective diagnostic features to identify photographs of living moths of the introduced *O. haemorrhoidalis* and the indigenous *O. abruptalis* in the Old World.

Variation in the two BINs comprising *O. haemorrhoidalis* and two BINs comprising *O. abruptalis* was assessed initially by collating the images of DNA barcoded specimens unequivocally allocated to a BIN, and supplementing this with images of live and pinned specimens allocated to BINs based on their area of origin. The guidance in Bippus (2019) was considered and diagnostic features for the recognition and identification of images of the two species of *Orphanostigma* were derived.

INATURALIST OBSERVATIONS

The shared images examined had been posted on iNaturalist (2024) by citizen scientists until the end of April 2024. iNaturalist records can be filtered by taxon and location. To locate records of *Orphanostigma* spp., this approach is dependent on iNaturalist identifiers having already recognised the genus. Where this is not the case, observations may be misidentified, unidentified, or identified to a higher taxonomic level, i.e. Spilomelinae or Crambidae. To check for such partially identified material for African and Pacific countries where the literature indicated releases had been made but no observations had subsequently been reported, we reviewed all observations for those countries identified as Spilomelinae. We did not attempt this for India because that would have involved screening more than 16,000 observations. Further, there were multiple observations of *O. abruptalis* from India, so had images of *O. haemorrhoidalis* been posted, at least some should have been identified as genus, or misidentified as *O. abruptalis*. We did not attempt to identify larvae (only one image was posted).

Correct identifications were added on iNaturalist for many of the partially identified or misidentified observations of *O. haemorrhoidalis* and *O. abruptalis* that were found, and misidentifications of *Orphanostigma* spp. were corrected or downgraded to Spilomelinae.

Results

DNA BARCODES

Searching BOLD for DNA barcodes identified as *Orphanostigma* spp., we found more than 200 sequences, and these (including some short sequences) were compiled in the BOLD dataset DS:ORPHAE. The dataset used for our analysis included an extra sequence from Hawai'i and excluded sequences shorter than 400 bp. The analysis used the Maximum-Likelihood method and the tree with the highest log likelihood (–1420.05) is shown in Supplementary Material 3. This analysis involved 33 nucleotide sequences, and there was a total of 655 positions in the final dataset. The bootstrap values for this analysis are shown in Table 1. The results aligned with the BINs allocated by BOLD. The illustrative tree based on the reduced dataset (Fig. 1) shows this arrangement, and Table 1 includes the differences found within and between groups.

The resultant evolutionary tree (as shown in Fig. 1) showed that *O. haemorrhoidalis* comprises a cluster of two BINs, 1.53% bp different and separate from other sequenced *Orphanostigma* spp. (Fig. 1, Table 1). BOLD:AAD3428 is indigenous to and widespread in the Neotropical Region (Costa Rica, Galapagos Islands, Argentina, Dominica (LNAUV1747-17)), whereas BOLD:ACE4975 is restricted to south-east USA (Florida (LNAUV1749-17, LNAUW1758-17) and Georgia).

Table 1. Results of phylogenetic analysis of *Orphanostigma* spp. barcodes. These were based on the full dataset DS:ORPHAE (less a sequence of 307bp, plus a yet to be published sequence from Hawai'i). Distances are given as the percentage of different nucleotides.

				Between group mean distance				
Taxon and BIN	**Number of sequences**	**Bootstrap value**	**Within group mean distances**	**BOLD: AAD3428**	**BOLD: ACE4975**	**BOLD: AAA0296**	**BOLD: AAO0477**	**BOLD: ABW3156**
Orphanostigma haemorrhoidalis BOLD:AAD3428	189	99	0.28	—				
Orphanostigma haemorrhoidlalis BOLD:ACE4975	7	88	0.75	1.75	—			
Orphanostigma eugeniephillipsia BOLD:AAA0296	5	99	0.00	4.91	4.33	—		
Orphanostigma abruptalis BOLD:AAO4775	4	99	0.21	7.01	6.64	6.64	—	
Orphanostigma abruptalis BOLD:ABW3156	11	96	0.49	6.98	6.77	6.77	2.86	—

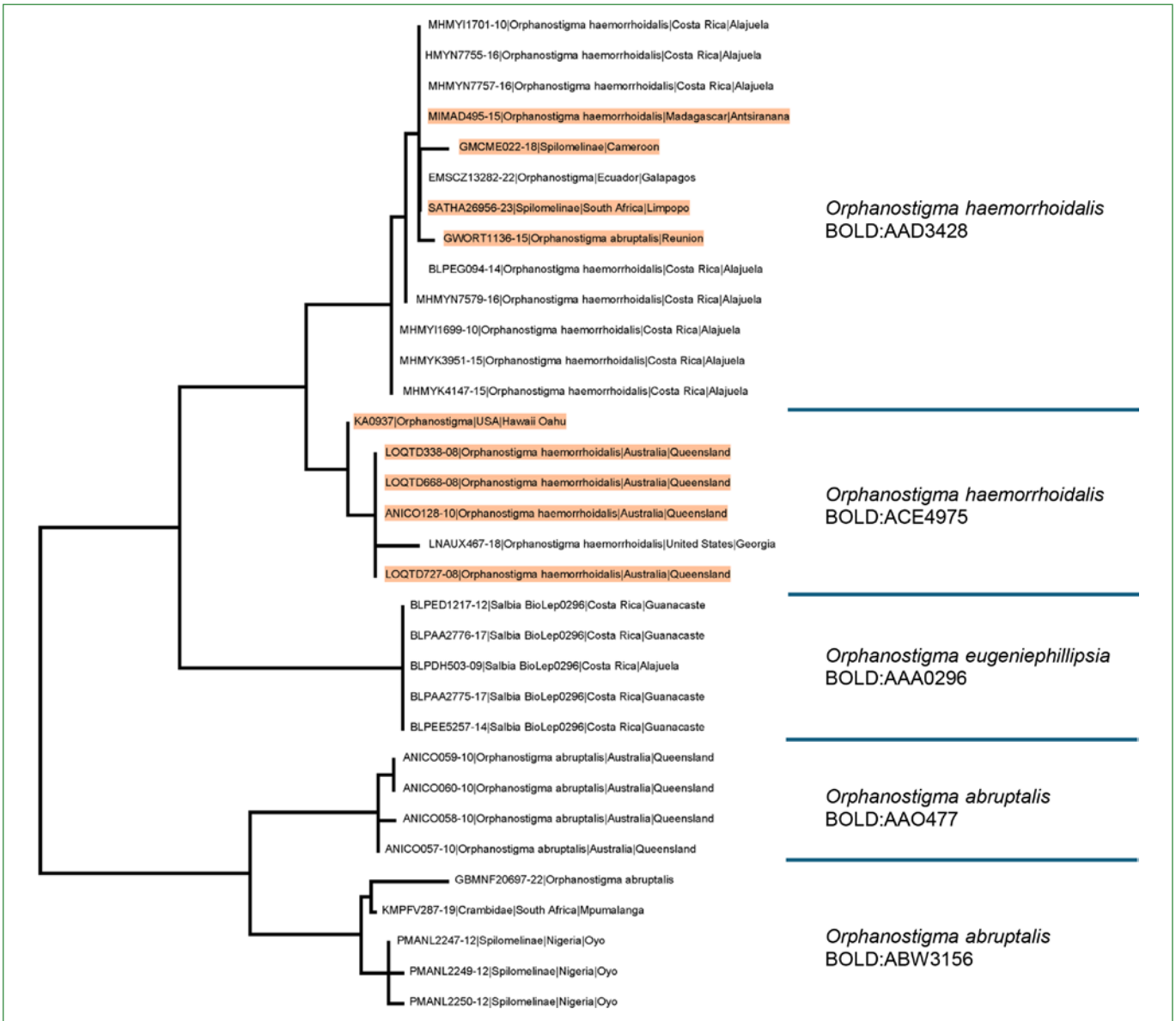

Fig. 1. Maximum likelihood tree of *Orphanostigma* barcodes and BINs based on 33 selected DNA barcodes. Introduced populations are highlighted. See Supplementary Material 3 for the complete tree.

The DNA barcodes comprising BOLD:AAD3428 in BOLD (Fig. 1, Table 2), reveal that *O. haemorrhoidalis* is present in Cameroon and Nigeria, although not previously reported from these countries (Supplementary Material 1). We also note that BOLD:AAD3428 includes an unpublished sequence from Ghana, but we have no details.

DNA barcodes in BOLD indicate that the name *O. abruptalis* has also been applied to material from two BINs: BOLD:AAO4775 from Australia only, and 2.81% bp distant BOLD:ABW3156 widespread in Africa from Côte d'Ivoire to Tanzania to South Africa and with one sequence from India. BOLD:AAO4775 and BOLD:ABW3156 are currently mutual nearest neighbours (Fig. 1, Table 1).

TAXONOMY

New dissections of *O. haemorrhoidalis* from Costa Rica, Cuba, Dominica, Dominican Republic, Fiji, Mexico, and the USA (Florida) (Figs. 2 and 3) were compared with published figures of dissections of *O. haemorrhoidalis* from the Galapagos Islands (Landry, 2016) and an unpublished dissection from Australia (Australian National Insect Collection). Dissections from Costa Rica represent BIN BOLD:AAD3428, while dissections from Australia, Florida and Fiji represent BIN BOLD:ACE4975. Those from Cuba, Dominica, Mexico and Galapagos are anticipated to represent the former, although no DNA barcodes are available from these localities. They were all found to be the same with no diagnostic features to separate the members of the two BINs.

DIAGNOSTIC FEATURES

Both *O. haemorrhoidalis* and *O. abruptalis* are predominantly shades of yellow, orange and brown (Figs. 4–9). The area distal to the post-medial line may be concolorous, slightly more intense or dark in colour, or significantly darker and browner. The pre-medial and post-medial lines are both well defined. In the area between the two lines, there is no basal spot in the cell, and the distal spot at the end of the cell is not rounded, but a streak or bar, near the end of the cell, reaching from close to the costa to close to the in-turned angle or step of the post-medial line. The margins of both wings are dark, as a single or double line. The hind wing has the marginal area broadly slightly darker, pre-medial and post-medial lines clearly marked and they are angled towards the tornus (although this may not be evident in resting moths with the dorsum of the hindwing compressed).

In general, *O. haemorrhoidalis* is a yellow-orange or pale brown species, with variable contrast between the area distal to the post-medial line and the area basal to that line, whereas *O. abruptalis* is more of an orange-brown or brown in colour with the area beyond the post-medial line contrastingly darker. Both species are variable in colour and contrast, so better, qualitative characters are needed. The step in the post-medial line is sharper, and slightly less than 90° in *O. haemorrhoidalis*, whereas it is more rounded in *O. abruptalis*, and the lines leading to the step are at a smaller angle to each other, or even near parallel. The section of the postmedial line from mid-wing to costa is smoothly continuous in *O. haemorrhoidalis*, whereas in *O. abruptalis*, there is a slight irregularity and thickening in the middle. Similarly, the section of the post-medial line from the step to the dorsum is smoothly continuous in *O. haemorrhoidalis*, whereas in *O. abruptalis* it bulges outwards, and then curves slightly towards the base of the wing as it meets the dorsum. The post-medial hindwing line of *O. abruptalis* is slightly curved, but straight in *O. haemorrhoidalis*.

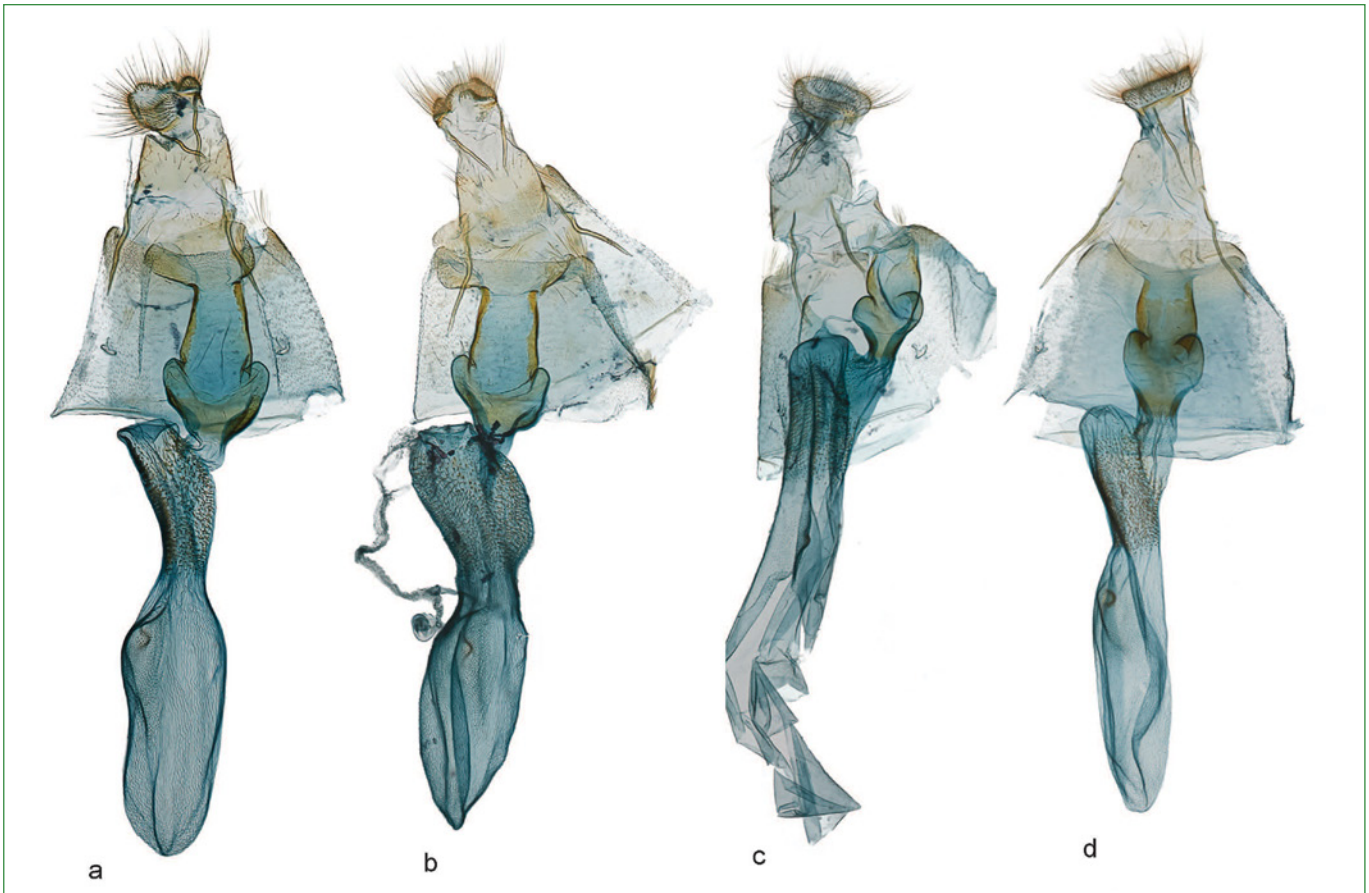

Fig. 2. Female genitalia (ventral view) of *Orphanostigma haemorrhoidalis* with locality, USNM barcode, and genitalia slide number. (a) Cuba, USNMENT01894133, #116371. (b) Dominican Republic, USNMENT01894006, #116368. (c) Florida, USA, USNMENT01343339, #116362 (genitalia damaged and distorted during preparation, corpus bursae torn and antrum mounted sideways and barely visible). (d) Costa Rica, USNMENT1894170, #116403.

Fig. 3. Male genitalia (ventral view and phallus lateral view) of *Orphanostigma haemorrhoidalis* with locality, USNM barcode, and genitalia slide number. (a–b) Fiji, USNMENT01068451, #116381. (c–d) Cuba, USNMENT01894025, #116370. (e–f) Costa Rica, USNMENT01894107, #116373. (g–h) Dominican Republic, USNMENT01894104, #116367 (valva costa folded and saccular knife-like elongated ridge not visible), and (i–j) Florida, USA, USNMENT01894031, #116363.

In both species, the dorsal abdominal segments are concolorous with the wings, apart from a pale, or dark then pale, posterior margin on each, which may be more pronounced in *O. abruptalis*, and dark individuals of *O. haemorrhoidalis* have the terminal abdomen segments dull orange. In the final segment of the male abdomen of *O. abruptalis,* the dorsum is concolorous, with a narrow white lateral margin, whereas the male of *O. haemorrhoidalis* has a dark spot on the posterior dorsum of the segment before the penultimate segment, with a white border anterior to this (broader laterally and thinner dorsally), the penultimate segment is concolorous, and the final segment is dark on the dorsum with a white border on each side. In the female of both species, the posterior dorsal margin of the distal segment is narrowly pale, or dark then pale, the penultimate segment is a more intense orange, and the final segment has a dark dorsal patch, its anterior margin white. Thus, the characters

of the distal dorsal abdomen are also useful to separate males of *O. abruptalis* and *O. haemorrhoidalis*.

There seems to be some geographically based variation in the colouring of *O. haemorrhoidalis*. BOLD:ACE4975 in Florida and where introduced (Fig. 7) is often darker brown than populations of BOLD:AAD3428, sometimes markedly so. BOLD:AAD3428 in Costa Rica is generally an intermediate shade of brown (Fig. 5a–h). BOLD:AAD3428 in South America and where introduced from Trinidad (Figs. 5i–p and 6) is often more yellow in colour (see also published figures for the Galapagos Islands and Brazil (Landry, 2016) and La Réunion (Bippus, 2019)). However, all populations are variable, and there is overlap, so we did not use colour to separate images of the two BINs.

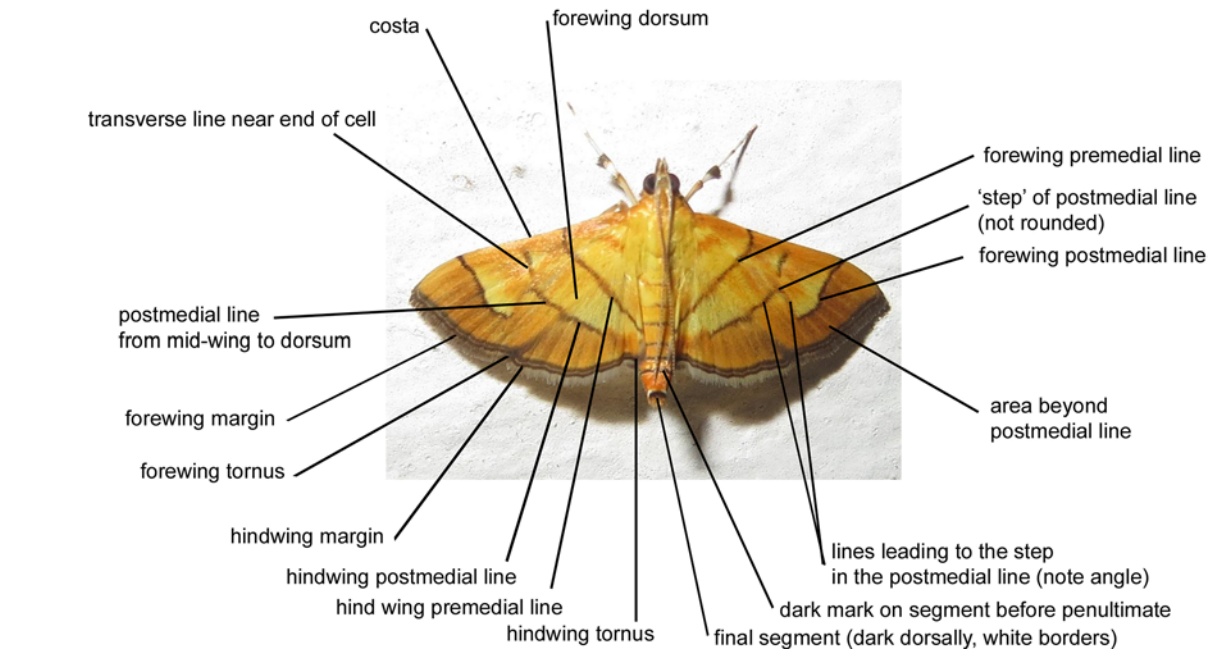

Fig. 4. Female *Orphanostigma haemorrhoidalis* with features labelled. Photograph: South Africa, St. Lucia, © magdaslucia, CC BY-NC (iNaturalist observation 11183313).

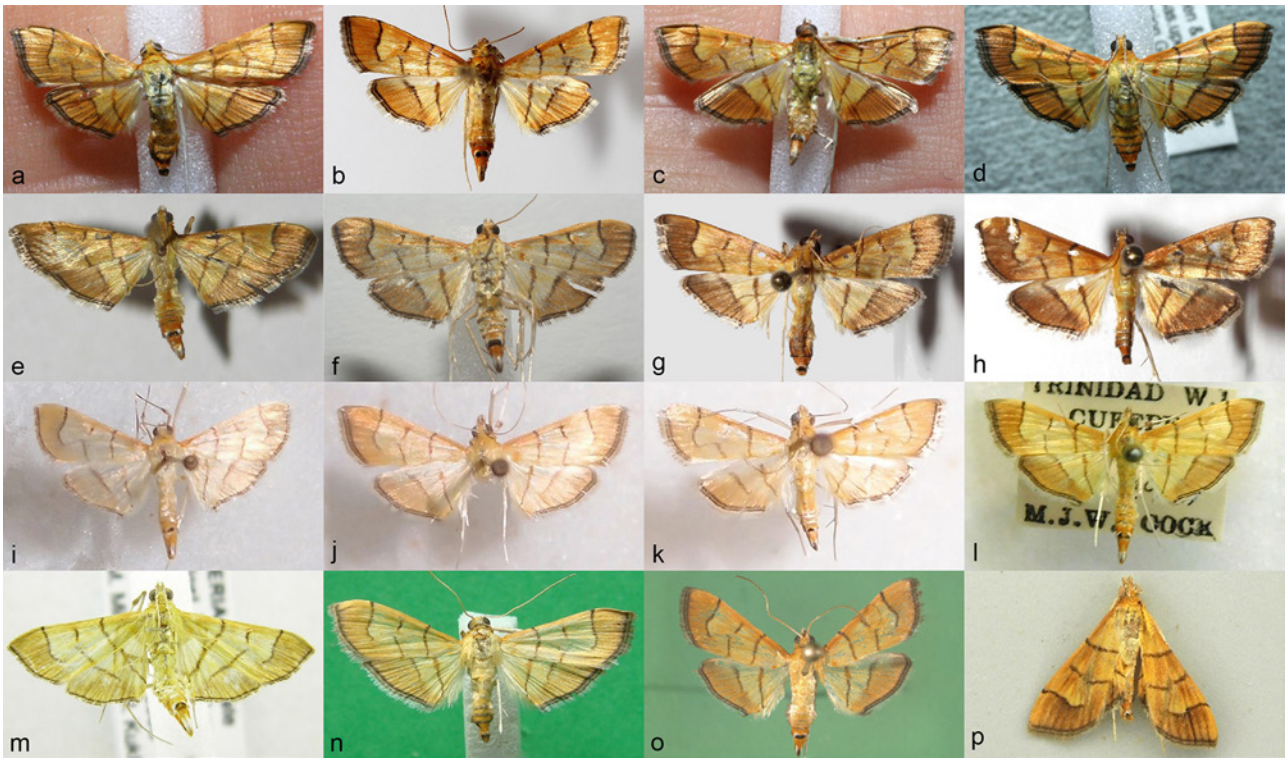

Fig. 5. Pinned *Orphanostigma haemorrhoidalis* (AAD34528). (a–h) Costa Rica (barcoded). (i–l) Trinidad (not barcoded). (m–p) Africa (barcoded: Nigeria, Madagascar, Madagascar, La Réunion). For details and picture credits see Supplementary Material 2.

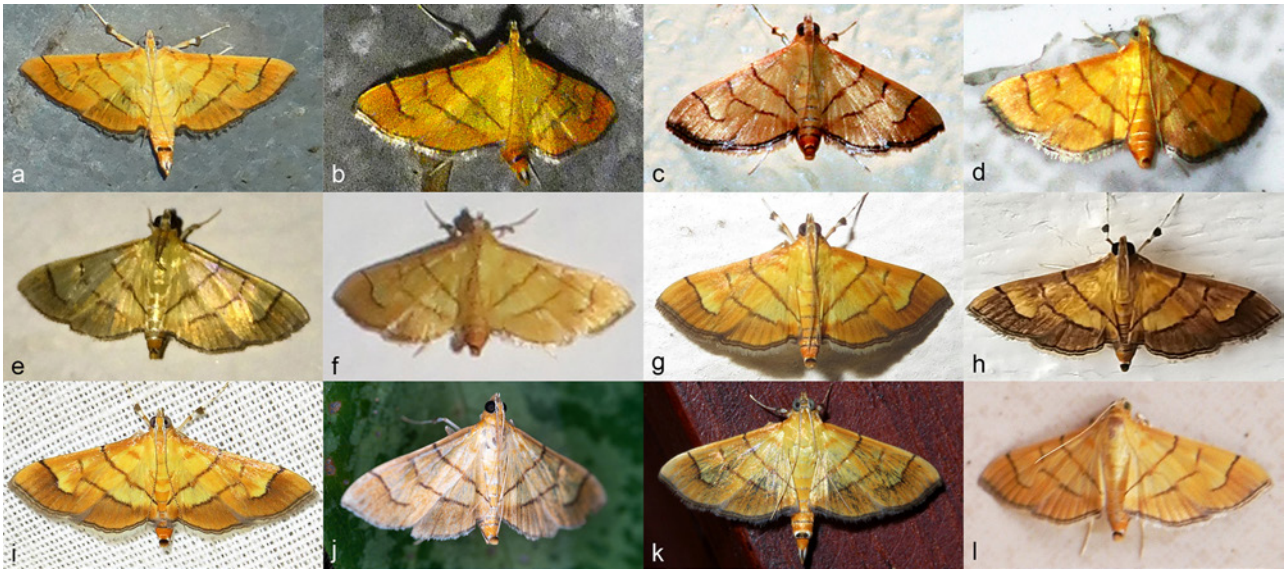

Fig. 6. Live *Orphanostigma haemorrhoidalis* (AAD34528) from iNaturalist. (a–d) Trinidad. (e–h) South Africa. (i–l) Africa (Eswatini, Malawi, Mozambique, Kenya). For details and picture credits see Supplementary Material 2.

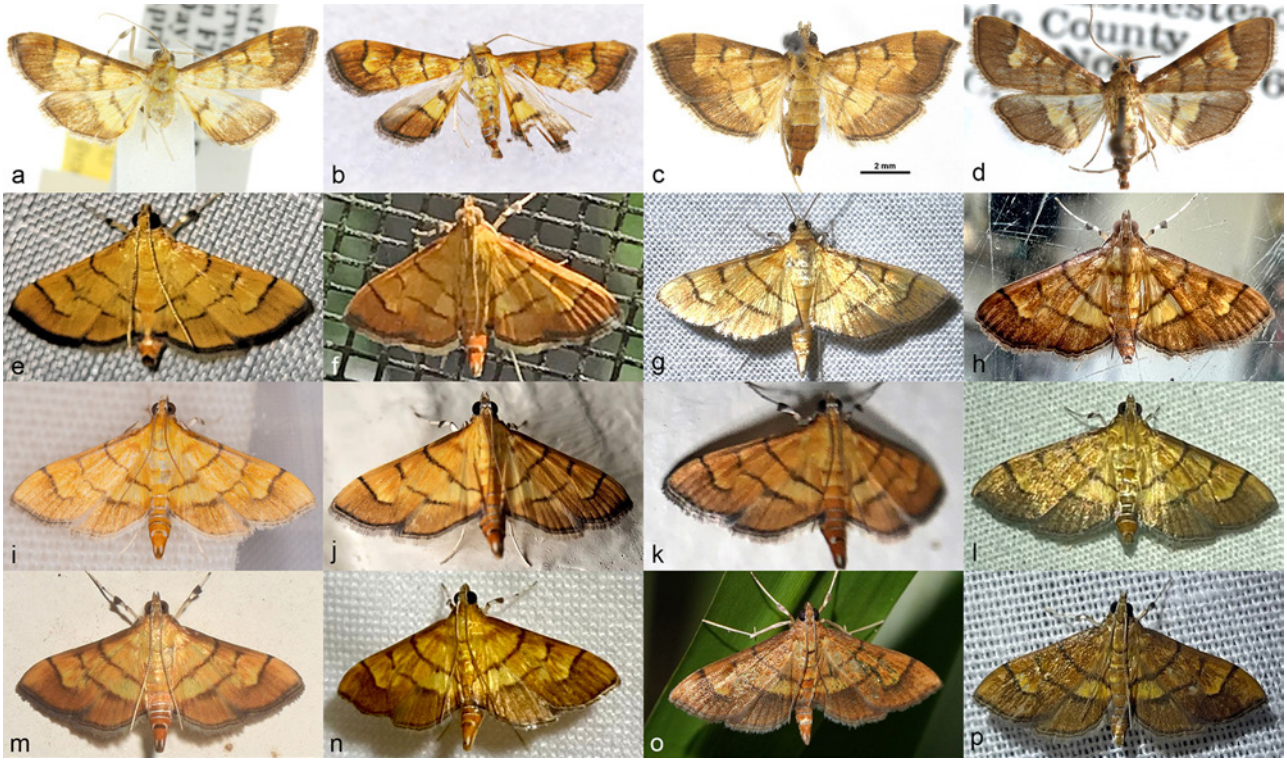

Fig. 7. *Orphanostigma haemorrhoidalis* (BOLD:ACE4975). (a–b) Australia (barcoded). (c–d) Florida (barcoded). (e–h) Florida (not barcoded). (i–l) Hawai'i (not barcoded). (m–p) Australia (not barcoded). For details and picture credits see Supplementary Material 2.

INATURALIST OBSERVATIONS

We used the diagnostic features that we developed to recognise *O. haemorrhoidalis* images and separate them from *O. abruptalis* images of living moths on iNaturalist. We were able to confirm the presence of *O. haemorrhoidalis* in areas where it had been released: Hawai'i (Hawai'i, Kaua'i, Maui, O'ahu), Australia (Queensland), Mauritius, South Africa (from Kwazulu-Natal: Hillcrest to Durban to St. Lucia and Mpumalanga: Hazyview), and Uganda (Holma), as well as areas where it was known to occur (Bippus, 2019; Winston *et al*., 2024) but had not been released: Malawi (Blantyre), Madagascar (Antsiranana, Toamasina) and La Réunion. In addition, we found observations from Eswatini, Mozambique and Kenya (Table 2, Fig. 6), where *O. haemorrhoidalis* had not previously been reported to be established (Supplementary Material 1).

Discussion

Currently, there is no known geographical overlap between the two BINs in either the indigenous or the introduced range. Based on

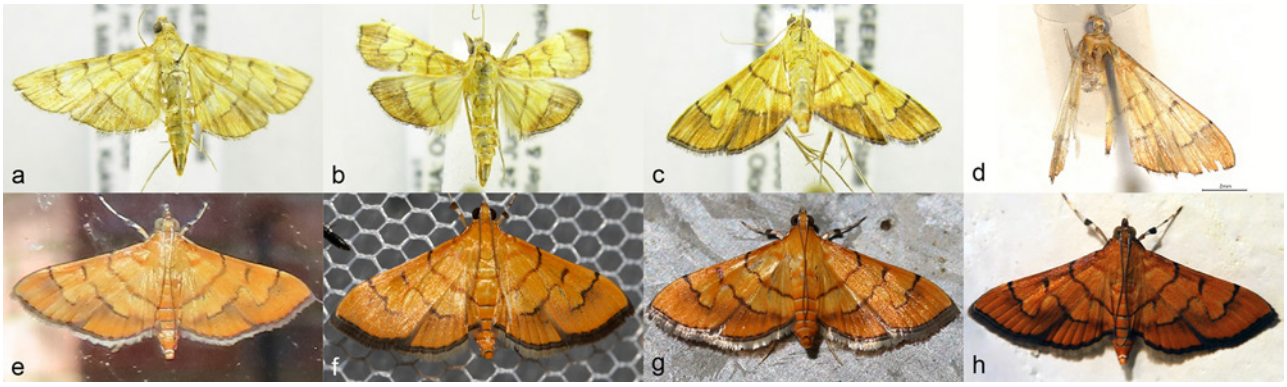

Fig. 8. *Orphanostigma abruptalis* (ADW3156) from Africa. (a–d) barcoded (3x Nigeria, Gabon). (e–h) not barcoded (South Africa, Malawi, 2x Tanzania). For details and picture credits see Supplementary Material 2.

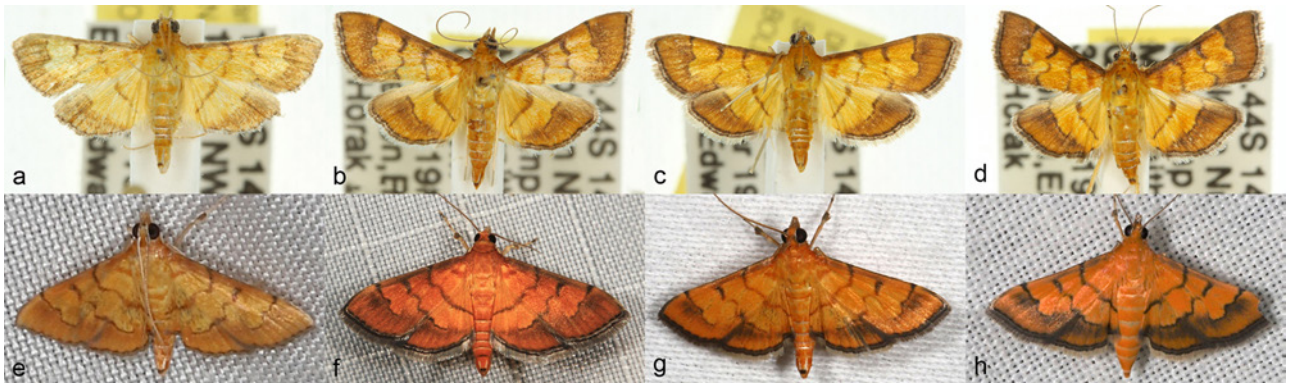

Fig. 9. *Orphanostigma abruptalis* (AAO4775) from Australia. (a–d) barcoded. (e–h) not barcoded. For details and picture credits see Supplementary Material 2.

the available information, the two BINs have the same biology. The host range of *O. haemorrhoidalis* BOLD:ACE4975 was studied in Hawai'i prior to release and found to be restricted to *Lantana* spp. (Harley, 1956). In their immense inventory programme of Lepidoptera on all food plants in Costa Rica, Janzen and Hallwachs (2024) amassed over 300 rearing records for *O. haemorrhoidalis* BOLD:AAD3428, all of which were *Lantana* spp. apart from four on *Lippia* spp. (Verbenaceae) (Janzen and Hallwachs, 2024; Solis *et al*., 2024). Where *O. haemorrhoidalis* (BOLD:ACE4975 and BOLD:AAD3428) has been released as a biological control agent, there have been no reports of feeding on plants other than *Lantana* spp.

Thus, members of the two BINs of *O. haemorrhoidalis* cannot be separated on differences in genitalia, biology, or other diagnostic traits. Based on our results (Fig. 1), the two BINs form a monophyletic group. They can only be differentiated by their barcode, and the difference between the two barcodes is less than the 2% bp threshold often used to separate species. Therefore, we choose to treat them as one species unless and until new research shows significant biological, ecological or genetic differences. However, the two BINs have been used separately as weed biological agents, and their DNA barcodes can be used to evaluate the source of different introduced populations in the Old World.

Material of *O. haemorrhoidalis* from Florida and Cuba was taken to Hawai'i, cultured, released and became established. From Hawai'i it was sent to other territories including Australia and Fiji. The Florida population is BIN BOLD:ACE4975. We have not located any DNA barcodes from Cuba, but because BOLD:AAD3428 occurs in Central America and Dominica, we suggest that the population in Cuba is more likely to be BOLD:AAD3428. We have no evidence as to how the two populations were reared and released in Hawai'i but they were probably pooled. The only available barcode for the established population of *O. haemorrhoidalis* on Hawai'i is BOLD:ACE4975; all eight barcodes from Australia are BOLD:ACE4975, and the only barcode available from Fiji is also BOLD:ACE4975. This sample size is not large enough or extensive enough to be conclusive, but it certainly points to only BOLD:ACE4975 being established in Hawai'i and moved onward to other countries (Supplementary Material 1).

There are several possible explanations. It might be that the material imported from Cuba was also BOLD:ACE4975, rather than BOLD:AAD3428. However, as explained above we consider this unlikely, and new samples from Cuba could readily test this. Alternatively, it may be that the culture established in Hawai'i was based only on the material from Florida, and the material from Cuba was not viable or died out. Given that the CO1 mitochondrial gene, on which the DNA barcode is based, is only transmitted through the maternal line, another possibility is that in any successful crosses, the female parent was always from the Florida strain BOLD:ACE4975, whether due to one-way incompatibility or chance. Finally, it is possible, but we think unlikely, that descendants of the female lines of both BINs are present but BOLD:AAD3428 has not yet been sampled. We suggest that establishment was obtained from descendants of the maternal line of the Florida population, with or without introgression from the male Cuba line.

O. haemorrhoidalis from Australia is BOLD:ACE4975. Populations from both Australia and Trinidad were sent to South Africa. We have not located DNA barcodes from Trinidad but given the Neotropical distribution of BOLD:AAD3428, the population in Trinidad is very likely to be BOLD:AAD3428. This

conclusion is supported by the recovery of BOLD:AAD3428 from several African countries following its introduction from Trinidad. In South Africa, *O. haemorrhoidalis* was cultured with difficulty and small releases were made (Supplementary Material 1). Most of the material received was from Australia (BOLD:ACE4975), but the timing of the releases suggests they are more likely to have been based on the material from Trinidad (BOLD:AAD3428). At the time, it was thought that the establishment failed. Material from Trinidad was also sent to Uganda and Mauritius, where it was cultured, released and reported to be established. Further releases descended from the same material from Uganda were made in Kenya and Tanzania but were reported to have failed to establish. The presence of *O. haemorrhoidalis* in Kenya and Tanzania documented here could be due to overlooked establishment from these releases, unassisted spread from Uganda, or less likely unassisted spread from South Africa.

At the time of the release of *O. haemorrhoidalis* (BOLD:AAD3428) in Africa in the 1950s and 1960s, establishment was only reported in Uganda. *O. haemorrhoidalis* was found to be established in KwaZulu-Natal, South Africa in 1984, and DNA barcoding of recent material confirms this to be BOLD:AAD3428. Hence, this established population was probably established from the 1962 releases derived from material imported from Trinidad, but the possibility that the population released in East Africa spread south cannot be ruled out. Today, *O. haemorrhoidalis* (BOLD:AAD3428) probably occurs from Kenya to South Africa in many areas where lantana is found.

The Uganda population is assumed to be the source of the populations now documented to the west in Cameroon, Nigeria (Table 2) and Benin (Winston *et al*., 2024); this indication that it has spread unaided to West Africa supports the possibility that it also spread within East Africa and south to South Africa.

The population established in Mauritius is assumed to be the source of the spread to nearby La Réunion, perhaps accidentally on inter-island boat traffic. The population in Madagascar may have derived from accidental spread from Mauritius (and/or La Réunion) or from South Africa. At least two Agromyzidae lantana biological control agents established in South Africa have subsequently been found in Madagascar, so this latter route is certainly possible. *Ophiomyia camarae* Spencer was introduced and established in South Africa in 2001, and recorded from Madagascar in 2009, and *Calycomyza lantanae* (Frick) was introduced and established in South Africa in 1982 and reported from Madagascar in 2010 (Winston *et al*., 2014, 2024).

The DNA barcodes for *O. abruptalis* form two different BINs, BOLD:ABW3156 and BOLD:AAO4775, 2.81% bp different, which are likely to represent two different species (Fig. 1, Table 1). Although there are no DNA barcodes available from the type locality, Sri Lanka, the inclusion of a sequence from India in BOLD:ABW3156 suggests this BIN will align with the true *O. abruptalis*. *Orphanostigma suffectalis* (Walker) was described from Queensland and is currently considered a synonym of *O. abruptalis*, so is available for the Australian population if needed. However, for our purposes, the two populations may be referred to as *O. abruptalis* (BOLD:ABW3156) and *O. abruptalis* (BOLD:AAO4775).

Our analysis of iNaturalist images was only practical because iNaturalist identifiers had already identified to at least genus almost all observations that we examined. In that sense, these are not new records but rather records that had not entered the biological control literature. Equally, some but not all of the DNA barcodes in BOLD considered here were already identified, and the use of BINs facilitated the grouping of these with unidentified or partially identified barcodes. In both cases, the sharing of information by citizen scientists and scientists respectively, has enabled us to gain new information and understanding regarding the use and spread of *O. haemorrhoidalis* as a biological weed control agent for lantana.

Interpretation of our results has depended upon a detailed historical record of the implementation of the biological control programmes against lantana. Accurate and detailed release records are of particular importance in all cases such as this where it has subsequently been shown that different genetic populations of a biological control agent were used, with potentially different results. For example, the weevil *Mecinus janthinus* Germar was introduced from Europe into North America for the biological control of toadflaxes (*Linaria* spp.; Plantaginaceae). The weevil initially appeared to have little impact on *L. vulgaris* Mill. infestations but was credited with dramatic, landscape-level control of *L. dalmatica* (L.) Mill. in both Canada and the USA (Toševski *et al*., 2018). Toševski *et al*. (2011) showed that this weevil actually comprised two species, *M. janthinus* and *M. janthiniformis* Toševski and Caldara. Further, *M. janthinus* introduced from northern Switzerland and southern Germany is associated with *L. vulgaris*, while *M. janthiniformis* introduced from southern Macedonia is associated with *L. dalmatica*. Detailed molecular and biology studies were interpreted in light of the documented collection and release programmes to understand the pattern of impact of *Mecinus* spp. on *Linaria* spp. in the North America and to guide future actions in the *Linaria* biological programme (Toševski *et al*., 2018). From this example and the results of this present study, it is clear that voucher material for all biological control agent releases needs to be kept, sequenced for DNA barcodes, and preserved for more detailed genetic analysis if needed.

Table 2. Records of *Orphanostigma haemorrhoidalis* (BIN BOLD:AAD3428) from countries where establishment has not been reported previously by Winston *et al*. (2024).

Country	Area	Deliberate release	Source
Cameroon	Bamenda	No	BOLD: GMCME022-18
Eswatini	Mbabane	No	iNaturalist 86452140
Kenya	Nairobi	Yes	iNaturalist 204357521
	Narok	Yes	iNaturalist 70440778
La Réunion		No	BOLD: GWORT1133-15, GWORT1134-15, GWORT1136-15 (see also Bippus, 2019)
Mozambique	Mtindiri	No	iNaturalist 42657565
Nigeria	Oyo	No	BOLD: PMANL2248-12|
Tanzania	Arusha	Yes	De Prins *et al*. (2024)

'Source' refers to the iNaturalist observation number, BOLD Process ID(s) or an online image (De Prins *et al*., 2024). There is a further unpublished record from Ghana in BOLD.

CONCLUSIONS

The DNA barcodes publicly shared by scientists on BOLD, and images shared by citizen scientists on iNaturalist, confirm the presence of *O. haemorrhoidalis* in much of its introduced range and several countries where it had not been previously reported. The use of BINs has enabled the introductions of *O. haemorrhoidalis* from Florida and Trinidad to be tracked separately.

Based on our findings, we conclude as follows. *O. haemorrhoidalis* (BOLD: AAD3428) derived from Trinidad is now widespread in Africa and the Mascarenes and can be expected to continue to spread throughout sub-Saharan Africa where lantana occurs. *O. haemorrhoidalis* (BOLD:ACE4975) derived from Florida is established in Hawai'i, Australia and Fiji and we assume is the BIN present on Norfolk Island and in the other Pacific countries where it is known to occur (Federated States of Micronesia, Niue, Tonga and Vanuatu). Based on the evidence available it appears that this is the only BIN established from the introductions into Hawai'i, and onward from there.

ACKNOWLEDGEMENTS

We acknowledge and thank iNaturalist, the observers who posted their images on iNaturalist, and the identifiers who made those images findable. Some of these images are shown in Figs. 4–9, and the details of each are given in Supplementary Material 2, together with the copyright details, and basis of use. We thank Kyhl Austin, Kate Braun, Cat Chang, Dianne Clarke, domf, dhfischer, Martin Grimm, Marc Henrion, Bastien Louboutin, magdastlucia, Tarran P. Maharaj, mz7, Rachel S, Reiner Richter, Ray Simpson, Luke Smith, Rebecca Stroud, Greg Tasney, Ricky Taylor, Stephanie Tran, foster_wine and Bart Wursten who shared their images under various Creative Commons licenses as set out in Supplementary Material 2, and Brian Waswala Olewe and Zach Pezzilo for personally allowing us to use their copyright images from iNaturalist.

We thank Ben Proshek, Systematic Entomology Laboratory (SEL, USDA) for dissection of specimens; the Área de Conservación Guanacaste (ACG)/Guanacaste Dry Forest Conservation Fund parataxonomist team that reared all of the ACG specimens and collected them from the light traps; the Australian National Insect Collection (David Yeates, Su You Ning) for sharing images of a dissection of *O. haemorrhoidalis* in the collection (especially while technically closed for a major relocation); The University of the West Indies Zoology Museum, St. Augustine, Trinidad and Tobago (Mike G. Rutherford) for allowing us to use images of specimens in the collection; David C. Lees for facilitating MJWC's visit to the Natural History Museum, London; Janis N. Matsunaga who helped with various enquiries in Hawai'i and liaised with Kyhl Austin to obtain a DNA barcode from the islands; and Alan Buddie (CABI) for advice on the barcode analysis.

We also thank BOLD, and the scientists who have shared their DNA barcodes, data and images on BOLD, including Carlos Lopez Vaamonde, Axel Hausmann and other data-managers who shared unpublished DNA barcodes with us.

CABI is an international intergovernmental organization, and MJWC gratefully acknowledge the core financial support from our member countries (and lead agencies) including the United Kingdom (Foreign, Commonwealth and Development Office), China (Chinese Ministry of Agriculture and Rural Affairs), Australia (Australian Centre for International Agricultural Research), Canada (Agriculture and Agri-Food Canada), Netherlands (Directorate-General for International Cooperation), and Switzerland (Swiss Agency for Development and Cooperation). See https://www.cabi.org/about-cabi/who-we-work-with/key-donors/ for full details (accessed 3 May 2024).

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA. USDA is an equal opportunity provider and employer.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

CONSENT FOR PUBLICATION

This article was approved for publication by the USDA-ARS.

ETHICS STATEMENT

Not applicable.

AUTHOR CONTRIBUTIONS

MJWC conceived the study, analysed the DNA barcodes on BOLD and iNaturalist observations. MAS led on the taxonomy and genitalia interpretation. MDD and RLW collated information on lantana biological control. All authors co-wrote the paper, and read and approved the final manuscript.

FUNDING STATEMENT

MJWC, MDD and RLW received no direct funding support for this research. MAS is supported by the United States Department of Agriculture – Agricultural Research Service.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article, its supplementary information files and the BOLD dataset DS:ORPHAE (apart from one DNA barcode from Hawai'i which is yet to be uploaded to BOLD).

References

Bippus, M. (2019) Pyraloidea of Mauritius and neighbouring islands (Lepidoptera). *Phelsuma* 27, 36–57. Available at: https://islandbiodiversity.com/Phelsuma27d.pdf (accessed 3 May 2024).

BOLD (2024) Barcode of Life Data System. Available at: http://www.boldsystems.org/ (accessed 3 May 2024).

Cock, M.J.W., Murphy, S.T., Kairo, M.T.K., Thompson, E., Murphy, R.J. and Francis, A.W. (2016a) Trends in the classical biological control of insect pests by insects: An update of the BIOCAT database. *BioControl* 61(4), 349–363. DOI: 10.1007/s10526-016-9726-3.

Cock, M.J.W., Day, R.K., Hinz, H.L., Pollard, K.M., Thomas, S.E. *et al.* (2016b) The impacts of some classical biological control successes. *CABI Review* 10(42), 1–58. DOI: 10.1079/pavsnnr201510042.

Cock, M.J.W., Day, M.D. and Winston, R.L. (2023) Citizen science to monitor the establishment and spread of a biological control agent: The case of *Pareuchaetes pseudoinsulata* (Lepidoptera, Erebidae) for the control of *Chromolaena odorata* (Asteraceae) in South and South-East Asia. *CABI Agriculture and Bioscience* 4(25), 1–6. DOI: 10.1186/s43170-023-00171-5.

De Prins, J., De Prins, W. and Goff, R. (2024) *Orphanostigma haemorrhoidalis*. Afromoths, Online Database of Afrotropical Moth Species (Lepidoptera). 2011–2024. Available at: https://www.afromoths.net/species/26472 (accessed 3 May 2024).

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39, 783–791.

Harley, K.L.S. (1956) Lantana investigations in Hawaii. Feeding Tests on Economic & Ornamental Plants with *Syngamia haemorrhoidalis* Guenee. Conducted by Ken L.S. Harley – April to June 1958. Unpublished Report. Queensland Department of Lands, n p. 8 + 5 (appendix).

Hebert, P.D.N., Cywinska, A., Ball, S.L. and deWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270, 313–321. DOI: 10.1098/rspb.2002.2218.

Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. (1991) *The World's Worst Weeds*. Reprint edn, Krieger Publishing Company, Malabar, Florida.

iNaturalist (2024) Available at: https://www.inaturalist.org/home (accessed 3 May 2024).

Janzen, D.H. and Hallwachs, W. (2024) Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids,

of Area de Conservacion Guanacaste (ACG), northwestern Costa Rica. Available at: http://janzen.sas.upenn.edu (accessed 1 May 2024).

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547–1549.

Landry, B. (2016) Taxonomic revision of the Spilomelinae (Lepidoptera, Pyralidae s. l.) of the Galápagos Islands. *Revue suisse de Zoologie* 123(2), 315–399.

McClay, A., Bourchier, R.S., Landry, J.-F., Morris, C.E. and Toševski, I. (2023) Citizen science reveals the establishment of *Chamaesphecia empiformis* (Esper) (Lepidoptera: Sesiidae), a long-lost biological control agent for *Euphorbia cyparissias* (Euphorbiaceae), in Ontario, Canada. *Canadian Entomologist* 155, e29. DOI: 10.4039/tce.2023.17.

Ratnasingham, S. and Hebert, P.D.N. (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. *PLoS One* 8(8), e66213. DOI: 10.1371/journal.pone.0066213.

Robinson, G.S. (1976) The preparation of slides of Lepidoptera genitalia with special reference to the microlepidoptera. *Entomologist's Gazette* 27, 127–132.

Shaffer, J.C. and Munroe, E. (2007) Crambidae of Aldabra Atoll (Lepidoptera: Pyraloidea). *Tropical Lepidoptera* 14(1-2), 1–110.

Solis, M.A., Cock, M.J.W., Janzen, D.H. and Hallwachs, W. (2024) New World *Orphanostigma* Guenée including a new species from Costa Rica (Lepidoptera: Crambidae: Spilomelinae). *Zootaxa* 5471(3), 383–390.

Tamura, K. and Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10, 512–526.

Toševski, I., Caldara, R., Jović, J., Hernández-Vera, G., Baviera, C., Gassmann, A. and Emerson, B.C. (2011) Morphological, molecular and biological evidence reveal two cryptic species in *Mecinus janthinus* Germar (Coleoptera, Curculionidae), a successful biological control agent of Dalmatian toadflax, *Linaria dalmatica* (Lamiales, Plantaginaceae). *Systematic Entomology* 36(4), 741–753.

Toševski, I., Sing, S., De Clerck-Floate, R., McClay, A., Weaver, D. *et al*. (2018) Twenty-five years after: Post-introduction association of *Mecinus janthinus* s.l. with invasive host toadflaxes *Linaria vulgaris* and *Linaria dalmatica* in North America. *The Annals of Applied Biology* 173(1), 16–34.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cook, M.J.W. and Julien, M.H. (eds.) (2014) *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*. 5th edn, USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia. FHTET-2014-04, p. 838.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cook, M.J.W. and Julien, M.H. (eds) (2024) Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds. Based on FHTET-2014-04, USDA Forest Service, Forest Health Technology Enterprise Team, Tifton, GA. Available at: https://www.ibiocontrol.org/catalog/ (accessed 1 May 2024).

Zahiri, R., Lafontaine, J.D., Schmidt, B.C., deWaard, J.R., Zakharov, E.V. and Hebert, P.D.N. (2014) A transcontinental challenge – A test of DNA barcode performance for 1541 species of Canadian Noctuoidea (Lepidoptera). *PLoS One* 9(3), e92797. DOI: 10.1371/journal.pone.0092797.