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ORIGINAL ARTICLE

Humble beginnings of the Australian giant wood moth (*Endoxyla cinereus*): A century-old mystery solved

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Abstract The Australian giant wood moth, *Endoxyla cinereus* (Cossidae), is a major forestry pest whose later instars bore into the trunks and branches of *Eucalyptus*, but the early larval stages have remained unknown for over a century. Using DNA analysis, we identified larvae collected from galls formed by scale insects (*Apiomorpha* spp.), as *En. cinereus*. This relationship was tested by rearing over 100 000 neonate larvae on alternative potential food resources including active galls of scale insects (*Apiomorpha munita* and *A. crispa*). Only larvae assigned to galls fed and developed into later instars. The complete life cycle of this moth was also confirmed by rearing a gall-fed larvae to adulthood over 2 years after establishment in a tree (*Eu. tereticornis*). This two-phase developmental strategy explains how larvae bridge the large size gap between hatching and tree establishment and provides a mechanism for the high reproductive output and dispersal bottlenecks of this species.

Key words *Apiomorpha*; Cossidae; Eriococcidae; galls; life cycle; wood-boring

Introduction

Key stages of the life history of the Australian giant wood moth, *Endoxyla cinereus* (Tepper, 1890) (Cossidae: Zeuzerinae), have remained unresolved despite over a century of observation. This species plays important ecological, cultural and economic roles across Australia. Larval tunneling initiates tree hollows that provide habitat for insects, frogs and birds (Mo, 2015; Thurman, 2022), and larvae form an important seasonal food resource for yellow-tailed black cockatoos (*Zanda funereus* Shaw, 1794) (McInnes & Carne, 1978; Dickinson *et al.*, 2003). *Endoxyla* larvae have long been valued culturally as a food source by Indigenous Australians, with some ceremonial songs and dances marking the seasonal harvest of their larvae commonly known as "witchetty grubs" (Dodd, 1916; Tindale, 1953; Thurman, 2022). Although there are several large species of *Endoxyla*, *En. cinereus* currently holds the record for the heaviest moth in the world at 31.2 g (Beccaloni, 2010). This species has also presented an economic challenge as a major forestry pest, where its larval tunneling and excavation by cockatoos causes substantial losses in commercial eucalypt plantations (McInnes & Carne, 1978; Griffiths *et al.*, 2004; King & Lawson, 2005).

Despite this importance, a fundamental gap persists in understanding early development of *En. cinereus*. Later larval stages have been well documented boring within trunks and branches of *Eucalyptus* since the 19th century (Froggatt, 1894; Dodd, 1916; McInnes & Carne, 1978), and adult emergence has been described more recently (Monteith, 1991a; Fearn & Leong, 2022), but the earliest larval stages remain largely unknown. Neonates measure

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∼1.5 mm in length, yet they are not observed entering trees until ∼25 mm in length (Monteith, 2011), indicating a distinct, unobserved development phase before tree establishment. This gap was first noted by Illidge & Quail (1903) and has remained unresolved.

Several hypotheses have been proposed to explain where early-instar larvae spend the first 8–10 months of development. Newly hatched larvae disperse by silk ballooning (Bell *et al.*, 2005; Monteith, 2011), a behaviour also observed in *En. lituratus* (Littler, 1904). After dispersal, larvae have been hypothesized to feed in leaf litter or on roots before entering trees (Monteith, 1991b, 2011; Fearn & Leong, 2022; Thurman, 2022). This is seen in ghost moth larvae like *Aenetus virescens* (Hepialidae), whose early instars feed on decaying wood and fungi before establishing in tree trunks or stems (Grehan, 1983, 1987). This subterranean hypothesis is plausible because other species of *Endoxyla* have life cycles that are entirely subterranean (Tindale, 1953; Fearn & Maynard, 2019). However, no early-instar larvae of *En. cinereus* have been documented in leaf litter, soil or roots.

Recent molecular evidence has provided the first indication of an alternative developmental pathway. DNA barcoding of lepidopteran larvae discovered within galls formed by scale insects (Hemiptera: Coccomorpha: Eriococcidae: *Apiomorpha*) on *Eucalyptus* were initially identified as *En. cinereus*, suggesting a previously undocumented association between neonate wood moths and these insect-induced plant structures. *Apiomorpha* galls are a plausible early-instar habitat for *En. cinereus* as they occur on the same host trees used by later-stage wood moth larvae, their apical openings are large enough to admit neonates (0.2–2 mm diameter), and they provide nutritious plant tissues (photosynthates) in a protected environment (Gullan, 1984), similar to the cambial tissues that later-stage larvae feed on within tree tunnels. The scale insect itself is also rich in sugars, proteins and fats. *Apiomorpha* is widespread in Australia (Gullan, 1984) and overlaps with the known range of *En. cinereus* (Thurman, 2022). Exploitation of scale insect galls could therefore resolve the long-standing discrepancy between hatching size and the size at which *En. cinereus* larvae establish in trees.

Here, we test this gall-feeding hypothesis for *En. cinereus* development through DNA analysis of field-collected larvae, controlled feeding trials with neonates, and rearing from egg to adulthood over multiple years. We compare survival and development across multiple potential food resources including scale insect galls and eucalypt bark, wood, and foliage. Through this process we also fill remaining knowledge gaps of the life history of the giant wood moth, including oviposition, hatching period, neonate larval behaviour and the transition from gall feeding to tree boring. These findings complete our understanding of *En. cinereus* life history and have implications for pest management in forestry plantations and ecological studies of wood-boring insects and their interactions with gall-forming species. This work also provides a novel example of multi-year development of an insect with high reproductive capacity, which may provide insights into the evolutionary ecology of extreme reproductive investment in long-lived insects.

Materials and methods

Adult specimens of the giant wood moth (*En. cinereus*) were obtained from Moore Park and Robertson Park, and from Herston Road in Queensland, Australia with permission from Brisbane City Council (Park Use Consent Reference: PC20211109) in late 2021. These specimens were used to record pupation period, adult lifespan and behaviour. Adults were kept alive post-emergence and larvae were maintained indoors under ambient, non-controlled conditions, which broadly reflected prevailing outdoor temperature and photoperiod. Existing collections of Lepidopteran larvae collected from scale insect galls by L.G. Cook and P.J. Mills were also reviewed for specimens of *En. cinereus*, and online records of the species were reviewed on Barcode of Life Data (BOLD) Systems database. DNA from these larvae was sequenced for *COI* to confirm their identity as *En. cinereus*.

Mitogenome and COI sequencing

DNA was extracted from each specimen using a DIY spin column method (Ridley *et al.*, 2016). Illumina DNA sequencing libraries were prepared by Novogene (Beijing, China) using the NebNext kit and 10 GB of paired-end 150 bp Illumina data was obtained for each of the samples, yielding 76–83 million sequences per sample to reconstruct mitogenomes. The sequence data were trimmed using BBDuk (https://jgi.doe.gov/data-and-tools/software-tools/bbtools/) using $k = 23$ to remove Illumina adapters, phiX, and trim the right end to remove any sequence with a quality score below 10. The sequences were mapped to the mitochondrial genome of *En. cinereus* from GenBank (accession OK644702.1) using the low sensitivity setting and three iterations in Geneious Prime 2022.1.1 (https://www.geneious.com). The trimmed sequence data were mapped again to the mitochondrial consensus sequence created from the initial mapping. In both cases this resolved the complete coding section of the mitochondrial genomes but did not fully resolve the control region, which is repeat-rich. Initial annotations were made using MITOS2 in galaxy (Bernt

et al., 2013), then refined by comparison to the *Endoxyla* mitogenome sequences available from GenBank.

The barcoding region of mitochondrial cytochrome c oxidase subunit I (*COI*) was amplified using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) in 25 μL reactions using 1 unit of MyTaq, 1 × MyTaq buffer (Meridian Bioscience, Ohio, USA) and 0.2 μmol/L of each primer. PCR reaction conditions were as follows: initial denaturation at 95 °C for 5 min, then 7 cycles of 95 °C denaturation for 30 s, 45 °C annealing for 45 s, and 72 °C extension for 1 min, followed by 35 cycles of 95 °C denaturation for 30 s, 50 °C annealing for 45 s, and 72 °C extension for 1 min, with a final extension at 72 °C for 5 min. PCR reactions were cleaned using one unit each of Exonuclease I and Antarctic Phosphatase. Sequencing was performed by Macrogen (Korea) and the sequences were edited using Codon Code Aligner (CodonCode, MA, USA) to remove low quality sequences and correct errors in base calling. Primers were then removed in Geneious Prime 2024.0.3, sequences were aligned using MAFFT (Katoh & Standley, 2013), and trees estimated using IQ-TREE 2 using the MFP+MERGE option (Nguyen *et al.*, 2015; Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017; Minh *et al.*, 2020). SH-like approximate likelihood ratio test values (SH-aLRT) (Guindon *et al.*, 2010) and ultrafast bootstrap approximation values (Hoang *et al.*, 2018) were obtained in IQ-TREE 2 using 10 000 replicates each. All available sequences for the genus *Endoxyla* were accessed on December 6, 2024 from BOLD and GenBank with the outgroup, *Xyleutes persona* included. An initial tree was used to identify closely related species to *En. cinereus*. Five records from three closely related species and one other genus were used as outgroups for the final tree. These records included *En. affinis* (ANICM205–10 and ANICM206–10), *En. edwardsorum* (ANICM218–10 and ANICM223–10), *En. magnifica* (ANICM207–10), and *Xyleutes persona* (HQ952091). For the final tree, the "TIM2+I+G4" model was chosen as the best fit according to the Bayesian information criterion (BIC).

Feeding tests

A mated adult female of *En. cinereus* (ECHB1) was obtained for this study to observe oviposition, neonate behaviour, and to test whether neonates can survive on scale insect galls (*Apiomorpha*) or alternative food resources from one of its known host trees (*Eu. tereticornis*) until they are ready to establish inside the trunks or branches. This female (ECHB1) was initially located by R. Coupland at a bus stop (Stop ID: 020523) in Herston, Queensland (−27.449747, 153.026279) on November 29, 2021. The condition of the female moth and weight upon collection (15.9 g) suggested that she had recently emerged. The adult female was allowed to lay eggs in a cage. Eggs were removed from the cage daily and placed into labeled Petri dishes for various feeding treatments and rearing. The total number of eggs laid by the female was estimated based on the weight of a known number of eggs (7642 eggs per gram).

Feeding trials consisted of exposure to three dietary treatments: (i) unfed controls (4 replicates), (ii) *Eu. tereticornis* plant material (10 replicates across 3 classes), or (iii) galls from active *Apiomorpha* spp. scale insects (2 replicates). Each replicate consisted of 30 recently hatched (2 d old) larvae. Plant material feeding treatments used larvae in Petri dishes with leaves (2 replicates) or bark with cambium tissue (2 replicates), bagged larvae on leaves and stems (2 replicates), and cage-enclosed larvae on trunks of blue gums (*Eu. tereticornis*) (4 replicates) in Moore Park, Indooroopilly, Queensland, Australia (−27.4993, 152.9636). Gall feeding treatments included two sets of bagged larvae with established scale insect galls. The first were bagged with eleven active and mature female galls of *A. munita* on epicormic stems of a Queensland grey gum tree (*Eu. major*), and the second were bagged with seven active and mature female galls of *A. crispa* on a potted broad-leaved ironbark tree (*Eu. melanophloia*). Because of the nature of scale insect galls, which can require years of establishing on a host plant, individual galls could not be separated and bagged cohorts had to be used. The apical orifices of the galls were ∼0.2 mm for both *A. munita* and *A. crispa*, serving as a potential entrance for the early-instar larvae of *En. cinereus*. Larvae in each feeding test were observed daily.

Life history observations

Once raised to later instars, larvae were either preserved in ethanol or allowed to establish on the trunk of a blue gum tree (*Eu. tereticornis*) in Robertson Park, St Lucia, Queensland, Australia (−27.50327, 152.98979) where other wild larvae of *En. cinereus* had been observed and where they could be regularly monitored. This second phase of larval development was recorded from the formation date of the larval pad and tunnel in the tree until the formation date of an exit hole which signals pupation. The pupation period was also recorded as the date of exit hole formation to adult emergence. The time spent in these phases of development inside the tree were recorded for both reared larva and wild larvae with daily checks of trees in Robertson Park, St Lucia, Queensland, and Moore Park, Indooroopilly, Queensland (Table 1).

Table 1 Records of larvae of *Endoxyla cinereus* found within scale insect galls (*Apiomorpha*) and host plants from public and private collections in Australia.

Gall inducer	Host plant	State	GPS	Date coll.	Collector	Record
Apiomorpha sp.	"mallee *Eucalyptus*"	SA	−34.059, 140.669	9 Dec 2011	P. Hebert & J. deWaard	BOLD: AUSGC212–12
A. ovicoloides	"mallee *Eucalyptus*"	SA	−34.059, 140.669	9 Dec 2011	P. Hebert & J. deWaard	BOLD: AUSGC213–12
A. pharetrata	*Eucalyptus globoidea*	NSW	−35.684, 150.163	5 Jan 2011	P.J. Mills	ECPJ1 (PJM00223)
A. floralis	*Eu. shirleyi*	QLD	−19.106, 145.304	29 Aug 2014	L.G. Cook	ECLG1 (LGC02785)
A. munita	*Eu. major*	QLD	−27.502, 152.961	19 Jan 2022	J.H. Thurman	ECHB1.1 (Reared in study)
A. crispa	*Eu. melanophloia*	QLD	N/A	17 Feb 2022	J.H. Thurman	ECHB1.2 (Reared in study)

Note: Public records included the Barcode of Life Data System (BOLD). Larval identifications were based on *COI* sequences.

Fig. 1 *Endoxyla cinereus* larvae found in scale insect galls from (A, B) *Apiomorpha ovicoloides* (Process ID: AUSGC213–12) and (C, D) an unidentifiable species of *Apiomorpha* (gall similar to multiple species) (Process ID: AUSGC212–12). Image (A) shows the live female scale insect with a larva while (D) reveals no scale insect currently present inside the gall. Images by CBG Photography Group from BOLD Systems and licensed under CC BY 4.0.

Results

Previous records and identification of specimens

Review of the images associated with records on BOLD Systems included two instances of *En. cinereus* larvae inside galls of scale insects (*Apiomorpha*) with frass suggesting they were feeding within the gall (Fig. 1). The scale insects in each record were both identified based on morphology visible in the photographs and were *A. ovicoloides* (Fig. 1A, B) and an unidentifiable species of *Apiomorpha* (Fig. 1C, D) (Table 1). A live scale insect

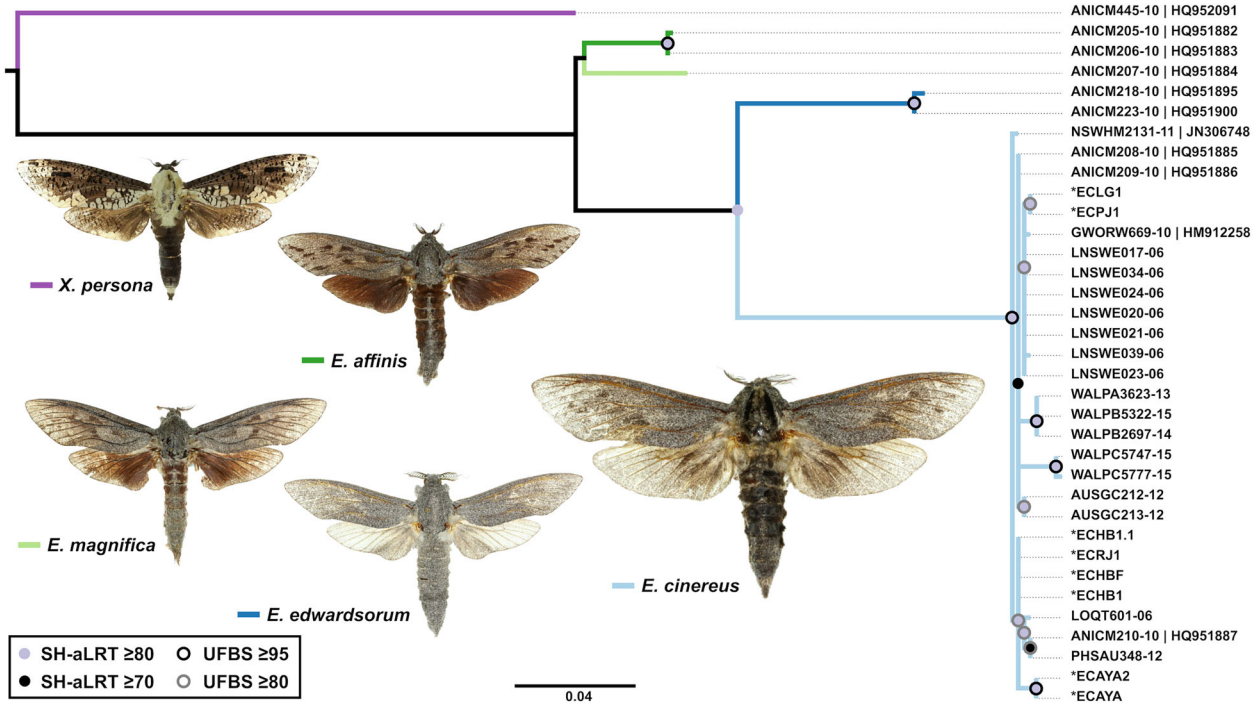

Fig. 2 Maximum-likelihood phylogenetic tree based on mitochondrial COI sequences showing relationships among *Endoxyla cinereus* and related species (*E. affinis*, *E. edwardsorum* and *E. magnifica*) using the outgroup (*Xyleutes persona*) to root the tree. Sequences were edited and aligned using MAFFT, and the tree was inferred in IQ-TREE under the TIM2+I+G4 substitution model. Node support values indicate SH-like approximate likelihood ratio test (SH-aLRT) support and ultrafast bootstrap support (10 000 replicates each). Scale bar indicates substitutions per site. All available *E. cinereus* records from BOLD ($n = 22$) and sequences generated in this study ($n = 8$, marked with *) are included. See Table 1 for record codes of specimens from galls. Additional adult and larval specimens were included from Ayr (ECAYA and ECAYA2) and (ECRJ1 and ECHB1) Brisbane, Queensland. Images were sourced from the BOLD Systems database and reproduced under a CC BY 4.0 license.

(*A. ovicoloides*) was observed in one record, whereas the other has no scale insect present in the gall (Fig. 1). Personal collections from L.G. Cook and P.J. Mills uncovered additional *En. cinereus* larvae collected from scale insect galls. These include *En. cinereus* in the gall of *A. floralis*, which was growing on *Eu. shirleyi*, and another larva in the gall of *A. pharetrata*, which was growing on *Eu. globoidea* (Table 1).

All records of *En. cinereus* available on BOLD ($n = 22$) and sequenced in this study ($n = 8$) were used to estimate a phylogeny (Fig. 2). All *COI* sequences of *En. cinereus* were within 2% pairwise similarity. This confirmed that the previous records of larvae found in galls, the adult female collected in this study (ECHB1) and her reared larvae (ECHB1.1, ECHB1.2) were all *En. cinereus* and that this species has a broad range of scale insect hosts (Table 1).

Eggs

The captured adult female of *En. cinereus* (ECHB1) began laying eggs in a mesh cage in the evening of November 29, 2021, but in relatively small clusters (~800 eggs) (Fig. S1A). Over the following 3 d, larger quantities of eggs were laid in both large (~10 000 eggs) and small clusters (~1000 eggs) (Fig. S1B). Based on the known weight of eggs per gram, an estimated total of 119 698 eggs were laid by ECHB1 over 4 d (29 November to December 2, 2021). ECHB1 weighed 15.9 g at collection and 5.3 g after this egg-laying period. The female perished after 5 d in captivity on December 4, 2021. This is thought to be the natural cycle for this species as adults do not feed and only survive long enough to mate and reproduce.

The eggs were opaque yellow with a thin casing and appeared susceptible to desiccation (Fig. S1B). The form

of the eggs and the long ovipositor (∼5 cm, Fig. S1A) of the female moth suggest that when laid in natural settings, the eggs are likely placed under flaking bark, like that found on *Eucalyptus*. The stripes of the larvae became visible in the eggs 11 d after being laid. Hatching began on day 12 and continued for each batch of eggs over the consecutive days.

Neonate larvae and feeding tests

An estimated 98% of the over 100 000 eggs hatched. The remainder were undeveloped and possibly unfertilized. Upon hatching, larvae were observed in Petri dishes consuming their egg casing, spinning silk and forming masses similar to the clusters of their eggs (Fig. S1C). The average head capsule width of neonate larvae was 0.27 ± 0.01 mm. Five days post-hatching, larvae were measured again with an average head capsule width of 0.48 ± 0.05 mm. When disturbed or uncovered from their silken covering, larvae would quickly disperse to another area where they would begin clustering and taking shelter. Samples of larvae 5 d post-hatching were placed on different potential food resources while the remaining cohorts were left in Petri dishes for further observation.

Larvae used as controls were kept together and unfed in Petri dishes until December 16, 2021. At this point, control larvae had been clustering for 3–5 d, and a mass dispersal event occurred (Fig. S1D). An environmental trigger for this dispersal event was not immediately apparent, but maximum temperatures and humidity peaked at 29 °C and 94% on this day, while wind speed (20 km/h), pressure (1012.5 hPa), and precipitation (0 mm) remained relatively constant compared to the previous 5 d [weather data accessed from Bureau of Meteorology (http://www.bom.gov.au)]. Larvae escaped Petri dishes and left trails of silk, which formed solid silk sheets after thousands of larvae followed the same course. Larvae were observed dispersing toward the nearest window, climbing up the wall and window, and silk-dropping. When they landed on the ground, the larvae would repeat this process of attempted dispersal.

No signs of feeding were observed over 5 d with larvae in any of the plant-feeding treatments including larvae in Petri dishes with leaves or bark, and those caged on trunks of *Eu. tereticornis*. Larvae in these treatments were found dead or missing. Larvae bagged with galls of *Apiomorpha* were not visible in the bag after 1 d of exposure, and the galls were checked for any signs of larvae daily. By January 2, 2022, frass was observed in the bags of both scale insect gall treatments. This frass was emptied and checked daily to monitor the activity of the larvae.

After 40 d in the scale insect treatment, reduced frass had been observed in the bag with *A. munita* galls and all eleven scale insect galls were then cut open (Fig. 3A). Of these, seven galls were still active with scale insects. A single larva of *En. cinereus* (ECHB1.1) was found in a gall full of frass and silk (Fig. 3B). The larva had grown from a head capsule width of 0.42 mm (Fig. 3A) to 1.35 mm (Fig. 3B). The growth of the larva and various sizes of frass pellets within the dead galls suggests that the larva consumed the plant tissue within the gall alongside the scale insect and potentially fed on multiple scale insects and their galls. No other larvae were present in the bag suggesting that they either escaped or were consumed by the larva recovered (ECHB1.1). This larva was photographed and preserved in ethanol.

Larvae bagged with active *A. crispa* continued to produce frass and by 19 January, frass was observed coming out of three different galls. The galls were allowed to continue to grow and monitored daily until February 17, 2022, when one larva of *En. cinereus* was observed outside of the galls and crawling in the bag (Fig. 3C). This larva (ECHB1.2) had grown from a head capsule width of 0.43 mm to 3.63 mm after 68 d in the scale insect treatment. The remaining galls of *A. crispa* were inspected and a second larva (ECHB1.3) was observed inside a gall with frass and silk. Only four out of the seven galls remained active with the other three having been hollowed out, full of frass and silk, and with no remains of the scale insects. Larger holes (∼4 mm wide) had been formed in the hollowed galls near the stem where the galls were attached to the plant (area above white dash in Fig. 3D). The larva outside the galls (ECHB1.2) is thought to have fed in one gall, left it and then moved into a new gall. The larva found inside a gall (ECHB1.3) may have still been forming its exit. One larva (ECHB1.2) was then exposed to a planted *Eu. tereticornis* to allow for larval establishment in a tree.

Larval establishment in trees

The second phase of development occurs when the larva establishes in a trunk or branch of *Eucalyptus*, where it will spend the next 1–2 years feeding on the callus tissue under the bark. One larva (ECHB1.2) from the cohort of eggs was allowed to establish in the trunk of a blue gum tree (*Eu. tereticornis*) at 18 : 45 on February 17, 2022. An additional wild larva (ECA7) was observed during a chance encounter, when it was found forming its silken webbing on the trunk of a flooded gum (*Eu. grandis*). The behaviour of both are described herein with photographs of larval establishment in trees (Fig. 4).

The raised larva (ECHB1.2) was placed at the base of an *Eu. tereticornis* tree. It then crawled up the trunk slowly, appearing to inspect the bark by pausing and moving its head from side-to-side before continuing crawl-

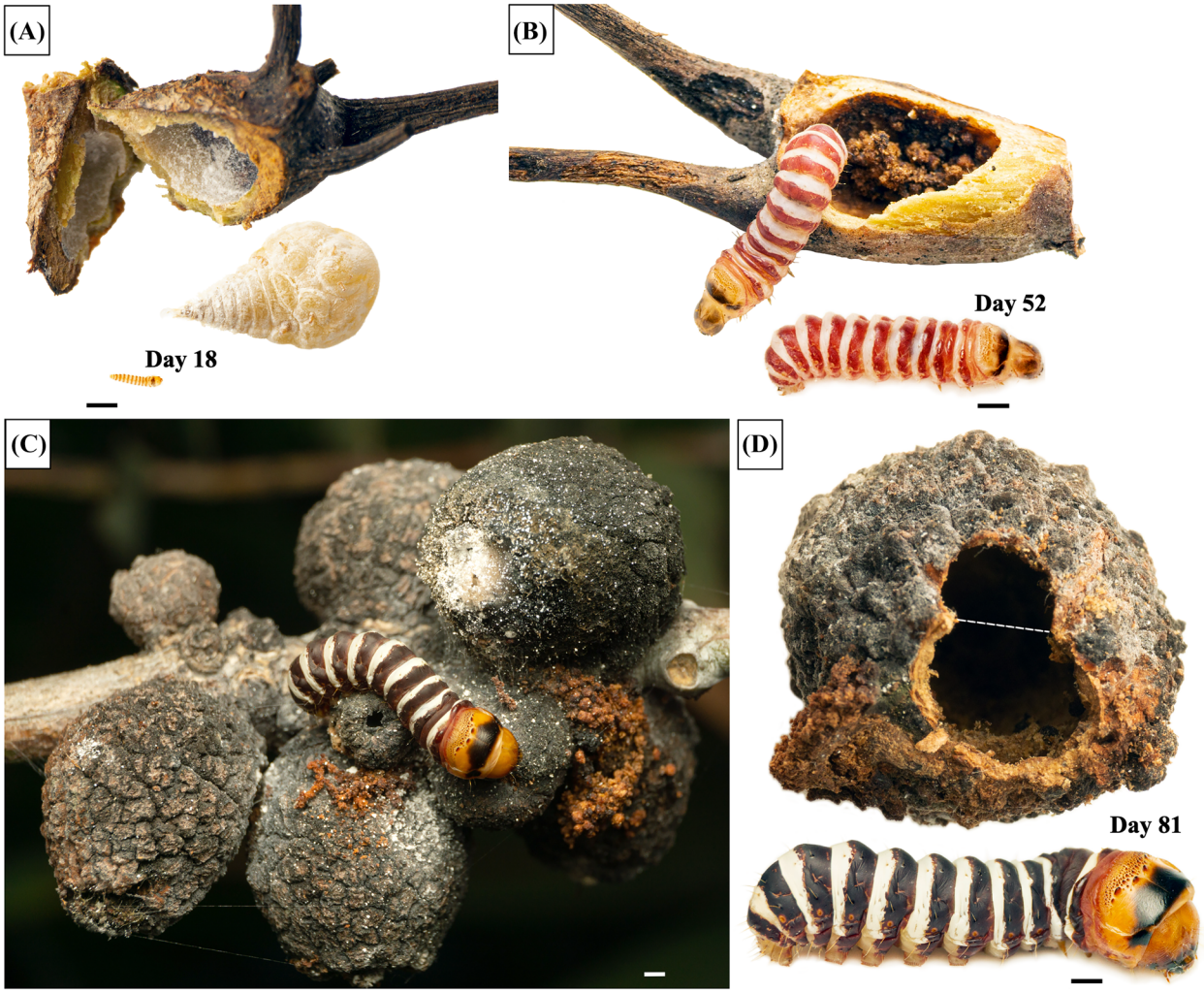

Fig. 3 Larvae of the giant wood moth (*Endoxyla cinereus*) were reared with scale insects (*Apiomorpha* spp.) and their galls. (A) Larvae were bagged with female *A. munita* and their galls on branches of a tree (*Eucalyptus major*) 18 d post-hatching. An opened gall is shown with the excised female scale insect. (B) One larva (ECHB1.1) was recovered inside of the female gall of *A. munita* 52 d post-hatching. (C) Other larvae were bagged with galls of females of *A. crispa* on a potted tree (*Eu. melanophloia*). (D) A larva (ECHB1.2) was found outside the galls at 81 d post-hatching. An excised gall shows the exit hole formed by the larva (above white dashed line), the area below the white dashed line indicates where the gall was attached to the branch. Scale bars represent 1 mm.

ing up. Within 20 min of being placed on the tree, the larva stopped ~0.7 m from the ground and began to lay silk strands over either side of its body, crossing dorsally (Fig. 4A). This continued for 25 min as the larva rotated until a circular silken hammock or pad was formed with the larva tightly curled inside of it. This silk held the larva in place as it began to tear strips of bark from the trunk, excavating a small circular hole, and incorporating the small bark strips into the silken pad (Fig. 4B). This continued until the larva then flipped to where its dorsal side was against the trunk and laid down more silk on the pad (Fig. 4C). The larva flipped back to face the trunk and resumed tearing small pieces of bark from the trunk, repeating this process as it further excavated the tree. Visibility of the larva's behaviour became limited after 40 min of this activity. The larva was observed excavating for a further 2 h, at which we lifted the silken pad and observed a tunnel one fourth of its body length excavated. The following morning, the silken pad was only partially attached to the tree trunk and was full of strips of bark (Fig. 4D). The larva was no longer visible and underneath the silken tent, a rigid wall was observed closing the entrance of the tunnel. This wall was made of dense silk and had a semi-transparent center (Fig. 4D). Two days after establishment in the tree, a hole in the center of this silken wall was formed by the larva and plugged with a

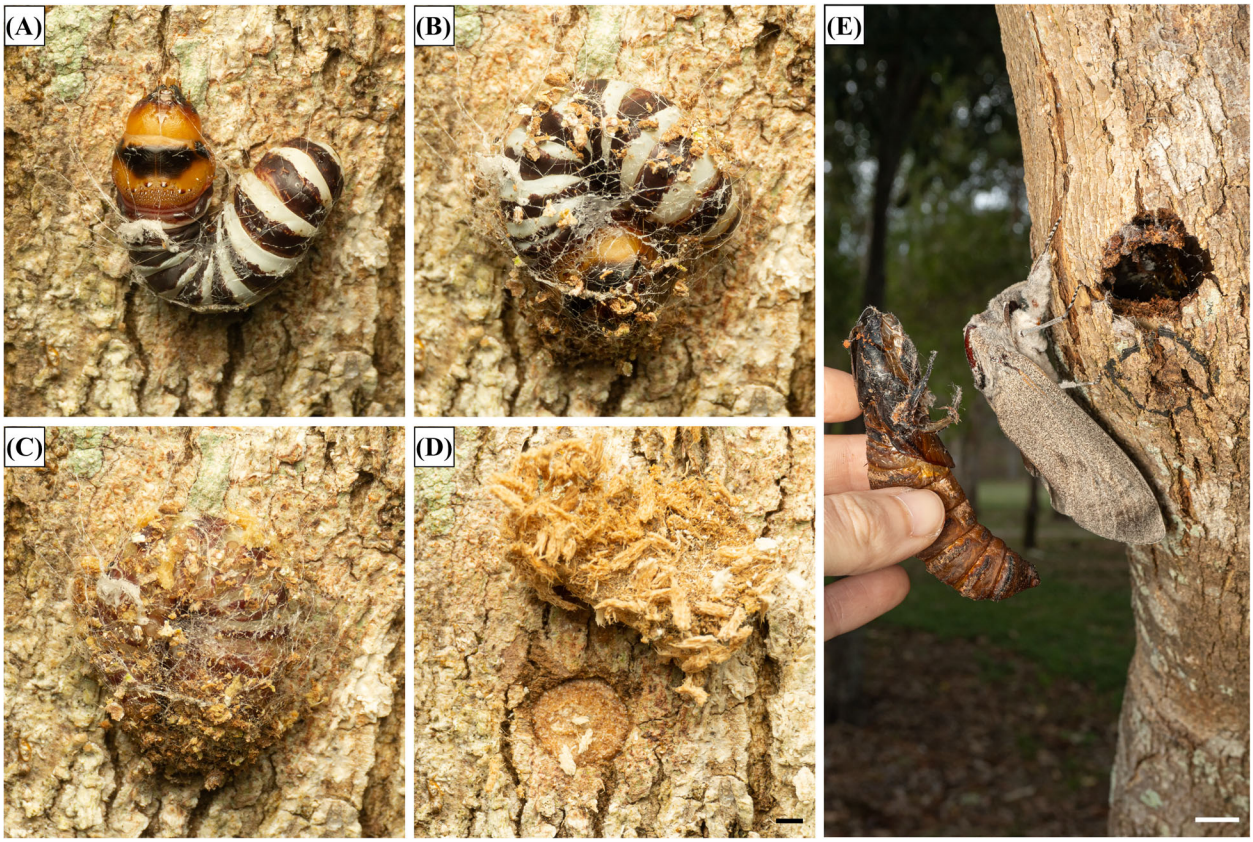

Fig. 4 Larval establishment in a tree in February 2022 and adult emergence in November 2023. (A) The larva first forms a hammock of silk to secure itself to the tree. (B) Excavation of the tunnel begins and material from the tree is incorporated into the silk enclosure the larva uses to secure itself in place. (C) As the excavation continues, the larva adds more silk to the covering and incorporates pieces of wood strips. (D) By the following day, the larva has excavated its tunnel and sealed it off with a plug made of silk. Scale bar represents 1 mm. (E) An adult male moth emerged on November 19, 2023, nearly 2 years after hatching from an egg. Its pupal case was removed from the exit hole (right) and held behind it (left) to demonstrate the size. Scale bar represents 1 cm.

strip of wood. This hole is used to discard frass and further excavated bark strips over time.

A wild *En. cinereus* larva (ECA7) was found during the early stages of this process where it had formed the silken pad, securing itself to the tree ~1.2 m from the ground at 12:30 on September 24, 2022. By 13:45 the larva was no longer visible beneath its silken pad. The pad was lifted up at 14:30, revealing a tightly packed mass of strips of bark and frass with the larva halfway into the trunk. By 18:15, the silken pad was lifted again to reveal the larva fully inside an excavated burrow with its head at the entrance. On the following day, the larva was checked in the morning, and it had excavated a tunnel large enough to be fully inside the tree, but no entrance plug to the tunnel was formed. A second check at 16:30 on the same day revealed an entrance plug like that observed in Fig. 4D.

Pupation period and adult emergence

Once established inside of the tree trunk or branch, the larva will remain here to feed (Fig. 5E, F), pupate (Fig. 5G), and later emerge as an adult (Fig. 5H). This second phase of development has been suspected to last 1–2 years and has been previously illustrated (McInnes & Carne, 1978; Monteith, 1991b; Thurman, 2022). The beginning of pupation includes a clearing of the lower initial entrance hole for the tunnel, which was once covered by the silken pad, and the formation of an upper, larger exit hole for the pupal case and adult to emerge out of (Fig. 5G).

The reared larva (ECHB1.2) spent 644 d in the tree from tunnel establishment (February 17, 2022) to adult emergence (November 23, 2023), with a combined prepupation and pupation period of 83 d—the longest recorded in this study (Table S1). Wild larvae averaged

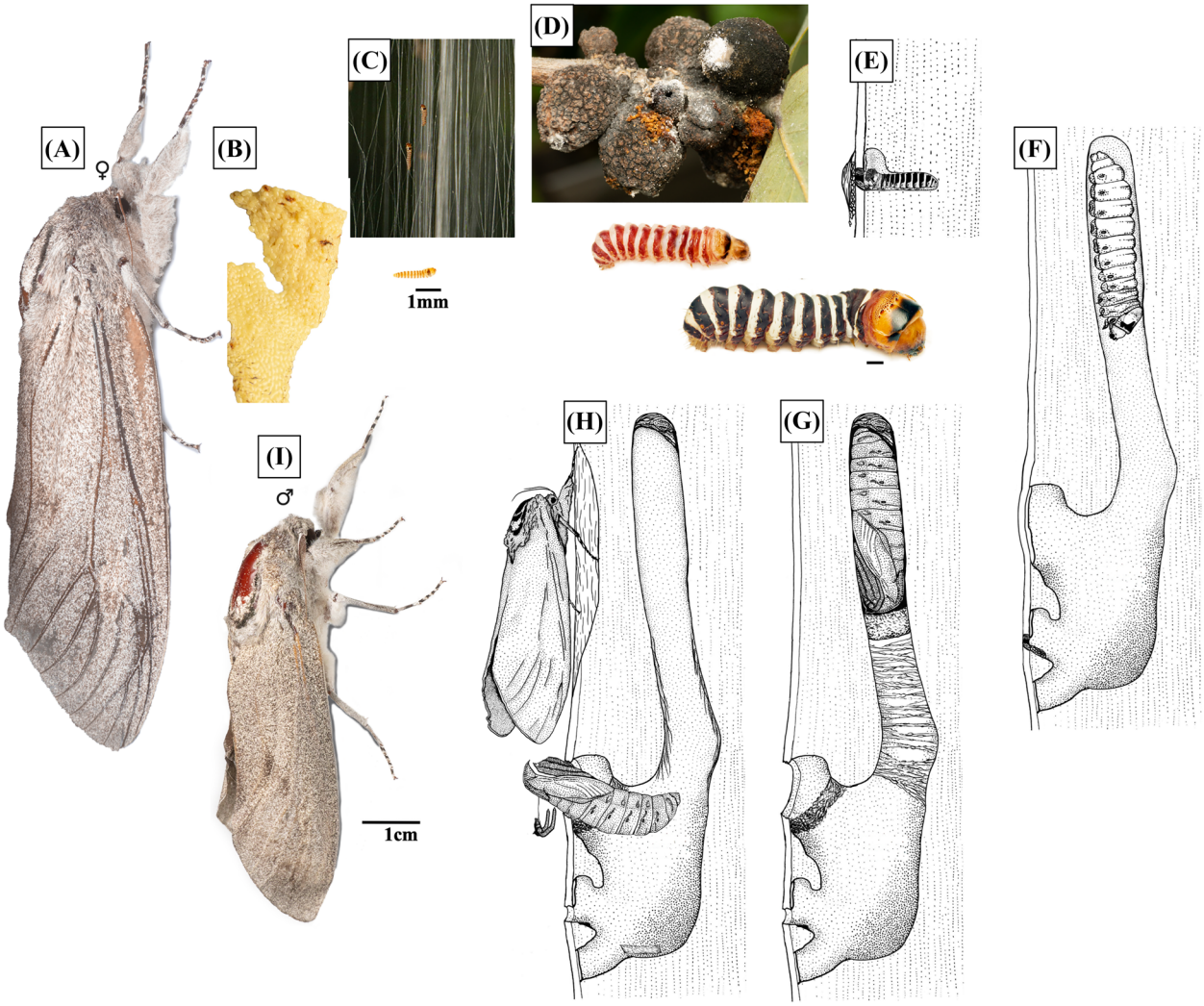

Fig. 5 The full life cycle of the giant wood moth (*Endoxyla cinereus*). (A) Adult females can lay (B) over 100 000 eggs. After hatching and sheltering, (C) larvae will silk disperse en masse. (D) Larvae are small enough to enter the opening of scale insect galls (*Apiomorpha*) where they feed on the gall tissue and the scale insect. (E) Once large enough, they move to the trunk or branch of a *Eucalyptus* tree to excavate a tunnel. (F) They feed on the callus tissue under the bark and further excavate their tunnel over multiple years. (G) The larva prepares to pupate by creating an exit hole, covering the hole with silk and frass, lining its tunnel with silk and mucus, then plugging itself in the top of the tunnel to pupate. (H) Pupae push through these barriers to emerge as adults, leaving their pupal case in the tree. (I) An adult male was reared through this life cycle to adulthood over a period of roughly 2 years after eggs were obtained from the female pictured in (A). Drawings represent historical knowledge while the photographs represent new information presented within this paper. Scale bars represent 1 mm for larvae and 1 cm for adults. Illustrations by J.H. Thurman and Geoff Thompson.

667 ± 54.3 d from larval establishment to adult emergence, ranging from 444 d (female, in 10 cm diameter *Eu. tereticornis*) to 794 d (female, in 18 cm diameter *Eu. tereticornis*). Development time showed no clear difference between sexes (males: 692 ± 48.0 d; females: 634 ± 77.2 d). Pupation averaged 56 ± 3.5 d overall, ranging from 31 to 83 d (both males), with no clear sex difference (males: 72 ± 4.9 d; females: 65 ± 4 d). However, half of pupation records ($n = 9$) could not be sex-assigned as they preceded the first author's ability to differentiate pupal cases by sex.

Discussion

Over a century ago, Illidge & Quail (1903) recognized that early larval development of *En. cinereus* does not occur within eucalypt trunks or branches, and that their

larvae must spend the first phase of their development elsewhere. This study demonstrates that early-stage larvae of *En. cinereus* can develop in scale insect galls before establishing in trees, resolving this long-standing gap in their life history. In feeding tests, only larvae exposed to scale insect galls survived, and one larva was reared to adulthood. Development from egg to adult took roughly 2 years, consistent with Dodd's (1916) predictions, but shorter than previous estimates of 4–8 years (Froggatt, 1894) or at least 3 years (Fearn & Leong, 2022).

This study also clarifies key aspects of *En. cinereus* reproduction and early development. Females can produce over 100 000 eggs, substantially exceeding previous estimates of 18000 based a congener (McInnes & Carne, 1978). Eggs were laid in multi-layered clusters rather than being coated in a glutinous secretion described by Common (1990), and egg incubation period was approximately two weeks. Eggs are likely laid under bark for protection, as seen in other cossid species like *Duomitus ceramicus* (Walker, 1865) (Gotoh *et al.*, 2007), and on particular host trees, but this has yet to be confirmed. Upon hatching, larvae consumed their egg casing and sheltered together for approximately 5 d. This gregarious behaviour may reduce predation risk through the dilution effect and may buffer environmental extremes with their shared silken structures (Despland & Le Huu, 2007). Larvae then dispersed by synchronized suspended ballooning, beginning their ontogenetic shift from gregarious early instars to solitary late instars. Ballooning is widespread among Lepidoptera, but shows no consistent association with habitat or diet (Bell *et al.*, 2005), suggesting that its adaptive significance is species-specific. In *En. cinereus*, ballooning likely assists in reducing sibling competition and locating the limited and spatially patchy resource of scale insect galls.

High mortality during this dispersal is expected, providing a strong selective basis for the extreme fecundity seen in *En. cinereus*. A similar strategy occurs in *Apiomorpha*, in which females produce thousands of crawlers (first-instar nymphs) that settle on their natal host plant, or disperse on wind currents or chance phoresy (Gullan & Cockburn, 1986). Dispersing crawlers must quickly locate a suitable host with the new growth needed for gall induction (Cook, 2000). *Apiomorpha* has a patchy distribution among suitable host trees, with few colonized and most trees hosting no galls (Gullan & Cockburn, 1986), a similar trend seen with *En. cinereus*. The patchy distribution for both groups is at least partially explained by the dispersal strategy used by each and may be further explained by host condition where stressed trees may be more likely to have galls and *En. cinereus*. The additional bottleneck applied to *En. cinereus* to find a suitable gall to feed in likely increases the need for thousands of offspring.

Together, these findings reveal a life history strategy built on high risk and high reproductive investment. Survival is constrained by sequential bottlenecks: successful dispersal to scale insect galls, establishment in a *Eucalyptus* host, successful pupation, and finding a mate within a short period. Prolonged larval development in this species likely results in the need to produce thousands of offspring and is made possible from feeding in environments protected from most predators and parasitoids. It also appears essential given the adult stage's severe temporal constraints. Adult males and females are aphagous, lacking functional mouthparts, and survive as adults for less than two weeks; females oviposit within 2 d regardless of mating, and eggs fail to hatch without fertilization. This is an extreme example of a capital breeding strategy, where reproduction is supported by using stored nutrients gained in earlier life stages but not in adulthood (Stephens *et al.*, 2009). Such a narrow reproductive window should theoretically limit population success, yet *En. cinereus* remains abundant across a wide geographic distribution, suggesting that adult emergence is highly synchronized. The mechanism for this synchronization likely involves environmental cues such as temperature and humidity and warrants further investigation.

The transition between the two larval phases also reveals distinctive adaptations. Larvae exhibit aposematic coloration with purple and white stripes and an orange-and-black head capsule, but toxicity has not been demonstrated. This conspicuous appearance may provide protection during the brief vulnerable period between leaving galls and establishing in trees. Larvae search for excavation sites during daylight hours, typically around noon, suggesting sun exposure may influence establishment site selection. Previous studies documented establishment on all tree aspects, with possible preference for north and west orientations (McInnes & Carne, 1978; Fearn & Leong, 2022).

These sun-exposed positions may promote callus tissue formation, providing optimal food resources, though this hypothesis requires experimental validation. Larvae may also establish more frequently near stressed trees where scale insect gall densities may be higher. It is most likely that larvae remain on the same trees as where they fed in scale insect galls, but they are usually observed crawling from the base of trees suggesting that they disperse between larval phases and could move between trees.

The discovery of gall-feeding in *En. cinereus* raises the question of whether other Australian cossids employ similar two-phase developmental strategies. *Endoxyla lituratus* and *En. encalypti* also produce large adults requiring substantial larval growth, raising the possibility that they

similarly exploit cryptic food sources before establishing in woody hosts. Wasp, fly, and fungal galls on Acacia species may serve analogous roles for these *Endoxyla* species. Systematic surveys and sequencing of gall inquilines would clarify whether gall-feeding represents a widespread strategy within *Endoxyla*.

From an applied perspective, dependence on scale insect galls during early development constrains the invasive potential of *En. cinereus*. Establishment outside Australia would require not only suitable *Eucalyptus* hosts but also compatible species of *Apiomorpha*. However, *En. cinereus* accepted multiple species of *Apiomorpha* in our trials, suggesting flexibility within this genus. Given the global cultivation of *Eucalyptus grandis*, co-introduction of appropriate scale insects could theoretically permit establishment of *En. cinereus*. Biosecurity risk assessments for overseas eucalypt forestry should therefore consider this previously unrecognized developmental requirement and its potential pathways.

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Disclosure

The authors declare that they have no relevant or material financial interests that relate to the research described in this paper.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 The pupation period and time spent in their tree phase for the giant wood moth (*Endoxyla cinereus*) was recorded from a reared larva (ECHB1.2) and from wild larvae across two sites: Robertson Park, St Lucia (RP, −27.503, 152.991) and Moore Park, Indooroopilly (MP, −27.500, 152.963), Queensland, Australia.

Fig. S1 Over 100 000 eggs were obtained from one female giant wood moth (*Endoxyla cinereus*, ECHB1).

