

Record of the Queen Victoria Museum and Art Gallery Launceston, Tasmania



Endoxyla cinereus (Tepper, 1890) (Lepidoptera: Cossidae: Zeuzerinae) in southeast Queensland.

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Cover photograph: Female Endoxyla cinereus shortly after eclosion, on trunk of Eucalyptus grandis. The Gap, Brisbane, Queensland. Photograph by Simon Fearn.

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# Ecological observations on the giant wood moth

*Endoxyla cinereus* is one of the world's largest insects and while it is widespread and locally common in eastern Australia, critical aspects of its biology are poorly understood. In this paper, important life history stages are described from the field for the first time. A three-phase larval cycle over a minimum of three years, including a second phase transfer larva, is confirmed. Behaviour of transfer and hatchling larvae and recently eclosed adults is described as well as weather conditions at time of eclosion. Seasonal timing of key developmental larval stages is identified as well as duration of pre-pupal and pupal stages. A north-west bias in larval bore placement on host tree stems by transfer larvae is identified and two possibly related explanations offered based on host stem exposure to solar radiation. A significant positive correlation between height above ground of bores as well as diameter of host stems at bore site and host tree height is identified and discussed. Variation in the size of adults as well as emergence times are documented. A previously undocumented host tree, *Eucalyptus punctata*, is recorded. Practical field notes on the collection and preservation of these giant insects are provided.

Plates of 10 set specimens of *E. cinereus* from two locations in south east Queensland are provided to assist future researchers with identification.

#### Introduction

The Cossidae are a cosmopolitan family of macro-moths comprising some 700 species in 95 genera (Schoorl 1990). Australia has a particularly rich fauna, especially in the subfamily Zeuzerinae, with approximately 100 described species and many more species awaiting description (Common 1990; Zborowski & Edwards 2007; P Marriott, pers. comm.). Nearly all known Cossidae live as larvae in the stems and roots of a wide variety of trees and shrubs (Schoorl 1990). Larvae of the Australian Zeuzerinae, and in particular the larger species of Endoxyla Herrich-Schäffer, 1854, bore singly in the main stems and branches of many species of Eucalyptus and Acacia (Common 1990). In some regions of Australia, the relatively large (>150 mm) larvae of Endoxyla moths were an important food source for Aboriginal peoples and in some arid zone environments, may have contributed a significant proportion of protein and fat in human diets (Tindale 1953). Endoxyla species are highly dimorphic as adults with females being typically much larger than males and with relatively massive abdomens (Plate 1). Several arid zone species have entirely brachypterous females (Tindale 1953; Common

1990). Most are relatively large moths and some of the larger Endoxyla species are among the largest insects on Earth with female wingspans exceeding 240 mm and weighing up to 30 g (Dodd 1916; Montieth 1991a, b; Plate 1). Females are highly fecund with a specimen of *E. encalypti* estimated to contain 18 000 eggs (Nielson & Common 1991 as Xyleutes encalypti). Eggs are deposited in a glutinous secretion in concealed sites such as cracks and splits in bark by a long flexible ovipositor. Hatchling larvae appear to be dispersed in the wind to alight on a suitable host tree by chance, perhaps explaining the extreme fecundity in this group (Common 1990). The biology and host plants for the majority of species are unknown (Common 1990), some are partially understood (Tindale 1953; McInnes & Carne 1978; Monteith 1991a, b) with only one, E. lituratus described in detail (Fearn 1985 as Xyleutes liturata; Fearn & Maynard 2019). Much of the past literature on Australian species contains many observations that are now largely of limited value because, due to name changes and misidentifications, it is not possible to determine which species was involved.

The distribution of the giant wood moth *Endoxyla cinereus* (Tepper, 1890) (= *Xyleutes boisduvali* in early literature) is not known with certainty. Common (1990) states that the distribution is coastal and near coastal from northern Oueensland to southern New South Wales (NSW), while Dodd (1916) encountered *E. cinereus* (as *Xyleutes boisduvalli*) in the Brisbane district of south east Oueensland (Old) and Kuranda in the north of the state. More recently, P. Marriott and A. Kallies (pers. comm.) have identified possible Victorian *E. cinereus* in private and public collections as well as two South Australian (SA) specimens in the Lyell collection at the Museum of Victoria. A sample of 11 specimens identified as this species based on their mitochondrial CO1 sequence were from Western Australia, SA, NSW and Qld (The Barcode of Life Data System (BOLD) 2021, Endoxyla cinereus). Superficially, the dorsal markings on the thorax were used as the main separator in a group of very large, predominately grey species that are poorly defined and may yet be found to comprise multiple species. In E. cinereus the thorax is light grey with the dorsal edge marked by a black inverted heart shape. The inside of the shape is not sharply defined and has tendrils extending further inside (P. Marriott pers. comm.; Plates 1, 7-8). In a recent work, McQuillan et al. (2019) record E. cinereus in Adelaide, South Australia, utilising red gum Eucalyptus camaldulensis as a larval host. The first author has examined bores that appear identical to Brisbane E. cinereus in smooth barked eucalypts growing along ephemeral water courses at Wilandra Station (-31.283 142.650) approximately 100 km eastnortheast of Broken Hill, NSW. Endoxyla cinereus may prove to be widespread in riparian corridors west of the Great Dividing Range. Clearly there is great scope for taxonomic and molecular studies to define species boundaries and distributions in this group.

Some aspects of the ecology of *E. cinereus* are well documented, particularly the form and construction of the larval bore in the main stems of *Eucalyptus* species (McInnes & Carne 1978; Monteith 1991a, b). However, the only peer-reviewed article on this species (McInnes & Carne 1978) failed to recognise that the first larval instars are not spent in the stems of host trees, but rather at another location that has yet to be determined with certainty. This oversight led to some ecological conclusions that require re-evaluation.

Endoxyla cinereus displays a three phase larval cycle and many other Endoxyla species that utilise eucalypt stems for larval bores probably do so as well. Where larvae spend their first eight to ten months before boring into tree stems is currently unknown but it is most likely subterranean in close proximity to host trees, perhaps associated with the roots (Illidge &Quail 1903; Monteith 1991a; data presented in this work). Larvae of Aenetus and Endoclita swift moths (Hepialidae) also display a three phase larval cycle with early instars living on the forest floor and feeding on decaying wood and fungi before moulting into a 'transfer larva' that migrates to host tree stems, establishes a bore and completes the larval cycle (see Grehan 1979, 1983 in Common 1990). In this work, we also adopt the term 'transfer larva' to describe the behaviour of migrating, second phase E. cinereus larvae.

In 2018 the first author rediscovered field note books, photographs and letters that had been placed in storage from a four-year period living in Brisbane. It became clear that much useful information on these gigantic but cryptic insects had been gathered and should be published. What follows is based on ecological field observations and data gathered in eucalypt woodland, parks and reserves in The Gap, Brisbane and Logan Village, southeast Queensland, during the spring and summer of 1993-94.

Specimens of moths relating to this work are in the first author's personal collection (SFColl-01-14) or lodged with the Queen Victoria Museum and Art Gallery (QVMAG) (registration numbers QVM.2021.12.3068-3095).



**Plate 1.** A pair of *Endoxyla cinereus* at life size. Female (right) from Logan Village (SFColl-01) and male from The Gap, Brisbane (SFColl-02). Photograph by David Maynard.

#### Study locations

The majority of the data presented in this work was collected at The Gap, a Brisbane suburb 8 km west of the city centre, at the base of Mt Coot-Tha. Most of the data and specimen collection took place along the Enoggera Creek bicycle and walking track that bisects a number of parks and riparian reserves between Waterworks Road and Payne Road. Additional data was collected at Wittonga Park on Alutha Road, in eucalypt woodland at the base of Mt Coot-Tha and a small number of adult *E. cinereus* reared from host tree billets collected from Logan Village, 45 km south of Brisbane.

#### Larval host trees

During this study, adult E. cinereus in The Gap were collected after eclosion from the trunks of the flooded gum (Eucalyptus grandis W. Hill) and forest red gum (E. tereticornis Sm.). E. cinereus from Logan Village were reared from billets of E. grandis and grey gum (E. punctata DC). I n Brisbane, Monteith (1991a, b) records E. grandis and 'blue gum' as host trees, and in the Coffs Harbour district of NSW McInnes and Carne (1978) record E. grandis, blackbutt (E. pilularis Sm.) and Sydney blue gum (E. saligna Sm.). In woodlands near Adelaide, South Australia, red gum (E. camaldulensis Dehnh) is a larval host (Tepper 1891; McQuillan et al. 2019). Endoxyla cinereus is known to only utilise smooth-barked eucalypts as stated by Common (1990).

#### Adult emergence

A total of 53 (24 males, 29 females) *E. cinereus* were collected in the summer of 1993-94, of which 42 were still available for examination (Table 2). A further 12 specimens were apparently predated upon shortly after eclosion, and three specimens (two males and one female) were too high up on host trees to be safely collected. Adults were collected shortly after eclosion in parks and reserves with the exception of one male and one female collected nocturnally at a black light and four females reared from eucalypt billets cut and collected at Logan Village. Most of the field-collected moths had emerged within approximately half

an hour of being discovered as indicated by the relative softness of their wings and relative pliability of pupal sheaths left protruding from the emergence hole. Four moths were initially discovered having only emerged moments beforehand and were still expanding their wings. These specimens were left to complete emergence before collection. One of these specimens was predated upon while the author was checking other nearby bores, presumably by magpies (Gymnorhina tibicen) which were the only conspicuous and likely predators observed. Apart from two notable exceptions, adults made no vertical or lateral movements on tree trunks after eclosion. This observation, as well as the relative freshness of protruding pupal sheaths, indicated that 12 adults had been predated upon shortly before the author had arrived at the scene. The two specimens that had travelled some distance on host tree stems were both males. One had emerged almost at ground level among long grass and so had to move 20 cm up the trunk in order to avoid grass and to expand its wings, while the second specimen emerged into full sun on very a hot afternoon (35°C) and moved laterally around the trunk into shade.

All active bores under observation were checked every evening from 7 November 1993 until the last known specimen emerged on 2 January 1994. All moths emerged in daylight and were collected between 1630-1845 hours. The first author's field note book states 'Small males emerged earlier in the afternoon than maximal males and all males emerged earlier than females.' This can be readily determined by the relative stiffness and dryness of the wings and pupal sheath. Because females emerged later in the afternoon, the first author was fortunate enough to witness the entire eclosion process on several occasions (Plates 2-5). One female pupa protruded from the emergence hole for 15 minutes engaged in a 'pumping action' before the adult emerged (Plate 2). Exactly the same behaviour has been documented in Tasmanian *E. lituratus*, with one female taking an hour to emerge after the pupa protruded from the emergence hole (Fearn 1985). This behaviour may be an attempt by the emerging moth to raise oxygen levels in its tissues before eclosion.

Dodd (1916) noted that all of the 27 species of Endoxyla that he had reared emerged in daylight hours between 11 am and 3 pm and that as a general rule, the hotter the day the earlier eclosion took place.

Analysis of Bureau of Meteorology weather data from Brisbane Aero (station 040223) for November 1993-January 1994 demonstrates that low humidity, less cloud cover and hence more hours of prolonged solar radiation were the common factors on all days when eclosion was documented. Conversely, no moths emerged on afternoons with high humidity (especially rainfall) and heavy cloud cover (Figure 1). Each climatic variable was analysed with a t-test to establish which were significantly different between days when eclosion took place and days that it did not.

Statistical analyses of data were conducted in R (version 3.5.3). Where appropriate, the means are displayed as bar graphs with confidence interval (CI) whiskers. When comparing meteorological data in relation to adult eclosion, an F-test for equality of variances was performed to determine which test to run: an independent two-tailed t-test assuming equal variance or Welch t-tailed t-test assuming unequal variances ( $\alpha$  = 0.05). Of the 20 meteorological variables, only eight were found to be significantly different and are presented here.

There was a significant difference found between eclosed moths present or absent on trunks for the following meteorological parameters: minimum temperature (t-value<sub>2 90</sub>=2.94, P=0.004); minimum terrestrial temperature (t-value<sub>290</sub>=3.63, t-value <0.001); 9 am relative humidity (t-value<sub>29</sub>0= 2.15, p-value= 0.034); 9 am wind speed (t-value\_\_\_\_=2.46, p-value = 0.015); 3 pm total cloud (t-value<sub>290</sub>=3.49, p-value <0.001); 3 pm wind direction (t-value<sub>2.90</sub>=2.70, p-value= 0.008); rainfall (t-value $_{2,67}$ = 2.50, p-value= 0.015); sunlight (t-value $_{2,67}$ = -2.57, p-value= 0.012).

The authors suggest these parameters are linked to the apparent northern bias of bore site selection on host trees (see larval bore location and discussion sections in this work).



Figure 1. Comparisons of the average values (±95% confidence intervals) for the following meteorological parameters a) minimum temperature, b) minimum terrestrial temperature, c) 9 am relative humidity, d) 9 am wind speed, e) 3 pm total cloud cover, f) 3 pm wind direction, g) rainfall, and h) sunlight, when eclosed moths were present or absent on tree trunks.

b)



**Plate 2.** Pupa of female *Endoxyla cinereus* protruding from emergence hole in *Eucalyptus grandis*. The Gap, Brisbane, Queensland. Photograph by Simon Fearn.



**Plate 3.** Female *Endoxyla cinereus* immediately after eclosion. The Gap, Brisbane, Queensland. Photograph by Simon Fearn.



**Plate 4.** Female *Endoxyla cinereus* expanding wings over back. The Gap, Brisbane, Queensland. Photograph by Simon Fearn.



**Plate 5**. Maximal sized female *Endoxyla cinereus* compared to a hand. Note that the moth made no lateral or vertical movement after eclosion. Also note the enlarged frass ejection hole below emergence hole, and the distinctive circular scar on tree trunk above moth that is an old emergence hole. The Gap, Brisbane, Queensland. Photograph by Simon Fearn.

### Adult size range

42 pinned and set specimens (24 females and 18 males) from south east Queensland were examined (Table 2). Wingspan was measured to the nearest millimetre (mm) by positioning pins at the fore wing-tip and measuring the distance between the pins. Costal length was determined with callipers to the nearest millimetre from right-hand wing-tip to the point where the wing joined the thorax. Both measurements are provided in this work as both have been used in various previous works and thus a direct comparison can be made. The largest female documented appears to be one set by Dodd (1916) with a wingspan of a little over 247 mm. Monteith (1991a, b) records female weights of 30 g and 27.6 g, so it is possible that maximalsized, fully-gravid specimens may exceed 30 g shortly after eclosion. Female E. cinereus are therefore among the largest insects on Earth both in terms of mass and wingspan (Kons 1998; Fearn 2018: Plate 1).

'Size of adults depends on diameter of trunk or stem. Without exception, maximal sized females emerge lower on trees than small females or males. Maximal size males emerge lower than small males. Without exception the smallest males and females have emerged from small trees or high on thin branches of large trees.' (extract from the first author's field notebook 22 December 1993).

This implies that either stem size or transfer larval 'choice' of appropriate sized stems, dictates the size individual *E. cinereus* can attain as adults.

## Egg laying and behaviour of hatchling larvae

Several authors have documented that *Endoxyla* larva hatchlings are minute (1.5 mm in length), spin large quantities of silk and appear to be dispersed by wind (Littler 1904; Coleman 1946; Kalshoven 1965 in McInnes & Carne 1978; Common 1990). Monteith (1991a) speculates that *E. cinereus* displays similar habits but provides no firsthand accounts to support this hypothesis. On 28 November 1993 the first author captured a female *E. cinereus* that flew to a black light and confined it in a cardboard box. Over the next two days before death the moth laid large numbers of eggs adhering together in a large clump. Sixteen days later, on the 16 December 1993, at ambient room temperatures, neonate larvae were observed within the egg mass, where they remained for a further four days. On 20 December 1993 the container was inspected and it was found the larvae had attempted to disperse, spinning great quantities of fine silk in the process. When the container was opened and exposed to a stiff breeze, the larvae became agitated and very active, immediately climbing any vertical surface, lowering themselves down from the container on strands of silk and blowing away on the wind. McInnes & Carne (1978) concluded that *E. cinereus* hatchling larvae were probably dispersed by wind based on the observations of Littler (1904), whom they may have mistakenly believed was writing about E. cinereus. However, Littler was reporting from Launceston on Tasmanian E. lituratus (as Zeuzera eucalypti), an Acacia feeding cossid with a different life history to E. cinereus (Fearn 1985). Female E. cinereus are equipped with a long flexible ovipositor which permits eggs to be laid in cryptic, confined spaces such as in crevices and under bark on trees. If larvae are dispersed randomly down wind, there may not be any particular host tree preference by females when ovipositing.

#### Behaviour of transfer larvae

It has been known for well over a century that the earliest stages of larval development of *E*. cinereus are not spent in the trunks of host trees. Illidge and Quail (1903) state 'The very earliest stage of the larva is not passed in the wood, and calls for special investigation, as to what is the exact habit when first hatched.' Little in our understanding has changed in the intervening 115 years. Monteith (1991a; 2011) logically suggests that the first year of larval development possibly takes place in the roots of host trees based on the fact that larval development in some smaller Endoxyla species is known to be entirely subterranean in root systems, particularly in arid portions of the continent (Dodd 1916; Tindale 1953; Simpson 1972; Fearn & Maynard 2019). The first author's observations in Brisbane may support Monteith's conjecture.

On 20 October 1994 at 1230 hours, a first year E. cinereus transfer larva, about 25 mm in length, was observed moving erratically up the trunk of a Eucalyptus grandis sapling with a trunk diameter at ground level of 17 cm. The larva when first observed was 8 cm up the trunk from the substrate and progressing rapidly while moving its head and fore body from side to side. As soon as the larva encountered smooth bark on a small offshoot stem with a diameter of 9 cm arising from the main trunk 1.4 m from the ground, it appeared to 'inspect' a portion of the stem, ceased moving forward and aligned its body vertically, head pointing downwards. The head and fore-body were then moved rapidly from side to side with the larva affixing strands of silk to the bark either side of its body. After five minutes the larva had constructed a rough silken tent over itself. Within the confines of this structure the larva then moved rapidly around in a circular motion, continuously adding silk and pushing on the walls of the tent to create a domed structure 22 mm in diameter. After approximately 10 minutes of this procedure the larva then began to bite off small pieces of outer bark and add these to the inside layers of silk that were steadily building up as the larva worked. This process continued for another 10 minutes with the larva becoming increasingly indistinct beneath the rapidly thickening and camouflaged silk cover. The larva then began to bore directly into the tree trunk, pushing wood flakes around the perimeter of the cover as well as attaching them with silk to the 'ceiling'. Time constraints led to cessation of observations after 35 minutes, when the larva was no longer visible beneath its cover but could still be detected moving beneath it. Once the larva has commenced its bore in the trunk of the host tree, the silken cover degrades and is easily dislodged in wind leaving only a small frass ejection hole measuring 1-2 mm in diameter at the centre of a glutinous plug 5-6 mm in diameter. This is formed by the larva to seal the tunnel formed when it first chewed its way into the trunk. Recently constructed shelters of the transfer larvae were found still attached to host trees in Brisbane parks as early as 4 August and up to 9 September 1993 indicating that transfer larvae are active through the early spring months. The ensuing two years of the larval

cycle are spent growing and preparing the large pupal bore in the centre heart wood of the host tree as described and illustrated by McInnes and Carne (1978) and Monteith (1991a, b).

Transfer larvae appear to be sensitive to stem diameter as they move up host trees from the ground. How this is done is a mystery but in the first author's experience bores are never located in stem diameters much greater than 30 cm. On very large, mature host trees, larvae may have to travel as much as 11 m from the substrate before initiating a bore. On the smallest host tree saplings, bores can be initiated just above ground level. The minimum stem diameter that can be utilised (around 6 cm) is probably a simple function of mechanical stem strength. The large pupal bore constructed in the centre of the tree in the third year of the larval stage must weaken smaller individuals and make them very prone to snapping off at the bore in strong winds, or render them too accessible to predators such as black cockatoos (McInnes & Carne 1978). Of the many bores examined by the first author, only one (a maximal-sized female; Plates 2-5) had been initiated in the rough barked portion of the lower trunk on mature trees, also necessitating the transfer larvae travel some distance up the stem to encounter smooth bark. Another mystery is how transfer larvae appear to avoid initiating bores where bores have already been established by previous transfer larvae. Active larval bores were never found in close proximity to each other in the same portion of trunk. Bores from the same cohort, and hence age, were spaced evenly up the trunk.

#### Larval bore location in host trees

*Endoxyla cinereus* transfer larvae at the first author's study sites initiated bores in host trees ranging from 2-12 m in height. Height of mature trees was estimated by comparison with smaller trees of known height, height of small trees as well as height above ground of bores was measured using a tape measure and/or a ladder and long fishing rod blank with metre graduations marked on it. Diameter of stems was determined directly with a flexible dress maker's tape. Compass aspect of each bore was documented to facilitate comparison with data provided by McInnes and Carne (1978) in relation to location of larval bores in host tree stems. Only the very largest trees with trunk diameters of 1 m or more appeared to display no recent larval bores even on thinner stems below the canopy. If a tree had more than one bore, each one was treated as a separate unit but the number of bores per tree was not always recorded. However, it was observed that some large, multi-stemmed eucalypts contained as many as nine recent bores. Data was collected for 115 recent bores (Table 1).

Bore placement in response to the dimensions of their host trees was summarised with regression analysis ( $\alpha = 0.05$ ). Prior to regression analysis, all variables were log-transformed to improve normality and homogeneity of variance. The data was then fitted with a linear regression equation and the R<sup>2</sup> reported as a measure of goodness of fit.

A significant positive correlation was found between height above ground of bore site (m)



and tree height (m) (F-value<sub>1,113</sub>=34.03, p-value <0.001). With increasing tree height, larval bores were situated higher off the ground. In addition, a significant positive correlation was found between stem diameter at bore sites (m) and tree height (m) (F-value<sub>1,113</sub>=39.54, p-value <0.001; Figure 2).

This evidence suggests that there is an optimal 'sweet spot' stem diameter for each gender based around the rather extreme female positive sexual size dimorphism in *Endoxyla* moths. On the smallest trees where it was not possible for transfer larvae to select optimum stem diameters, bores were initiated close to the ground in the thickest part of the stem and always resulted in undersized adult moths.

> **Figure 2:** Regression analysis with linear trend line of a) tree height and height of bore site above the ground; b) tree height and trunk diameter at the emergence hole.

A very high percentage (75.4%) of larval bores were initiated between the northern and western aspects of host tree stems (Table 1). This result is remarkably similar for E. cinereus utilising Eucalyptus grandis plantations in the Coffs Harbour region of NSW where 83% of larval bores were present on the northern and western portions of tree trunks (McInnes & Carne 1978). Those authors were unaware that *E. cinereus* larvae do not enter host tree stems until nine to ten months of age and postulated that the observed northern and western bias of larval bore placement in their study was due to ballooning hatchling larvae alighting on tree trunks at night in prevailing winds originating in the north and west. They further suggested that if this scenario was correct, hatchling larvae must have bored into trunks at the point they landed with little if any lateral movement. Clearly, an alternative explanation is required. As reported in this work, transfer larvae are very mobile and able to climb as much as 11 m up host trees to locate a suitable stem diameter in which to initiate a bore. There would appear to be no compelling reason why these larvae could not initiate bores on any compass aspect of host stems. We suggest a possible explanation may lie in the amount of sunlight that different aspects of the trunk receive, especially in the cooler winter months. Higher stem temperatures may facilitate larval development or metamorphosis in the pupal stage. In addition, exposure to afternoon sunshine may be important in efficient drying of the relatively thick and leathery wings of these giant moths. If this scenario is correct it may explain why transfer larvae appear to be active in daylight hours as they need to use the position of the sun to identify the best aspect for bore initiation.

#### Pupation

Because the same trees were being examined regularly it was possible for the first author to record the precise dates when full-term larvae opened emergence holes by chewing through the covering bark. Ten observation dates were recorded between 19 August and 30 November 1993 (nine emergence holes opened in August, six in September, three in October and two in November). In addition, during the same period, larvae greatly increase the size of the frass ejection hole, presumably to allow rain water to drain away that may enter the large emergence hole during frequent spring and summer storms in Brisbane (McInnes & Carne 1978). It is during this period of preparation for pupation that large amounts of coarse woody frass accumulate rapidly on the ground below the tree. On 27 August 1993 the head capsule of a full-term larvae could be easily observed as it snipped off pieces of wood around the enlarged frass ejection hole and dropped them to the ground. The final preparations for pupation including the construction of several plugs of silk and wood scrapings dividing the bore into inner and outer sections, are described and illustrated by both McInnes and Carne (1978) and Monteith (1991a). The period from opening of the emergence hole to adult emergence was recorded for five larval bores. The pre-pupal and pupal stage for three females was 52, 105 and 112 days (7.4-16 weeks) and for two males were 89 and 104 days (12.7-14.8 weeks).

Table 1: Orientation of 115 larval bores of Endoxyla cinereus in stems of host trees.

|  | West   | North-<br>west | South-<br>west | North  | North-<br>east | East  | South-<br>east | South  |
|--|--------|----------------|----------------|--------|----------------|-------|----------------|--------|
| Number in<br>cardinal and<br>intercardinal<br>directions     | 17     | 21             | 14             | 26     | 9              | 7     | 6              | 15     |
| Percentage in<br>cardinal and<br>intercardinal<br>directions | 14.7 % | 18.2 %         | 12.1 %         | 22.6 % | 7.8 %          | 6.0 % | 5.2 %          | 13.0 % |

Table 2: Adult Endoxyla cinereus wingspan and costal length data (based on 18 males and 24 females).

|                    | Wing Sp | an (mm) | Costal Length (mm) |         |  |  |
|--------------------|---------|---------|--------------------|---------|--|--|
|                    | Males   | Females | Male               | Females |  |  |
| Minimum            | 142     | 184     | 63                 | 83      |  |  |
| Mean               | 158     | 211     | 70                 | 95      |  |  |
| Maximum            | 173     | 237     | 78                 | 107     |  |  |
| Standard Deviation | 9.98    | 14.12   | 4.80               | 6.53    |  |  |

#### Discussion

Many aspects of the life history and habits of *E. cinereus* are now reasonably well understood (McInnes & Carne 1978: Monteith 1991a, b: data presented in this work) with the exception of the whereabouts and trophic ecology of phaseone larvae and bore site selection of phase-two transfer larvae. It is only assumed that phase-one larvae are associated with the roots of host trees (Monteith 1991a, b) but there appears to be little doubt that phase-one larvae are subterranean or near subterranean dwellers close to the trunks of host trees. Based on its orientation and rate of progression up the host tree, the first author must only have missed the transfer larvae described in this work emerging from the ground by moments. It is unlikely that transfer larvae have to travel any great distance to host trees, as the first author observed several host specimens of *Eucalyptus grandis* growing in suburban foot paths with bitumen sealing the

substrate to within 10 cm of the trunk. Whatever the trophic habits of the phase-one larvae turn out to be. they would appear to impart distasteful properties to the larva to discourage ingestion by potential predators. The transfer larva is prominently banded in purple and white (illustrated by Monteith 2011), which fades significantly with growth once secreted away inside the host tree. The author has collected insects extensively up the east coast of Australia in *E. cinereus* habitat and has never seen a species that could act as a plausible Batesian model for transfer larvae if they had no distasteful properties. The fact that transfer larvae are prominently coloured and active on smooth eucalypt trunks in daylight hours, when they would be highly vulnerable to detection by sharp-eyed predators such as birds and reptiles, indicates they may have toxic or distasteful properties. Once safely enclosed within the host tree stem, any distasteful properties appear to dissipate or disappear as the larger instars of *E. cinereus* larvae are a valuable food source for black cockatoos

(McInnes & Carne 1978) and formerly for humans (Tepper 1891; Dodd 1916).

Data presented in this work indicates that the transfer larval phase may only exist at all to facilitate larval bore location on host trees. This is clearly not random, either in terms of orientation or stem diameter. Previous researchers also identified this phenomenon but failed to understand that the earliest larval instars were not spent in host tree stems (see McInnes & Carne 1978). Hatchling larvae are minute, 1.5 - 2.0 mm in length, and possibly prone to desiccation. They would therefore be incapable of extensive movement over the trunks and stems of host trees or by tunnelling directly into smooth barked sections of host stems at that size. The transfer larval stage therefore serves to counter the randomness of hatchling larval dispersal and allow 'choice' of larval bore sites. While the mechanisms at this stage are unclear, data presented in this work indicates that the gender of transfer larvae may be already established and that a suitable stem diameter is 'chosen' (presumably around mechanical strength) to accommodate the pupal bore, which is more than twice the diameter for maximal-sized females compared to males. On larger, mature host trees this necessitates male larvae having to travel further up the trunk and main stems to initiate bores in optimal stem diameters. Because of the large variation in size of adult E. cinereus (Table 2) it was not possible to differentiate between maximal-sized male and small female emergence holes when measuring stem diameter at the bore site. Quantifying gender differences in bore site selection will necessitate measurements of trees and stems being taken when moths are collected after eclosion on host trees.

The preference for *E. cinereus* larval bore orientation being between the north and west cardinal directions on host tree stems is clear from our data but the reasons for it are unknown. It is reasonable to speculate that it may aid in larval development, metamorphosis in the pupal stage or efficient drying of the large wings of this species through elevated temperatures/ solar radiation on the most sun-exposed portions of host stems. The giant species of

Endoxyla moths probably represent the most massive insects occurring well south of the equator anywhere on Earth and may be related to the extreme fecundity of this group owing to positive correlations between adult size, egg size and number (Harrison et al. 2010). Egg size would seem to be unusually small for the size of the moth and such extreme fecundity is probably an adaptation to offset the very low probability of larval establishment. Giant size may also be facilitated by the extended feeding period of multi-year larvae (P. McQuillan pers. comm.). Developmental temperatures, even in the relatively mild and brief sub-tropical winters of Brisbane at 27° S 153° E, may require bores to be situated on the warmest aspect of stems. The first author's field note book from this period records that bores that were not orientated to the north or west were on trees where that portion of trunk was entirely in shade all day due to vegetation or human structures. The only other situation where bores appeared to be haphazardly placed was on mature, multi-stemmed trees growing in isolation with spreading canopies and thus largely shaded stems at all times of the day. Solar semi-diurnal arcs (the arcs which the sun appears to trace across the sky from sunrise to the meridian in the morning and the equivalent arc in the afternoon between the meridian and sunset) are essentially identical, except that one is east of the meridian and the other is west of it. The part of the trunk of a tree exposed to sunlight for the greatest period each day is the northern point on the trunk. It would appear logical that in mid-morning and mid-afternoon there would be a peak in solar intensity on the tree trunk centred on the azimuth of the sun. However, asymmetry in the heating effects could be expected due to the fact that the 'morning side' of the trunk has a cooler initial temperature whereas the 'afternoon side' has had the benefit of warmer air circulating for several hours. Thus the peak afternoon temperature would be expected to be higher (M. George, pers. comm.). Presumably, if any part of this hypothesis is correct, it may explain why transfer larvae are active during the day so they can use the position of the sun to orientate themselves on host tree stems.

Further support for these hypotheses may come

from Endoxyla bores in colder climates, which should be orientated on the part of host stems expected to be the warmest. For comparative purposes, the author collected compass quadrant data for 74 E. lituratus bores in the stems of black wattle (*Acacia mearnsii* De Wild) in the Trevallyn Reserve, Launceston, Tasmania (centered on GDA94 0508640 mE 5410861 mN), approximately 1700 km south of Brisbane and found that 84% of bores were situated on the northern and western portions of tree trunks. Tasmanian E. lituratus have a very similar eclosion time (1600-2000 hours Eastern Daylight Savings Time) to *E. cinereus* (as reported here). Females are the largest insects in Tasmania and a second phase transfer larval stage cannot be ruled out. The smallest Tasmanian E. lituratus larva ever discovered boring in the stem of a host tree was found in spring, was 15 mm long and was brightly coloured in pinkish purple (Fearn 1985). This may well represent a transfer larva, based on the apparent lack of randomness in the placement of bores. There clearly remains much scope for future research on the earliest stages of Endoxyla larval behaviour.

Regardless of the causes for observed bore orientations and timing of eclosion, there must be a selective advantage for these large insects to emerge during daylight hours, when the greatest number of potential visual predators is active.

Undoubtedly, the difficulties involved with locating subterranean first phase larvae have resulted in this aspect of *Endoxyla* biology remaining undocumented. Perhaps the only way forward would be careful and systematic exposure and examination of the lower trunk and major roots of eucalypt saplings in locations with high *Endoxyla* densities, such as plantations, as described by McInnes and Carne (1978). Such excavations would be best undertaken in winter when phase one larvae are at their maximum size (approx. 25 mm) and just before they migrate upwards to host tree stems as a transfer larvae in spring.

## Field techniques: rearing, collection and preservation

The larger eucalypt-feeding *Endoxyla* species are rather plain and very similar in appearance, which can result in misidentifications and uncertainties around distribution. Subtle differences in thoracic blotches and wing markings between species can be consistent but difficult to quantify on worn or damaged specimens (first author, pers. obs.; Marriott, pers. comm.). The wings of Endoxyla moths are relatively lightly scaled and easily rubbed or damaged unless handled carefully. Obtaining perfect specimens shortly after eclosion is therefore desirable but difficult. The difficulties involved have not improved since first identified by Dodd (1916) more than a century ago. Emergence holes in host trees or collected billets must be checked every afternoon during the emergence period until eclosion occurs. Bores discovered in the field that cannot be easily revisited must be collected. This may involve felling large trees with a manual saw while balanced on a ladder to avoid vibration damage to pre-pupal stage larvae or pupae, which is inevitable if a chain saw is used. The tree must initially be felled above the emergence hole (at least 40 cm for larger species that pupate head downwards) with the billet secured by a second cut below the frass ejection hole. Collected billets must not be allowed to dry out, as this can result in pupae failing to push out or cut through the various plugs created by full-term larvae as they retreat to the top end of the bore for pupation (Dodd 1916). McInnes and Carne (1978) recommend standing billets in containers of damp sand and covering the upper end in thick plastic. The author can confirm that this method resulted in success under Brisbane conditions. Freshly eclosed moths emerging from billets in controlled circumstances can be left until nightfall before euthanasia to ensure their wings are fully dried and hardened. This is not always possible in the field and moths collected and euthanased after the wings are fully expanded, but not yet fully dry, must be left for several hours before setting. If the forewings are still soft they will buckle close to where the wing joins the thorax when drawn forward into

position on the setting board (Plate 6). Great care needs to be taken when securing moths for euthanasia. The author had complete success by gently sliding thumb and forefinger up under the wings of resting moths (while being careful not to rub fluff (hair-scales) from the side of the abdomen) and then firmly gripping the moth by the sides of the thorax. This action also raises the wings over the back of the specimen. The moth is then turned upside down, an insulin needle inserted directly into the thorax between the hind legs and an injection of a saturated solution of oxalic acid administered. This results in instant death and the moth can be carefully placed in an appropriate container for transportation. Moths are generally reluctant to move, relying on their resemblance to exfoliating bark to avoid predation, so careful, unhurried movements are required. If a moth does begin to move off, it is best to let it settle down again before attempting another capture. If moths are allowed to become agitated by repeated unsuccessful attempts at capture they can move off more rapidly while 'flicking' their wings and eventually, in some cases, falling to the ground while clumsily flapping around. Moths high on trees were secured with a step ladder and fishing rod blank. The tip of the rod was gently pushed under the moth and forward until the moth became agitated, relinquished its grip on the bark and grasped the rod. The moth was then lowered to the ground. These moths are comparatively powerful insects and need to be confidently and securely held to avoid damage. In addition, waste fluids or meconium that accumulated during pupation can be expelled by moths with great force from the end of the abdomen as a lastresort deterrent to predation. This fluid stains clothing and can irritate the eyes so care should be taken.

The abdomens of all moths collected by the senior author were eviscerated before setting. Modern collectors usually do not bother with evisceration as *Endoxyla* moths, especially the males, eventually go greasy with the breakdown of fats and have to be placed in a degreasing agent such as ethyl acetate for at least 48 hours, whether they have been eviscerated or not. However, the author found that in the subtropical climate of Brisbane evisceration was desirable

as it greatly reduced drying time on setting boards and prevented rotting and distortion of abdomens. Placing the set specimens in a drying cabinet or oven at a temperature no greater than 38°C as outlined by Braby (2000) may also produce good results, although the author has not tried this with such large moths. The author found the best way to eviscerate the abdomen without damage to the fluff and scales that clothe the abdomen was to partially set moths with the abdomen hanging clear of the end of the setting board. The whole board was then turned upside down on a makeshift platform of appropriate height (books of various thicknesses are ideal). The abdomen is then allowed to lie flat on a surface covered in cellophane to reduce friction and a longitudinal incision made along the mid-line of the abdomen through all but the terminal sternite plates. Once eviscerated the abdominal cavity is then loosely filled with cotton wool and gently pressed back into a natural shape. The moth is then reset in the usual way and left to dry.

Standard 38 mm entomological pins are inadequate for very large *Endoxyla* moths as the great depth of the thorax leaves too little of the pin protruding above and below the thorax.

**Plate 6.** Female *Endoxyla cinereus* (SFColl-04) showing buckling and deformation of fore wing base by setting the specimen before wings have fully hardened. Photograph by David Maynard.



This causes problems with affixing labels but more importantly, leads to fingertip damage to delicate but important taxonomic features of thoracic scales through routine collection handling. The author solved this problem by cutting 51 mm, size 7 entomological pins down to 45 mm. This leaves plenty of pin above and below the specimen and will still be accommodated by commercially available cabinets, unit trays, display cases and store boxes. An effective pin head can be created with hard drying glues if required. A less aesthetic but functional response is to pin the moth at an angle with a size seven pin. Finally, the early literature on *Endoxyla* moths is often difficult to interpret because of misidentifications and name changes. The collection of undamaged and accurately labelled voucher specimens is crucial for future research on this group. In particular, rearing these moths from collected billets of trees and shrubs that have been identified by botanists will be vital in better defining species, their distribution and biology. Ideally, examples of host plants, split billets revealing bores and the moths themselves should be cross-referenced and deposited at appropriate institutions. Once host trees and larval bores can be reliably identified in the field, larvae and pupae can be collected and preserved. It is also important to retain pupal exuviae and cross-reference or stage these with reared moth specimens. In the absence of adult moths, reliably identified pupal exuviae can be used to confirm a species presence during field surveys, where pupal sheaths can be found protruding from emergence holes sometimes for months after the moth has eclosed.



**Plate 7.** Thoracic splotch and fore wing markings variation in female *Endoxyla cinereus*. Photograph by David Maynard.



**Plate 8.** Thoracic splotch and fore wing markings variation in male *Endoxyla cinereus*. Photograph by David Maynard.

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