The biology of *Epiplema albida* (Hampson, 1891) (Lepidoptera, Uraniidae, Epipleminae) on Sri Lankan Privet, *Ligustrum robustum* (Roxb.) Blume subsp. *walkeri* (Decne.) P. S. Green (Oleaceae)

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Abstract: The biology of *Epiplema albida* (Hampson, 1891) (Lepidoptera: Uraniidae, Epipleminae) from Sri Lanka, was studied as part of an evaluation of its suitability for use as a biological control agent for Sri Lankan Privet, *Ligustrum robustum* (Roxb.) Blume subsp. *walkeri* (Decne.) P. S. Green (Oleaceae) in the Mascarene Islands. The life cycle of *E. albida* is documented: females lay an average of 80 eggs, the larva has four instars and feeds on leaves, and the life cycle from egg to imago is usually completed in 34 days.

Keywords: Epipleminae, Oleaceae, Sri Lanka, parasitoid

INTRODUCTION

Sri Lankan Privet (or Ceylon Privet), *Ligustrum robustum* (Roxb.) Blume subsp. *walkeri* (Decne.) P. S. Green (Oleaceae), is a woody shrub or small tree indigenous only to Sri Lanka, but a serious invasive alien plant on the Mascarene Islands (Lavergne *et al*., 1999; CABI, 2016). These islands have international conservation significance as biodiversity hotspots because of their high levels of endemicity (Myers *et al.*, 2000). The indigenous distribution of the species *L. robustum* is restricted to Sri Lanka, India and Bangladesh to Vietnam. Within this range, subspecies *robustum* was described from Bangladesh (Silhet) and is found from northeast India to Thailand and Vietnam (Green, 2003) and subspecies *walkeri* is restricted to Sri Lanka (Green, 1988, 2003). The southern Indian population was treated as a subspecies of *L. robustum*, but is now considered a separate species, *L. perrottetii* A. DC. (Green, 1990, 2003). Sri Lankan Privet can be found in wet and intermediate low montane regions, often near a stream, between 450-2000 m (Green, 1988). In our previously reported surveys, populations were found in the hill country of Sri Lanka including Kandy, Matale, Nuwara Eliya, Badulla, Monaragala and Ratnapura districts. It was always a relatively minor component of the natural flora (Sakalasooriya *et al.*, 2000).

Our field surveys of natural enemies of *L. robustum* in its indigenous range in India and Sri Lanka have been published for Sri Lanka (Sakalasooriya *et al.*, 2000). Based on these surveys, a defoliating moth, *Epiplema albida* (Hampson, 1891) (Uraniidae, Epipleminae), was selected for evaluation as a potential biological control agent for introduction to the island of La Réunion, a French Department and one of the Mascarene islands. Hampson (1891, p. 102-3, plate 150.10) described and illustrated *Erosia albida* Hampson, 1891 from the Nilgiri District, southern India, and *Erosia lanigera* Hampson, 1893 from Ceylon, now Sri Lanka (Hampson, 1893, p. 137, plate 157.13). He subsequently treated the latter as a synonym of the former and placed it in the genus *Epiplema* Herrich-Schäffer, 1855 (Hampson, 1895, p. 129). There have been no subsequent changes in nomenclature. The type species of the genus *Epiplema* is *E. acutalangulara* Herrich-Schäffer, 1855, a Neotropical species, so the generic placement of *E. albida* and other Old World *Epiplema* species are expected to change in the future when the south Asian genera of this subfamily are revised (Holloway *et al.*, 1987; Holloway, 1998).

The status and classification of the subfamily Epipleminae has varied over the years, but it is now accepted as a member of the superfamily Geometroidea, family Uraniidae (Minet and Scoble, 1999; Nieukerken *et al.*, 2011). Minet & Scoble (1999) summarized information on the Epipleminae as follows: it is the largest subfamily of Uraniidae with more than 550 mainly tropical species found in the Americas, and Africa to Australia; young larvae tend to have gregarious habits and usually live in a web. Recorded host plants belong to various families: e. g. Bignoniaceae, Caprifoliaceae, Daphniphyllaceae, Loganiaceae, Oleaceae, Rosaceae, Rubiaceae and Verbenaceae.

Detailed studies on host-specificity of *E. albida* reported elsewhere show that it is restricted to species of *Ligustrum* (Shaw and Cock, 2017). Knowledge of the early stages of Asian Epipleminae is limited: Holloway (1988) includes brief descriptions of larvae from four out of 13 genera found in Borneo; Sugi (1987) includes photographs and notes on three species from Japan. Hence, the current study on the biology of *E. albida* will add valuable information to our knowledge of the subfamily.

METHODS

Field collected larvae and newly formed pupae of *E. albida* were taken from Sri Lanka to the UK and held in the CABI UK quarantine facility, in a controlled environment room at 20ºC (+/- 2.2 ºC) with a 12hr daylight: 12hr darkness regime and 60-80% humidity. A culture was established and all experiments were carried out under these conditions in quarantine.

The progeny of individual females were kept separately until it was clear that no obvious parasites or diseases were present in the population. For oviposition, up to 15 adult moths of mixed sexes were exposed for a period of up to 6 days to

individual Sri Lankan Privet plants growing in John Innes No. 3 soil in 15 cm pots with matching, fitted, plastic cloches with a cloth insert in the top. Fungal contamination was minimized by placing dry capillary matting at the base of the plant stem over the soil, whilst humidity was maintained each day with a spray of water and Nipagin™ (an antifungal agent used in food production). For food, the adults were provided with a 1:5 dilute honey solution that was soaked into cotton wool placed on the cloth mesh on the top of the cloche, replaced daily.

The eggs laid were monitored and after hatching, the larvae were harvested by tilting the potted plant and gently tapping the leaves causing the larvae to drop and hang by silk threads. Larvae were gently transferred using a fine artists' paintbrush into round transparent plastic butter dishes, 15 cm in diameter and 7 cm deep, lined with filter paper. They were fed with new bunches of *L. ovalifolium* Hassk. foliage, harvested from the field in Berkshire, UK, which were changed every seven days or when the leaves showed signs of deterioration. Up to 25 fourth instar larvae were transferred to a larger rectangular transparent plastic box, 28 x 18 x 10 cm, lined with damp tissue paper covered with Vermiculite® to provide a soil-like substrate for pupation. Attempts to surface sterilise the pupae using sodium hypochlorite to minimise any fungal/bacterial contamination resulted in the loss of the colony, so cultures were maintained in separate lines to minimise risk. Emergence took place in the same rectangular ventilated boxes described above with folded cardboard for the emerging adult moth to rest on, and honey solution for them to feed.

A sample of the adults that emerged were killed by freezing, pinned (e.g., Holloway *et al.*, 1987), and identified by comparison with the reference collection of The Natural History Museum, London. Since, as noted above, *Epiplema* is considered to be a genus correctly limited to the New World, adults were dissected to assess whether the structure of the genitalia could be used to assign *E. albida* to an appropriate Old World genus by comparison with the information and images in Holloway (1998) and Sohn and Yen (2005). Parasitoids that emerged from rearing were identified by Gavin Broad.

In addition to laboratory studies in the UK, natural history observations of the early stages were made in the field during survey visits to Sri Lanka. Larvae were collected by beating and searching foliage showing signs of feeding. Larvae of *E. albida* were found at all sites that we surveyed in Sri Lanka, from 800–1625 m (Sakalasooriya *et al.*, 2000). The distribution of the larvae was not uniform with some sites apparently devoid of larvae whilst others produced up to 30 per man hour of collection. Observations of the biology and life history were noted in the field surveys and during laboratory rearing.

Oviposition sites were documented by recording the number and position (upper or lower leaf) of all eggs laid on Sri Lankan Privet in 50 cm x 40 cm x 50 cm ventilated perspex cages in oviposition tests reported by Shaw and Cock (2017). To establish life history parameters, a trial with 46 newly-hatched larvae was set up in quarantine, holding larvae individually with excised Sri Lankan Privet leaf material in 5 cm Petri dishes over a period of 60 days. The dishes were stored in a large sandwich box with mesh-covered ventilation holes above moistened tissue paper. Rearing containers were cleaned every 1-2 days, and soiled tissue paper replaced. Feeding damage was observed and any developmental changes recorded daily when possible, but at least every second day if weekends prevented daily sampling. If a moult could have taken place on either of two days, the mean was used to estimate the date of the moult. Pupation took place amongst the leaves and tissue paper. The development of each individual was followed through to death or adult emergence.

As adults emerged from culture, their wingspans (wing tip to wing tip in normal resting position as in Fig. 2I) and body lengths (frons to abdomen tip without antennae) recorded using a micrometer as they rested against the outer wall of the clear plastic vessels in which they were held. Student's t-tests (using R version 2.3.1) were used to test the significance of the difference between males and females.

To measure fecundity, ten replicates of mating adults (two males and one female) were held under normal rearing conditions in a cage with a potted Sri Lankan Privet host plants. Plants were changed weekly until the moths had all died, and cumulative egg production was recorded for each female.

In an adult survival study, adult females were collected immediately after emergence and placed in individual butter dishes containing small vials filled with cotton wool soaked in a honey solution. Vials were replaced every three days and the number of females still alive was recorded daily or every other day until all had died. The median survival is used rather than mean as recording was not daily, and so would be misleading.

RESULTS

Initial comparison of adults with the specimens at The Natural History Museum, London, indicated that adults from the current study were within the range of variation shown by the museum series of *E. albida*. The series includes specimens from India, Sri Lanka and 'Upper Burma'. Our identification was confirmed by J. D. Holloway. Further investigation showed that *E. albida* adults (Fig. 1A-B) resemble species of *Dysaethria* in wing markings and key out as this genus using Sohn & Yen (2005), but the male genitalia (Fig. 1C) key out as a species

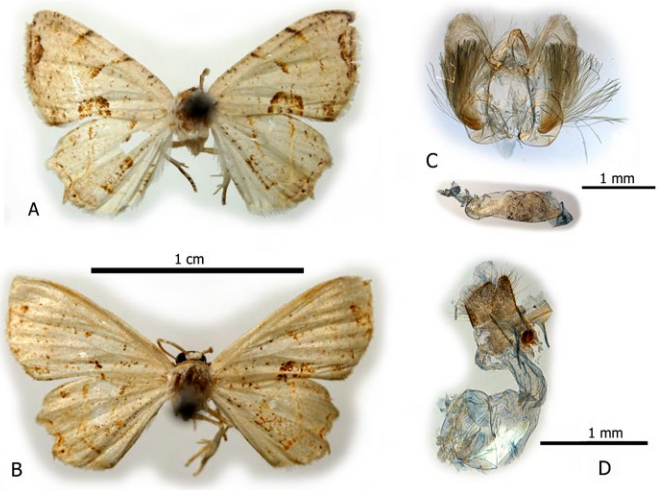

Fig. 1. Adults and genitalia of *Epiplema albida* from Sri Lanka. A, male; B, female, C, male genitalia, with penis in lateral view below; D, female genitalia.

of *Phazaca*, having the valves differentiated into a dorsal and a ventral part with a bunch of scent hairs (Holloway, 1998; Sohn & Yen, 2005). We refer to Sohn and Yen's (2005) work on the Korean fauna as it is the only key available to Asian genera, although not intended to cover the Sri Lanka fauna. We further note that Sugi (1987, Plate 47.11) illustrates a larva of *D. moza* (Butler, 1878) similar to that which we illustrate for *E. albida* (Figs. 2C-E). Based on the larvae, but not the male genitalia, *D. moza* and *E. albida* might be congeneric. In view of these inconsistencies, we do not propose a new generic placement for *E. albida* at this time, and this will need to be addressed in a review of the Indian and Sri Lankan species and genera.

Our observations in the field indicated that *E. albida* merited evaluation as a biological control agent for Sri Lankan Privet. The larva of *E. albida* was found only on Sri Lankan Privet during surveys in Sri Lanka. It was not found on other plants of the Oleaceae that were growing nearby such as *Jasminum officinale* L. and *Chionanthus albidiflorus* Thwaites, nor was it found during our surveys in southern and north-east India (R. H. Shaw unpublished data, September 2000), although it would be expected to occur on *L. perrottetii* in southern India. Larvae were found throughout the range of Sri Lankan Privet in Sri Lanka, but most frequently collected from the Central Highlands. Larvae were most commonly collected during the dry season (February) but not restricted to this season.

Parasitism was commonly observed when rearing field-collected specimens, 30-50% of young larvae being attacked. Two species of Braconidae were reared out and have been provisionally identified as *Canalirogas spilonotus* (Cameron, 1905) (= *Pseudogyroneuron spilonotum*) (Rogadinae) and an

Fig. 2. Biology of *Epiplema albida*. **A**, ova laid on midrib of privet leaf, 1.3 mm diameter. **B**, first instar larva, 2.2 mm long. **C**, mature larvae, 14 mm long, dorsal view. **D**, mature larvae and a pupa, 12 mm and 7 mm long respectively. **E**, prepupa in partially formed, weak cocoon. **F**, pupa removed from cocoon, 7 mm long. **G**, *Dolichogenidea* sp. dead adult with its cocoon. **H**, *Canalirogas spilonotus* dead adult. **I**, adult male; wing span 18 mm.

unidentified species of *Dolichogenidea* Viereck, 1911 (Microgastrinae) (G. Broad, pers. comm.) (Figs. 2G-H).

Canalirogas spilonotus was described from Sri Lanka, but is also found from India to China and south to Malaysia and Indonesia (Long and Achterberg, 2015). It has been reared from an unidentified species of Lymantriinae (Erebidae) in Sumatra (Quicke and Shaw, 2005). The use of *E. albida* by *C. spilonotus* is the second record of a host for the parasitoid. Rogadinae, including species of *Canalirogas* mummify their host larva or prepupa and pupate within (Quicke and Shaw, 2005), and we also observed this for *C. spilonotus*.

Dolichogenidea is a genus of more than 200 described species, closely related to *Apanteles* Foerster, 1862 (Fernández-Triana and Ward, 2016). The species whose biology are known are parasitoids of Lepidoptera, usually Microlepidoptera (Rousse and Gupta, 2013). We found that *Dolichogenidea* sp. is a solitary parasitoid of young larvae of *E. albida;* the mature parasitoid larva emerges from a second or third instar host larva to spin a solitary white cocoon (Fig. 2G).

Epiplema albida lays its 1.3 mm diameter circular, flattened eggs singly or in groups (Fig. 2A). The normal position is along the upper mid-vein of the leaf with the eggs touching or slightly overlapping each other in a line. Based on observations made during the multiple and single-choice oviposition studies reported by Shaw and Cock (2017), almost twice as many eggs were laid on the upper surface of the leaf (1,323) as on the lower surface (781). It was often the case that those eggs laid underneath the leaf were clumped together in crenulations in the leaf.

The larval stage of *E. albida* has four instars, compared to the five typical of many families of Lepidoptera. The newly hatched larvae began feeding by gnawing patches in the lower epidermis of the leaf. From the second or third instar the larvae ate through the leaves, making holes in the lamina. Fourth instar larvae fed mainly from the leaf edge, and were observed to feed both by day and by night. Larvae dropped and dangled by threads when disturbed. Fourth instar larvae grew to a maximum length of 12 mm; the head was plain light brown, the body translucent grey with a darker dorsal line, and subdorsal, dorsolateral, lateral and ventrolateral rows of dark tubercles, each surmounted with a single pale seta (Fig. 2C). The larvae could not be confused with any other Lepidoptera larvae found on Sri Lankan Privet.

Pupae were never found on plants of Sri Lankan Privet in the field. In the laboratory, pupation occurred amongst leaves and tissue paper in a flimsy silk cocoon (Fig. 2E) at the bottom of the rearing container. Because *E. albida* makes a cocoon with available debris, we assume that pupation is normally amongst leaf litter on the ground surface or at a shallow depth in the soil, rather than deeper in the soil, where a cocoon is not typical. The pupa is not distinctive, being uniformly smooth and brown, and estimated at c. 7 mm long (Fig. 2F).

The development results are summarised in Table 1. Ova normally hatched 7 days after being laid, although some took as long 10 days. This gives a total development time from oviposition to adult emergence of 33.6 days (±3.13, n=46).

This species shows moderate sexual size dimorphism, females being larger than males. Adult females showed significantly greater mean wingspan than males: females averaged 19.3 mm (range 16.4-22.0 mm, n=63), males averaged 18.0 mm (range 15.4-20.0 mm, n=59) (Student's $t = 5.3$, $df = 120$, $p<0.001$). Adult females were also found to have significantly longer mean total body length: females averaged 6.8 mm (range 5.8-8.0 mm), males averaged 6.6mm (range 5.5-7.6 mm) (Student's $t = 2.3$, $df = 120$, $p < 0.05$).

Table 1. Timing of the life cycle stages in the development of *Epiplema albida* (if a molt could have taken place on either of two days the mean was taken)

Stage	Number of replicates	Range in days from egg hatch to completion of stage	Mean in days to completion of stage from eclosion +/- 95% confidence interval	Mean duration of stage in days
Instar 1	30	3-6	3.4 ± 1.3	3.4
Instar 2	34	5-10.5	7.0 ± 1.3	3.6
Instar 3	35	7-14	10.2 ± 1.25	3.2
Instar 4	30	12-27	15.7 ± 1.7	5.5
Pupa	29	22-31.5	25.6 ± 1.2	9.9

Females laid a mean of 80.3 ± 13.1 (95% confidence interval) eggs (range 43–152, n=7). Fig. 3 shows the mortality of 43 adult females over time. Median female longevity in captivity was 7 days although some females survived as long as 14 days.

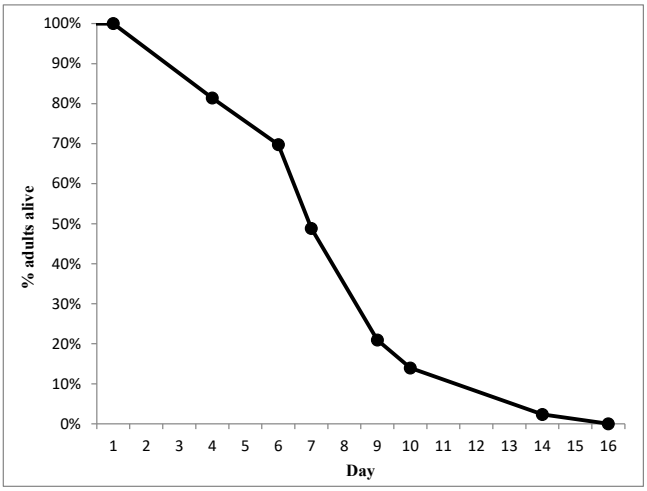

Fig. 3. Percentage of 43 newly emerged adult females of *Epiplema albida* surviving over time.

DISCUSSION

The species-level identification of the moth was relatively straightforward, although its generic placement was not resolved. In contrast to published reports that young larvae of Epipleminae tend to have gregarious habits and usually live in a web (Minet and Scoble, 1999), we found the larvae of *E. albida* to be solitary and without a web. The use of *Ligustrum* spp. as a larval food plant is not surprising as Oleaceae have been recorded as food plants for Epipleminae (Holloway *et al.*, 1987; Holloway, 1998).

Two parasitoids were recorded to commonly attack larvae of *E. albida* in Sri Lanka, but parasitism of eggs and pupae was not investigated. The high level of larval parasitism suggests that parasitoids may have an important role in keeping the populations of *E. albida* in check in Sri Lanka. This also suggests that *E. albida* could build up high population densities if introduced into La Réunion where it is presumed these parasitoids are absent.

A satisfactory rearing technique was developed, enabling host range testing to be carried out in quarantine over a period of 21 months (Shaw and Cock, 2017). The life cycle of *E. albida* was short in culture, taking just 29–42 days, and the adult fecundity was an average of 80 eggs per female. Thus, under favourable conditions, this species has the potential to increase in numbers very rapidly if unchecked and it is likely that multiple annual generations would be possible in La Réunion.

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