

# The first record of the parasitic myrmecophilous caterpillar *Liphyra brassolis* (Lepidoptera, Lycaenidae) inside Asian weaver ant (*Oecophylla smaragdina*) nests in oil palm plantations

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## Abstract

Asian weaver ants (*Oecophylla smaragdina*) are an important biocontrol agent in agricultural habitats. We conducted surveys in oil palm plantations in Riau, Indonesia for an obligate myrmecophilous butterfly larvae, *Liphyra brassolis* (Lepidoptera, Lycaenidae), that is known to consume weaver ant larvae in other habitat types. We found *L. brassolis* larvae in five of the twenty nests surveyed, with larval presence not being related to weaver ant nest size. We also observed *L. brassolis* larvae in a weaver ant mass rearing facility. This is the first report of *L. brassolis* from oil palm plantations and may have implications for the use of weaver ants as biological control agents.

## Keywords

Formicidae, parasitism, biocontrol, bagworm, Riau

## Introduction

The Asian weaver ant, *Oecophylla smaragdina* (Fabricius, 1775), is widespread from western Asia to northern Australia (Wetterer 2017) and maintains arboreal territories

through aggressive behaviour. Worker ants patrol tree branches and attack other animals because they are generalist predators, preying on insects and other arthropods and also attack humans who disturb their nests (Crozier et al. 2010). The Asian weaver ant has several ecological and economic functions regarding its ecological interactions: enhancing potential nitrogen (Pinkalski et al. 2015, Vidkjær et al. 2015, Vidkjær et al. 2016), shaping plant-pollinator interactions (Rodríguez-Gironés et al. 2013) and acting as biocontrol agent (Peng et al. 1999, Peng and Christian 2005, Peng and Christian 2013, William et al. 2015, Anato et al. 2017). Weaver ants are commonly used as biocontrol agents in several agricultural systems with potential to control major pests such as true bugs (Coreidae and Miridae), beetles (Chrysomelidae), aphids (Aphididae), caterpillars (Lepidoptera), leaf miners (Coleoptera), leafrollers (Lepidoptera), fruit flies (Drosophilidae), leafhoppers (Cicadellidae) and shoot borers (Lepidoptera) (Van Mele 2007).

The majority of the species in the butterfly family Lycaenidae are associated with ants, either mutualistically or parasitically, with interactions either being facultative or obligate (Pierce et al. 2002). Parasitic interactions usually involve myrmecophagy, with butterfly larvae consuming the ant brood. In the family Lycaenidae, 74 species show this behaviour during some stage of their life cycle (Pierce 1995). These parasitic-myrmecophagy interactions can be found between Africa and northern Australia (Fiedler 1991, Eastwood and Fraser 1999, Pierce et al. 2002, Terblanche and Van Hamburg 2003, Crozier et al. 2010, Table 1).

*Liphyra brassolis* (Westwood, 1864), is obligately myrmecophilous, with larvae being dependent on weaver ants (*Oecophylla smaragdina*) and eating the ant brood (Dodd 1902, Eastwood and Fraser 1999, Pierce et al. 2002). As *L. brassolis* feeds on the larvae of weaver ants, it might have the potential to affect ant populations. Hence, it is important to understand its occurrence in widespread crops, such as oil palm plantation, where *O. smaragdina* has the potential to control pests, such as leaf-eating caterpillars.

## Materials and Methods

Sampling of weaver ant nests was conducted in an oil palm plantation located in the Province of Riau in Sumatra, Indonesia (0°56'35.3"N, 101°11'56.4"E) in August 2021. A localised outbreak of *Clania tertia* Templeton, 1847 (Lepidoptera, Pyschidae) bagworm was ongoing when the sampling was conducted. Twenty active weaver ant nests were chosen randomly on twenty individual palms. Only nests in which ants were present (confirmed using binoculars), constructed from live green palm leaflets, were sampled. The nest was collected, wrapped in a plastic bag and all connecting leaflets and fronds were cut using machete and pruning shears. Nest dimensions (length, width and depth) were measured. On the ground, nests were thoroughly dissected and checked for the presence of *L. brassolis* larva. Dissected nests were removed from bags and replaced at the base of the palm to allow ants to return.

During this period, as part of another study, we were conducting mass-rearing of weaver ants with the aim of propagation and release as biocontrol agent against leaf-eating

caterpillar pests. All the colonies were fed on a 30% sugar solution and live insects or fish as a protein source. Observations of *L. brassolis* were conducted in this mass-rearing activity in 25 ant colonies that were being propagated in plastic bottles (Offenberg 2014) for integrated pest management (IPM).

*Liphyra brassolis* larvae, found in ant nests, were identified morphologically (Crozier et al. 2010, Eastwood et al. 2010, Dupont et al. 2016). The larva of this species is distinguished by its carapace-like structure with a hard cuticle both dorsally and laterally (Fig. 1), which is used to defend against ants attacks (Dupont et al. 2016). All specimens of *L. brassolis* larvae and *O. smaragdina* were housed in Smart Research Institute, Riau, Indonesia.

A generalised linear model (GLM) with binomial errors was used to investigate the relationship between the weaver ant nest size and *L. brassolis* occurrence. The occurrence of *L. brassolis* larvae was used as a binary response variable with one nest sample per observation. Nest size was calculated as an ellipsoid volume using the three measured nest dimensions. Correction for overdispersion was conducted using the quasibinomial family since the residual deviance was larger than the degree of freedom (Crawley 2015). All statistical analyses were performed using the R statistical program version 4.0.2 (R Core Team 2020) in the R Studio version 1.1.423 (RStudio Team 2020) environment. Graphs were created using “ggplot2” and “tidyverse” R packages (Wickham et al. 2019). The generalised linear model was performed using the “lme4” package (Bates et al. 2015). The dataset of observation is deposited in GBIF, Global Biodiversity Information Facility (<https://doi.org/10.15468/upzkak>).

## Results and Discussion

*Liphyra brassolis* larvae were present inside both weaver ant nests from the field (5 of 20 nests sampled) and mass-rearing observations (2 of 25 nests). In all seven nests with *L. brassolis* larvae present, only a single larvae was found (Suppl. material 1). A similar rate of occurrence of *L. brassolis* was reported during the ant harvesting season in Khao Chong, Thailand (this study: 16%, Eastwood et al. 2010: 21%). We found no evidence for a relationship between ant nest volume and *L. brassolis* larva presence (GLM, binomial errors, d.f. = 19,  $t = 0.78$ ,  $p = 0.44$ ; Fig. 2). This contrasts with a previous anecdotal observation mentioning that *L. brassolis* was found in larger weaver ant nests (Itterbeeck 2014).

We observed the larvae in the mass-rearing facility actively following the weaver ants during migration from the original disturbed leaf nest into the plastic bottle used to house the colony (Fig. 3). The presence of *L. brassolis* larvae in mass-rearing facilities should be avoided since consumption will reduce the number of ant brood. Mass-rearing operators should be trained to notice the *L. brassolis* larvae, so that larvae can be removed before the ant colony becomes established in the new nest. Education is needed here, since often local people misidentify *L. brassolis* larvae as a large ant or queen ant (Eastwood et al. 2010). This is important because the weaver ant mass-rearing is commonly used to produce ant brood for use as bird feed (Prayoga 2015), a protein source for human

consumption (Sribandit et al. 2008) or as an augmentation strategy for integrated pest management (IPM) programmes (Peng et al. 2014).

Although adult *L. brassolis* have been reported in West Java, Indonesia (Peggie 2012), this is the first report of this species in an oil palm plantation to our knowledge, despite multiple other butterfly surveys in this habitat in Sumatra (Panjaitan et al. 2020, Panjaitan et al. 2021). In Thailand, the larva of *L. brassolis* is commonly reported in weaver ant nests on orchard plantations, being first documented during entomophagy by local people (Eastwood et al. 2010). Furthermore, the presence of *L. brassolis* larva in weaver ant nests has not yet been reported during brood harvesting activity by local people in Indonesia (Césard 2004).

Implementation of biological control against leaf-eating caterpillars in an oil plantation is needed to support sustainable palm oil practices. However, only a small number of studies have investigated the ecological function of *O. smaragdina* as a biological control agent in oil palm plantation (Pierre and Idris 2013). With specific interaction with *O. smaragdina*, the butterfly *L. brassolis* can be a bioindicator for weaver ant colony in oil palm plantations. *Liphyra brassolis* larvae are only ever found associated with *O. smaragdina*. However, other Lycaenid larvae have been observed in the nests of *Oecophylla* spp, such as *Liphyra grandis* and *Zesius chrysomallus* (Pierce et al. 2002).

Our finding indicates that further investigations of weaver ant symbionts in oil palm plantations could be useful, in particular, because this ant species has potency for controlling leaf-eating caterpillar pests. It would be worth conducting larger-scale observations over greater numbers of colonies in order to measure any potential impacts of *L. brassolis* larvae on colony fitness, both in the field and in mass-rearing facilities. Furthermore, this observation represents an additional data point for lepidopteran biodiversity in Sumatra, especially in oil palm landscapes.

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## Hosting institution

Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah; Smart Research Institute, PT Smart Tbk, Indonesia

## Ethics and security

The data in the paper were collected as part of an ongoing leaf-eating caterpillar pest and its natural enemy monitoring in the plantations, following standard industry operating procedures.

## Author contributions

ADA, KMY and TMF contributed to the conception and design of the study. ADA collected the data. ADA, KMY and TMF wrote the first draft of the manuscript. All authors contributed to manuscript revision and have read and approved the submitted version.

## Conflicts of interest

The authors declare no conflict of interest.

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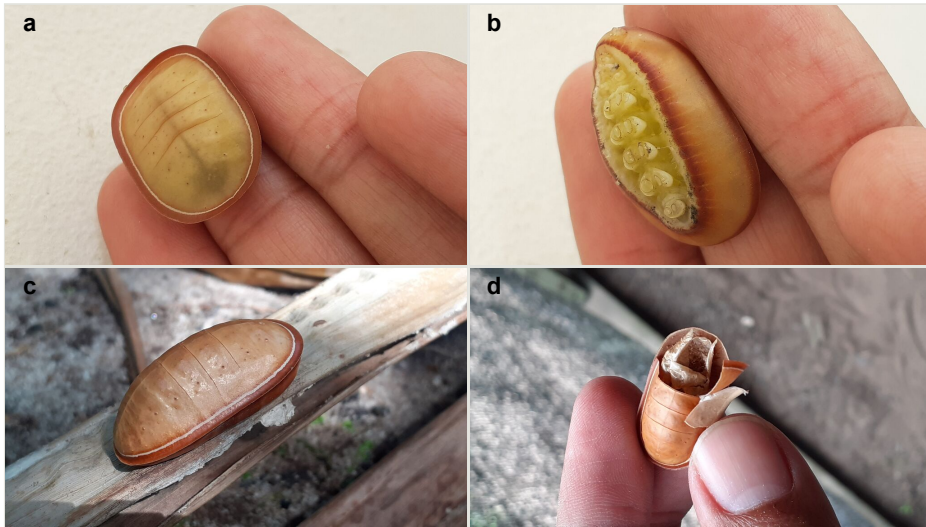


Figure 1.

Morphology of *Liphya brassolis* that were collected from weaver ant nests during field observations in an oil palm plantation in Riau Province, Indonesia.

- a: dorsal side of larva
- b: ventral side of larva
- c: pupal stage
- d: pupal exuvia



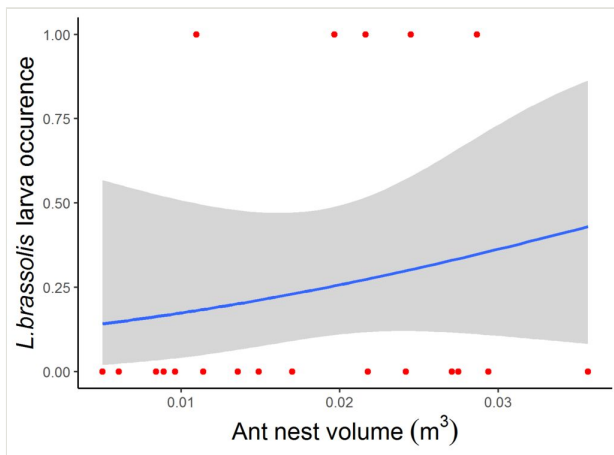


Figure 2.

Relationship between *Liphyra brassolis* larvae occurrence and weaver ant nest volume. Fitted line comes from a GLM with binomial errors.



Figure 3.

Presence of *Liphyra brassolis* (Lepidoptera: Lycaenidae), an obligate-parasitic myrmecophily larva, inside a weaver ant (*Oecophylla smaragdina*) colony in the mass-rearing facility.

Table 1.

List of lycaenid lepidopteran with myrmecophagy association

Family	Subfamily	Tribe/Subtribe	Species	Associated ant	Distribution
Lycaenidae	Miletinae	Liphyrini	<i>Liphyra brassolis</i>	<i>Oecophylla smaragdina</i>	Australia, Thailand, Malaysia, Indonesia
Lycaenidae	Miletinae	Liphyrini	<i>Liphyra grandis</i>	<i>Oecophylla</i> spp.	Papua, Australia, Pacific islands
Lycaenidae	Miletinae	Lachnocnemini	<i>Lachnocnema bibulus</i>	<i>Camponotus</i> spp., <i>Crematogaster</i> spp., <i>Pheidole</i> spp.	Africa continent
Lycaenidae	Miletinae	Lachnocnemini	<i>Lachnocnema brimo</i>	<i>Camponotus</i> spp., <i>Crematogaster</i> spp., <i>Pheidole</i> spp.	Africa continent
Lycaenidae	Miletinae	Lachnocnemini	<i>Lachnocnema durbani</i>	<i>Camponotus</i> spp., <i>Crematogaster</i> spp., <i>Pheidole</i> spp.	Africa continent
Lycaenidae	Lycaeninae	Theclini/ Arhopaliti	<i>Arhopala wildei</i>	<i>Polyrachis</i> spp.	Papua, Northern Australia
Lycaenidae	Lycaeninae	Theclini/ Luciti	<i>Acrodipsas aurata</i>	<i>Crematogaster</i> spp., <i>Papyrius</i> spp.	Australia
Lycaenidae	Lycaeninae	Theclini/ Luciti	<i>Acrodipsas brisbanensis</i>	<i>Crematogaster</i> spp., <i>Papyrius</i> spp.	Australia
Lycaenidae	Lycaeninae	Theclini/ Luciti	<i>Acrodipsas cuprea</i>	<i>Crematogaster</i> spp., <i>Papyrius</i> spp.	Australia

Lycaenidae	Lycaeninae	Theclini/ Luciti	<i>Acrodipsas illidgei</i>	<i>Crematogaster</i> spp., <i>Papyrius</i> spp.	Australia
Lycaenidae	Lycaeninae	Theclini/ Luciti	<i>Acrodipsas myrmecophila</i>	<i>Crematogaster</i> spp., <i>Papyrius</i> spp., <i>Iridomyrmex</i> spp.	Australia
Lycaenidae	Lycaeninae	Theclini/ Zesiiti	<i>Zesius chrysomallus</i>	<i>Oecophylla</i> spp.	South Asia
Lycaenidae	Lycaeninae	Theclini/ Ogyriti	<i>Ogyris idmo</i>	<i>Camponotus</i> spp.	Australia
Lycaenidae	Lycaeninae	Aphnaeini	<i>Cigaritis [Apharitis] acamas</i>	<i>Crematogaster</i> spp.	Africa continent, Japan
Lycaenidae	Lycaeninae	Aphnaeini	<i>C. [Spindasis] japanesenyassae</i>	<i>Crematogaster</i> spp.	Africa continent, Japan
Lycaenidae	Lycaeninae	Aphnaeini	<i>C. [Spindasis] takanonis</i>	<i>Crematogaster</i> spp.	Africa continent, Japan
Lycaenidae	Lycaeninae	Aphnaeini	<i>Trimenia agyroplaga</i>	<i>Anoplolepis</i> spp.	Africa continent
Lycaenidae	Lycaeninae	Aphnaeini	<i>T. wallengrenii</i>	<i>Anoplolepis</i> spp.	Africa continent
Lycaenidae	Lycaeninae	Aphnaeini	<i>T. [Argyrocupha] malagrida</i>	<i>Anoplolepis</i> spp.	Africa continent
Lycaenidae	Lycaeninae	Aphnaeini	<i>Oxychaeta dicksoni</i>	<i>Crematogaster</i> spp., <i>Myrmicaria</i> spp.	South Africa
Lycaenidae	Lycaeninae	Polyommagini/ Polyommatis	<i>Phengaris daitozana</i>	<i>Myrmica</i> spp.	Asia
Lycaenidae	Lycaeninae	Polyommagini/ Polyommatis	<i>P. atroguttata</i>	<i>Myrmica</i> spp.	Asia

Lycaenidae	Lycaeninae	Polyommagini/ Polyommati	<i>Lepidochrysops</i> spp.	<i>Camponotus</i> spp.	Africa continent
Lycaenidae	Lycaeninae	Polyommagini/ Polyommati	<i>Maculinea</i> spp.	<i>Myrmica</i> spp., <i>Aphaenogaster</i> spp.	Europe, Asia

## Supplementary material

### Suppl. material 1: *L. brassolis* field observation

**Authors:** Andreas Dwi Advento

**Data type:** Observation data

**Brief description:** These data are an observational census for *L. brassolis* occurrence in an oil palm plantation. They cover date sampling, location (plantation block), time sampling, nest dimension, larva number per nest and larva condition. (<https://doi.org/10.15468/upzkak>)

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