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The first record of the myrmecophilous caterpillar *Liphyra brasollis* in weaver ant nests in oil palm plantations

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Abstract

Asian weaver ants are an important biocontrol agent in agricultural habitats. We conducted surveys in oil palm plantations for an obligate myrmecophilous butterfly larvae *Liphyra brassolis* 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 has implications for the use of weaver ants as biological control agents.

Keywords

Liphyra brassolis, weaver ants, predator, pest, oil palm

Introduction

The Asian weaver ant, *Oecophylla smaragdina,* is widespread from Asia to Northern Australia and maintains territories through aggressive behaviour. Worker ants patrol tree branches and attack other animals as well as humans. They are generalist predators, preying on insects and other arthropods. The Asian weaver ant has several beneficial functions: enhancing potential nitrogen (Pinkalski et al. 2015, Vidkjær et al. 2014, Vidkjær et al. 2016), shaping plant-pollinator interactions (Rodríguez-Gironés et al. 2013), increasing fruit production (Anato et al. 2017, Peng and Christian 2013, William et al. 2015)

and conducting pest control (Peng et al. 1999, Peng and Christian 2005). Weaver ants are commonly used as biocontrol agents in agriculture with potential to control major pests such as true bugs (Coreidae and Miridae), beetles (Chrysomelidae), aphids (Aphididae), caterpillars (Lepidoptera), leafminers (Coleoptera), leaffrollers (Lepidoptera), fruit flies (Drosophilidae), leafhoppers (Cicadellidae), and shoot borers (Lepidoptera) in several agricultural systems (Van Mele 2007).

Belonging to the family Lycaenidae, *Liphyra brassolis* is obligately myrmecophilous, with larvae being dependent on weaver ants (*Oecophylla smaragdina*) and eating the ant brood (Eastwood and Fraser 1999). Consumption of ant brood is typical in the family Lycaenidae, with at least 55 species showing this behavior during some stage of their life cycle (Pierce 1995). *Liphyra brassolis* has the potential to affect populations of *Oecophylla* (Crozier et al. 2009) and hence it is important to understand its occurrence in widespread crops such as oil palm plantation.

Materials and Methods

Sampling of weaver ant nests was conducted in a large oil palm plantation located in the province of Riau in Sumatra (Indonesia). The oil palm plantations cover around 4000 ha and lie on mineral soil. An localized outbreak of *Clania tertia* bagworm was ongoing when the sampling was conducted. Twenty active weaver ant nests were chosen randomly on 20 individual palms. The nests were selected using a binocular to confirm an active population of ants. Only nests in which ants were present and green palm leaflets were used to construct the nest were selected and dissected. Only one nest was collected from any one palm. The nests were collected, wrapped in a plastic, and all connecting leaflets and fronds were cut using a 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 trophobionts and parasites because the weaver ants associate with it generally. Dissected nests were removed from bags and replaced at the base of the palm to allow ants to return.

In parallel research, there was a mass-rearing activity for weaver ants. Observations of *L. brassolis* were also conducted in a weaver ant mass-rearing facility where 25 ant colonies were being propagated in plastic bottles (Offenberg 2014) for integrated pest management (IPM). The twenty-five colonies in the mass-rearing facility were observed for *L.brassolis* presence. All the colonies were fed on a 30% sugar solution and live insect or fish as a protein source. *L.brassoli*s larva that found in ant nests were identified morphologically (Crozier et al. 2009, Dupont et al. 2015, Eastwood et al. 2010). The larva has a peculiar carapace-like structure with a hard cuticle both dorsally and laterally Fig. 1, which is used to defend against ants attacks.

A generalized linear model (GLM) with binomial errors was used to investigate the relationship between the weaver ant nest size and trophobiont or parasite occurrence. The occurrence of *L. brasollis* 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 quasibinomial family when 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 generalized linear model was performed using the "lme4" package (Bates et al. 2015).

Results and Discussion

Liphyra brassolis larvae were present inside both weaver ant nests from the field (4 of 20 nests sampled) and mass-rearing observations (2 of 25 nests;). 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.brasollis* larvae in mass-rearing facilities should be avoided since consumption of ant larvae will reduce the number of ant brood. The weaver ant mass-rearing is common to produce ant brood for use as bird feed (Prayoga 2015), a protein source (Sribandit et al. 2008), or as an augmentation strategy of integrated pest management (IPM) programme.

Although adult butterflies of *L. brassolis* have been reported in Ujung Kulon, West Java (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. 2021, Panjaitan et al. 2020). Other lepidopteran larvae can be found in weaver ant nests ranging between Africa and Northern Australia, including many lycaenids, which are often myrmecophilous (Pierce 1995, Eastwood and Fraser 1999, Crozier et al. 2009, Braby et al. 2018; Table 1).

Our finding indicates that further investigations of weaver ant symbionts in oil palm plantations could be useful, in particular, because this ant species has potential for controlling leaf-eating caterpillar pests. Furthermore, this observation represents an additional data point for lepidopteran biodiversity in Sumatra, especially in oil palm landscapes.

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- Smart Research Institute, PT Smart Tbk, Indonesia

Ethics and security

The data in the paper were collected as part of ongoing 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.

References

- Anato FM, Sinzogan AAC, Offenberg J, Adandonon A, Wargui RB, Deguenon JM, Ayelo PM, Vayssières J-F, Kossou DK (2017) *Oecophylla longinoda* (Hymenoptera: Formicidae) lead to increased cashew kernel size and kernel quality. Journal of Economic Entomology 110 (3): 1133-1137. https://doi.org/10.1093/jee/tox054
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67 (1): 1-48. https://doi.org/10.18637/jss.v067.i01
- Braby MF, Franklin DC, Bisa DE, Williams MR, Williams AA, Bishop CL, Coppen RA (2018) Atlas of butterflies and diurnal moths in the monsoon tropics of northern Australia. ANU Press https://doi.org/10.22459/ABDM.12.2018

- Crawley M (2015) Statistics: An Introduction Using R. second. John Wiley and Sons, Chichester, 357 pp. [ISBN 9781118941096]
- Crozier R, Newey P, Schluns E, Robson SA, et al. (2009) A masterpiece of evolution *Oecophylla* weaver ants (Hymenoptera: Formicidae). Myrmecological News, 13 (May): 57-71. URL: ISSN 1997-3500
- Dupont S, Zemeitat D, Lohman D, Pierce N (2015) The setae of parasitic *Liphyra brassolis* butterfly larvae form a flexible armour for resisting attack by their ant hosts (Lycaenidae: Lepidoptera). Biological Journal of the Linnean Society 117 (3): 607-619. https://doi.org/10.1111/bij.12656
- Eastwood R, Fraser A (1999) Associations between lycaenid butterflies and ants in Australia. Australian Journal of Ecology 24 (5): 503-537. https://doi.org/10.1046/j.1440-169x.1999.01000.x
- Eastwood R, Kongnoo P, Reinkaw M, et al. (2010) Collecting and eating *Liphyra brassolis* (Lepidoptera: Lycaenidae) in southern Thailand. The Journal of Research on the Lepidoptera 43 (January): 19-22.
- Fiedler K (2012) The host genera of ant-parasitic Lycaenidae butterflies: a review. Psyche 2012 (Article ID 153975): 1-10. https://doi.org/10.1155/2012/153975
- Itterbeeck JV (2014) Prospects of semi-cultivating the edible weaver and *Oecophylla smaragdina*. Wageningen University, Wageningen, 112 pp. [ISBN 978-94-6257-040-5].
- Offenberg J (2014) The use of artificial nests by weaver ants: a preliminary field observation. Asian Myrmecology, 6(1) 6 (1): 119-128. URL: ISSN 1985-1944
- Panjaitan R, Drescher J, Buchori D, Djunijanti P, Harahap IS, Csheu S, Hidayat P (2020) Diversity of butterflies (Lepidoptera) across rainforest transformation systems in Jambi, Sumatra, Indonesia. Biodiversitas Journal of Biological Diversity 21 (11). https://doi.org/10.13057/biodiv/d211117
- Panjaitan R, Hidayat P, Peggie D, Buchori D, Scheu S, Drescher J (2021) The Butterflies of Jambi (Sumatra, Indonesia): An EFForTS Field Guide. LIPI Press, Jakarta, 101 pp. [ISBN 978-602-496-270-8]
- Peggie D (2012) A list of the butterflies of Ujung Kulon National Park, Java, Indonesia. Treubia 39 (December): 67-76.
- Peng R, Christian K (2005) Integrated pest management in mango orchards in the Northern Territory Australia, using the weaver ant, *Oecophylla smaragdina*, (Hymenoptera: Formicidae) as a key element. International Journal of Pest Management 51 (2): 149-155. https://doi.org/10.1080/09670870500131749
- Peng R, Christian K (2013) Do weaver ant (Hymenoptera: Formicidae) marks affect mango internal quality and storage life? Journal of Economic Entomology 106 (1): 299-304. https://doi.org/10.1603/ec12162
- Peng RK, Christian K, Gibb K (1999) The effect of colony isolation of the predacious ant, *Oecophylla smaragdina* (F.) (Hymenoptera: Formicidae), on protection of cashew plantations from insect pests. International Journal of Pest Management 45 (3): 189-194. https://doi.org/10.1080/096708799227789
- Pierce N (1995) Predatory and parasitic lepidoptera: carnivores living on plants. Journal of the Lepidoterists' Society 49 (4): 412-453.
- Pinkalski C, Damgaard C, Jensen K, Peng R, Offenberg J (2015) Quantification of ant manure deposition in a tropical agroecosystem: implications for host plant nitrogen acquisition. Ecosystems 18 (8): 1373-1382. https://doi.org/10.1007/s10021-015-9906-5

- Prayoga B (2015) Kupas Tuntas Budidaya Kroto Cara Modern. 4th Edition. Penebar Swadaya, Jakarta, 124 pp. [ISBN 978-979-002-589-9]
- R Core Team (2020) R: A Language and Environment for Statistical Computing. Vienna, Austria. URL: https://www.r-project.org/
- Rodríguez-Gironés M, Gonzálvez F, Llandres A, Corlett R, Santamaría L (2013) Possible role of weaver ants, *Oecophylla smaragdina*, in shaping plant-pollinator interactions in South-East Asia. Journal of Ecology 101 (4): 1000-1006. https://doi.org/10.1111/1365-2745.12100
- RStudio Team (2020) RStudio: Integrated Development Environment for R. Boston, MA. URL: http://www.rstudio.com/
- Sribandit W, Wiwatwitaya D, Suksard S, Offenberg J (2008) The importance of weaver ant (*Oecophylla smaragdina* Fabricius) harvest to a local community in Northeastern Thailand. Asian Myrmecology 2 (1): 129-138. https://doi.org/10.20362/am.002015
- Van Mele P (2007) A historical review of research on the weaver ant *Oecophylla* in biological control. Agricultural and Forest Entomology 10 (1): 13-22. https://doi.org/10.1111/j.1461-9563.2007.00350.x
- Vidkjær N, Wollenweber B, Gislum R, Jensen K, Fomsgaard I (2014) Are ant feces nutrients for plants? A metabolomics approach to elucidate the nutritional effects on plants hosting weaver ants. Metabolomics 11 (4): 1013-1028. https://doi.org/10.1007/s11306-014-0757-4
- Vidkjær N, Wollenweber B, Jensen K, Ambus P, Offenberg J, Fomsgaard I, et al. (2016) Urea in weaver ant feces: quantification and investigation of the uptake and translocation of urea in *Coffea arabica*. Journal of Plant Growth Regulation 35 (3): 803-814. https://doi.org/10.1007/s00344-016-9586-1
- Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the Tidyverse. Journal of Open Source Software 4 (43). https://doi.org/10.21105/joss.01686
- William JG, Hella J, Lars E, Offenberg J, Mwatawala M, Rwegasira G, et al. (2015) Benefit-cost analysis of alternative insect pests management in cashew and mango orchards in Tanzania. Quarterly Journal of Econometrics Research 1 (2): 32-44. https://doi.org/10.18488/journal.88/2015.1.2/88.1.32.44

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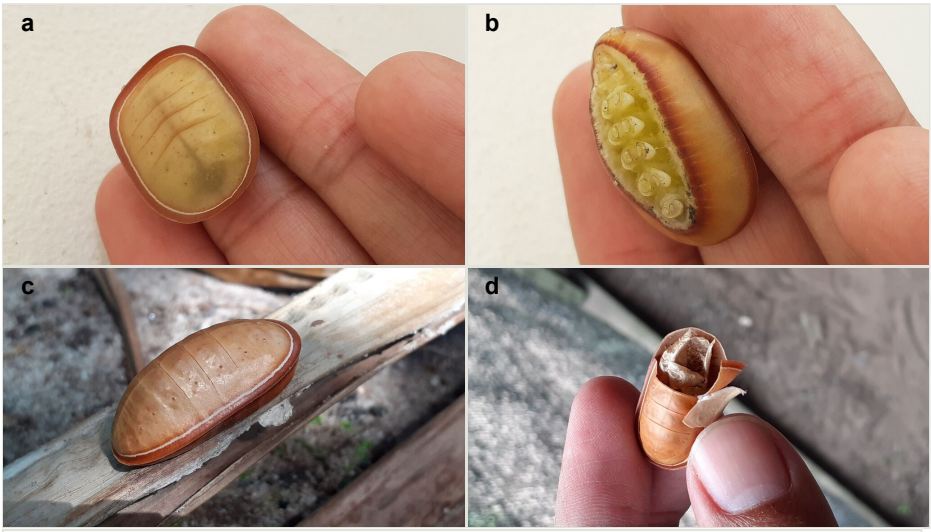

Figure 1.

Morphology of *Liphyra brassolis* that were collected during field observations.

a: ventral side of larvae
b: dorsal side of larvae
c: pupae stage
d: the exuviae

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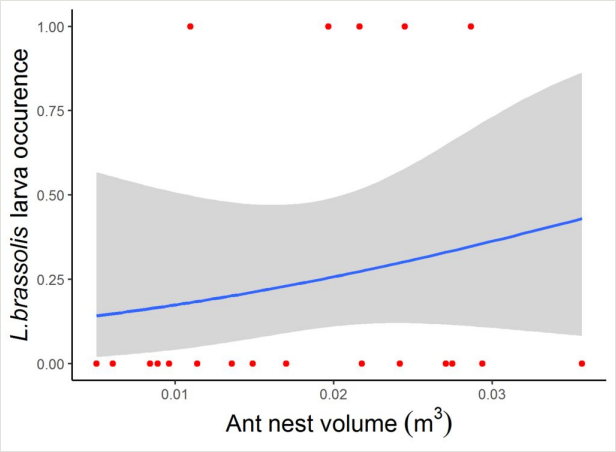

Figure 2.

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

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Figure 3.

Presence of *L. brassolis* larva in a weaver ant colony in the mass-rearing facility.

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Table 1.

Lepidoptera is known to associate with weaver ants.

No	Family	Subfamily	Tribe	Species	Degree of myrmecophily	Location	References
1	Lycaenidae	Liphyrinae	Liphyrini	*Liphyra brassolis*	obligate-parasitic	Australia, Thailand, Malaysia, Indonesia	Eastwood and Fraser 1999, Crozier et al. 2009
2	Lycaenidae	Liphyrinae	Liphyrini	*Liphyra grandis*	obligate-parasitic	Papua, Australia and Pacific islands	Crozier et al. 2009, Pierce 1995
3	Lycaenidae	Liphyrinae	Liphyrini	*Euliphyra mirifica*	obligate-parasitic with trophollaxis	Africa continent	Pierce 1995, Fiedler 2012
4	Lycaenidae	Liphyrinae	Liphyrini	*Euliphyra leucyania*	obligate-parasitic with trophollaxis	Africa continent	Pierce 1995, Fiedler 2012
5	Lycaenidae	Theclinae	Arhopalini	*Arhopala wildei*	obligate-parasitic	Papua, Northern Australia	Eastwood and Fraser 1999
6	Lycaenidae	Theclinae	Arhopalini	*Arhopala centaurus*	obligate-mutualistic	Northen Australia	Eastwood and Fraser 1999, Crozier et al. 2009
7	Lycaenidae	Theclinae	Arhopalini	*Arhopala madytus*	obligate-mutualistic	Northen Australia	Eastwood and Fraser 1999, Crozier et al. 2009
8	Lycaenidae	Theclinae	Arhopalini	*Arhopala micale*	obligate-mutualistic	Northen Australia	Eastwood and Fraser 1999, Braby et al. 2018
9	Lycaenidae	Theclinae	Ogyrini	*Ogyris zosine*	obligate-mutualistic	Australia	Eastwood and Fraser 1999

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10	Lycaenidae	Theclinae	Hypolycaenini	*Hypolycaena phorbas*	obligate-mutualistic	Northen Australia	Eastwood and Fraser 1999, Crozier et al. 2009, Braby et al. 2018
11	Lycaenidae	Theclinae	Deudorigini	*Deudorigini smilis*	weakly	Southeast Asia, Australia	Eastwood and Fraser 1999
12	Lycaenidae	Polyommatinae	Lycaenesthini	*Anthene seltuttus*	obligate-mutualistic	Northen Australia	Eastwood and Fraser 1999, Crozier et al. 2009, Braby et al. 2018
13	Lycaenidae	Polyommatinae	Lycaenesthini	*Anthene lycaenoides*	moderately (facultative)	Northen Australia, Jambi Indonesia	Eastwood and Fraser 1999, Crozier et al. 2009, Panjaitan et al. 2020
14	Lycaenidae	Polyommatinae	Lycaenesthini	*Anthene emolus*	obligate-mutualistic	Northen Australia	Crozier et al. 2009
15	Lycaenidae	Polyommatinae	Polyommatini	*Nacaduba berenice*	moderately	Northen Australia	Eastwood and Fraser 1999, Crozier et al. 2009
16	Lycaenidae	Polyommatinae	Polyommatini	*Theclinesthes miskini*	steadily	Northen Australia	Eastwood and Fraser 1999, Crozier et al. 2009
17	Lycaenidae	Theclinae	Zesiini	*Zesius chrysomallus*	parasitic	South Asia	Pierce 1995
18	Pyralidae	Spilomelinae	Wurthiini	*Niphopyralis myrmecophila*	obligate-parasitic	Java, Indonesia	Pierce 1995