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SHORT COMMUNICATION

Oviposition of *Minstrellus grandis* (Lepidoptera: Riodinidae) in a harmful ant-plant symbiosis

Lucas A. KAMINSKI[1*], Eduardo CARNEIRO[2], Diego R. DOLIBAINA[2], Mirna M. CASAGRANDE[2], Olaf H. H. MIELKE[2]

[1] Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Zoologia, Av. Bento Gonçalves 9500, 91501-970, Porto Alegre, RS, Brazil

[2] Universidade Federal do Paraná, Departamento de Zoologia, Laboratório de Estudos de Lepidoptera Neotropical, Avenida Cel. Francisco H. dos Santos, 100, 81531-980, Curitiba, Paraná, Brazil

* Corresponding author: lucaskaminski@yahoo.com.br; https://orcid.org/0000-0002-6468-0960

ABSTRACT

The oviposition behavior of the rare butterfly *Minstrellus grandis* (Callaghan, 1999) (Lepidoptera: Riodinidae) is recorded for the first time. Two females laid eggs on the old leaves of an unidentified *Triplaris* Loefl. ex L. (Polygonaceae), a myrmecophytic plant typically known as 'Triplaria' or 'novice' tree, inhabited by aggressive 'taxi' ants of the genus *Pseudomyrmex* Lund. 1831 (Hymenoptera: Formicidae). These observations suggest that *M. grandis* caterpillars live associated with one of the most harmful types of Amazon ant-plant symbiosis.

KEYWORDS: Carnivory, myrmecophily, host plant selection, Pachythonina, social parasitism

Oviposição de *Minstrellus grandis* (Lepidoptera: Riodinidae) em uma perigosa simbiose formiga-planta

RESUMO

O comportamento de oviposição da rara borboleta *Minstrellus grandis* (Lepidoptera: Riodinidae) é registrado pela primeira vez. Duas fêmeas depositaram ovos sobre folhas velhas de uma *Triplaris* (Polygonaceae) não identificada, uma planta mirmecofítica conhecida popularmente como 'pau-formiga' ou 'novateiro', habitadas por formigas 'taxi' agressivas do gênero *Pseudomyrmex* (Hymenoptera: Formicidae). Estas observações sugerem que as lagartas de *M. grandis* vivem associadas com um dos mais perigosos tipos de simbiose formiga-planta da Amazônia.

PALAVRAS-CHAVE: Carnivoria, mirmecofilia, seleção de planta hospedeira, Pachythonina, parasitismo social

The systematics of Riodinidae has advanced in recent years, and robust phylogenetic hypotheses supported by fossil-calibrated dating are now available (Seraphim *et al*. 2018). This backbone is essential to understand the evolutionary history of this remarkable group of butterflies (Kaminski *et al*. 2013). The main impediment for the advancement on the knowledge on this family, however, is the lack of information on natural history of species in this group, especially for rare and/or restricted species (DeVries 1997). This is the case for members of the subtribe Pachythonina (Nymphidiini), which comprises 33 species in five genera (Callaghan and Lamas 2004; Hall 2007, 2018; Dias *et al.* 2015; Gallard 2017; Gallard and Fernandez 2017). Only recently, the first life cycle information for this lineage was revealed, indicating that their caterpillars are myrmecophilous (i.e., live associated with ants), present armored larval morphology and are carnivorous (Medina 2014; Mota *et al*. 2020). During a collection expedition to the Serra do Divisor National Park [Parque Nacional da Serra do Divisor (PNSD)], in the western Amazon region (state of Acre, Brazil), we observed, for the first time, the female reproductive behavior of the poorly known riodinid *Minstrellus grandis* (Callaghan, 1999). This Pachythonina species is rare, known from only five localities in the western Amazon region in Brazil, Bolivia and Peru (Callaghan 1999; Hall 2007). Here we describe its oviposition behavior.

The observations were made in the riparian forest on the right margin of the Moa River, in a frontier-protection deployment area of the Brazilian military forces (Destacamento Especial de Fronteira de São Salvador, 61º Batalhão de Infantaria de Selva) (7°24'47.72"S, 73°12'46.55"W, 200 m), about 8 km east of the border of the PNSD. This region is characterized by a high biological diversity, including riodinid butterflies (Brown and Freitas 2002; Dolibaina *et al*. 2012, 2015, 2016). Our behavioral observations were

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made on June 19, 2013, between 13:50 and 14:15 h, when two females of *M. grandis* were observed inspecting a *Triplaris* Loefl. ex L. (Polygonaceae) myrmecophytic plant inhabited by *Pseudomyrmex* Lund, 1831 ants (Hymenoptera: Formicidae). The females flew slowly and walked along the plant branches, at a height of about three meters from the ground, without attracting the attention of ants (Figure 1a,b). Three oviposition events were observed in both the abaxial and adaxial surfaces of old leaves (Figure 1c). The adult butterflies were collected and are deposited (DZ 51.398 and DZ 51.408) in the collection of the Department of Zoology of Universidade Federal do Paraná, Curitiba, Paraná, Brazil (DZUP). Due to the ant aggressiveness (see Haddad *et al.* 2009), we were unable to search the *Triplaris* plant to find possible caterpillars, nor were the eggs and ant vouchers collected. The ants were identified based on behavior, photographs and taxonomist opinion (see Acknowledgments).

Our observations confirm that *M. grandis* uses the myrmecophytic *Triplaris* plant as oviposition substrate. Although we cannot confirm whether *M. grandis* caterpillars are myrmecophilous, nor whether the type of interaction with ants is commensal, mutualistic or parasitic, the observed behavior suggests a unique life cycle, which may represent the first known case of butterfly caterpillars specialized in *Pseudomyrmex* ants (Pseudomyrmecinae). These large-eyed arboreal ants have stingers and are quite aggressive, especially those that live associated with myrmecophytes, such as those of the *Pseudomyrmex triplarinus* (Weddell, 1850) group (sensu Chomicki *et al.* 2015), that are specialized on *Triplaris* (Benson 1985; Ward 1999; Chomicki *et al.* 2015; Sanchez 2015). Known associations between myrmecophilous caterpillars and *Pseudomyrmex* ants are rare and facultative or antagonic (Fiedler 2001; Kaminski *et al.* 2012).

The *Triplaris-Pseudomyrmex* ant-plant symbiosis is widely distributed in the Neotropical lowland forests, but the hotspot for these associations is the western part of the Amazon Basin (Sanchez 2015). Interestingly, the four species of *Minstrellus* Hall, 2007 are also restricted to the western Amazon (Hall 2007). Although some species of *Triplaris* and *Pseudomyrmex* are widely distributed, others have restricted distribution ranges (Ward 1999; Sanchez 2015). Thus, the precise identification of *Triplaris-Pseudomyrmex* associations is essential to understand their evolutionary history and their relationship with the distribution range and apparent rarity

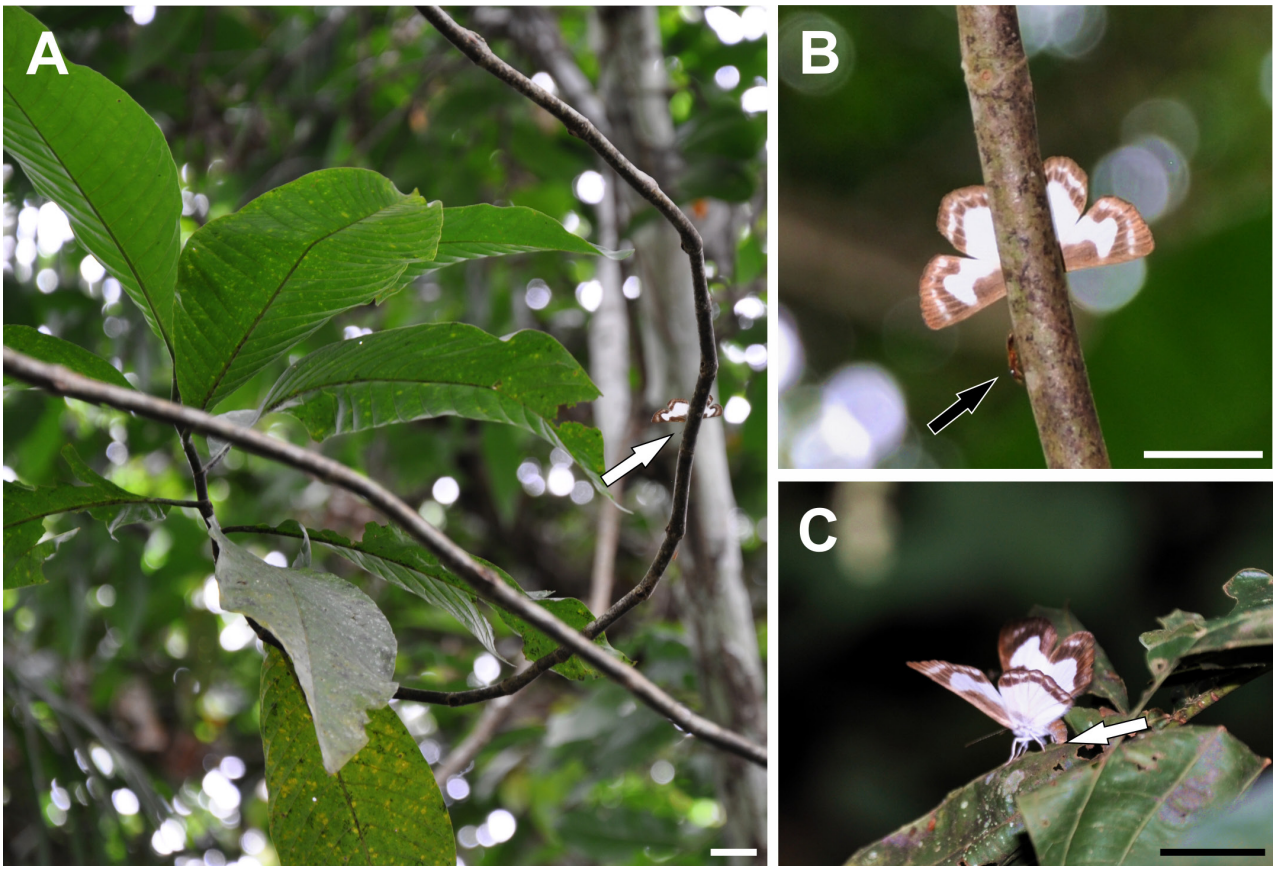

Figure 1. Female behavior of *Minstrellus grandis* (Lepidoptera: Riodinidae) on a *Triplaris* (Polygonaceae) plant inhabited by *Pseudomyrmex* ants by the Moa River, Mâncio Lima, Acre, Brazil. A – host plant, showing female walking on *Triplaris* branch (arrow); B – detail of female on a branch of the host plant near an ant worker (arrow); C – female laying eggs on an old leaf of the host plant (arrow). Scale bars = 1.5 cm. This figure is in color in the electronic version.

of *Minstrellus* species. It is worth mentioning this was our only record of *M. grandis* in a total of four expeditions to the PNSD, totaling 35 days of sampling effort in September 2011, June 2013, August 2014, and October 2018. This suggests that the occurrence of *M. grandis* may be seasonal, with adults flying for a short period, as reported for some social parasitic species (Fiedler 1998).

Myrmecophilous caterpillars that exploit ant-plant symbiosis are known to exist (Maschwitz *et al*. 1984). For instance, *Triplaris* trees inhabited by *Azteca* Forel, 1878 (Dolichoderinae) ants are attacked by *Theope pieridoides* C. Felder & R. Felder, 1865 (Riodinidae) caterpillars (Kaminski *et al*. 2013). In general, myrmecophilous caterpillars are herbivorous and receive protective benefits from ant partners by inhabiting an enemy-free space (e.g., Kaminski *et al*. 2010). From herbivorous ancestors, however, some species evolved caterpillars with carnivorous habit and even social parasitism, when caterpillars directly exploit the ants' resources (Pierce *et al*. 2002). In Pachythonina, it is known that caterpillars of *Pachythone xanthe* H. Bates, 1868 and *Pachythone gigas* Godman & Salvin, 1878 are predators of scale insects associated with aggressive *Azteca* ants (Medina 2014; Mota *et al.* 2020). In common, the caterpillars have an armored morphology, perforated cupola organs and tentacular nectary organs with possible appeasing function (Mota *et al.* 2020). These morphological traits can be preadaptations to live with aggressive ants (see Fiedler 1998; Dupont *et al*. 2016).

Therefore, it would not be unlikely that *M. grandis* caterpillars are also able to exploit resources from the harmful *Triplaris-Pseudomyrmex* ant-plant symbiosis. Confirming this hypothesis may add another piece to understand the evolution of carnivory and social parasitism in Pachythonina. Thus, we hope that our report will encourage future fearless efforts to reveal the life cycle of this rare Amazon butterfly.

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