ENTOMOLOGICAL Communications

Entomological Communications, 6, 2024: ec06021
doi: 10.37486/2675-1305.ec06021
e-ISSN: 2675-1305
Open Access Full Text Article

Scientific Note

First record of parasitoidism in *Danaus erippus* (Cramer, 1775) pupae (Lepidoptera, Nymphalidae): A possible mediation by an exotic plant species

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Edited by: Daniel Aquino

Received: July 16, 2024. Accepted: July 29, 2024. Published: August 06, 2024.

Abstract. Monarch butterflies have a close relationship with plants of the Apocynaceae family, especially with the genus *Asclepias* Linnaeus, 1753, using their toxic cardenolides as a defense against predators. *Calotropis procera* (Aiton) W.T. Aiton, 1811, native from Africa and Asia and introduced in Brazil as an ornamental plant, is a food alternative for monarchs but contains fewer cardenolides than *Asclepias*, which may make the butterflies more vulnerable to parasitoids. The interaction between wasps of the genus *Brachymeria* Westwood, 1829 and butterflies of the genus *Danaus* Kluk, 1802 is seldom reported. This study reports the first case of parasitism by *Brachymeria pandora* (Crawford, 1914) in pupae of *Danaus erippus* (Cramer, 1775) in Brazil, collected in the city of Cuiabá, Mato Grosso. Five butterfly pupae were collected on *C. procera*; three were parasitized, with 34 emergences of parasitoids. We suggest that the relationship between *D. erippus* and *B. pandora* may be facilitated by the lower toxicity of *C. procera* compared to *Asclepias*, possibly increasing susceptibility to parasitoidism. The high rate of parasitoidism observed suggests that this possible new interaction could be detrimental to the conservation of *D. erippus*. Further studies are needed to confirm whether this parasitoid-host interaction also occurs with native *Asclepias* plants and to investigate the impacts of exotic plants on these types of interactions and on butterfly conservation.

Keywords: Apocynaceae, Cardenolides, Chalcididae, Milkweed Butterflies, Monarch Butterfly.

Monarch butterflies (Nymphalidae: Danainae: Danaini) have an intimate relationship with plant species of the family Apocynaceae, mainly from the genus *Asclepias* Linnaeus, 1735 (Oberhauser et al. 2015). These plants are known for containing cardenolides in their leaves and latex, which are toxic to animals (Malcolm 1994). Butterflies' caterpillars accumulate these compounds in their bodies, using them as a chemical defense against predators and parasites (Tan et al. 2018; Stenoien et al. 2019).

Calotropis procera (Aiton) W.T. Aiton, 1811 (Apocynaceae) is a native shrub from Africa and Asia, used as a food resource by monarch butterflies (Dhileepan 2014). The plant was introduced in Brazil for ornamental purposes in the early 20th century (Rangel & Nascimento 2011), where it became invasive due to its adaptability to the country's climatic conditions (Sobrinho et al. 2013). Additionally, *C. procera* is a strong competitor compared to native plants because of its reproductive strategy, which is independent of seasonality and involves the production of a large amount of anemochoric seeds (Sobrinho et al. 2013).

Like others Apocynaceae species, *C. procera* produces several cardenolides (Mohamed et al. 2015; Ramos et al. 2022). However, when compared with species from the genus *Asclepias*, *C. procera* have a less diverse defence compounds (Ramos et al. 2022). *Danaus plexippus* (Linnaeus, 1758) (Lepidoptera, Nymphalidae) that feed on plants with few chemical defense compounds are more vulnerable to parasitoids compared to those that feed on plants with a higher amount of compounds (Oberhauser et al. 2015; Stenoien et al. 2019).

Chalcididae is a family of parasitoid wasps with a wide distribution (Arias & Delvare 2003), which is specialized in parasitize caterpillars and pupae of Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera, and Strepsiptera (Zache et al. 2012). In Brazil, 262 species are found, divided into 19 genera (Tavares 2024). Species from the genus *Brachymeria* Westwood, 1829 are specialized in parasitizing pupae, mainly from lepidopterans and dipterans (Burks 1960).

The parasitoidism of *Danaus* Kluk, 1780 species by *Brachymeria* species seems to be uncommon. The few recorded cases are restricted to the association of *Brachymeria jambolana* Gahan, 1942 attacking *Danaus* sp. in Dhaka (India: Bangladesh) (Narendran & Van Achterberg 2016); *Brachymeria albicrus* (Klug, 1834) in *Danaus chrysippus* (Linnaeus, 1758) in United Arab Emirates (Delvare 2017); *Brachymeria ovata* (Say, 1824) in *Danaus gilippus thersippus* (H. Bates, 1863) and *D. plexippus* in the United States of America (Halstead 1988; Prudic & Olson 2005). There are no records of *Brachymeria* associated with *Danaus* species in the neotropics. Here, we discuss the first report of parasitoidism of *Brachymeria pandora* (Crawford, 1914) in pupae of *D. erippus* collected on the exotic plant *C. procera*.

We collected five pupae of *D. erippus* in the urban perimeter of Cuiabá (Brazil: Mato Grosso) at coordinates 15°35'29.9" S, 56°04'13.6" W; 15°35'24.81" S, 56°4'19.35" W and 15°35'2.52" S, 56°4'8.89" W on April 3, 2024. The specimens were collected by active searching through the leaves of *C. procera*. One of the pupae was found dead and presented holes characteristic of parasitoid emergence (Fig. 1); the other four were brought to the laboratory. Each specimen was kept isolated in transparent plastic containers, maintained in the laboratory at room temperature (~25 °C) until the emergence of butterflies or parasitoids. The natural light-dark cycle was maintained, and no treatments were applied to the individuals.

From the four collected pupae, three were parasitized by *B. pandora* (Fig. 2). The emergence dates of the parasitoids were April 6th, 9th, and 15th, with 15 (five males; 10 females), 10 (two males; eight females), and nine (one male; eight females) individuals, respectively (Fig. 2). The identification of the parasitoid genus was based on the keys provided by Bouček (1992) and Andrade & Tavares (2009). The identity of the *Brachymeria* species was reached by analyzing the type specimens deposited at the Entomological Collection of National Museum of

Natural History (Type nº 18175). From the unparasitized pupa, emerged on April 9th a healthy specimen of *D. erippus* with normal size, showing no signs of parasitoidism. The butterfly was identified by comparison with specimens from the Coleção Entomológica de Mato Grosso Eurides Furtado (CEMT). All specimens (butterfly, parasitoids, and pupae) were prepared and will be deposited in the CEMT as vouchers.

Figure 1. The dead pupae of *Danaus erippus* collected on *Calotropis procera* in Cuiabá, MT, Brazil containing several holes characteristic of parasitoid emergence.

Figure 2. Individual of *Brachymeria pandora* (male) emerged from *Danaus erippus* pupae collected in Cuiabá, MT, Brazil.

The parasitoidism by *B. pandora* has been recorded to the lepidopterans *Argon lota* (Möschler, 1877) (Hesperiidae), *Historis odius* (Fabricius, 1775) (Nymphalidae), and *Saliana* sp. (Hesperiidae) in Brazil (Gil-Santana & Tavares 2005; Salgado-Neto et al. 2010; Tinôco et al. 2012). On the other hand, the only parasitoid recorded in *D. erippus* is the Tachinidae fly *Masicera brasiliensis* (Moreira, 1915) (Almeida et al. 1986). In this study, we record for the first time the relationship between *B. pandora* and *D. erippus*. Compared to other butterfly species, the number of parasitoid emergences was higher in the present study. In *H. odius*, one to six parasitoids per pupa were reported (Gil-Santana & Tavares 2005), in *A. lota*, seven to twelve individuals were recorded (Salgado-Neto et al. 2010), and for *Saliana* sp. an average of three emergences per pupa was observed (Tinôco et al. 2012).

Most interestingly, all the parasitized pupae were found on *C. procera*. This indicates that the caterpillars fed on this plant, given the behavior of *D. erippus* to pupate on the same plants they feed on. Therefore, we raise an important question: Would the parasitoidism relationship recorded here occur without the presence of the exotic plant *C. procera*? *Calotropis procera* has fewer toxic cardenolides compared to native species of *Asclepias* (Ramos et al. 2022). Studies with *D. plexippus*, a sister species of *D. erippus*, have shown that individuals feeding on less toxic plant species are more susceptible to parasites or parasitoids than those that feed on more toxic ones (Oberhauser et al. 2015; Tan et al. 2018). Therefore, we speculate that the presence of the invasive species *C. procera* may facilitates parasitoidism by *B. pandora* in *D. erippus*, as its chemical composition is less toxic. Another piece of evidence that the relationship reported here might be evolutionarily recent is the fact that parasites that cause host mortality are not expected to have high prevalence (Poulin & Randhawa 2015). Given that we found 80% of the individuals infected, the introduction of the parasitoid poses a significant threat to the conservation of *D. erippus*, as it necessarily causes host mortality and may negatively affect their populations sizes.

Finally, we cannot rule out the hypothesis that the relationship between *D. erippus* and *B. pandora* could also occur without the presence of the exotic plant. To confirm this, it would be necessary to collect individuals that have used native *Asclepias* species as a food resource or to conduct laboratory experiments. This note presents the first record of *B. pandora* in *D. erippus*, being the first record of parasitoidism of *Brachymeria* in *Danaus* species in the neotropics, opening possibilities for studies on the impacts of exotic plants on parasitic relationships and the conservation of butterfly species.

Acknowledgments

We thank the Entomological Collection of Mato Grosso for providing its space and resources for the development of this work, especially for the photographic equipment [Leica motorized stereomicroscope system model M205C with image capture system MC190 HD]. We would like to give special thanks to the curator Fernando Zagury Vaz-de-Mello, Bruna Bordin and Jorge Arias for their assistance with the photographic equipment. We thank Amanda Galdi Boaretto for providing insights on the manuscript. Finally, we thank Michael Angelo for assisting in the collection of the pupae.

Funding Information

Entomological Collection of Mato Grosso: (subproject EECBio UFMT/Finep #01.12.0359.00); MTT: Instituto Nacional de Ciência e Tecnologia dos Hymenoptera Parasitoides (INCT HYMPAR) (CNPq Proc. # 465562/2014-0); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Proc. #2014/50940-2) and Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES) (Proc. #51665797178233009).

Authors' Contributions

WVL: Conceptualization, Methods, Writing - original draft preparation; AE: Conceptualization, Methods, Writing - review and editing; Supervision; MTT: Methods, Writing - review and editing.

Conflict of Interest Statement

The authors have no competing interests to declare that are relevant to the content of this article.

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