

## RESEARCH ARTICLES

## ОРИГИНАЛЬНЫЕ СТАТЬИ

SYSTEMATIC POSITION AND CONSERVATION ASPECTS OF  
*MELINAEA MNASIAS THERA* (LEPIDOPTERA: NYMPHALIDAE: DANAINAE)Augusto H. B. Rosa<sup>1,\*</sup> , Eduardo P. Barbosa<sup>1</sup> , Niklas Wahlberg<sup>2</sup> , André V. L. Freitas<sup>1</sup> <sup>1</sup>Universidade Estadual de Campinas, Brazil\*e-mail: [augustohbrosa@hotmail.com](mailto:augustohbrosa@hotmail.com)<sup>2</sup>Lund University, Sweden

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The tribe Ithomiini (Nymphalidae: Danainae) includes nearly 400 species of butterflies distributed from Mexico to Northern Argentina, and adults of all species are aposematic and the main models in several Neotropical mimicry rings. The subtribe Melinaeina, a small group composed of five genera of large ithomiines, is the sister group of all remaining groups in the tribe Ithomiini. With 14 recognised species, the genus *Melinaea* is the most species rich, and also the most widespread within the Melinaeina. From all species of the genus, *Melinaea mnasias* is considered very rare and a little known one. This is also true for *Melinaea mnasias thera*, a subspecies from the Atlantic Forest with less than 20 specimens known in all world museums. Studies combining systematics, ecology, biogeography and natural history are priority in tropical areas, especially when focusing on threatened species. Thus, the aim of this study was to compile all available knowledge on the threatened *M. mnasias thera*, providing information to future management plans focusing on the conservation of this butterfly and its habitats. Data were compiled from scientific collections and personal observations, and the systematics of species of *Melinaea* was assessed by DNA sampling and analysis. The obtained phylogeny recovered the subtribe Melinaeina organised in two clades, the first composed by *Olyras* + *Paititia* and the second by *Eutresis* + (*Athyrtis* + *Melinaea*). *Melinaea mnasias thera* was recovered as a sister to *M. mnasias lucifer*. A total of only 17 specimens of *M. mnasias thera* from four Brazilian localities were found in all revised collections. However, well-preserved forests are present only at one of these localities, in the southern Bahia state, from where a recent specimen has been collected, suggesting that the last populations of *M. mnasias thera* are restricted to this region. If this is true, the real conservation status of this species could be much more critical than the estimated.

**Key words:** Atlantic Forest, Brazil, butterfly, Ithomiini, endangered species

### Introduction

The tribe Ithomiini (Lepidoptera: Nymphalidae: Danainae) includes about 400 butterfly species distributed from Mexico to Northern Argentina (Doré et al., 2023). Adults of all species are aposematic and considered the main models in many Neotropical mimicry rings (Brown & Benson, 1974; Beccaloni, 1997). In a study combining morphological and molecular data, Brower et al. (2014) recovered the tribe Ithomiini divided in ten subtribes, nine of which are well supported by both types of characters, a result congruent with subsequent molecular studies (Garzón-Orduña et al., 2015; Chazot et al., 2019). The subtribe Melinaeina is composed of five genera of large ithomiines and was recovered as a sister group of all remaining ithomiines in the listed above studies. With 14 recognised species, the genus *Melinaea* Hübner, 1816 is the most species rich, and also the most widespread within

the Melinaeina (Brown, 1979; McClure et al., 2018). From those 14 species, only three taxa occur in the Atlantic Forest, namely the endemic *Melinaea ethra* (Godart, 1819) and two subspecies of two widely distributed species, namely *Melinaea ludovica paraiya* Reakirt, 1866 and *Melinaea mnasias thera* C. Felder & R. Felder, 1865 (Brown, 1979).

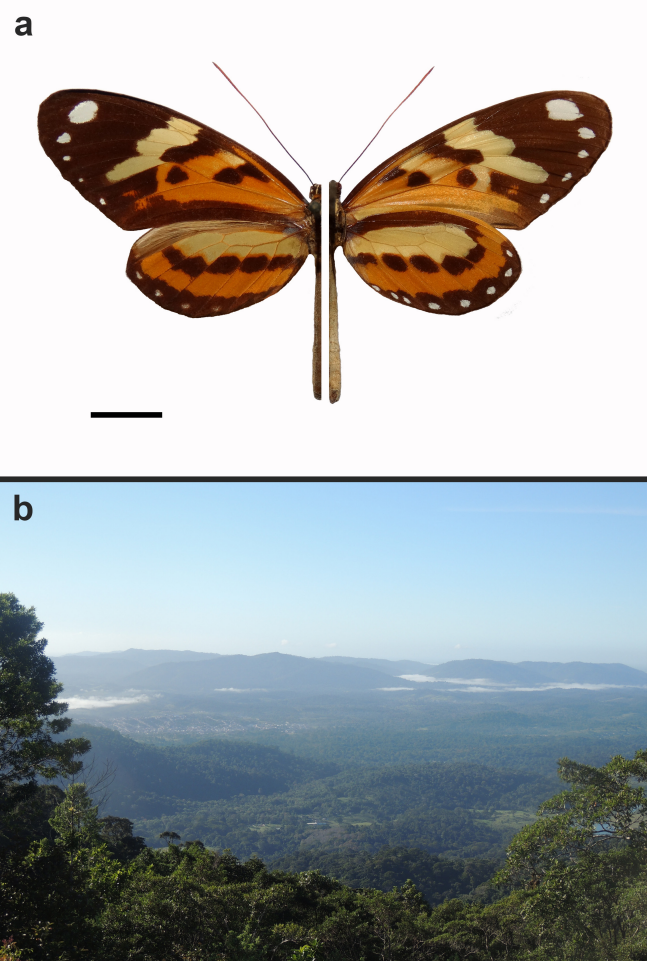
*Melinaea mnasias* (Hewitson, [1856]) is divided into 11 described subspecies, and it is considered very rare and little known (Brown, 1977). Except for *M. mnasias comma* W. Forbes, 1927 and *M. mnasias lucifer* H. Bates, 1862, which are locally common, all other subspecies are scarce in collections. This is also true for the Atlantic Forest subspecies *Melinaea mnasias thera*, known from only 17 specimens so far (Brown & Freitas, 2008; Freitas et al., 2018; Rosa et al., 2023).

*Melinaea mnasias thera* occurs in a narrow region of the coastal Atlantic Forest in the Brazil-

ian state of Bahia, at only two localities. Accordingly, this taxon was included in the Brazilian Red List, being assessed as «Critically Endangered» (CR) (MMA, 2003, 2014, 2022; Freitas et al., 2018). The compiling of all available information on any threatened butterflies is an important task, especially for the species which are scarce in museum collections (Freitas & Marini-Filho, 2011). In this specific case, *M. mnasias thera* occurs in a critical portion of the Atlantic Forest, a global biodiversity hotspot for conservation (Myers et al., 2000). Thus, the aim of this study was to investigate the systematic position of *M. mnasias thera*, its conservation aspects, and compile all available information about this rare butterfly. Studies combining systematics, ecology, biogeography and natural history are priority in tropical areas, especially when focusing on threatened species. Moreover, the information obtained for a given group of herbivores can be useful in studies of diversity and health of a given plant assemblage (Gilbert, 1980; Freitas, 1996). Finally, studies like this one are important for providing information to future management plans focusing on the conservation of threatened butterflies and their habitats.

### Material and Methods

Specimens of *M. mnasias thera* (Fig. 1a) were found in the following nine public collections: Coleção Entomológica do Instituto Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil (CEIOC); Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil (DZUP); Muséum National d’Histoire Naturelle, Paris, França (MNHN); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM); Museu de Zoologia, Universidade de São Paulo, São Paulo, São Paulo, Brazil (MZUSP); Natural History Museum, London, England, United Kingdom (NHMUK); Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ) (these specimens were lost by the fire that destroyed the Museum in 2018); Museum für Naturkunde, Berlin, Germany (ZMB); Zoological Collection, Museu de Diversidade Biológica da Universidade Estadual de Campinas, Campinas, São Paulo, Brazil (ZUEC). The search for data on *M. mnasias thera* followed the same databases and methods described in Rosa et al. (2023). Data labels of all specimens were compiled; they are available in Table S1.



**Fig. 1.** Adult specimen and the habitat of *Melinaea mnasias thera*. Designations: A – *Melinaea mnasias thera* (left – dorsal view, right – ventral view); B – general view of forest habitats in Serra Bonita, Camacan, Bahia state, Brazil.

### DNA sampling and analysis

All sequences used were downloaded from Genbank, except for the museum samples EB19-049 (MNHN, Muséum National d’Histoire Naturelle, Paris, France) and AHBR119 (ZUEC, Zoological Collection, Museu de Diversidade Biológica da Universidade Estadual de Campinas, Campinas, Brazil), which had their genomic DNA extracted from two legs using the QIAamp DNA MicroKit (QIAGEN®, USA) protocol, adapted with columns from MinElute PCR Purification Kit (QIAGEN®, Düsseldorf, Germany). The whole genome shotgun sequencing was performed on the two DNA extractions of museum specimens, following protocols described in Twort et al. (2021), which were modified from protocols in Meyer & Kircher (2010). Briefly, DNA was first blunt-end repaired, after which custom-made indexed Illumina adapters were ligated. The library was then pooled with 59 other Lepidoptera specimens and run

on the Illumina NovaSeq platform. Raw reads were checked with FASTQC v. 0.11.8 (Andrews, 2010), and reads were cleaned with Prinseq v. 0.20.4 (Schmieder & Edwards, 2011) and Trimmomatic v. 0.38 (Bolger et al., 2014). The genome was then *de novo* assembled with spades v. 3.13.0 (Bankevich et al., 2012) with a kmer value of 21. Orthologs of the five standard genes for Lepidoptera (COI, EF1- $\alpha$ , CAD, wingless and GAPDH) were identified from the fragmented genome assembly using MESA v. 1.3 (Neethiraj et al., 2017). See Twort et al. (2021) for more detailed descriptions of the bioinformatic pipelines. For AHBR119, we were able to identify all five genes (COI, EF1- $\alpha$ , CAD, wingless and GAPDH). For EB19-049 we were only able to find COI. All sequences were aligned on CIPRES Science Gateway portal v. 3.1 (Miller et al., 2010) using MAFFT v. 7 (Kato & Standley, 2013).

The final concatenated matrix comprised 37 specimens, representing most of *Melinaea* species and more close related genera, with a total of 4698 base pairs and five genes. The type species of all genera are represented in the final matrix. *Tellervo zoilus* (Fabricius, 1775) was used to root the tree (see Table S3 for voucher numbers). Analyses to find the best-fit substitution models and partition subsets for the dataset (see Table S2 for best model selected for each partition), using «merge» option, were done using ModelFinder (Kalyaanamoorthy et al., 2017) with edge-linked partition model + FreeRate heterogeneity in IQ-TREE v. 1.6.12 (Nguyen et al., 2015).

The maximum likelihood tree was inferred using ten likelihood searches in IQ-TREE v. 1.6.12. The support was calculated using the ultrafast bootstrap (UFBoot) (Hoang et al., 2018), with 2000 replications, in addition to assessing node support through 1000 replications of Shimodaira Hasegawa approximate Likelihood Ratio Test (SH-aLRT) (-alrt 1000) (Guindon et al., 2010; Hoang et al., 2018) and approximate Bayes branch test (aBayes; Anisimova et al., 2011).

## Results

### Systematic position

In the obtained phylogeny, the subtribe Melinaeina was organised in two clades, the first composed by *Olyras* + *Paititia* (type species: *Olyras crathis* E. Doubleday, 1847 and *Paititia neglecta* Lamas, 1979) and the second by

*Eutresis* + (*Athyrtis* + *Melinaea*) (type species: *Eutresis hypereia* E. Doubleday, 1847, *Athyrtis mechanitis* C. Felder & R. Felder, 1862 and *Melinaea ludovica* (Cramer, 1780)). The genus *Melinaea* was recovered as monophyletic and divided in two major clades, the first composed by *Melinaea mnasias* + *Melinaea ludovica* and the second formed by *Melinaea ethra* as the sister taxon to a large clade composed by nine species of *Melinaea*. The two individuals of *Melinaea mnasias thera* clustered together forming a clade with strong support, sister to *M. mnasias lucifer* (Fig. 2).

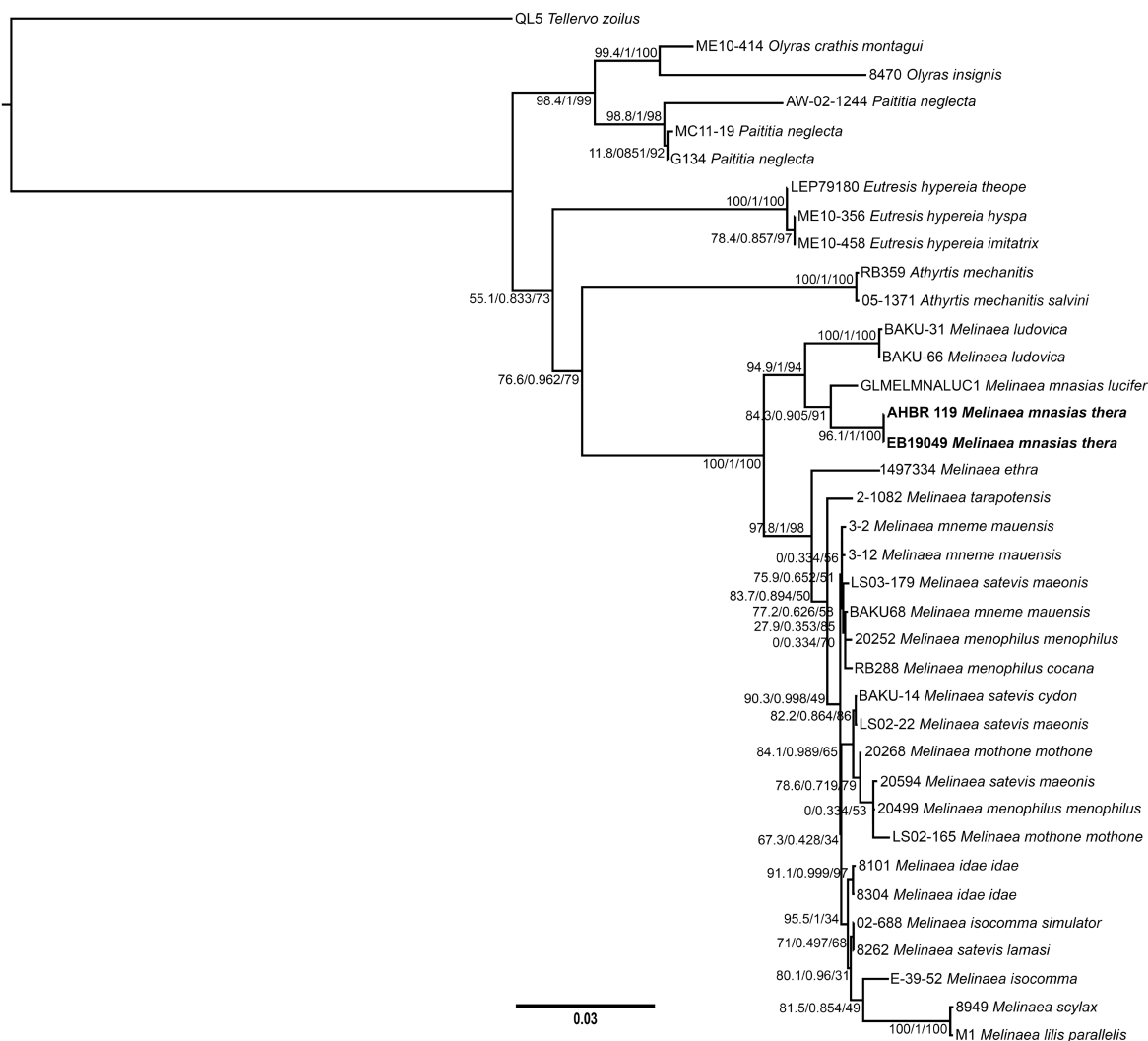
### General information and conservation aspects

A total of only 17 specimens of *M. mnasias thera* were found in nine public collections (Table S1). These specimens represent geographical records of four localities in the Brazilian states of Rio de Janeiro, Espírito Santo, and Bahia (records from the state of São Paulo, Paraguay and Colombia are mistakes, see Rosa et al., 2023) (see Table S1, Fig. 3). Most individuals of *M. mnasias thera* are either very old or without any information on collecting dates, and a single individual has been collected in the XXI century (in 2016, Table S1). Morphology (mostly wing pattern) was presented and discussed by Fox (1960), and Brown (1977) presented additional data on taxonomy and distribution, anticipating by a decade the presence of *M. mnasias* in coastal Bahia state.

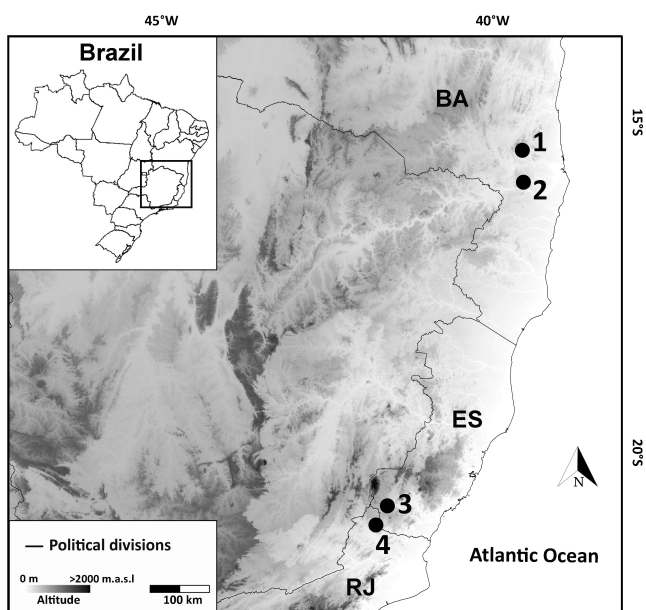
Based on the trustworthy geographical data, *M. mnasias thera* occurs in lowland coastal forests (Fig. 1b), at altitudes of 100–400 m a.s.l. (Fig. 3). At both localities, where *M. mnasias thera* was reported in the Bahia state, at least half of the area is covered by cocoa plantations locally known as «cabruca», where the cocoa trees are planted in the shade of larger trees.

The known distribution of *M. mnasias thera* is disjunct. The two localities in Bahia state (Camacan and Itapebi) are relatively close to each other (about 60 km of distance), as well as the two localities in the Espírito Santo state (Alegre) and Rio de Janeiro state (Bom Jesus do Itabapoana) are about 50 km of distance to each other. These two pairs, however, have a large gap of about 700 km between them. Based on these data, the extent of occurrence (EOO) of *M. mnasias thera* was estimated as at 7264.35 km<sup>2</sup> and its area of occupancy (AOO) as 16 km<sup>2</sup> (Rosa et al., 2023).





**Fig. 2.** Phylogenetic relationships of *Melinaea* species based on COI, EF1- $\alpha$ , CAD, wingless, and GAPDH genes and obtained by a maximum likelihood analysis. Numbers near the nodes are SH-aLRT/aBayes/Ultrafast bootstrap support values.



**Fig. 3.** Map showing the four known localities of *Melinaea mnasiae thera*. Designations: 1 – Itapebi, Bahia state; 2 – Reserva Serra Bonita, Camacan, Bahia state; 3 – Alegre, Espírito Santo state; 4 – Bom Jesus de Itabapoana, Rio de Janeiro state.

## Discussion

### *Phylogeny of subtribe Melinaeina and position of M. mnasiae thera*

Although composed of only five genera, the internal relationships of the subtribe Melinaeina has not reached a consensus based on previous studies. Common points include the monophyly of *Melinaea* and the clade formed by *Olyras* + *Paititia* (Brower et al., 2006, 2014; Willmott & Freitas, 2006; Garzón-Orduña et al., 2015; Chazot et al., 2019: Fig. S2.1, Fig. S2.2). The position of the genus *Athyrtis* is not congruent with previous studies. Although recent molecular studies recovered it as a sister group of all remaining Melinaeina (Garzón-Orduña et al., 2015; Chazot et al., 2019), the present study recovered *Athyrtis* as a sister group of *Melinaea*. This result is similar to that obtained in the morphological study by Willmott & Freitas (2006), in combined analyses by Brower et al. (2014), and in an early molecu-

lar study by Brower et al. (2006). In addition, together with *Melinaea*, *Athyrtis* is the only other genus of Melinaeina that presents a tiger wing pattern; *Olyras*, *Paititia* and *Eutresis* have translucent wing patterns. A second divergence is the position of *Eutresis*: in the present study it was the sister group of *Athyrtis* + *Melinaea*, while it is the sister to *Olyras* + *Paititia* in previous morphological and molecular studies (Willmott & Freitas, 2006; Chazot et al., 2019).

The systematic position of *Melinaea mnasias thera* confirms the placement of this taxon as a subspecies of *M. mnasias*, as proposed by Fox (1965) based on wing patterns. In addition, the present results show that *M. mnasias* is sister to *M. ludovica* (Cramer, 1780), contrasting with the results of Chazot et al. (2019: Fig. S2.1, Fig. S2.2), where *M. mnasias* was recovered as the sister group to other *Melinaea* species. The lack of resolution among the large clade composed by nine species of *Melinaea* confirm the results found by McClure & Elias (2017), and is a result of the absence of mitochondrial divergence, suggesting a recent radiation for these nine species.

Only three species of *Melinaea* are present in the Atlantic Forest. There are no discernible reasons why none of the remaining nine taxa have reached the Atlantic Forest, as many of them are extremely common, locally abundant and present in several forested habitats (Brown, 1977; Freitas, 1996). McClure & Elias (2017) suggested that these nine taxa could have originated from a recent and rapid radiation. In fact, the dating provided by Chazot et al. (2019: Fig. S2.2) showed that this clade originated about one million year ago, and most species are much younger than that. In this case, it is possible that these species just have not had enough time to reach and colonise the Atlantic Forest domain yet.

### **Geographical distribution and conservation**

Based on the reliable data, *M. mnasias thera* has been reported from four localities from south Bahia to north Rio de Janeiro states (Fig. 3). However, it is important to call attention to two important factors. First, most of the forests in the northern Rio de Janeiro state and southern Espírito Santo state have been destroyed and persists as small remnants (MMA, 2000), and it is hard to believe that populations of *M. mnasias thera* are still present in this region. Second, not a single individual of *M. mnasias thera* has been collected or sighted in the last large forest remnants in the

central and northern Espírito Santo state, including the low mountains near the municipality of Santa Teresa and the large tableland forests near the municipality of Linhares, both intensively censused in the last three decades (Brown & Freitas, 2000; Freitas et al., 2016). Accordingly, it is not impossible that the last populations of *M. mnasias thera* are restricted to the wet forests in south Bahia state. If this is true, the actual EOO should be much smaller than that presented in Rosa et al. (2023), and the estimate of EOO, which is now on the threshold to Vulnerable (VU) category, is much more optimistic than the reality. However, the fact that *M. mnasias thera* may persist in areas with cocoa plantation opens several possibilities for conservation, since there are still several areas where cocoa is cultivated in the «cabruca» system (Cocoa plantation shaded by native trees). The reasons for this can be explained in its biology. So, although the host plant and immature stages of *M. mnasias thera* are unknown, it likely uses *Dyssochroma* spp. (Solanaceae); the same host plants are used by the other two *Melinaea* in the Atlantic Forest (Brown, 1987; Drummond & Brown, 1987; Brown & Freitas, 1994). These plants are epiphytic, growing in tall trees near the canopy, exactly the trees that are preserved to provide the shade for the cocoa plantations in south Bahia state.

Based on this scenario, it is very important that potentially additional localities of populations of this rare butterfly should be surveyed. Suggested areas in the South Bahia state include the Serra das Lontras National Park and the region of Una Biological Reserve. In addition, the large areas of cocoa plantations in the Ilhéus-Itabuna region could harbour populations of *M. mnasias thera*. It should not be difficult to obtain eggs from wild caught females, as for other species of *Melinaea* (McClure & Elias, 2017; A.V.L. Freitas, unpublished). Details of its life cycle could provide clues to a better understanding of its rarity, as well as important information for conservation actions for this taxon.

### **Conclusions**

The present study is the most complete concerning the threatened Brazilian butterfly *Melinaea mnasias thera*. This is one of the rarest and the most threatened *Melinaea* taxa, so far known from only 17 specimens deposited in nine museum collections. Most of the potential historical range of this species is now deforested. The species is presently restricted to the large forest remnants in

the southern part of the Bahia state, in northeastern Brazil. In fact, given this scenario, only two localities are possibly maintaining populations of *M. mnasia's thera*, meaning that the current IUCN status of Vulnerable could be much more optimistic than the reality. Additional populations should be located, as these would provide more opportunities for conservation of this rare butterfly.

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### Supporting Information

Additional data for the paper of Rosa et al. (2024) may be found in the [Supporting Information](#).

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## СИСТЕМАТИЧЕСКОЕ ПОЛОЖЕНИЕ И ПРИРОДООХРАННЫЕ АСПЕКТЫ *MELINAEA MNASIAS THERA* (LEPIDOPTERA: NYMPHALIDAE: DANAINAE)

А. Э. Б. Роза<sup>1\*</sup> , Э. П. Барбоса<sup>1</sup> , Н. Вахлберг<sup>2</sup> , А. В. Л. Фрейтас<sup>1</sup> 

<sup>1</sup>Университет Кампинаса, Бразилия

\*e-mail: [augustohbrosa@hotmail.com](mailto:augustohbrosa@hotmail.com)

<sup>2</sup>Лундский университет, Швеция

Триба Ithomiini (Nymphalidae: Danainae) включает около 400 видов чешуекрылых, распространенных от Мексики до Северной Аргентины. При этом взрослые особи всех видов являются апосематическими и выступают основными моделями в нескольких кольцах неотропической мимикрии. Небольшая подтриба Melinaeina, состоящая из пяти родов крупных чешуекрылых, является сестринской группой всех остальных групп трибы Ithomiini. Род *Melinaea*, насчитывающий 14 видов, является самым крупным, а также самым распространенным в пределах подтрибы Melinaeina. Из всех видов рода *Melinaea mnasias* считается очень редким и малоизученным. Это справедливо и для *Melinaea mnasias thera*, подвида из Атлантического леса, менее 20 экземпляров которого известно во всех музеях мира. Исследования, сочетающие систематику, экологию, биогеографию и естественную историю, являются приоритетными в тропических регионах, особенно когда основное внимание уделяется видам, находящимся под угрозой исчезновения. В связи с этим целью этого исследования было собрать все доступные данные о *M. mnasias thera*, находящемся под угрозой исчезновения, предоставив информацию для будущих планов управления, направленных на сохранение этой бабочки и ее среды обитания. Информация была собрана на основе научных коллекций и личных наблюдений, а систематика видов *Melinaea* оценена путем отбора и анализа ДНК. Полученные данные о филогении восстановили подтрибу Melinaeina, организованную в две клады: первую составляют *Olyras* + *Paititia*, а вторую – *Eutresis* + (*Athyrtis* + *Melinaea*). *Melinaea mnasias thera* был обнаружен как сестринский подвид к *M. mnasias lucifer*. Всего во всех изученных коллекциях обнаружено 17 экземпляров *M. mnasias thera* из четырех местонахождений в Бразилии. Однако хорошо сохранившиеся леса присутствуют только в одном из этих мест, на юге штата Баия, откуда недавно был собран экземпляр. Это позволяет предположить, что последние популяции *M. mnasias thera* ограничены этим регионом. Если это действительно так, то реальный природоохранный статус этого таксона может быть гораздо более критичным, чем предполагалось.

**Ключевые слова:** Ithomiini, атлантический лес, бабочка, Бразилия, исчезающий вид