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A new genus and endangered species of euptychiine butterfly from isolated mountains in southeastern Brazil (Lepidoptera: Nymphalidae: Satyrinae)

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Abstract

Agojie rupicola **gen. et sp. nov.**, a new genus and species of Euptychiina from the *campos rupestres* and granitic inselbergs of eastern Minas Gerais, southeastern Brazil, is herein described using comparative morphology and ecological data. Diagnoses, photos, and illustrations are also provided, in addition to a discussion on the putative systematic placement of the new taxa and its conservation status. Considering the collecting points, the estimated value for the extent of occurrence (EOO) is 100.263 km^2 and the area of occupancy (AOO) is 20 km^2, which combined with the reduced number of localities in which the species is known to occur, and the observed continuing decline of the quality of the habitat, we recommend that *A. rupicola* **gen. et sp. nov.** should be formally assessed as Endangered based on criteria B1+2(a,biii) of the International Union for Conservation of Nature (IUCN). The results presented here add to the increasing faunistic and floristic novelties that justify the priority for the conservation of these still scientifically underexplored Brazilian mountains.

Key words: Atlantic Forest, Campo rupestre, Conservation, Endangered species, Euptychiina, *Megisto* clade, *Moneuptychia*, Morphology, *Pharneuptychia*, Taxonomy

Resumo

Agojie rupicola **gen. et sp. nov.**, um novo gênero e espécie de borboleta Euptychiina dos campos rupestres e inselbergs graníticos do leste de Minas Gerais, sudeste do Brasil, é aqui descrita com base em morfologia comparada e dados ecológicos. Diagnose, fotos e ilustrações também são apresentadas, além de uma discussão sobre a possível posição sistemática do novo táxon e seu status de conservação. Com base nos pontos de amostragem, o valor estimado para a extensão de ocorrência (EOO) é 100.263 km^2 e a área de ocupação (AOO) é 20 km^2 que, associados com o número reduzido de localidades até então conhecidas para a espécie e o continuado declínio observado da qualidade do habitat, permite a recomendação do status de *A. rupicola* **gen. et sp. nov.** como Ameaçada, com base no critério B1+2(a,biii) da União Internacional para Conservação da Natureza (IUCN). Os resultados do presente estudo somam-se às crescentes novidades da fauna e flora que justificam a prioridade para a conservação dessas montanhas brasileiras ainda pouco exploradas cientificamente.

Palavras-chave: Mata Atlântica, Campo rupestre, Conservação, Espécie ameaçada, Euptychiina, clado *Megisto*, *Moneuptychia*, Morfologia, *Pharneuptychia*, Taxonomia

Introduction

The monophyletic Euptychiina comprises about 450 species and 63 described genera (Zacca *et al.* 2018; Espeland *et al.* 2023). These numbers keep drastically increasing in the last years, driven mainly by the efforts made within

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the scope of the international collaborative project "ARTS: Phylogeny and systematic revision of the diverse and cryptic Euptychiina (Lepidoptera, Nymphalidae, Satyrinae)" (see more details here: https://www.floridamuseum.ufl.edu/neotropica/research/euptychiina/). Some estimates indicate an increase of at least 30% of the current known diversity of euptychiine butterflies in the next few years (K. R. Willmott *et al.* 2021, unpublished data).

Brazil harbors an impressive diversity of Euptychiina with 52% of the known species, especially in the three most studied biomes in the country (i.e., Amazon, Atlantic Forest, and Cerrado). The high altitudinal, latitudinal, climatic, and vegetational variation combined with the fact there are a few Lepidopteran taxonomists in the country still favors the discovery of new taxa year by year (Silva-Neto *et al.* 2021), mainly in places very few explored regarding its biodiversity.

This is the case of some mountains in southern Brazil, especially those with rock outcrops harboring rupicolous vegetation. Some mountains of east Minas Gerais, within the Doce River basin, have been received attention recently from botanists by yielding several new plant taxa, many of them endemic to the *campos rupestres* of the João Pinto formation (Gonella *et al.* 2015; Mello-Silva 2018; Andrino & Gonella 2021; Antar *et al.* 2021; Couto *et al.* 2023), but also from the surrounding granitic inselbergs (Goldenberg *et al.* 2022; Mezzonato-Pires *et al.* 2021). Despite such increasing numbers of new and microendemic taxa, most of these mountains are currently unprotected, with the exception of Serra da Onça which lies within the limits of Sete Salões State Park. Once covered by an exuberant vegetation, the Doce River valley has historically suffered from the intensive conversion of its original forests into pastureland, especially after 1920 (Espindola 2015), and its few natural remnants are still under pressure from constant fires for pasture renovation, and from invasive species that benefit from these interventions (Mapbiomas 2023; Gonella *et al*. 2015).

Currently, there is an effort to investigate and describe the fauna and flora of these mountainous formations, from which some new insect taxa have already been described (Reategui *et al*. 2022; Cordeiro & Camico 2023). It is expected that these studies can subsidize conservation actions, including the proposition of new Protected Areas.

During expeditions to these mountains, an unidentified euptychiine butterfly associated with rock outcrop formations was collected. After thorough study of the specimens, here we propose and describe a new genus and species of Euptychiina based on morphology, ecological and distributional data. We further discuss the findings in a phylogenetic context by comparing the morphology of the new and other putative closely related taxa. Finally, we comment on the conservation status and threats to the species.

Material and Methods

The collecting sites were in the municipalities of Conselheiro Pena and Santa Rita do Itueto, east Minas Gerais, southern Brazil. This region is inserted in the "Sugarloaf Land" (de Paula *et al.* 2020) of the granitic inselbergs within the Atlantic Forest. Nevertheless, the mountains of this particular region represent a unique encounter of these granitic monoliths with the quartzites of the João Pinto geological formation (Oliveira 2000), which harbors the *campos rupestres* at elevations usually above 800 m. Specimens of the new taxa were collected on rock outcrops of both granite (Pedra de Santa Rita) and quartzite (Serra do Padre Ângelo, Serra do Parado, Serra da Palha Branca) using an entomological net (Fig. 1).

All specimens, including the types, are deposited at the new Entomological Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), which has been built after the fire in September 2018, and the collection of the Museu de Biologia Prof. Mello Leitão, Instituto Nacional da Mata Atlântica (MBML/INMA).

The morphological comparative study was based on external characters often used for the species delimitation in most euptychiine, such as wing patterns, venation, and male genitalia. Females were not found in fieldwork. The morphology of the new taxon was compared with the descriptions of other phenotypically resembling euptychiine species, mainly those from the '*Megisto* clade', '*Amphidecta* clade' and '*Archeuptychia* clade' sensu Espeland *et al.* (2019, 2023). Genitalia dissections were performed by detaching the abdomen from the body and soaking it in a heated test tube with 10% potassium hydroxide solution for about five minutes in a water bath to dissolve fat and soft tissues. After inspection, the genitalia were rinsed in water and stored permanently in glycerol inside microvials with the respective labels. Specimens dissected are indicated with an asterisk after the voucher number. Images of genitalia and wing venation were obtained with a stereoscopic microscope Leica EZ4W with an attached camera. Plates were made using the free license software GIMP 2.10.32.

FIGURE 1. Habitat of *Agojie rupicola* **gen. et sp. nov.** (a–c) Conselheiro Pena, Serra do Padre Ângelo, Pico da Bela Adormecida, quartzitic campo rupestre: (a) Overview of the mountain, (b) Collecting site at 1250 m a.s.l., (c) Uppermost part of the mountain, known as Pico da Bela Adormecida, at 1500 m a.s.l., (d) Conselheiro Pena, Serra do Parado, nearby Vista Alegre village, quartzitic campo rupestre. (e–f) Santa Rita do Itueto, Pedra de Santa Rita, granitic inselberg: (e) Overview and (f) Specific spot where most of the individuals of *A. rupicola* **gen. et sp. nov.** were observed.

The following abbreviations are used throughout the text: DW—dorsal wings, VW—ventral wings, DFW—dorsal forewings, DHW—dorsal hindwings, VFW—ventral forewings, VHW—ventral hindwings, HT—holotype, PT—paratypes, N—number of examined specimens. The terminology of the wing venation follows Comstock & Needham (1898–99) and Comstock (1918). For genitalia, we follow the terminology proposed by Klots (1970) with the term 'gnathos' (= brachia) applied *sensu* Pierce (1914), 'fultura superior' and 'fultura inferior' (= juxta) *sensu* Petersen (1904) and 'combination of ventral arm of tegumen and dorsal arm of saccus' (= vinculum) *sensu* Casagrande (1979).

The map with the known occurrence was developed on QGIS software (QGIS Development Team 2023) using satellite images from the Google Earth plugin, geographical limits from IBGE (2023), land use and cover from SISEMA (2023), and fire record and frequency from Projeto MapBiomas (2023). The preliminary conservation

status assessment was based on IUCN (2012) categories and criteria and values of Area of Occupancy (AOO) and Extent of Occurrence (EOO) were generated using the georeferencing tool GeoCAT described in Bachman *et al*. (2011) and employing the standard values for cell size (4 km2) for the estimation of AOO.

Taxonomy

Agojie Zacca, gen. nov.

urn:lsid:zoobank.org:act:BF81B0E9-8B2D-46CA-9A10-C1A34B5F782C

Type species. *Agojie rupicola* Zacca, **gen. et sp. nov.**

Diagnosis. Among the Euptychiina, species of *Agojie* can be superficially confused with *Pharneuptychia innocentia* (C. Felder & R. Felder, 1867) by its small size (15–18 mm), absence of ocelli on the dorsal wings and presence of them on the ventral wings. However, the ocelli number, shape and location can easily distinguish both taxa (see details below under Diagnosis of *Agojie rupicola* **sp. nov.**) in addition to the distinct morphologies of the wing venation and male genitalia.

Etymology. The generic name is after the elite women warriors of the Daomé Realm, called 'agojie', in the fictional Hollywoodian movie 'The Woman King'. The generic name is feminine and should be considered indeclinable (ICZN 1999, Article 30.2.2).

Agojie rupicola Zacca, gen. et sp. nov.

(Figs 2–7)

urn:lsid:zoobank.org:act:C13CC777-B3C0-4B95-9DD8-7A74D72D9512

Type specimens (Figs 2). Holotype male with the following labels (separated by transverse bars): /HOLOTYPUS/ BRASIL, Minas Gerais, Conselheiro Pena, Pedra da Antena, 1237m, 19°20'46"S 41°33'0"W, 19.VIII.2020, D. P. Cordeiro *leg.*/ MN-LEP 0004962/ Holotypus *Agojie rupicola* Zacca det. 2022/. Deposited at MNRJ.

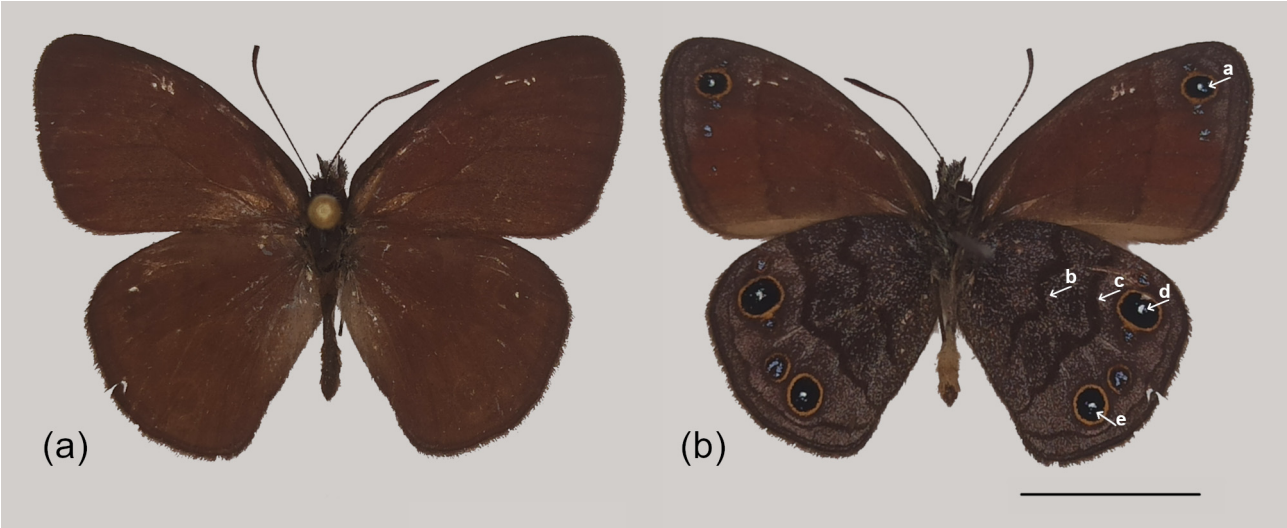

FIGURE 2. Holotype male of *Agojie rupicola* **gen. et sp. nov.**: (a) Dorsal and (b) Ventral. White arrows indicate key features on the wing pattern to easily recognize the species: a, d, e = monopupillated ocelli; b, c = irregular submedian and median lines. Scale bar = 1 cm.

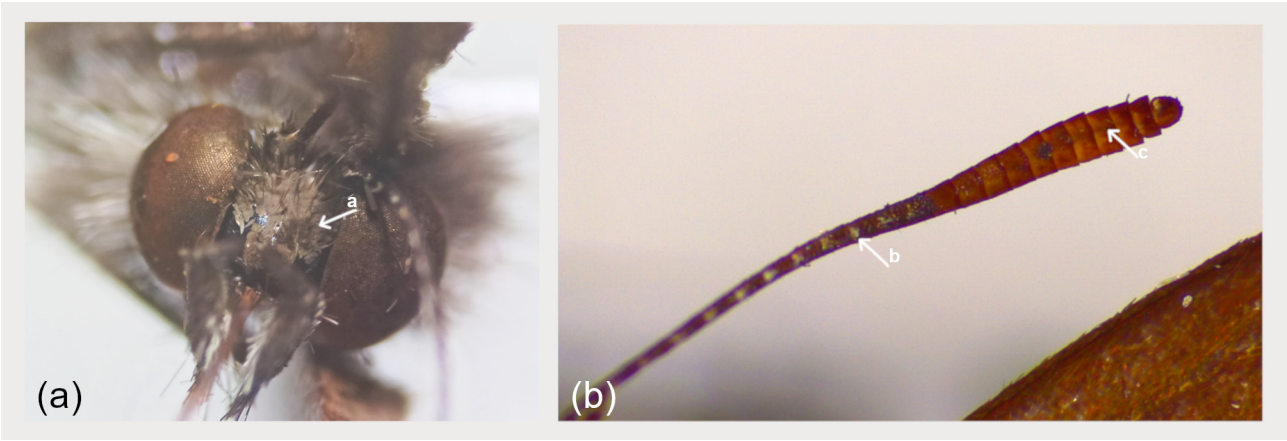

FIGURE 3. Morphology of *Agojie rupicola* **gen. et sp. nov.**: (a) Head in frontal view and (b) apex of the clavate antennae. Scale bar = 1 cm.

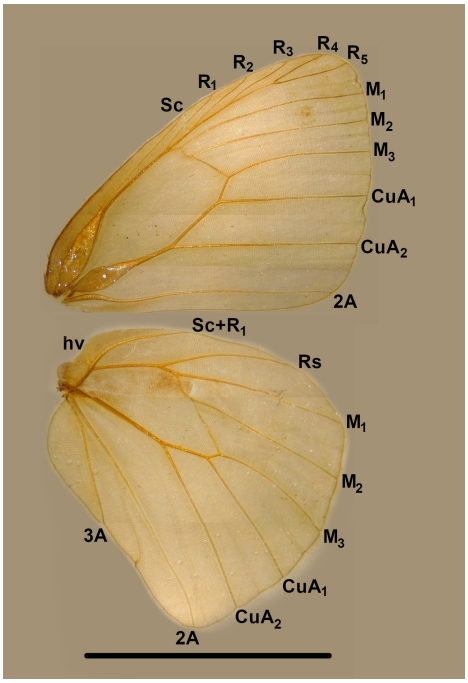

FIGURE 4. Wing venation of *Agojie rupicola* **gen. et sp. nov.**. Sc = subcostal vein; R = radial vein; M = median vein; CuA = cubitus anterior vein; A = anal vein; Rs = radial sector vein; hv = humeral vein. Scale bar = 1 cm.

Paratypes (17 specimens)—1 male, BRASIL, Minas Gerais, Conselheiro Pena, Serra do Padre Ângelo, Pico Bela Adormecida, 1400m, 19°19’08.9”S 41°34’43.9”W, 21.VIII.2020, D. P. Cordeiro *leg.* (MN-LEP 0004620); 2 males, BRASIL, Minas Gerais, Conselheiro Pena, Serra do Padre Ângelo, 1° platô (acampamento), 900–1300m, 19°18’45.9”S 41°34’38.1”W, 04.V.2021, D. P. Cordeiro *leg.* (MN-LEP 0004963, MN-LEP 0004964); 1 male, BRASIL, Minas Gerais, Conselheiro Pena, Serra do Parado, 850–980m, 19°24’00.15”S 41°33’04.76”W, 27.II.2021, D. P. Cordeiro *leg.* (MN-LEP 0004618); 6 males, BRASIL, Minas Gerais, Santa Rita do Itueto, Trilha Pedra de Santa Rita, 700m, 19°22’34”S 41°22’04”W, 06.V.2021, D. P. Cordeiro *leg.* (MN-LEP 0004619, MN-LEP 0004617, MN-LEP 0004621, MN-LEP 0004961*; MN-LEP 0004965*, MN-LEP 0004966); 7 males, BRASIL, Minas Gerais, Santa Rita do Itueto, Trilha Pedra de Santa Rita, 700m, 19°22’34”S 41°22’04”W, 13.X.2022, D. P. Cordeiro *leg.* (MBML-INV 5280–5286).

Etymology. The specific name is an allusion to the habitat where the species occurs, *campos rupestres* and granitic inselbergs. It is formed by the combination of the Latin words *rupi* (= rocks) and *cola* (= inhabitant).

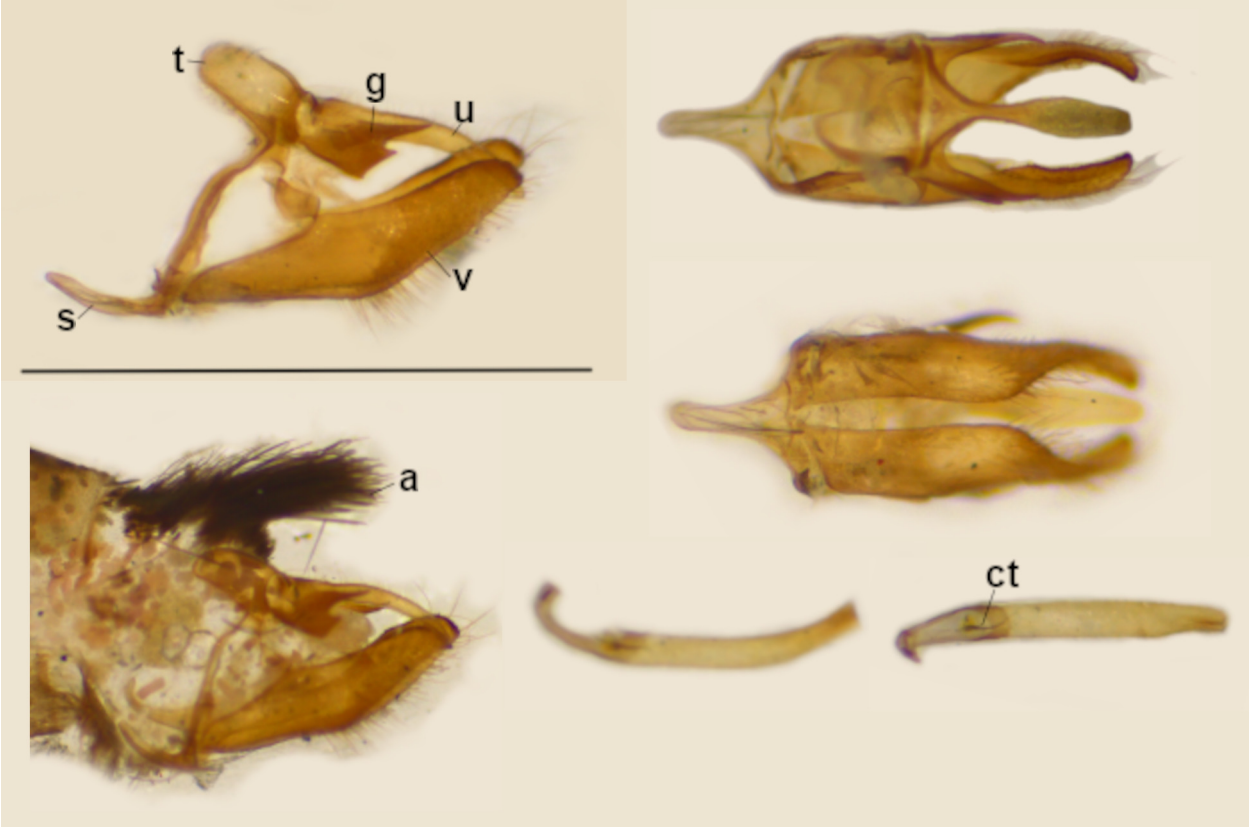

FIGURE 5. Male genitalia of *Agojie rupicola* **gen. et sp. nov.**: (a) Lateral, (b) Dorsal, (c) Ventral, (d) Aedeagus, lateral, (e) Aedeagus, dorsal. t = tegume; u = uncus; g = gnathos; v = valva; s = anterior projection of the saccus; ct = cornutus; a = androconial tuft. Scale bar = 0.2 mm.

Vernacular name: Borboleta-guerreira-das-pedras (Portuguese) / Rock warrior satyr (English)

Diagnosis. Compared with other Euptychiina, this species is easily recognized by the following set of characters: reddish brown wing on ventral forewing, irregular shape of the median and submedian lines on ventral hindwing together with the monopupillated ocelli in M_1-M_2 and CuA_1-CuA_2, and three small bipupillated ocelli in Sc+R_1-M_1, M_2-M_3 and M_3-CuA_1 (Fig. 2). It also has a unique shape of gnathos and cornutus (Fig. 5) among other euptychiine species.

Description. Eyes brown and glabrous. Frons with short light brown scales mixed with brown elongated scales (Fig. 3a). Antennae clavate (Fig. 3b), tricarinate, brown with sparse whitish scales mid-ventrally located in each flagellomere except in the glabrous apex (Fig. 3b); 36 flagellomeres with ventral carina from the 17th flagellomere to the apex (N = 5). Labial palpi with mixed creamy, brown, and greyish scales; third segment almost the same length as the first with short scales. Thorax covered by short light brown scales mixed with brown elongated scales. Legs brown, with spines ventrally on tarsus and tibia, pair of tibial spurs at distal end of tibia. Abdomen brown in dorsal view, lighter in ventral view, with the sclerotized portion of the eight tergite reduced; androconial black elongated scales on the distal region (Fig. 5b).

Wings size, shape, color, venation and variation (Figs 2 and 4). Average size of the forewings of the males is 15–18 mm (PT; HT = 16 mm). FW subtriangle and HW rounded. DFW and DHW entirely brown. VFW reddish brown with dark brown median, submedian, submarginal and marginal lines with a monopupilled ocelli in M_1-M_2 (Fig. 2). VHW brown with dark brown and large irregular median and submedian lines, slight irregular submarginal line, straight marginal line with two large monopupilled ocelli in M_1-M_2 and CuA_1-CuA_2, and three small bipupilled ocelli in Sc+R_1-M_1, M_2-M_3 and M_3-CuA_1 (Fig. 2). Wing venation as in Figure 4.

Male genitalia (Fig. 5). Tegumen rectangular and projected posteriorly in lateral view, with a median concavity posteriorly in dorsal view. Uncus twice longer than tegumen, slightly curved downwards at the apex in lateral view, dorsally ovoid from the mid region to apex. Gnathos half-length of the uncus, robust and with ventral projections in

lateral view. Combination of the ventral arms of tegumen and dorsal arms of saccus sinuous. Appendices angulares short. Anterior projection of saccus as longer as gnathos, cylindrical and not dilated in dorsal view. Fultura inferior like a thin stripe. Valva elongated, covered by long hairy-like setae latero-ventrally, and short ones at the inner side, costa developed, apex rounded and serrated. Aedeagus as long as the valva, cylindrical, with both anterior and posterior regions curved upwards, anterior region digitiform, posterior region twice longer than anterior region with bipartite apex in dorsal view, distal opening ventral and the same length as the proximal opening. Vesica with an elongated cornutus.

Female. Unknown.

Ecology, distribution, and conservation (Figs. 1, 6, 7). *Agojie rupicola* **gen. et sp. nov.** was collected in February, May, August, and October, but it is very likely to be a multivoltine species as other euptychiine butterflies. So far, the species is endemic to the east mountains of Minas Gerais (municipalities of Conselheiro Pena and Santa Rita do Itueto), southeast Brazil (Fig. 6). In the region, *Agojie rupicola* **gen. et sp. nov.** was found between 700–1400 m of elevation and observed very active, flying over the exposed rock in the inselberg Pedra de Santa Rita between islands of bromeliads and other rupicolous vegetation (Fig. 1e–f). The species was found on mountains of both granitic and quartzitic rock, each harboring different vegetation compositions (lowland inselberg and *campo rupestre*, respectively), but sharing the presence of exposed rock and the predominance of herbs and shrubs. These mountains were once inserted in a matrix of seasonal forests of the Atlantic Forest domain but are now mainly surrounded by pastures and plantations (Fig. 7), which results in several pressures for these remnant areas of native vegetation and associated fauna.

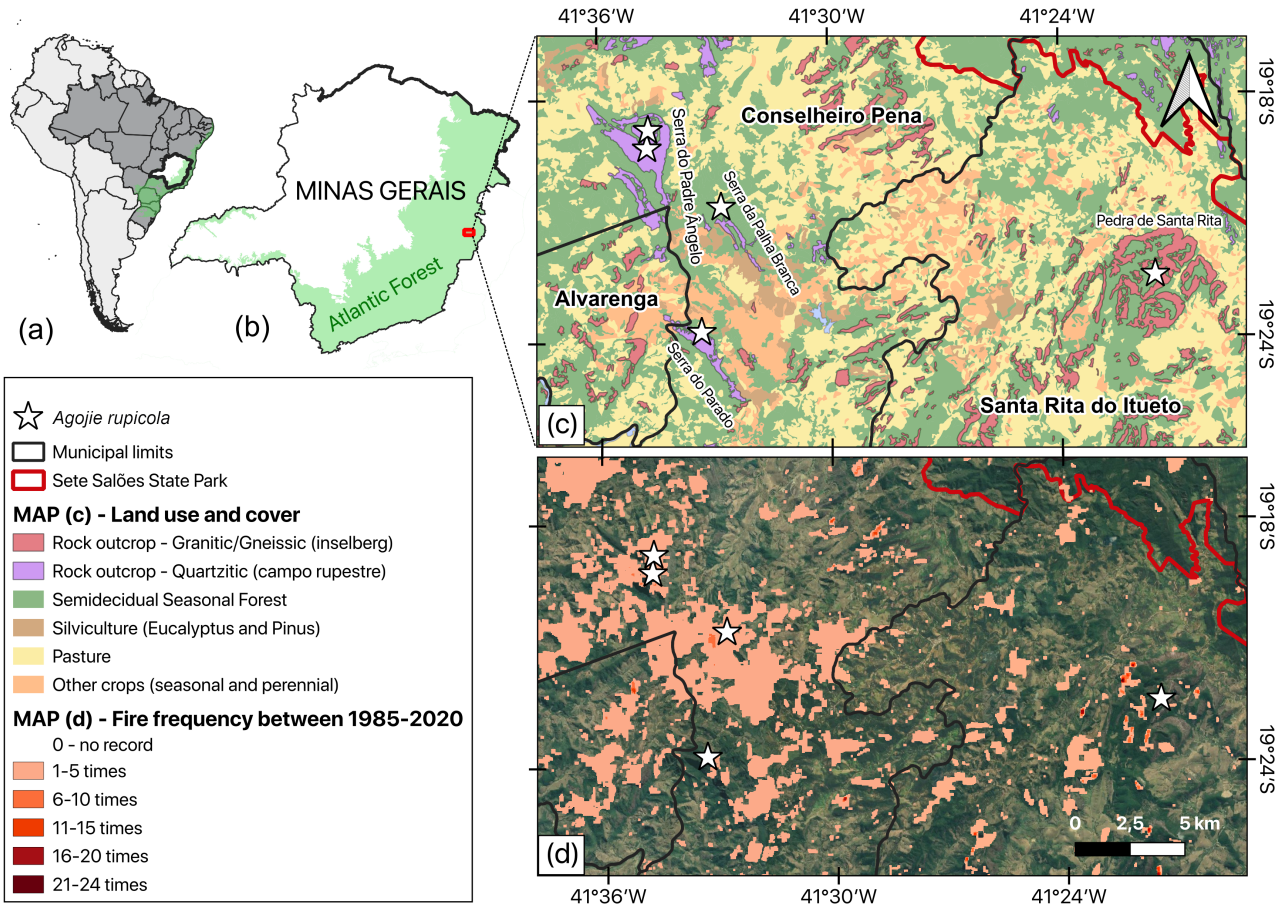

FIGURE 6. Map of known occurrence of *Agojie rupicola* **gen. et sp. nov.** (a) Map of South America with Brazil highlighted in dark grey. (b) Map of Minas Gerais State, Brazil, highlighting the Atlantic Forest domain. (c) Map of the land use and cover of the sampled area. (d) Map of the fire frequency between 1985–2020 in the sampled area.

The main pressure for the species is the occurrence of anthropogenic large-scale wildfires during the dry season which has been locally changing the composition of the vegetation and benefiting the dominance of invasive grass and fern species (Gonella *et al.* 2015; Couto *et al.* 2023). Such alteration of abundance and composition of the

native grassland vegetation changes negatively the quality of the habitat, possibly impacting the population of the host and feeding plants and, therefore, the population of *A. rupicola* **gen. et sp. nov.**. As an example, the subpopulation at Pico da Bela Adormecida was affected by an extensive criminal fire in September/October 2020, which consumed most of the natural grassland and also affected small forest remnants (Fig. 7). After the fire, it was observed the expansion of areas invaded by molasses grass (*Melinis minutiflora* P. Beauv.) and bracken fern (*Pteridium aquilinum* (L.)) Kuhn, with a reduction in the abundance of native plants, which are slower in recovering from that fire event. Considering the collecting points, the estimated value for EOO is 100.263 km^2 and the AOO is 20 km^2, which combined with the reduced number of localities (less than 5) in which the species is known to occur, and the observed continuing decline of the quality of the habitat, we recommend that *A. rupicola* **gen. et sp. nov.** should be formally assessed as Endangered based on criteria B1+2(a,biii) of IUCN (2012). We cannot disregard the pressure of uncontrolled tourism, which can also facilitate fires and expand the area affected by invasive species, as the only protected area with its occurrence is the Área de Proteção Ambiental de Santa Rita, but this protected area has no management plan or regulation of visitation.

Hostplant and immature stages. Unknown.

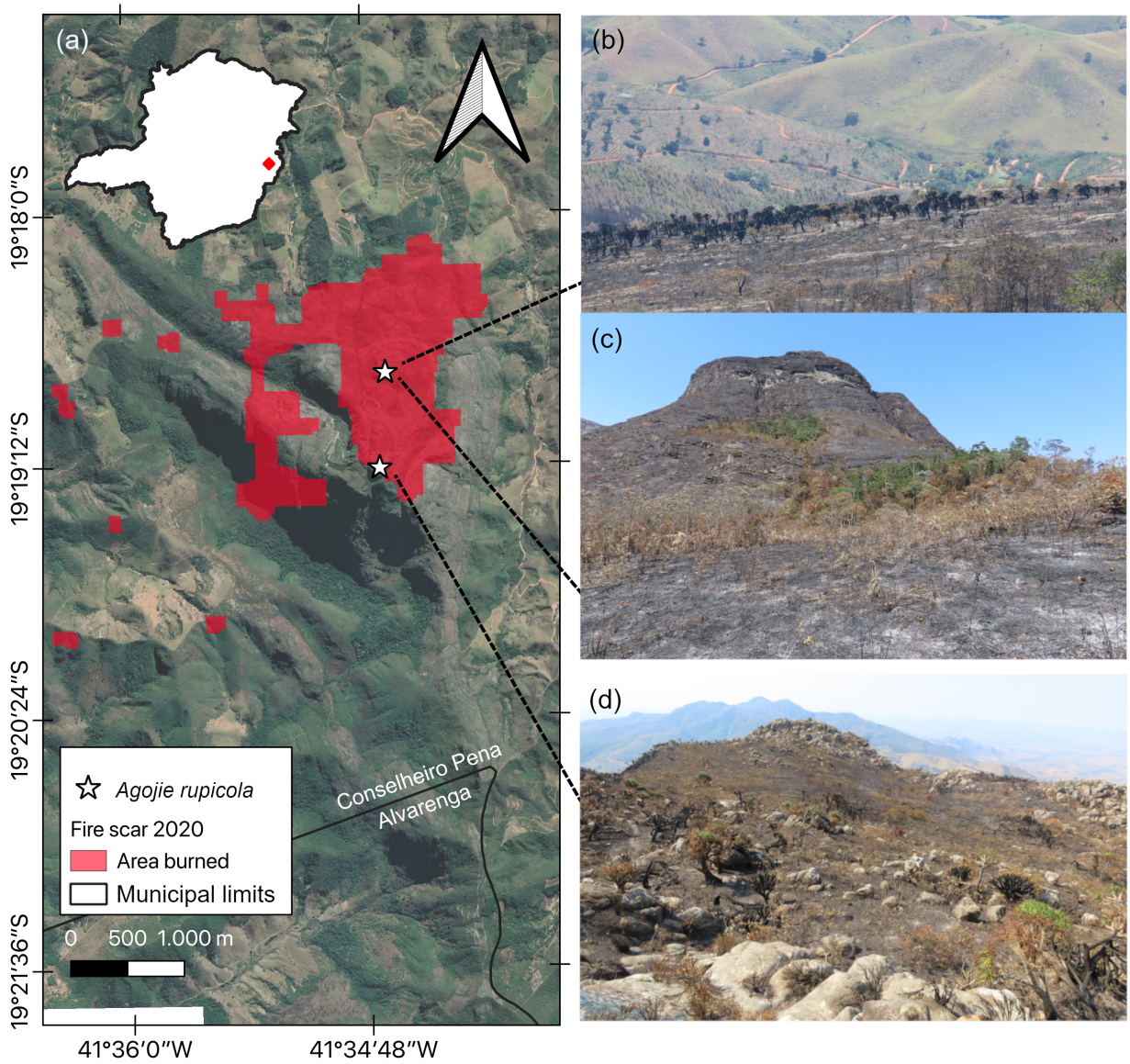

FIGURE 7. Map of (a) site known as Pico da Bela Adormecida (Serra do Padre Ângelo), showing the recorded fire scar of the large-scale fire of September/October 2020, in one of the collecting sites of *Agojie rupicola* **gen. et sp. nov.** (b) Part of the burned area with crops of *Eucalyptus*, coffee and pastures in the background. (c) Burned area at collecting site of *A. rupicola* **gen. et sp. nov.**, at 1250m a.s.l. (d) Burned area at the uppermost part of the mountain, another collecting site of *A. rupicola* **gen. et sp. nov.**

Discussion

The interpretation and identification of the new genus and species of Euptychiina is primarily based on the external morphology of the wings and genitalia. Although DNA sequencing and analysis were not possible in the present study due to financial constraints, the wing pattern and habitat preference of *A. rupicola* **gen. et sp. nov.** might suggest its close affinities with other taxa in the '*Megisto* clade' sensu Espeland *et al.* (2019, 2023), such as species of *Moneuptychia* Forster, 1964 and *Pharneuptychia* Forster, 1964, all typical of montane natural grasslands in southern Brazil. However, compared to *Moneuptychia* species, *A. rupicola* **gen. et sp. nov.** can be distinguished by the absence of any ocellus on dorsal wings, absence of the callus on the forewing Sc vein, shape of the transverse veins of the discal cell, origin of R_1 on discal cell (see Freitas *et al.* 2015, fig. 3), in addition to the reduced appendices angulares (well-developed in *Moneuptychia* species, see Freitas *et al.* 2015, fig. 4), particular shape of the gnathos, absence of fultura superior and presence of an elongated cornutus. The three latter characters also distinguish *A. rupicola* **gen. et sp. nov.** from *Pharneuptychia* species (T. Zacca 2023, unpublished data; Henao-Bañol & Meneses 2017, fig. 5).

One might argue the similarity between *A. rupicola* **gen. et sp. nov.** and some species of *Pseudeuptychia* Forster, 1964 (i.e. *P. hemileuca* (Staudinger, [1886]) and *P. cuzquenya* Nakahara & Lamas, 2018) in the *'Archeuptychia* clade' by the distribution of the ocelli on VW. Nevertheless, while *A. rupicola* **gen. et sp. nov.** has large monopupilled ocelli in M_1-M_2 and CuA_1-CuA_2, ocelli in *Pseudeuptychia* species are monopupilled in M_1-M_2 and bipupilled CuA_1-CuA_2 in addition to the differences on their male genitalia, mainly the gnathos, valvae and cornuti (see illustrations of *Pseudeuptychia* in Forster (1964, p. 86, fig. 58) and Nakahara *et al.* (2018, p. 35, fig. 13). The absence of ocelli on DW, the number and distribution of ocelli on VW and the wing colour also might suggest some relationship between *A. rupicola* **gen. et sp. nov.** and *Pharneuptychia innocentia* (C. Felder & R. Felder, 1867), a species that will be transferred to a new genus (E. P. Barbosa *et al*. 2022, unpublished data) in the '*Amphidecta* clade'. However, *A. rupicola* **gen. et sp. nov.** differs from *P. innocentia* by the shape of the basal and median lines on VHW, male genitalia with gnathos short and bifid (same length of uncus and not bifid in *P. innocentia*—TZ, personal observation), absence of fultura superior (present in *P. innocentia*—TZ, personal observation) and elongated cornuti (absent in *P. innocentia*—TZ, personal observation). These relationship hypotheses should be tested in the future since the main purpose of the present study is to describe and make the new generic and specific names available for science.

We also recommend that future fieldworks concentrate on finding the females and immature stages of *Agojie rupicola* **gen. et sp. nov.** since the morphological data from those semaphoronts are highly informative in a phylogenetic context of Euptychiina. It is also important to highlight that protecting the occurrence areas is crucial to maintain the populations of *A. rupicola* **gen. et sp. nov.**, as well as its conservancy also depends on the knowledge of its biology and host plant, in addition to a better understanding on the human impacts in the habitat where these organisms live.

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