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Dasyophthalma (Lepidoptera: Nymphalidae: Satyrinae): systematics, distribution, and conservation perspectives of a butterfly genus endemic from the Brazilian Atlantic Forest

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Received 17 October 2022
Accepted 30 March 2023
Published 23 May 2023

Academic Editors Ângelo Pinto, Martin Wiemers

Citation: Rosa AHB, Barbosa EP, Machado PA, Siewert RR, Freitas AVL (2023) *Dasyophthalma* (Lepidoptera: Nymphalidae: Satyrinae): systematics, distribution, and conservation perspectives of a butterfly genus endemic from the Brazilian Atlantic Forest. Arthropod Systematics & Phylogeny 81: 455–473. https://doi.org/10.3897/asp.81.e96397

Abstract

Dasyophthalma includes five species of medium-sized butterflies, all endemic from the Atlantic Forest of Brazil. All known species are univoltine and are dayflying, differently from other Brassolini that are mostly crepuscular. In despite of recent advances little is known about their natural history. Three out of the five species are included in the Brazilian Red List of threatened fauna and are of conservation concern. The present study provides for the first time a phylogenetic assessment of all *Dasyophthalma* species based on a molecular approach based on three loci. Also, the taxonomic status of *D. rusina delanira* was revised based on molecular data. In addition, up-to-date distributional data and conservation aspects of the threatened species from the genus are presented and discussed. The molecular phylogenetic analysis supports the monophyly of *Dasyophthalma*, with *Dynastor darius* as its sister-group, and, combined with a genetic divergence analysis, supported *Dasyophthalma delanira* **stat. rest.** as a valid name to species-level, sister-group to *D. geraensis* (and not a subspecies of *D. rusina*). The geographical range (extent of occurrence and area of occupancy) for all five species are presented, showing that these are very restricted for *D. delanira* **stat. rest.** and *D. geraensis*, following the distributions of the high-altitude forests. As much biological information about the genus is lacking, the present study can serve as a starting point for future studies on *Dasyophthalma*, adding information that can be crucial for future conservation actions and essential to assure the future of the threatened species in this genus.

Keywords

Brassolini, fruit-feeding butterflies, Neotropical, phylogeny, endangered species.

1. Introduction

The tribe Brassolini (Nymphalidae: Satyrinae) is a clade of exclusively Neotropical butterflies that currently includes 108 species distributed in 17 genera (Penz 2007; Shirai et al. 2016). Brassolini butterflies are notorious by being large sized, including some of the largest Neotropical butterflies, and by presenting several species with crepuscular behavior, flying at dawn or in the first hours of the day (DeVries 1987; Freitas et al. 1997; Penz 2007).

The genus *Dasyophthalma* Westwood, 1851 includes species of conspicuous medium sized butterflies, all endemic from the Brazilian Atlantic Forest. All known species are univoltine, with adults usually flying during the summer season and unlike most brassolines, which have crepuscular habits, all species of *Dasyophthalma* fly at day time, being especially active just after noon (Brown 1992; Casagrande and Mielke 2000, 2003).

According to Casagrande (2004), *Dasyophthalma* includes four species and five subspecies: the type species, *Dasyophthalma rusina* (Godart, [1824]) — (with three subspecies, *D. r. rusina* (Godart, [1824]), *D. r. delanira* Hewitson, 1862 and *D. r. principesa* Stichel, 1904) — *Dasyophthalma geraensis* Rebel, 1922, *Dasyophthalma creusa* (Hübner, [1821]) — (with two subspecies, *D. c. creusa* (Hübner, [1821]) and *D. c. baronesa* Stichel, 1904) — and *Dasyophthalma vertebralis* Butler, 1869. In previous studies concerning the phylogenetic relationships of *Dasyophthalma* (Penz 2009; Matos-Maravi et al. 2021) two well supported clades were recovered, the first including *D. creusa* + *D. vertebralis* (hereafter "*creusa*-group") and the second including *D. rusina* + *D. geraensis* (hereafter "*rusina*-group") (clade names following Penz 2009).

Among the taxa included in the "*rusina*-group", *D. r. delanira* is quite distinct from all other species and subspecies by both size and coloration of wing marks, showing a distinct pattern of postmedial bands in dorsal forewings. In addition, this subspecies is known from only three localities in the Rio de Janeiro State, restricted to areas above 1200 meters of altitude (Casagrande and Mielke 2008; Penz 2009; Freitas et al. 2018a). Due to these differences, Penz (2009) in her morphological revision of the genus suggested that the status of *D. r. delanira* should be verified.

Very little natural history information, such as morphology of the immature stages and host plants, is available for the genus, and this is basically restricted to the two more common and widespread species, *D. creusa* and *D. rusina* (Casagrande et al. 2000, 2003). This results in a knowledge gap for the three taxa in this group that are included in the Brazilian list of threatened fauna, namely *D. r. delanira*, *D. geraensis*, and *D. vertebralis*, (Casagrande and Mielke 2008; Freitas et al. 2018a, 2018b, 2018c; MMA 2022).

Thus, the present study aims to provide for the first time a phylogenetic assessment of all *Dasyophthalma* species based on molecular data. In addition, the taxonomic status of *D. r. delanira* is revised based on morphological and molecular data. Furthermore, up-to-date distributional data and conservation aspects of the threatened species from the genus are presented and discussed.

2. Methods

2.1. Examined specimens and compilated data

Adult specimens of *D. r. delanira* (Figs 1A, 2), *D. geraensis* (Fig. 1B) and *D. vertebralis* (Fig. 1F, G, Supplementary Material 1) were examined from the following collections: **AMNH**, American Museum of Natural History, New York, New York, USA; **CEIOC**, Coleção Entomológica do Instituto Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil; **CGCM**, Carlos Guilherme Costa Mielke research collection, Ponta Grossa, Paraná, Brazil; **DZUP**, Coleção Entomológica Pe. Jesus de Santiago Moure, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; **FLMNH**, McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA; **MCZ**, Museum of Comparative Zoology, Cambridge, Massachusetts, USA; **MNRJ**, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; **MZUSP**, Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil; **NBCN**, Naturalis Biodiversity Center, Leiden, The Netherlands; **NHMUK**, The Natural History Museum, London, UK; **NHMW**, Naturhistorisches Museum Wien, Vienna, Austria; **NHRS**, Swedish Museum of Natural History, Stockholm, Sweden; **RBINS**, Royal Belgian Institute of Natural Sciences, Brussels, Belgium; **USNM**, Smithsonian Institution National Museum of Natural History, Washington, D.C., USA; **ZMUC**, Zoological Museum, University of Copenhagen, Copenhagen, Denmark; **ZUEC**, Coleção Zoológica do Museu de Diversidade Biológica da Universidade Estadual de Campinas, Campinas, São Paulo, Brazil (Table 1).

Data from some collections were compiled from the Global Biodiversity Information Facility (GBIF) (https://www.gbif.org). Data from field observations were also compiled and divided in two categories: with and without photographs. Photographs of live specimens were also searched and found on two websites, iNaturalist (www.inaturalist.org), and Facebook (www.facebook.com), which provided useful data on the geographical distribution of these species. Data without photographs came from butterfly specialists (personal communication) (see Table 1). In addition, geographical data were also compiled from Freitas et al. (2018b) and Freitas et al. (2018c) (in these cases, data was not linked to voucher specimens). Geographical data from *D. rusina* (Fig. 1C) and *D. creusa* (Fig. 1D, E, Supplementary Material 1) came exclusively from the datasets of Santos et al. (2018) and Shirai et al. (2019) and also from the iNaturalist website (Fig. 3B, C). The dates of the records of *D. creusa* and *D. rusina* were searched on the iNaturalist website (see Rosa et al. 2023 for details).

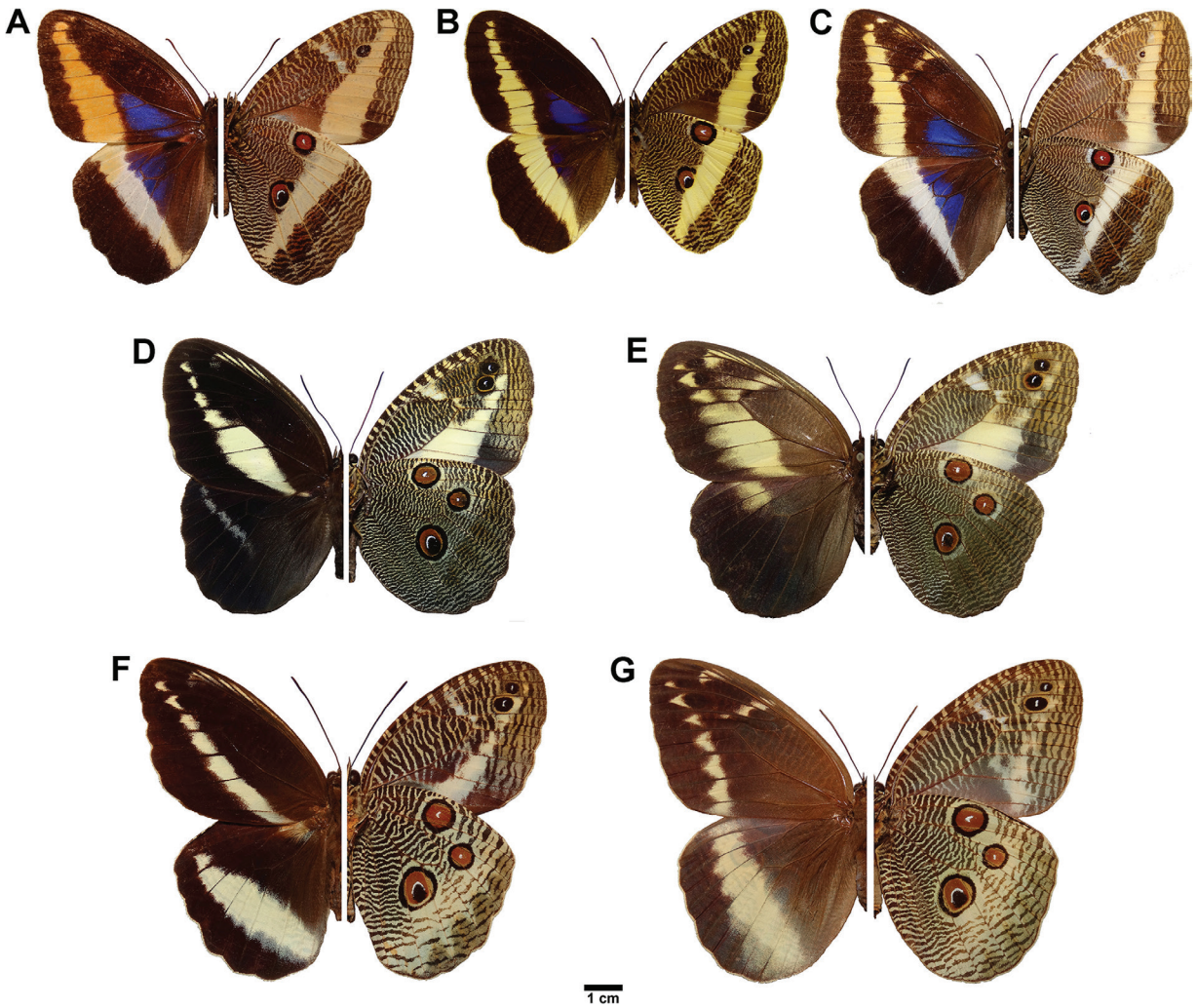

Figure 1. Adult *Dasyophthalma* species. **A** *D. delanira* **stat. rest.**; **B** *D. geraensis*; **C** *D. rusina*; **D**, **E** *D. creusa*, male and female, respectively; **F**, **G** *D. vertebralis*, male and female, respectively; in all pictures, dorsal view at left and ventral view at right. Scale bar = 1 cm.

Table 1. Data for studied individuals of the three threatened species of *Dasyophthalma* from 16 public/private collections (see text or acronyms), literature, and field observations. PA = Protected area (if pertinent).

Taxon	Code	N and Sex	Date	Country or State	Municipality	Site	PA	Altitude (m)
D. delanira	BMNH (type)	1 female	—	—	—	—	—	—
D. delanira	CEIOC	1 male	31.I.1988	RJ	Nova Friburgo	Morro São João, Mury	MCEPA	—
D. delanira	CEIOC	1 male	9.II.1992	RJ	Nova Friburgo	Morro São João, Mury	MCEPA	—
D. delanira	CGCM	1 female	—	RJ	Nova Friburgo	—	—	—
D. delanira	DZUP	3 individuals	30.I.1993	RJ	Nova Friburgo	Morro São João	MCEPA	—
D. delanira	DZUP	1 male	9.II.1992	RJ	Nova Friburgo	Morro São João	MCEPA	—
D. delanira	DZUP	1 individual	14.II.1957	RJ	Nova Friburgo	Mury	MCEPA	—
D. delanira	DZUP	1 individual	13.II.1957	RJ	Nova Friburgo	Mury	MCEPA	—
D. delanira	DZUP	1 female	8.II.1957	RJ	Nova Friburgo	Mury	MCEPA	—
D. delanira	DZUP	10 individuals	9.II.1987	RJ	Nova Friburgo	Pico São João, Mury	MCEPA	1200–1600
D. delanira	DZUP	1 male	—	RJ	Nova Friburgo	—	—	—
D. delanira	MNRJ	3 males	31.I.1988	RJ	Nova Friburgo	Morro São João	MCEPA	—
D. delanira	MNRJ	1 individual	31.I.1988	RJ	Nova Friburgo	P. de São João	MCEPA	1780
D. delanira	ZUEC	1 female	17.III.2023	RJ	Nova Friburgo	RPPN Bacchus	MCEPA	1460
D. delanira	ZUEC	1 female	26.III.2023	RJ	Nova Friburgo	RPPN Bacchus	MCEPA	1460
D. delanira	CGCM	1 individual seen	1996	RJ	Nova Friburgo	Pico do Caledônia	TPSP	—
D. delanira	JS	1 individual seen	10.III.2019	RJ	Nova Friburgo	Pico do Caledônia	TPSP	2050
D. delanira	JMSB	1 individual seen	—	RJ	Cachoeiras de Macacu	GER	BRMEPA	—

Taxon	Code	N and Sex	Date	Country or State	Municipality	Site	PA	Altitude (m)
D. delanira	MP	1 individual seen	9.II.2019	RJ	Nova Friburgo	Pico do Caledônia	TPSP	—
D. geraensis	AMNH	1 male	—	MG	—	—	—	—
D. geraensis	BMNH (type)	1 male	3.II.1916	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	CEIOC (type)	1 individual	26.I.1921	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	CEIOC	1 individual	15.I.1920	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	CEIOC	1 individual	4.II.1916	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	CEIOC	1 individual	28.I.1923	—	Virgínia	Serra dos Cochos	SMEPA	—
D. geraensis	CEIOC	1 female	20.2.1922	—	Virgínia	Serra dos Cochos	SMEPA	—
D. geraensis	CEIOC	1 individual	15.I.1930	—	—	km 5	—	—
D. geraensis	CEIOC	1 individual	15.I.1936	—	—	km 8	—	—
D. geraensis	CEIOC	1 individual	5.XII.1919	—	—	—	—	—
D. geraensis	CEIOC	1 individual	7.I.1941	—	—	—	—	—
D. geraensis	CGCM	1 male	26.I.1996	MG	Itajubá	—	SMEPA	1400
D. geraensis	DZUP	1 male	17–18.I.2004	MG	Delfim Moreira	15 Km SE	SMEPA	1500–1700
D. geraensis	DZUP	4 individuals	15.II.1984	MG	Delfim Moreira	Barreira de Piquete	SMEPA	1400–1600
D. geraensis	DZUP	1 male	6–11.I.1961	MG	Delfim Moreira	Barreira de Piquete	SMEPA	1500
D. geraensis	DZUP	1 individual	6–11.I.1961	SP	Piquete	Barreira de Piquete	SMEPA	1500
D. geraensis	DZUP	1 male	—	MG	Delfim Moreira	—	SMEPA	—
D. geraensis	DZUP	1 male	—	MG	Virgínia	Fazenda dos Campos	SMEPA	1500
D. geraensis	DZUP (type)	1 male	15.I.1920	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	DZUP	1 individual	6.II.1919	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	DZUP	1 individual	18.II.1916	MG	Virgínia	Fazenda dos Campos	SMEPA	1600
D. geraensis	DZUP	1 individual	26.I.1923	MG	Virgínia	Serra dos Cochos	SMEPA	—
D. geraensis	DZUP	1 individual	II.1922	MG	—	Sul de Minas	—	—
D. geraensis	DZUP	1 individual	27.XII.1929	RJ	Itatiaia	Estação biológica	INP	1200
D. geraensis	DZUP	1 individual	12.I.1973	RJ	Itatiaia	INP	INP	1400
D. geraensis	DZUP	1 individual	17.I.1979	RJ	Itatiaia	INP	INP	1750
D. geraensis	DZUP	2 individuals	22.I.1967	RJ	Itatiaia	INP	INP	—
D. geraensis	DZUP	1 individual	12.I.1973	RJ	Itatiaia	INP	INP	1400
D. geraensis	DZUP	1 individual	21.I.1969	RJ	Itatiaia	INP	INP	1300
D. geraensis	DZUP	1 female	6.2.1919	RJ	Itatiaia	—	INP	—
D. geraensis	DZUP	1 individual	—	RJ	Itatiaia	—	INP	—
D. geraensis	DZUP	1 individual	22.III.1969	RJ	—	Serra do Itatiaia	INP	1400
D. geraensis	DZUP	1 individual	15.I.1969	RJ	—	Serra do Itatiaia	INP	1650
D. geraensis	FLMNH	1 male	21.II.1922	ES	Castelo*	—	—	—
D. geraensis	FLMNH	1 male	21.III.1972	RJ	Itatiaia	—	INP	—
D. geraensis	FLMNH	1 male	13–14.I.1973	RJ	—	INP	INP	1000–2000
D. geraensis	MCZ	1 individual	—	MG	—	—	—	—
D. geraensis	MNRJ	2 individuals	—	MG	Passa Quatro	—	SMEPA	—
D. geraensis	MNRJ	1 individual	—	MG	Virgínia	Fazenda dos Campos	SMEPA	—
D. geraensis	MNRJ	1 individual	—	MG	Virgínia	Fazenda dos Campos	SMEPA	1500
D. geraensis	MNRJ	3 males	31.I.1970	RJ	Itatiaia	INP	INP	1650
D. geraensis	MNRJ	1 male	20.I.1953	RJ	Itatiaia	—	INP	—
D. geraensis	MZUSP	1 male	—	MG	Virgínia	Fazenda dos Campos	SMEPA	1500
D. geraensis	MZUSP	1 male	15.I.1936	RJ	Itatiaia	Itatiaia, km 11	INP	—
D. geraensis	MZUSP	1 male	—	RJ	Itatiaia	—	INP	—
D. geraensis	NHMW	1 male (type)		MG	Passa Quatro	—	SMEPA	—
D. geraensis	USNM	2 individuals	26.II.	MG	Virgínia	Fazenda dos Campos	SMEPA	—
D. geraensis	USNM	1 individual	—	MG	—	—	—	—
D. geraensis	ZUEC	1 male	30.II.1969	—	—	—	—	—
D. geraensis	ZUEC	2 males	20.I.1969	RJ	Itatiaia	INP, above Maromba	INP	1400–2000
D. geraensis	ZUEC	2 males	3.II.1968	RJ	Itatiaia	INP, above Maromba	INP	1600
D. geraensis	ZUEC	4 males	31.I.1970	RJ	Itatiaia	INP, above Maromba	INP	1400–1700
D. geraensis	ZUEC	1 female	20.I.1969	RJ	Itatiaia	INP, above Maromba	INP	1400–2000
D. geraensis	ZUEC	1 male	3.II.1968	RJ	Itatiaia	INP, above Maromba	INP	1400–1700
D. geraensis	CGCM	1 individual seen	2010	SP	Piquete	Pico dos Marins	SMEPA	—

Taxon	Code	N and Sex	Date	Country or State	Municipality	Site	PA	Altitude (m)
D. geraensis	CRSS	1 individual seen	11.III.2019	MG	Baependi	SPSP	SPSP	1777
D. geraensis	KSBJr.	1 female seen	15–16. II.1988	MG	Delfim Moreira	11 km SE. of Itajubá	SMEPA	—
D. geraensis	KSBJr.	1 individual seen	—	MG	Itamonte	—	INP	—
D. geraensis	KSBJr.	1 individual seen	15.II.1983	RJ	Itatiaia	PNI, Maromba to Macieiras	INP	—
D. geraensis	Freitas et al. 2018b	—	—	SP	Campos do Jordão	CJSP	CJSP	—
D. vertebralis	BMNH (type)	1 male	—	PA*	—	—	—	—
D. vertebralis	CEIOC	2 females	II–III.1948	ES	Sooretama	Parque Sooretama, Cupido	SBR	—
D. vertebralis	DZUP	1 male	—	ES	—	—	—	—
D. vertebralis	DZUP	1 female	—	ES	—	—	—	—
D. vertebralis	DZUP	1 male	—	Peru*	Iquitos	—	—	—
D. vertebralis	DZUP	1 female	—	Peru*	Iquitos	—	—	—
D. vertebralis	MCZ	1 male	—	ES	—	—	—	—
D. vertebralis	MCZ	1 female	—	ES	—	—	—	—
D. vertebralis	MNRJ	1 individual	—	ES	—	—	—	—
D. vertebralis	MNRJ	1 male	—	—	—	—	—	—
D. vertebralis	MNRJ	1 female	—	Peru*	Iquitos	—	—	—
D. vertebralis	MNRJ	1 individual	—	Peru*	Iquitos	—	—	—
D. vertebralis	MZUSP	1 female	—	AM*	—	E. Amazonas	—	—
D. vertebralis	MZUSP	1 female	—	ES	—	—	—	—
D. vertebralis	MZUSP	2 males	—	ES	—	—	—	—
D. vertebralis	NBCN	1 individual	—	—	—	Zuid Amerika	—	—
D. vertebralis	NBCN	1 individual	—	—	—	Zuid Amerika	—	—
D. vertebralis	NHMD	1 male	—	ES	—	—	—	—
D. vertebralis	NHMD	1 male	—	—	—	—	—	—
D. vertebralis	NHMD	1 female	—	—	—	—	—	—
D. vertebralis	NHRS (type)	1 female	—	ES	—	—	—	—
D. vertebralis	RBINS	1 male	—	—	—	—	—	—
D. vertebralis	USNM	1 female	—	ES	Santa Leopoldina	Leopoldina	—	—
D. vertebralis	USNM	1 male	—	—	—	S. Braz.	—	—
D. vertebralis	Freitas et al. 2018c	—	—	ES	Alegre	—	—	—
D. vertebralis	Freitas et al. 2018c	—	—	ES	Muqui	—	—	—
D. vertebralis	Freitas et al. 2018c	—	—	ES	Santa Teresa	—	—	—
D. vertebralis	Freitas et al. 2018c	—	—	MG	Teófilo Otoni	—	—	—

* Individuals possibly mislabeled. Abbreviations for Brazilian states: AM = Amazonas, ES = Espírito Santo, MG = Minas Gerais, PA = Pará, RJ = Rio de Janeiro, SP = São Paulo; Protected areas: BRMEPA = Bacia do Rio Macacu Environmental Protection Area, CJSP = Campos do Jordão State Park, GER = Guapiaçu Ecological Reserve, INP = Itatiaia National Park, MCEPA = Macaé de Cima Environmental Protection Area, SBR = Sooretama Biological Reserve, SMEPA = Serra da Mantiqueira Environmental Protection Area, SPSP = Serra do Papagaio State Park, TPSP = Três Picos State Park; Unpublished data: CGCM = Carlos Guilherme Costa Mielke, CRSS = Carlos Roberto Silva Silva, JS = Jalmirez Silva, JMSB = Jorge Manuel Saraiva Bizarro, MP = Max Peters, KSBJr. = Keith Spalding Brown Jr.

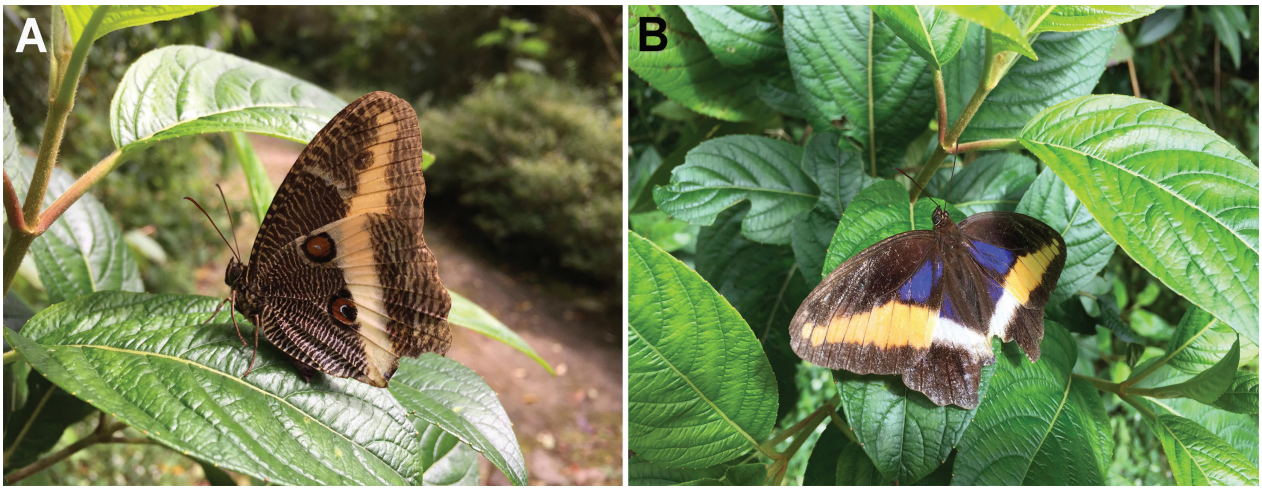

Figure 2. Adult of *Dasyophthalma delanira* **stat. rest.** in Nova Friburgo, Rio de Janeiro state, Brazil. **A** Adult with closed wings perched on a leaf; **B** adult with open wings perched on a leaf (photos courtesy of Max Torres Peters).

2.2. Geographical range (area of occupancy and extent of occurrence)

Geographical range (extent of occurrence EOO and area of occupancy AOO) was estimated based on all known sites for each species of *Dasyophthalma*. The EOO is the area contained within the shortest continuous imaginary boundary that includes all known distribution points of a species, and the AOO is the area within its EOO that is really occupied by a taxon (IUCN 2012; IUCN Standards and Petitions Committee 2022). Both EOO and AOO were estimated using the online open-source program GeoCAT (Geospatial Conservation Assessment Tool, available at http://geocat.kew.org) (Bachman et al. 2011). As recommended by IUCN for AOO analyses, a 2 km grid (cell area of 4 km^2) was used (IUCN 2012; IUCN Standards and Petitions Committee 2022). Doubtful geographical data were not used in these analyses.

2.3. DNA purification, amplification, and sequencing

The mitochondrial cytochrome c oxidase subunit I (COI) and the nuclear glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal protein S5 (RpS5) genes were selected for this study.

Total genomic DNA was purified from one or two legs per individual using the DNeasy Blood & Tissue Kit protocol (QIAGEN, Düsseldorf, Germany). DNA was stored in TE buffer at –20°. PCR amplification of cytochrome c oxidase subunit I (COI) partial sequences (ca. 658 bp) were performed using the LCO 1490 (Forward; 5' GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (Reverse; 5' TAAACTTCAGGGTGACCAAAAAATCA) primers by Folmer et al. (1994). Reactions were done in 26 µL µL and contained 15,6 µL H_2O Milli-Q, 2,5 µl buffer solution (500 Mm KCl), 0,4 µL of dNTP (25 Mm), 2,0 µL of MgCl2 (2 Mm), 2,5 µL of DMSO 5%, 0,5 µL of each initiator (10 Mm), 0,2 µL of Taq DNA-Polymerase (Promega, Madison, WI, USA) (1 U), and 2,0 µL of DNA extracted. The amplification program included an initial denaturation step at 94°C for 2 min, followed by 34 cycles of denaturation at 94°C for 45s, annealing at 45°C for 45s, and polymerization at 72°C for 1 min, followed by an extension step at 72°C for 5 min (Silva-Brandão et al. 2005). PCR products were purified of primers and deoxynucleotides with ExoSAP-IT (GE Healthcare, Bucks, UK), and then sequenced by BigDye Kit protocol in an ABI 3500 automated sequencer, with primers used for amplification.

In the case of two specimens of *D. vertebralis* (voucher code: EB-19009 and EB-19033), DNA was extracted from old museum specimens deposited at the Natural History Museums at Stockholm and Copenhagen, respectively. The DNA extraction was carried out using QIAamp DNA MicroKit (QIAGEN®, USA) protocol, adapted with columns from MinElute PCR Purification Kit (QIAGEN®, Düsseldorf, Germany).

The standard steps for NGS library preparation (e.g., end repair, adaptor ligation and fill-in, and indexing PCR) were used, using Blunt-End Illumina Libraries, which consists in single index blunt-end Illumina library construction for ancient and historical samples, following a modified protocol of Meyer and Kircher (2010) as described in Twort et al. (2021). The pooled libraries were sent to sequencing at National Genomics Infrastructure (NGI) in Stockholm, Sweden on an Illumina NovaSeq machine.

All libraries were successfully sequenced using Whole Genome Shotgun Sequencing (WGSS) and the raw data were analyzed and each genome has been cleaned and assembled using de novo assembly techniques, as described in Twort et al. (2021). The COI and GAPDH genes were then successfully pooled from the two genomes and incorporated in the matrix.

Sequences generated in this work and from Matos-Maraví et al. (2021) were aligned with the sequences from COI and the nuclear genes GAPDH and RpS5 (Table 2 for accession numbers) using the program Geneious v. 11.1.5 (Kearse et al. 2012).

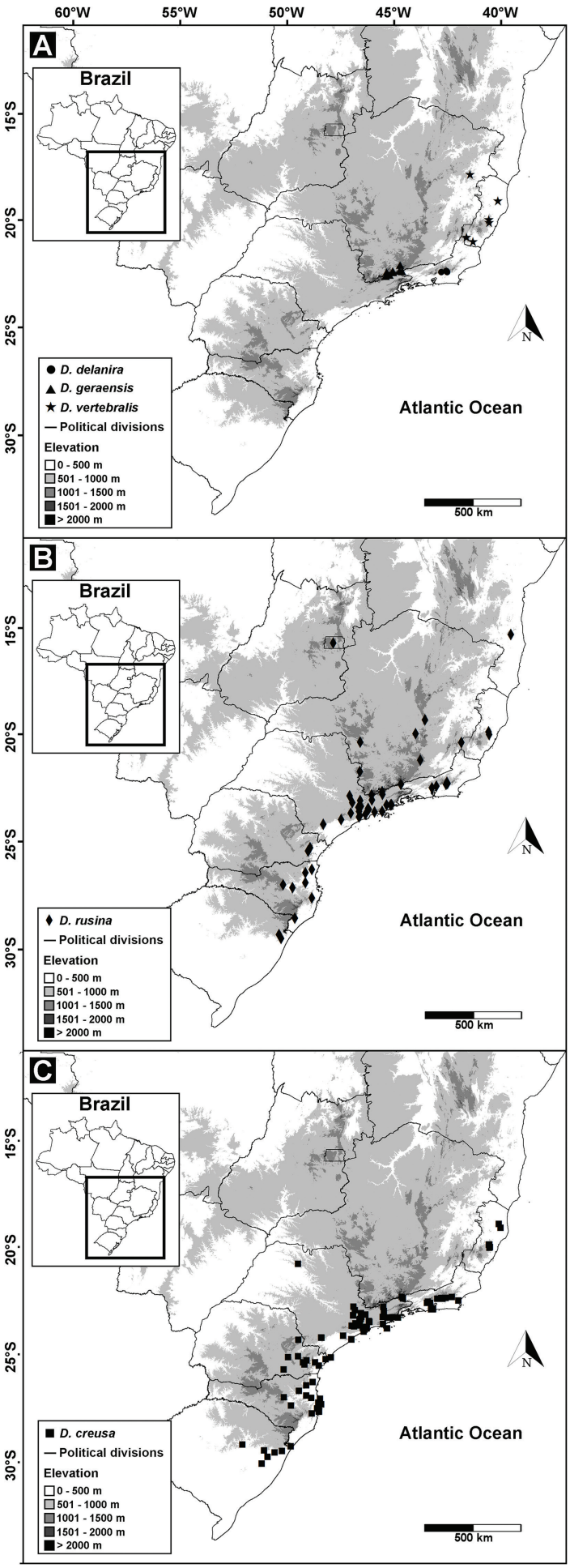

Figure 3. Distribution map showing the known geographic distribution of *Dasyophthalma* species in Brazil. **A** Distribution of *D. delanira*, *D. geraensis* and *D. vertebralis*; **B** distribution of *D. rusina*; **C** distribution of *D. creusa.*

Table 2. Samples of Satyrinae (Morphini and Brassolini) used in the present study with codes, localities and GenBank/Boldsystems accession numbers for COI, GAPDH and RpS5 sequences.

Taxon	DNA voucher	Locality	COI	GAPDH	RpS5
Morpho helenor	NW127-12	Águas da Prata, São Paulo, Brazil	JN696174	JN696220	JN696282
Bia actorion	PM14-28	Phil DeVries Collection	MK551389	MK551492	MK551469
Bia actorion	NW17842	Xingu, Pará, Brazil	MK551366	—	MK551450
Blepolenis bassus	NW155-6	Floresta Nacional, São Francisco de Paula, Rio Grande do Sul, Brazil	JF508389	MK551479	JF508405
Blepolenis bassus	BC-CGCM-8.908	São Bento do Sapucaí, Brazil	JX215903	—	—
Blepolenis bassus	BC-CGCM-9.339	São Bento do Sapucaí, Brazil	JX215904	—	—
Blepolenis batea	BC-CGCM-18.416	Boca do Mato, Cachoeiras de Macacu, Rio de Janeiro, Brazil	JX215905	—	—
Blepolenis batea	NW155-3	Paranapiacaba, Santo André, São Paulo, Brazil	JF508388	KM013289	JF508404
Blepolenis batea	BPU172	Parque Nacional Serra da Bocaina, São José do Barreiro, São Paulo, Brazil	OQ720958	—	—
Blepolenis catharinae	PM02-09	Lagoa do Peri, Florianópolis, Santa Catarina, Brazil	JF508390	—	JF508406
Orobrassolis ornamentalis	PM25-05	Bairro Novo Capivari, Campos do Jordão, São Paulo, Brazil	MK551395	—	MK551473
Orobrassolis ornamentalis	BLU-785	Campos do Jordão, São Paulo, Brazil	MK551348	—	—
Orobrassolis ornamentalis	BPU-169	Campos do Jordão, São Paulo, Brazil	OQ720956	—	—
Orobrassolis ornamentalis	BPU-202	Campos do Jordão, São Paulo, Brazil	OQ720963	—	—
Opsiphanes bogotanus	NW118-6	Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JF508393	MK551476	JF508408
Opsiphanes bogotanus	08-SRNP-40116	Sector Rincon Rain Forest, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ537131	—	—
Opsiphanes cassiae	PM06-11	Misahualli, Napo, Equador	KM012972	—	KM013196
Opsiphanes cassina	NW118-5	Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JF508392	—	—
Opsiphanes cassina	YB-BCI24435	Barro Colorado, Panamá, Panamá	KP849172	—	—
Opsiphanes invirae	MACN-Bar-Lep-ct-02127	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF547146	—	—
Opsiphanes invirae	MACN-Bar-Lep-ct-02129	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF547363	—	—
Opsiphanes quiteria	NW109-10	Area de Conservacion Guanacaste, Guanacaste, Costa Rica	DQ018957	KM013296	EU528451
Opsiphanes quiteria	BLU263	Serra do Japi, Jundiaí, São Paulo, Brazil	KX858943	—	—
Opsiphanes quiteria	BLU322	Serra do Japi, Jundiaí, São Paulo, Brazil	KX858944	—	—
Opsiphanes tamarindi	08-SRNP-56847	Sector Mundo Nuevo, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ538545	—	—
Opsiphanes tamarindi	07-SRNP-33627	Sector Pitilla, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ537147	—	—
Opsiphanes tamarindi	NW118-3	Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JF508391	—	JF508407
Dynastor darius	06-SRNP-60108	Sector Mundo Nuevo, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ548386	—	—
Penetes pamphanis	NW155-5	Trilha Pedra de Amolar, Maquiné, Rio Grande do Sul, Brazil	KM012973	KM013304	KM013219
Dasyophthalma creusa	NW126-4	Serra do Japi, Jundiaí, São Paulo, Brazil	EU528318	EU528387	EU528431
Dasyophthalma delanira	BC-CGCM-7.561	Pico São João, Nova Friburgo, Rio de Janeiro, Brazil	JX215914	—	—
Dasyophthalma delanira	BC-DZ-9.911	Pico São João, Mury, Nova Friburgo, Rio de Janeiro, Brazil	CGCM377-08	—	—
Dasyophthalma geraensis	BC-CGCM-3.035	Itajuba, Minas Gerais, Brazil	JX215915	—	—
Dasyophthalma geraensis	BC-DZ-9.903	Delfim Moreira, Minas Gerais, Brazil	CGCM381-08	—	—
Dasyophthalma rusina	BC-CGCM-9.492	Boca do Mato, Cachoeiras de Macacu, Rio de Janeiro, Brazil	JX215917	—	—
Dasyophthalma rusina	BC-CGCM-20.515	Conceição dos Ouros, Minas Gerais, Brazil	JX215916	—	—

Taxon	DNA voucher	Locality	COI	GAPDH	RpS5
Dasyophthalma rusina	BC-DZ-9.887	Pico São João, Mury, Nova Friburgo, Rio de Janeiro, Brazil	CGCM380-08	—	—
Dasyophthalma rusina	BC-DZ-9.919	Rio Natal, São Bento do Sul, Santa Catarina, Brazil	CGCM382-08	—	—
Dasyophthalma rusina	NW155-8	Paranapiacaba, Santo André, São Paulo, Brazil	MK551359	MK551480	MK551443
Dasyophthalma rusina	BPU187	Vossoroca, Tijucas do Sul, Paraná, Brazil	OQ720959	—	—
Dasyophthalma rusina	BPU191	Parque Natural Municipal do Trabijú, Pindamonhangaba, São Paulo, Brazil	OQ720960	—	—
Dasyophthalma rusina	BPU192	Serra do Japi, Jundiaí, São Paulo, Brazil	OQ720961	—	—
Dasyophthalma rusina	BPU193	Parque Nacional do Caparaó, Alto Caparaó, Minas Gerais, Brazil	OQ720962	—	—
Dasyophthalma rusina	BPU208	Parque Nacional do Itatiaia, Itatiaia, Rio de Janeiro, Brazil	OQ720964	—	—
Dasyophthalma rusina	BPU268	RPPN Bacchus, Nova Friburgo, Rio de Janeiro, Brazil	OQ720965	—	—
Dasyophthalma vertebralis	EB19-009	Espírito Santo, Brazil	OQ720966	OQ722368	—
Dasyophthalma vertebralis	EB19-033	Espírito Santo, Brazil	OQ720967	OQ722369	—
Catoblepia amphirhoe	MACN-Bar-Lep-ct-01007	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF546750	—	—
Catoblepia amphirhoe	MACN-Bar-Lep-ct-01178	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF546831	—	—
Catoblepia berecynthia	NW155-14	Trap 25 understory, Garza Cocha, Sucumbios, Ecuador	MK551355	—	MK551439
Catoblepia berecynthia	NW178-38	Xingu, Pará, Brazil	MK551363	MK551483	MK551447
Catoblepia berecynthia	YB-BCI64698	Gamboa, Colón, Panamá	KP848752	—	—
Catoblepia berecynthia	YB-BCI64818	Gamboa, Colón, Panamá	KP848753	—	—
Catoblepia xanthus	BLU024	Itacoatiara, Amazonas, Brazil	KX858937	—	—
Catoblepia xanthus	BLU025	Itacoatiara, Amazonas, Brazil	KX858938	—	—
Catoblepia xanthus	NW155-13	Trap 14 understory, Garza Cocha, Sucumbios, Ecuador	JF508395	—	JF508410
Catoblepia soranus	NW178-39	Xingu, Pará, Brazil	MK551364	—	MK551448
Catoblepia versitincta	NW178-40	Xingu, Pará, Brazil	MK551365	MK551484	MK551449
Selenophanes cassiope	YPH-0585	Foz do Iguaçu, Paraná, Brazil	KX858949	—	—
Selenophanes cassiope	YPH-0587	Foz do Iguaçu, Paraná, Brazil	KX858948	—	—
Selenophanes orgetorix	NW109-15	Area de Conservacion Guanacaste, Guanacaste, Costa Rica	DQ338754	—	EU528427
Opoptera aorsa	MACN-Bar-Lep-ct-01206	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF545789	—	—
Opoptera aorsa	MACN-Bar-Lep-ct-01208	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF545409	—	—
Opoptera aorsa	NW137-21	Cotia, São Paulo, Brazil	KM012970	—	MK551437
Opoptera syme	BLU511	Serra do Japi, Jundiaí, São Paulo, Brazil	KX858935	—	—
Opoptera syme	NW126-3	Serra do Japi, Jundiaí, São Paulo, Brazil	EU528323	EU528403	EU528450
Opoptera arsippe	NN33	Peru	MK551352	—	MK551436
Narope cyllabarus	PM01-24	Três Lagoas, Mato Grosso do Sul, Brazil	KM012968	KM013295	KM013211
Narope cyllene	NW127-27	Extrema, Minas Gerais, Brazil	DQ338755	EU528401	EU528447
Caligo brasiliensis	10-SRNP-21447	Sector Del Oro, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ539210	—	—
Caligo brasiliensis	10-SRNP-21446	Sector Del Oro, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ539211	—	—
Caligo illioneus	06-SRNP-2481	Area de Conservacion Guanacaste, Alajuela, Costa Rica	JQ548328	—	—
Caligo illioneus	MACN-Bar-Lep-ct-02571	Parque Nacional Rio Pilcomayo Seccional Estero Poi, Pilcomayo, Formosa, Argentina	MF547002	—	—

Taxon	DNA voucher	Locality	COI	GAPDH	RpS5
Caligo illioneus	PM06-17	Puente Chinchavito, Leoncio Prado, Peru	MK551377	MK551488	MK551461
Caligo uranus	PM06-15	Quintana Roo, Othon P. Blanco, Mpio, Bacalar, Mexico	MK551375	MK551487	MK551459
Eryphanis aesacus	MAL-02690	La Union, Rio Hondo, Quintana Roo, Mexico	GU659596	—	—
Eryphanis aesacus	MAL-02692	Chetumal, Quintana Roo, Mexico	GU659598	—	—
Eryphanis reevesii	MACN-Bar-Lep-ct-00287	Parque Nacional Iguazú, Departamento de Iguazu, Misiones, Argentina	MF546004	—	—
Eryphanis lycomedon	INB0004269656	Nogal, Puerto Viejo, Sarapiqui, Heredia, Costa Rica	ASARD2471-12	—	—
Eryphanis lycomedon	16-SRNP-31641	Area de Conservacion Guanacaste, Alajuela, Costa Rica	BLPAA5850-17	—	—
Eryphanis lycomedon	PM06-06	Pichincha, Tinalandia, Peru	MK551368	MK551485	MK551452
Eryphanis lycomedon	PM06-07	Pichincha, Tinalandia, Peru	MK551369	—	MK551453
Eryphanis automedon	NW155-18	CICRA, Peru	KM012950	KM013287	KM013193
Brassolis sophorae	NW122-21	Campinas, São Paulo, Brazil	EU528314	GQ357384	EU528425
Brassolis sophorae	BLU617	UNICAMP, Campinas, São Paulo, Brazil	OQ720957	—	—
Brassolis sophorae	BC-DZ-9.863	Cambé, Paraná, Brazil	CGCM373-08	—	—
Brassolis isidrochaconi	MHMYJ1180-11	Sector Rincon Rain Forest, Area de Conservacion Guanacaste, Alajuela, Costa Rica	JQ539205	—	—
Brassolis isidrochaconi	07-SRNP-6120	Sector Santa Maria, Area de Conservacion Guanacaste, Guanacaste, Costa Rica	JQ536912	—	—

Table 3. Best-fit substitution models by partition derived from ModelFinder on IQTree 2.1.2.

Partition	Name	Best fit-model
1	COI_pos1	TIM2+F+G4
2	COI_pos2	TIM2+F+I+G4
3	COI_pos3_GAPDH_pos3_RPS5_pos3	TPM2+F+I+G4
4	GAPDH_pos1_RPS5_pos1	TPM2+F+G4
5	GAPDH_pos2_RPS5_pos2	TN+F+I+G4

2.4. Phylogenetic analysis

The concatenated matrix comprised three genes (mitochondrial COI and nuclear GAPDH and RpS5) and 88 specimens of 40 Satyrinae species (39 Brassolini and one Morphini, used to root the tree), representing all five *Dasyophthalma* species, with a total of 2,776 base pairs (COI – 1475bp; GAPDH – 691bp; RpS5 – 610bp) (see Table 2 for voucher numbers and information on the newly generated sequences). Data was partitioned by codon position and model selection (see Table 3 for details) was done using ModelFinder (Kalyaanamoorthy et al. 2017) with edge-linked partition model + FreeRate heterogeneity in IQ-TREE v. 2.1.2. (Minh et al. 2020) on CIPRES portal (Miller et al. 2010). The concatenated data set with aligned sequences is provided in the Supplementary Material (Supplementary Material 2).

The maximum likelihood tree was inferred using 10 independent likelihood searches in IQ-TREE v. 2.1.2, and support was calculated using the ultrafast bootstrap (UFBoot) (Hoang et al. 2018), with 2000 replications. In addition to assess node support through 1000 replications of Shimodaira Hasegawa-like approximate Likelihood Ratio Test (SH-aLRT) (-alrt 1000) (Guindon et al. 2010; Hoang et al. 2018) and Approximate Bayes test (aBayes) (Anisimova et al. 2011). The tree associated with the highest likelihood score (LnL = –16552.4484) was rooted as described above.

The genetic distances (Table 4) among species of *Dasyophthalma* were determined by using the nucleotide substitution model Kimura-2-parameters (Kimura 1980) by using the program MEGA X v.10.1.7 (Kumar et al. 2018).

3. Results

3.1. Compiled data

A total of 28 adult specimens of *D. r. delanira*, 67 of *D. geraensis* and 26 of *D. vertebralis* were found in 16 public/private collections (Table 1). Data from field observations "without photographs" (pers. comm.) are two of *D. r. delanira*, and four of *D. geraensis.* Data "with photographs" came from Facebook, two of *D. r. delanira*, one of *D. geraensis*. Literature data (Freitas et al. 2018b,c) are three of *D. vertebralis* and 1 of *D. geraensis.* Data of *D.*

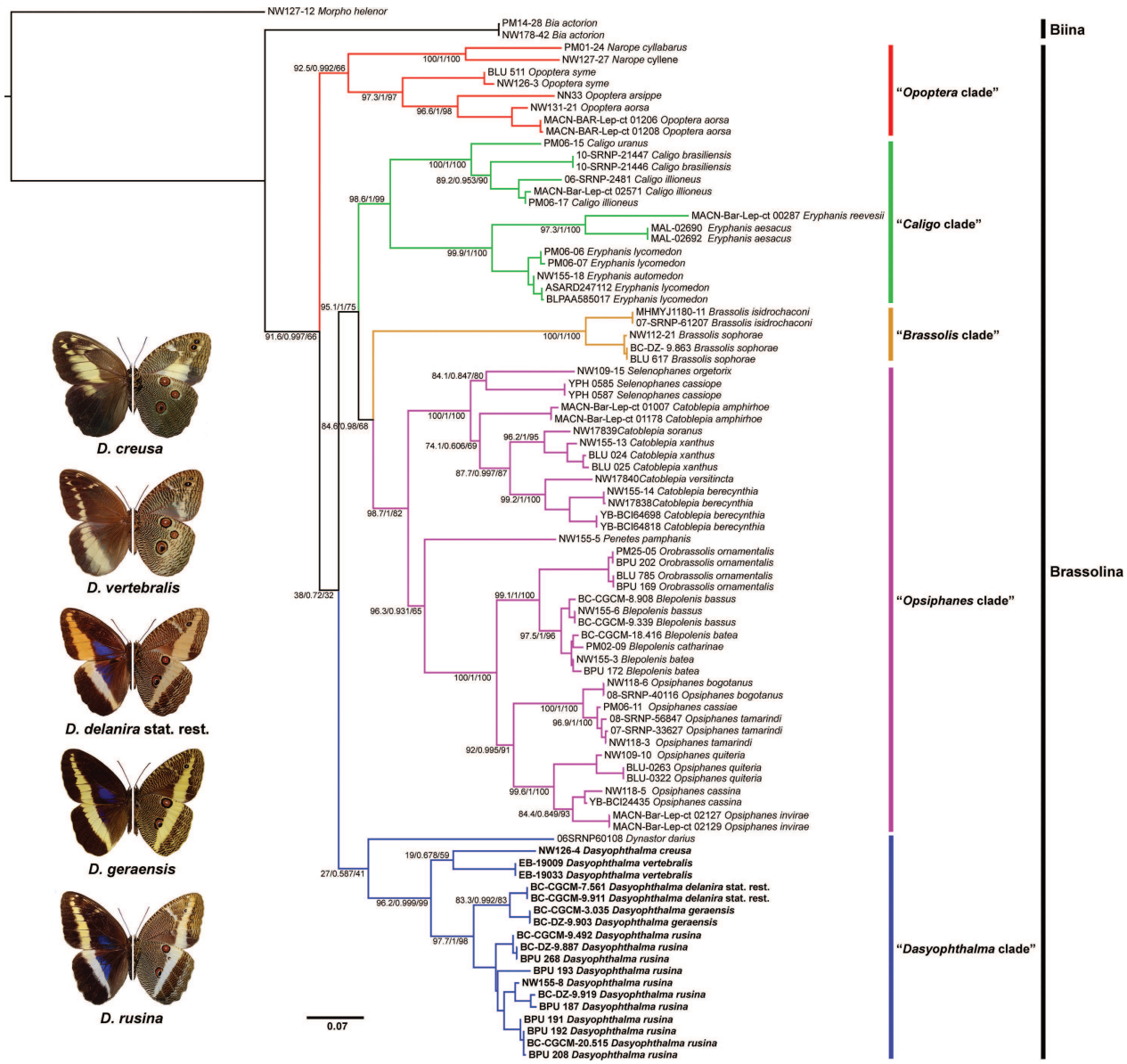

Figure 4. Phylogenetic relationships of *Dasyophthalma* species and other Satyrinae taxa based on COI, GAPDH and RpS5 genes and obtained by a maximum likelihood analysis. Numbers near the nodes are SH-aLRT/aLRT/UFBoot support values.

creusa are 41 from datasets and 76 from iNaturalist and for *D. rusina* 34 from datasets and 27 from iNaturalist website.

3.2. Phylogenetic assessment and taxonomy

The obtained molecular phylogeny (Fig. 4) recovered the genus *Bia* (subtribe Biina) as the sister group of all remaining Brassolini (subtribe Brassolina). In the subtribe Brassolina, five main clades were identified: 1) *Narope* + *Opoptera* (hereafter "*Opoptera* clade") as the sister group of all remaining Brassolina; 2) *Dynastor* + *Dasyophthalma* ("*Dasyophthalma* clade"); 3) *Caligo* + *Eryphanis* ("*Caligo* clade"); 4) the genus *Brassolis* ("*Brassolis* clade"), as the sister group of 5) a large clade with all remaining genera ("*Opsiphanes* clade"). All brassoline genera were recovered as monophyletic, including *Dasyophthalma*, which have *Dynastor darius* as sister group. The two species groups of *Dasyophthalma* previously defined (Penz 2009) were also recovered as sister to each other: (1) the "*creusa* group", including *D. creusa* + *D. vertebralis*, and (2) the "*rusina*-group", including *D. delanira* **stat. rest.** + *D. geraensis*, this clade sister to *D. rusina* (Fig. 4).

Based on the COI, the mean K2P distance within species of *Dasyophthalma* was 0.017 (range 0 to 0.03), and the mean among species distance was 0.071 (range 0.02 to 0.10). The average distance between *D. delanira* **stat. rest.** and *D. rusina* varied from 0.05 to 0.07 (mean 0.056), and between *D. delanira* **stat. rest.** and *D. geraensis* was 0.02, both above the mean distance within species (Table 4).

In short, the present results recovered *D. delanira* **stat. rest.** as the sister species of *D. geraensis*, and this clade sister to *D. rusina*. This, combined with the genetic distances validate the decision of reinstating the specific status of the former, and not as a subspecies of *D. rusina*.

Table 4. Pairwise Genetic distances (Kimura 2-parameter distance) among sampled *Dasyophthalma* species.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Dasyophthalma vertebralis* EB19009																	
2	*Dasyophthalma vertebralis* EB19033	0.00																
3	*Dasyophthalma creusa* NW126-4	0.06	0.06															
4	*Dasyophthalma delanira* BC-CGCM-7.561	0.09	0.09	0.09														
5	*Dasyophthalma delanira* BC-DZ-9.911	0.09	0.09	0.09	0.00													
6	*Dasyophthalma geraensis* BC-CGCM-3.035	0.09	0.09	0.10	0.02	0.02												
7	*Dasyophthalma geraensis* BCDZ9.903	0.09	0.09	0.10	0.02	0.02	0.00											
8	*Dasyophthalma rusina* BC-CGCM-9.492	0.09	0.09	0.08	0.05	0.05	0.05	0.05										
9	*Dasyophthalma rusina* BC-CGCM-20.515	0.09	0.09	0.09	0.06	0.06	0.06	0.06	0.02									
10	*Dasyophthalma rusina* BC-DZ-9.887	0.09	0.09	0.08	0.05	0.05	0.05	0.05	0.00	0.02								
11	*Dasyophthalma rusina* BC-DZ-9.919	0.10	0.10	0.09	0.06	0.06	0.06	0.06	0.03	0.02	0.03							
12	*Dasyophthalma rusina* NW155_8	0.09	0.09	0.08	0.05	0.05	0.05	0.05	0.02	0.01	0.02	0.01						
13	*Dasyophthalma rusina* BPU191	0.09	0.09	0.09	0.06	0.06	0.06	0.06	0.02	0.00	0.02	0.02	0.01					
14	*Dasyophthalma rusina* BPU192	0.09	0.09	0.09	0.06	0.06	0.06	0.06	0.02	0.00	0.02	0.02	0.01	0.00				
15	*Dasyophthalma rusina* BPU208	0.10	0.10	0.09	0.07	0.07	0.06	0.06	0.02	0.00	0.02	0.02	0.02	0.00	0.00			
16	*Dasyophthalma rusina* BPU268	0.09	0.09	0.08	0.05	0.05	0.05	0.05	0.00	0.02	0.00	0.03	0.02	0.02	0.02	0.02		
17	*Dasyophthalma rusina* BPU187	0.10	0.10	0.09	0.06	0.06	0.06	0.06	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	
18	*Dasyophthalma rusina* BPU193	0.10	0.10	0.09	0.05	0.05	0.06	0.06	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03

3.3. Distribution and habitat

Based on the available geographical data, the genus *Dasyophthalma* is endemic from Brazil and has its distribution mostly in the Atlantic Forest domain, with only a few records in the Cerrado domain (Fig. 3). The most restricted species is *D. delanira* **stat. rest.**, known from only four localities of well-preserved montane forest at altitudes above 1200 meters of two municipalities (Nova Friburgo and Cachoeiras de Macacu) in the Rio de Janeiro State (Fig. 3A, Table 1). Another high-altitude montane forest species, *D. geraensis*, was reported in 11 localities above 1000 meters of altitude along the Mantiqueira mountain range, in the states of Minas Gerais, São Paulo, and Rio de Janeiro (Figs 3A, 5C, D; Table 1). There is a single record of *D. geraensis* for the municipality of Castelo in the State of Espírito Santo that needs to be confirmed and could represent a mistake. The most enigmatic of the species of the genus is *D. vertebralis*; although the available data suggests a relatively wide distribution, in lowland Tableland forests in six localities in the states of Minas Gerais and Espírito Santo (Fig. 3A, Supplementary Material 1, Table 1), no individuals were observed since 1948, when two individuals were captured in Sooretama Biological Reserve, Espírito Santo State (Supplementary Material 1). In addition, the occurence of this species in the Brazilian state of Pará, in the Amazon region (mentioned in the original description) and in Iquitos (Peru), are both considered as mislabeled specimens (Freitas et al. 2018c). Two species, *D. creusa* and *D. rusina*, are common and show a wider geographical range, occurring in sympatry in great part of its distribution (Fig. 3B, C). However, *D. rusina* is more widespread and common in altitudes above 500 m, also occurring in forest areas within the Cerrado in Central Brazil (in Minas Gerais state and the Federal District), (Figs 3B, 5A, B), while *D. creusa* is more common on lowland coastal forests (on all states of Southeastern and South Brazil) (Figs 3C, 5A, B).

3.4. Natural history

Species of *Dasyophthalma* fly during the day, including the warmer hours (from 11:00 to 14:00), a pattern distinct of most Brassolini, which present crepuscular behavior (Brown 1992; Freitas et al. 1997; Casagrande and Mielke 2000, 2003). Adults are commonly observed in an erratic and fast flight in the forest understory, occasionally leaving to forest edges along rivers, dirty roads and trails. Based on all available data and field observations, all species of *Dasyophthalma* are univoltine, with adults flying during the summer months (records are mostly from December to March, with few records in November, April and May) (Table 1; see also Brown 1992; Penz 2009: Appendix 1; data from iNaturalist and personal observations of the authors also indicate the same flight period). Territorial behavior was not observed, and courtship behavior is unknown.

Some species occur in sympatry in several localities, such as *D. creusa* with *D. rusina* (Fig. 5A, B) and *D. creusa* with *D. vertebralis* (Supplementary Material 1). Others occurs in a parapatry as a result of different altitudinal preferences; the most notable example is *D. rusina* and *D. delanira* **stat. rest.**, where the latter occurs in altitudes higher than the former, resulting in a very narrow line of sympatry around 1200–1300 m.

As most Brassolini, all species of *Dasyophthalma* belong to the fruit-feeding guild, and the most common species (i.e., *D. creusa* and *D. rusina*) are usually sampled in studies using traps baited with fermented fruits (e.g. Uehara-Prado et al. 2007), however, the other species in the genus are also potentially attracted to bait traps (Freitas and Marini-Filho, 2011; Freitas et al. 2018a, 2018b,

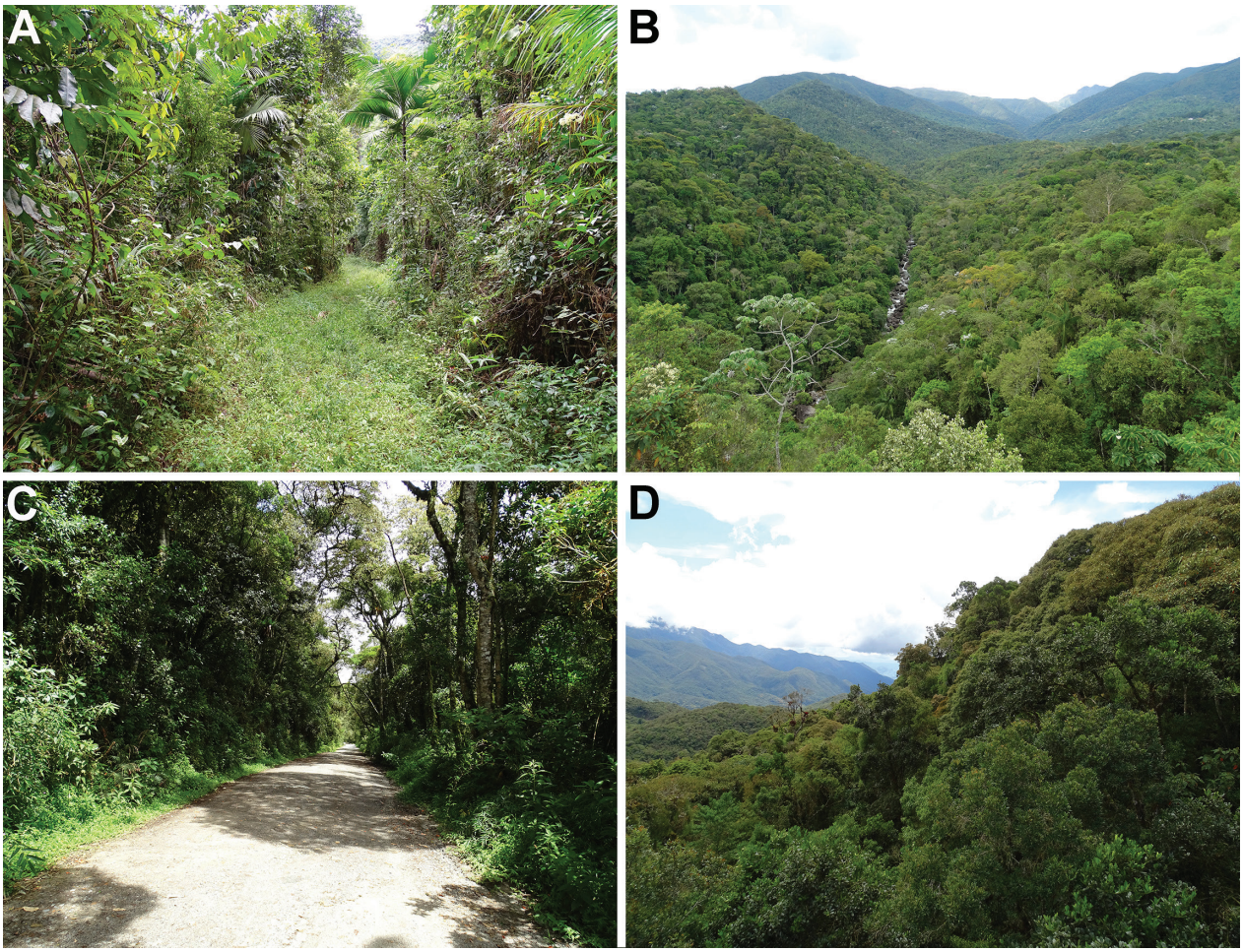

Figure 5. Habitat of *D. rusina*, *D. creusa* and *D. geraensis* in Itatiaia National Park (INP). **A** Closer view of *D. rusina* and *D. creusa* habitat; **B** general view of *D. rusina* and *D. creusa* habitat; **C** closer view of *D. geraensis* habitat; **D** general view of *D. geraensis* habitat.

2018c). It is worth mentioning that fieldwork to sample *D. vertebralis* using attractive traps was carried out at Sooretama Biological Reserve on March 2020 without success (Supplementary Material 1). Also, a previous three-month sampling of fruit-feeding butterflies in the same area (April, July and August) captured only *D. creusa* in baited traps (Nogueira 2012).

Immature stages and host plants have been described (partially) only for the two most common species, *D. creusa* and *D. rusina*. For *D. geraensis*, its host plant "uricana/uricangas" (*Bactris tormentosa*, Arecaceae) is mentioned in some old studies, but immature stages are unknown (d'Araújo e Silva et al. 1968; Zikán and Zikán 1940; Beccaloni et al. 2008). For *D. creusa*, the fourth and fifth instars and the pupa were described (Casagrande and Mielke 2003) and larvae were fed with *Geonoma schottiana* (Arecaeae) as host plant. Other host plants mentioned in the literature include "taquara" (Poaceae) (Hoffmann 1936), *Astrocaryum aculeatum*/*vulgare*? (Arecaceae) (Schmith and Hoffmann 1931) and *Bactris* sp. (Arecaceae) (Brown 1992). For *D. rusina*, the fifth instar and pupa were described, and its host plant is *Geonoma schottiana* (Arecaeae) (Casagrande & Mielke 2000). Other reported host plants include *Euterpe edulis* (Arecaceae) (Zikán and Zikán 1940; d'Araújo e Silva et al. 1968) and *Bambusa* sp. (Poaceae) (d'Araújo e Silva et al. 1968; Brown 1992).

Population data is limited and once again restricted to the two common and widespread species, *D. creusa* and *D. rusina*. Available data using baited traps reveal a large variation in number of sampled individuals in different areas. For example, for *D. creusa*, a minimum of one individual and a maximum of 81 individuals are reported in several short-term studies (Supplementary Material 1). For *D. rusina*, the few short-term studies sampled from one to 11 individuals (Table S1). In the only long-term study, in a montane area in southeastern Brazil, numbers were low, with only 35 individuals of *D. creusa* (30 males and five females) and three individuals of *D. rusina* (one male and two females) captured over 10 years of sampling (Supplementary Material 1).

3.5. Conservation

As previously mentioned, three out of the five species of *Dasyophthalma* are of conservation importance, namely *D. delanira* **stat. rest.**, *D. geraensis* and *D. vertebralis* (MMA 2003, 2014, 2022; Freitas et al. 2018a, 2018b, 2018c). In these three cases, the inclusions on the Bra-

Table 5. Conservation status of all three threatened species of *Dasyophthalma* species along different assessments from 1989 to 2022 (from the Official Brazilian Red List).

Species	1989[a]	2003[b]	2014[c]	2022[d]	Future[e]
D. delanira	Threatened	Threatened	CR	EN	EN
D. geraensis	Threatened	Threatened	CR	CR	VU
D. vertebralis	Threatened	Threatened	CR-PEX	CR-PEX	DD
D. creusa	—	—	—	LC*	LC
D. rusina	—	—	—	LC*	LC

CR = Critically endangered, CR-PEX = Critically endangered, probably extinct; EN = Endangered; DD = Data Deficient; LC = Least Concern; LC* = Least Concern (Freitas et al. 2022a,b: Freitas AVL, Brant A, Rosa AHB, Ribeiro DB, Barbosa EP, Dias FMS, Bizarro JMS, Prado UM, Cardoso MZ, Filho OJM, Taumaturgo TZB (2022a). *Dasyophthalma rusina* (Godart, [1824]), Apr/2018. Sistema de Avaliação do Risco de Extinção da Biodiversidade – SALVE. https://salve.icmbio.gov.br/salve/ and Freitas AVL, Brant A, Rosa AHB, Ribeiro DB, Barbosa EP, Dias FMS, Bizarro JMS, Prado UM, Cardoso MZ, Filho OJM, Taumaturgo TZB (2022b) *Dasyophthalma creusa* (Hübner, [1821]), Apr/2018. Sistema de Avaliação do Risco de Extinção da Biodiversidade – SALVE. https://salve.icmbio.gov.br/salve/). [a] = IBAMA 1989; [b] = MMA 2003; [c] = MMA 2014; [d] = MMA 2022; [e] = Unpubl. Data.

Table 6. Geographic range of *Dasyophthalma* species

Species	EOO (km^2)	AOO (km^2)
D. delanira	89.90	16
D. geraensis	2,336.36	40
D. vertebralis	30,556.44	24
D. creusa	662,297.69	408
D. rusina	917,612.14	224

EOO = Extent of occurrence; AOO = Area of occupancy.

zilian Red list were all based on the IUCN B criterion (geographical range), since there is no data on population trends for them. The changes in the conservation status of each three species over time are shown in Table 5.

In addition of being on the national Red List, these three species were/are also listed on several regional Red lists, such as for the state of Minas Gerais (COPAN 1996; Casagrande et al. 1998; COPAN 2010), Espírito Santo (Espírito Santo 2005; Azevedo et al. 2007; Fraga et al. 2019), Rio de Janeiro (SEMA 1998; Otero et al. 2000), and São Paulo (São Paulo 2008, 2010, 2014, 2018).

However, for at least two threatened species of *Dasyophthalma* the situation is optimistic, since several known populations are located inside protected areas (PAs) (IUCN Protected Area Management Categories I–VI (I–V = fully protected areas, and VI "Protected area with sustainable use of natural resources", see Dudley 2008)). For *D. delanira* **stat. rest.,** all four known localities of occurrence are inside three protected areas (two in two PAs VI category and one in a fully PA) and for *D. geraensis*, all 11 localities of occurrence are inside four protected areas (seven in one PA VI category and four in three fully PAs). For *D. vertebralis*, however, only one out of the six known localities of occurrence is inside a fully protected area (Table 1).

Based on geographic distribution records, the geographical ranges (EOO and AOO) were estimated and are presented in Table 6. Based only on the thresholds in km^2 of the IUCN "B" criterion (geographic range), *D. delanira* **stat. rest.** and *D. geraensis* present values of EOO that would keep them as threatened by the subcriterion "B1", the others present values greater than 30,000,00 km^2 and are not able to figure under "B1" (Table 6). However, the AOO threshold values would include all species under the sub-criterion "B2" (Table 6).

4. Discussion

4.1. Phylogeny and taxonomy

The present phylogeny recovered five main clades that exactly correspond to the ones obtained by Matos-Maraví et al. (2021) based also on molecular data. Accordingly, the "*Dasyophthalma* clade" corresponds to 'clade A' of Matos-Maravi et al. (2021), the "*Opoptera* clade" corresponds to 'clade B', the "*Caligo* clade" corresponds to 'clade C', the "*Opsiphanes* clade" corresponds to 'clade D' and the "*Brassolis* clade" corresponds to an unnamed clade including only the genus *Brassolis*. The main difference is the position of the "*Opoptera* clade"; in the present study this clade is the sister group of all remaining Brassolina, a position occupied by the "*Dasyophthalma* clade" (as 'clade A') in Matos-Maraví et al. (2021). The results are also somewhat congruent with those presented by Penz et al. (2013), based on morphology. In that study, three out of the five main clades have correspondence with the present study, namely, the "*Caligo* clade", the"*Brassolis* clade" and the "*Opsiphanes* clade"; the "*Dasyophthalma* clade" was not recovered, with *Dynastor* appearing as sister to *Brassolis* (Penz et al. 2013) and *Dasyophthalma* as sister to *Opoptera* in some analyses. Concerning the genus *Dasyophthalma*, the present results are virtually identical to those obtained in previous morphological and molecular studies, with two well supported clades (Penz 2009; Matos-Maravi et al. 2021), the "*creusa*-group" and the "*rusina*-group" (*sensu* Penz 2009). However, our study is the first to include samples of *D. delanira* **stat. rest.**

Penz (2009) presented a detailed description of the morphology of wings and genitalia of four species of

Dasyophthalma (except *D. delanira* **stat. rest.)** and the monophyly of the "*rusina*-group" was supported by seven morphological synapomorphies: (1) dorsal forewings and hindwings with a blue iridescence below discal cell and on the submedial area, respectively; (2) dorsal forewings with yellow scales located inside cell R5 with bifid edge; (3) dorsal forewings of males without yellow markings across costal margin; (4) dorsal hindwings of males with a brown hairpencil at base of discal cell; (5) hindwings with brown androconial patch on Rs-M1; (6) ventral hindwings with postmedial band with well-defined edges; and (7) a presence of a small midline extension on the posterior portion of sterigma. Six out of the seven above synapomorphies are observed in *Dasyophthalma delanira* **stat. rest.** (the seventh was not observed since no females of *D. delanira* **stat. rest.** were available for the present study).

In addition, *D. delanira* **stat. rest.** can be distinguished from the other two species of the "*rusina*-group" by the following morphological characters: (1) dorsal forewings of males with postmedial yellow band broader than in *D. geraensis* and in *D. rusina*; and (2) dorsal hindwings with postmedial band broader than in *D. rusina*, and not as yellowish as in *D. geraensis*. The overall morphology of male genitalia is very similar to all species of the genus, but the valva has a dorsal row of spines slightly different to those illustrated by Penz (2009). However, the number and shape of the spines present high intraspecific variability, and since a single male specimen of *D. delanira* **stat. rest.** was available to study, this character was not analyzed in further details.

4.2. Conservation

The genus *Dasyophthalma* can be considered of high conservation interest for three main reasons: (1) the genus is endemic to the Atlantic Forest, with very few records in riparian forests of the Cerrado savannas, (2) three out of the five described species are of conservation interest, and (3) these are large dayflying butterflies, with a large appeal to be used in citizen science. Accordingly, getting photographic records in social media or from butterfly watchers is relatively easy, since these large butterflies call attention when present (as can be seen in this work). The geographical data is abundant and covers possibly the actual geographic distribution of all five species, allowing adequate niche modelling (except maybe for *D. delanira* **stat. rest.**), including past and future scenarios. Moreover, most species in the genus present a strong potential to be used as models in studies of molecular population ecology and phylogeography in the Atlantic Forest.

However, as previously mentioned, there are few data on natural history for all species *Dasyophthalma* (except for the two more widespread species). A good start would be describing the early stages of all species in detail (including complementing data for *D. rusina* and *D. creusa*). As for other species of Brassolini, females easily oviposit in plastic bags with leaves of the host plants; this method resulted in dozens of fertilized eggs of *D. creusa* and *D. rusina* in previous attempts and larvae easily accepted leaves of the palm trees *Bactris*, *Astrocarium* and *Euterpe* (the records on bamboos need to be confirmed) (AVLF unpublished results). Accordingly, rearing the other three species relies only on obtaining wild-caught fertilized females.

An important action would be searching for additional localities for the three threatened species in the genus (as suggested in Freitas and Marini-Filho 2011) in order to improve the calculations of AOO and EOO. For *D. delanira* **stat. rest.**, candidate areas are all in the hilltops above 1300 m in the area between Nova Friburgo, Casimiro de Abreu and Cachoeiras de Macacu (in the state of Rio de Janeiro). For *D. geraensis*, the recent records in the "Serra do Papagaio" in Alagoa municipality and Baependi municipality, both localities in the state of Minas Gerais, suggest that this species could be more widespread through the west slopes of the Mantiqueira mountain range, in some more drier and seasonal areas. Finally, for *D. vertebralis* it is very important to find any extant population within the potential geographical range; based on museum specimens, this species was last collected in 1948 (more than 80 years ago) and since then no additional individual or photo/image has been reported so far. The Sooretama Biological Reserve is the first candidate area, since the forests are virtually the same as when the two females have been collected there in 1948. Other candidate areas in the state of Espírito Santo include the lowland forests in the center of the state, in the vicinities of Santa Teresa and Santa Leopoldina and Cariacica, and some of the last larger forest remnants in the west, in region of Castelo, São Vicente and Burarama.

5. Conclusion

As much biological information about the genus *Dasyophthalma* is lacking, the present work contributes with updated data on the phylogeny, taxonomy, geographic distribution and geographic range estimates for the genus. Therefore, by including molecular data for all five species of the genus, the present study confirms the monophyly of *Dasyophthalma* and the organization of its species in two clades. In addition, data on genetic divergence supported the decision of reinstating the specific status of *D. delanira* **stat. rest.**, as the sister species of *D. geraensis* and not as a subspecies of *D. rusina.* All five species of *Dasyophthalma* are endemic to the Atlantic Forest (with *D. rusina* also present in some riparian forests in areas of Cerrado savannas adjacent to the former domain), making this an excellent model group to be studied in terms of diversification in the Atlantic Forest. In addition, two out of the five species in the genus present narrow distribution ranges and are threatened (*D. delanira* **stat. rest.** and *D. geraensis*), with huge potential to be included in monitoring programs in the protected areas where they are present. In short, by combining most of the available information, the present study can serve as a starting point for future

studies on *Dasyophthlalma*, adding information that can be crucial for future conservation actions and essentially assuring the future of the threatened species in this genus.

6. Competing interests

The authors have declared that no competing interests exist.

7. Acknowledgments

We thank Mirna Casagrande, Eduardo Carneiro, Olaf Mielke for the access to material deposited in DZUP collection; Alexandre Soares for the access to material deposited in MNRJ collection; Márcio Félix, Felipe Moreira, and Claudia Rodrigues for the access to material deposited in CEIOC collection; Marcelo Duarte and Renato Silva for the access to material deposited in MZUSP collection; Thomas Pape for the access to material deposited in ZMUC; Tobias Malm for the access to material deposited in NHRS; To Carlos Guilherme Costa Mielke, Max Peters, Carlos Roberto Silva Silva and Jalmirez Silva that provided personal observation / and or photos of some live species in their habitats. To all people who provided data of *D. creusa* and *D. rusina* in iNaturalist website. To Alexandre Antonelli, Thomas Berg and RPPN Bacchus staff for sharing their place to our field work. To Junia Carrera and André Tacioli for their help in field work. To an anonymous reviewer and Dr. Ângelo Pinto for their valuable comments and suggestions to improve the manuscript; RRS was supported by FAPESP (2020/09595-0). EPB was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (2012/03750-8, 2016/15873-8 and 2018/21432-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (162673/2020-5). AHBR and PAM thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. PAM thanks Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (2021/13396-6). AVLF thanks (FAPESP) (BIOTA-FAPESP grants 2013/50297-0, 2021/03868-8) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (421248/2017-3, 304291/2020-0). This publication is part of the "RedeLep – Rede Nacional de Pesquisa e Conservação de Lepidópteros" – SISBIOTA-Brasil/CNPq (563332/2010-7). Butterfly species are registered in the SISGEN (A842AC0).

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Supplementary Material 1

Figure S1, Table S1

Authors: Rosa AHB, Barbosa EP, Machado PA, Siewert RR, Freitas AVL (2023)
Data type: .docx
Explanation note: Figure S1. Adults and habitat of *Dasyophthalma vertebralis* and *D. creusa* from Sooretama Biological Reserve (SBR), Sooretama, Espírito Santo state. **A**, **B** The two *D. vertebralis* female specimens from CEIOC collection; **C**, **D** two *D. creusa* female specimens from CEIOC collection; **E** closer view of *D. vertebralis* and *D. creusa* habitat on a road of SBR; **F** attractive traps in the interior of forest of SBR. — **Table S1**. Abundance data of *D. creusa* and *D. rusina*.

Link: https://doi.org/asp.81.e96397.suppl1

Supplementary Material 2

Concatenated dataset with aligned sequences

Authors: Rosa AHB, Barbosa EP, Machado PA, Siewert RR, Freitas AVL (2023)
Data type: .phy
Explanation note: Concatenated dataset with aligned sequences.

Link: https://doi.org/asp.81.e96397.suppl2