


ARTICLE

# Uncovering the Brazilian Orthocentrinae (Hymenoptera: Ichneumonidae) fauna: high diversity at high elevation

Luiza Figueiredo Camargo<sup>1\*</sup> , Eduardo Mitio Shimbori<sup>2</sup>, and Angélica Maria Pentead-Dias<sup>3</sup>

<sup>1</sup>Instituto de Biologia, Universidade Estadual de Campinas (IB-Unicamp), CP 6109, 13083-970 Campinas, São Paulo, Brazil,

<sup>2</sup>Departamento de Entomologia e Acarologia, Escola Superior de Agricultura “Luiz de Queiroz” Universidade de São Paulo, 13418-900 Piracicaba, São Paulo, Brazil, and <sup>3</sup>Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, CEP 13, 565-905, São Carlos, São Paulo, Brazil

\*Corresponding author. Email: [luiza.figueiredoc@gmail.com](mailto:luiza.figueiredoc@gmail.com)

(Received 11 August 2020; accepted 17 December 2021)

## Abstract

The Orthocentrinae is one of the least-studied groups within Ichneumonidae and possibly a major component of the tropical fauna, encompassing a rich but still obscure diversity. In this first attempt to uncover these biota in Brazil, we surveyed a restricted area within mountain chains in the southeastern region immersed in the Atlantic tropical forest, using several sampling methods along an elevational gradient. Using integrative taxonomy, we found a richness eight times higher than the current number of species known in Brazil, including 13 genera, of which 10 are new to the country. The 127 molecular taxonomic units found represent an increase of at least 50% in species for the Neotropical fauna, confirming the immense gap in taxonomic knowledge of this group in the Neotropics. Analyses of elevational gradient patterns of distribution indicated an increase in species elevational ranges with increasing elevation, supporting Rapoport's rule. A key including all 14 genera occurring in Brazil is proposed, aiming to facilitate future taxonomic reviews and encourage research on this group. Orthocentrinae is a major component of the fauna and likely to be more diverse in wet lowland tropical forests; however, taxonomic and faunistic studies in tropical areas, including dry forests and savannahs, are needed.

## Introduction

Orthocentrinae (Hymenoptera, Ichneumonidae) is a group of small-bodied, koinobiont endoparasitoids of fungus gnats (Diptera: Sciaroidea) (Broad *et al.* 2018). Some of their hosts are known to cause damage on orchid roots and shiitake crops, which might have their populations reduced by the action of these parasitoids (Humala *et al.* 2017; Mukai and Kitajima 2019). Despite recent efforts studying the diversity of this subfamily in the Neotropical region (Humala 2010, 2014, 2017, 2019; Veijalainen *et al.* 2012, 2014a; Zwickhals and Diller 2015; Humala and Ruiz-Cancino 2017; Camargo *et al.* 2020), a large taxonomic gap regarding this fauna remains, mainly as a result of a high diversity combined with small body size (Quicke 2012).

Orthocentrinae may represent one of the most abundant and species-rich groups of ichneumon wasps in moist tropical forests (Quicke 2015). For example, Orthocentrinae and Cryptinae were the two most abundant subfamilies in Amazonian Ecuador and Peru, with Orthocentrinae comprising 15–34% of the collected ichneumonids (Veijalainen *et al.* 2013), and in Central America, Orthocentrinae was third in abundance and fourth in species richness within

Subject editor: Alex Smith

© The Author(s), 2022. Published by Cambridge University Press on behalf of the Entomological Society of Canada.

Ichneumonidae subfamilies (Veijalainen *et al.* 2014b). The first publication highlighting the diversity of the Neotropical Orthocentrinae was a faunistic assessment in which 1549 specimens were analysed using morphological and molecular approaches, resulting in 177 morphospecies from Ecuador and Central America (Veijalainen *et al.* 2012).

Such studies are instrumental to unravelling the diversity of ichneumonids in the Neotropics, and they corroborate the perception that theories regarding latitudinal patterns of distribution (e.g., Owen and Owen 1974; Janzen 1981; Askew and Shaw 1986; Gauld 1986, 1987) depend on substantial accumulation of information from tropical regions to be relevant (Quicke 2012). In fact, recent studies on latitudinal patterns repeatedly show higher diversity in the tropics for most ichneumonids (Veijalainen *et al.* 2012, 2013; Timms *et al.* 2015; Shimizu *et al.* 2020). Local intensive faunistic assessments of the Ichneumonoidea along elevational gradients have been helpful in understanding diversity patterns in this group, which most commonly peaks at mid-elevations in tropical mountains (e.g., van Noort 2004; Peck *et al.* 2008; Veijalainen *et al.* 2014b), a pattern predicted by the “mid-domain effect” (Colwell and Lees 2000). High diversity in lowlands has also been reported (Sääksjärvi *et al.* 2004; Aguirre *et al.* 2018). Richness peaks at low elevations may be predicted by Rapoport’s rule, as a result of increasing range size with increasing elevations or latitudes (Stevens 1992). The rule was first formulated after observation of patterns of species distribution along latitudinal gradients (Stevens 1989) and later applied for elevational patterns (Stevens 1992). According to the rule, both for latitude and elevation, the lower bands would present higher species richness as a result of narrower ranges (explained by their physiological constrain) of the species evolving in warmer and more stable climate. Despite these general patterns, some degree of idiosyncrasy is expected, and richness peaks may vary depending on the group studied (e.g., Gauld 1988, 1991, 1997, 2000; Gauld *et al.* 2002; Aguirre *et al.* 2018).

The taxonomic history of the Orthocentrinae is complex, with several changes in classification and synonyms at genus and subfamily level. The genera that currently belong to Orthocentrinae include taxa previously classified as Microleptinae, Oxytorinae, and Helictinae (Townes 1971; Gauld 1984, 1991; Gupta 1988; Wahl 1990; Dasch 1992). Whether the Cyloceriinae would be better placed as a tribe within Orthocentrinae or as a separate subfamily continues to be debated (Broad *et al.* 2018). In fact, Klopstein *et al.* (2019) recently confirmed a close relationship between Cyloceriinae and Orthocentrinae, which form a clade including the Diacritinae. Klopstein *et al.* (2019) also transferred the genus *Hemiphanes* Förster from Orthocentrinae to Cryptinae.

Currently, the approximately 500 described Orthocentrinae species are distributed among 29 genera (Villemant *et al.* 2016; Yu *et al.* 2016; Klopstein *et al.* 2019) that are separated into two groups: the *Orthocentrus* genus group and the *Helictes* genus group (Broad *et al.* 2018). In the Neotropical region, 109 species in 17 genera are known; of these, only seven species in four genera have been recorded in Brazil (Veijalainen *et al.* 2012; Yu *et al.* 2016; Humala 2017, 2019; Humala and Ruiz-Cancino 2017; Camargo *et al.* 2020). Here, we present the first comprehensive survey of the Orthocentrinae in Brazil that focuses on high-elevation sites (for Brazilian standards), characterising the fauna at the genus level and estimating its diversity along an elevational gradient based on molecular operational taxonomic units inferred by DNA barcoding analysis. An illustrated identification key to the Brazilian genera of Orthocentrinae is provided.

## Material and methods

### Field sampling

Samples were taken for one week, during the rainy season of 2015 (between October and December), using sweep nets, light traps, Malaise traps, and Moericke traps, as part of a research project on elevational gradients in parasitoid wasp faunas. Sampling sites were

located in four conservation units in mountainous regions of the Brazilian Atlantic forest (Table 1): Reserva Privada do Patrimônio Cultural de Itamonte, Itamonte, Minas Gerais state (1400–2150 m); Reserva Biológica da Serra do Japi, Jundiá, São Paulo state (896–1190 m); Parque Nacional da Serra da Bocaina, São José do Barreiro, São Paulo state (1149–1970 m); and Parque Nacional do Itatiaia, Itatiaia, Rio de Janeiro state (786–2451 m). Additional material, collected with Moericke traps at Parque Estadual da Serra do Mar, São Luis do Paraitinga São Paulo state, was also examined. There, samples were taken during one week per month for one year between April 2012 and April 2013 at elevations ranging from 961 to 1004 m. A map (Fig. 1) with the study locations was generated using SimpleMapp (Shorthouse 2010).

### Identification

The material was identified to genus level using keys presented by Townes (1971), Dasch (1992), and Broad (2010). The morphological terminology follows Gauld (1991). Identification of *Pantisarthrus* Förster, 1871 at species level follows Camargo *et al.* (2020). The specimens were preserved in ethanol and dried using a critical point dryer (Leica EM-CPD030; Leica, Wetzlar, Germany). Digital scanning electron microscopy photographs were taken with a FEI Quanta 250 scanning electron microscope (Thermo Fisher Scientific, Waltham, Massachusetts, United States of America) in a low-vacuum mode of uncoated specimens. Colour digital photographs were taken with a Leica MC 170 high-definition video camera attached to a Leica M205C with a planapo 1.0× objective and edited using Adobe Photoshop (Adobe, San Jose, California, United States of America). The specimens are deposited at the DCBU Collection (Departamento de Ecologia e Biologia Evolutiva da Universidade Federal de São Carlos, campus São Carlos, Brazil). The genus *Sphingozona* Townes, 1971 was not collected, despite being known from Brazil. It was included in the key based on the original description (Townes 1971).

### Laboratory procedures and analysis

A total of 459 randomly selected specimens had whole-body genomic DNA extracted in a nondestructive method using the DNeasy Blood & Tissue Kit (QIAGEN Inc., Valencia, California, United States of America) and following the manufacturer's instructions. Subsequent DNA purification was performed by ethanol precipitation (Sambrook and Russell 2001), and then DNA extracts were resuspended in 50 mL of Tris–EDTA buffer. A cytochrome oxidase 1 (*COI*) mitochondrial DNA fragment belonging to the barcoding locus was amplified using the universal LCO 1490 and HCO 2198 primers (Folmer *et al.* 1994). Polymerase chain reaction was carried out in 25-μL final volume (2.5 mM MgCl<sub>2</sub>, 2.5 mM dNTP Mix, 0.2 M each primer, 1× HOT FIREPol Buffer B1, and 1 U HOT FIREPol DNA Polymerase (Solis Biodyne, Tartu, Estonia)) and followed a cycling process of initial denaturation at 95 °C for 15 minutes; 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 1 minute, and then a final extension at 72 °C for 5 minutes. Polymerase chain reaction products were purified by polyethylene glycol precipitation (Lis and Schleif 1975) and sequenced at Macrogen, Inc. (Teheran-ro, Gangnam-gu, Seoul, Republic of Korea). Sequences were edited using the programme Sequencher 4.14 and aligned using MAFFT, version 7 (<https://mafft.cbrc.jp/alignment/software/>), and the phylogenetic analysis was performed using the program Mega X (Kumar *et al.* 2018).

### Data analyses

A neighbour-joining tree using the bootstrap method with 500 replicates was reconstructed, and a genetic distances table was generated based on the *P*-distance model to cluster the

**Table 1.** Sampling sites, including geographic coordinates, elevations, and methods. Conservation units are abbreviated: PNI, Parque Nacional de Itatiaia; RBSJ, Reserva Biológica da Serra do Japi; PESM, Parque Estadual da Serra do Mar; RPPCI, Reserva Privada do Patrimônio Cultural de Itamonte; PNSB, Parque Nacional da Serra da Bocaina; followed by the state abbreviation: MG, Minas Gerais; RJ, Rio de Janeiro; SP, São Paulo.

Location	Geographic coordinates	Elevation (m)	Sampling methods
PNI, RJ	S 22° 27' 09" W 44° 36' 35"	781	Light; sweeping net
PNI, RJ	S 22° 27' 06" W 44° 36' 35"	786	Light; Moericke
PNI, RJ	S 22° 27' 08" W 44° 36' 35"	788	Light
PNI, RJ	S 22° 27' 04" W 44° 36' 40"	790	Moericke
PNI, RJ	S 22° 27' 03" W 44° 36' 40"	798	Light
PNI, RJ	S 22° 27' 11" W 44° 36' 26"	807	Moericke
PNI, RJ	S 22° 26' 55" W 44° 36' 40"	881	Malaise
RBSJ, SP	S 23° 13' 53" W 46° 55' 24"	895	Light; Malaise; Moericke
RBSJ, SP	S 23° 13' 47" W 46° 55' 23"	896	Malaise; sweeping net
RBSJ, SP	S 23° 13' 49" W 46° 55' 23"	896	Malaise
PNI, RJ	S 22° 26' 45" W 44° 36' 38"	921	Malaise
PNI, RJ	S 22° 26' 42" W 44° 36' 39"	934	Malaise
RBSJ, SP	S 23° 13' 53" W 46° 56' 05"	1036	Light
PESM, SP	S 23° 20' 37" W 45° 7' 47"	961	Moericke
PESM, SP	S 23° 20' 15" W 45° 6' 22"	1004	Moericke
RBSJ, SP	S 23° 14' 33" W 46° 56' 10"	1082	Malaise; Moericke; sweeping net
RBSJ, SP	S 23° 14' 30" W 46° 56' 14"	1134	Malaise
RBSJ, SP	S 23° 14' 32" W 46° 56' 12"	1136	Malaise
PNSB, SP	S 22° 46' 59" W 44° 36' 09"	1149	Malaise; Moericke; sweeping net
PNSB, SP	S 22° 47' 04" W 44° 36' 07"	1161	Malaise
RBSJ, SP	S 23° 13' 56" W 46° 56' 20"	1177	Light
RBSJ, SP	S 23° 13' 55" W 46° 56' 27"	1183	Moericke
PNSB, SP	S 22° 47' 05" W 44° 36' 09"	1184	Malaise; Moericke; sweeping net
RBSJ, SP	S 23° 13' 56" W 46° 56' 29"	1190	Malaise
RBSJ, SP	S 23° 13' 54" W 46° 56' 22"	1191	Malaise
RBSJ, SP	S 23° 13' 55" W 46° 56' 29"	1191	Malaise
PNI, RJ	S 22° 25' 41" W 44° 37' 34"	1389	Malaise; sweeping net
RPPCI, MG	S 22° 21' 13" W 44° 47' 49"	1399	Light; Moericke; Sweeping net
RPPCI, MG	S 22° 21' 12" W 44° 47' 47"	1403	Light
RPPCI, MG	S 22° 21' 11" W 44° 47' 55"	1412	Sweeping net; Moericke
RPPCI, MG	S 22° 21' 16" W 44° 47' 56"	1443	Malaise; Moericke
PNI, RJ	S 22° 25' 44" W 44° 37' 43"	1446	Malaise
PNSB, SP	S 22° 43' 57" W 44° 36' 58"	1446	Moericke
PNSB, SP	S 22° 44' 04" W 44° 36' 55"	1473	Moericke
PNSB, SP	S 22° 44' 22" W 44° 37' 01"	1480	Light

(Continued)

Table 1. (Continued)

Location	Geographic coordinates	Elevation (m)	Sampling methods
PNSB, SP	S 22° 44' 02" W 44° 37' 04"	1489	Malaise
PNSB, SP	S 22° 44' 02" W 44° 37' 03"	1495	Malaise
RPPCI, MG	S 22° 21' 29" W 44° 47' 63"	1498	Malaise
PNI, RJ	S 22° 25' 35" W 44° 37' 52"	1499	Malaise
PNSB, SP	S 22° 44' 01" W 44° 36' 56"	1504	Malaise
PNSB, SP	S 22° 44' 02" W 44° 36' 59"	1504	Light
PNSB, SP	S 22° 43' 59" W 44° 36' 59"	1528	Light
RPPCI, MG	S 22° 21' 35" W 44° 47' 57"	1605	Malaise
RPPCI, MG	S 22° 21' 46" W 44° 48' 01"	1612	Malaise
RPPCI, MG	S 22° 21' 51" W 44° 48' 00"	1637	Malaise
RPPCI, MG	S 22° 21' 52" W 44° 48' 02"	1652	Malaise
PNSB, SP	S 22° 45' 17" W 44° 38' 36"	1943	Malaise
PNSB, SP	S 22° 45' 16" W 44° 38' 35"	1961	Malaise
PNSB, SP	S 22° 45' 12" W 44° 38' 32"	1969	Malaise
PNSB, SP	S 22° 45' 12" W 44° 38' 32"	1969	Sweeping net
RPPCI, MG	S 22° 22' 24" W 44° 48' 56"	2131	Malaise
RPPCI, MG	S 22° 22' 26" W 44° 48' 59"	2133	Malaise
RPPCI, MG	S 22° 22' 25" W 44° 48' 41"	2149	Malaise
PNI, RJ	S 22° 22' 25" W 44° 42' 08"	2449	Malaise
PNI, RJ	S 22° 22' 28" W 44° 42' 01"	2449	Malaise
PNI, RJ	S 22° 22' 24" W 44° 42' 14"	2451	Malaise

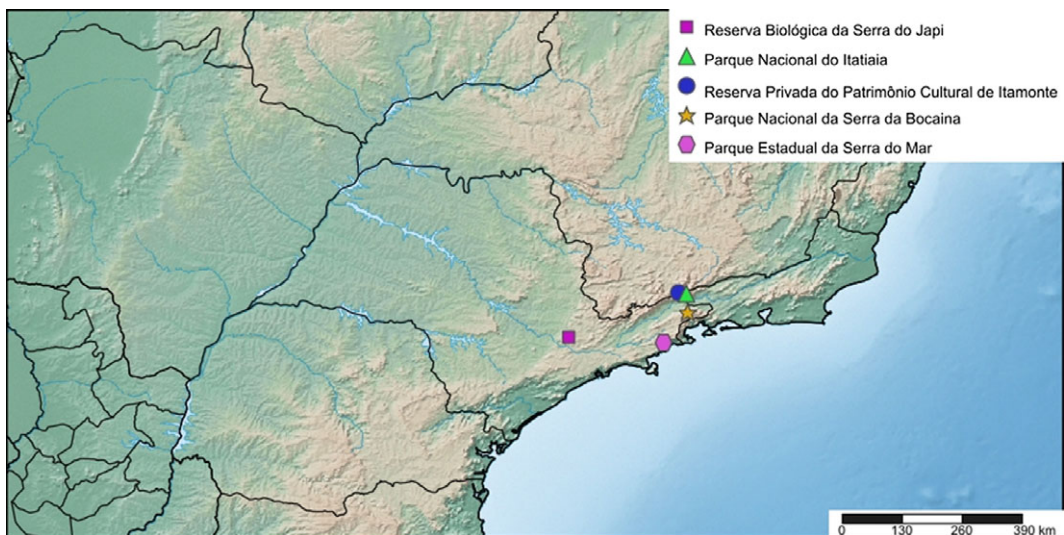


Fig. 1. Map with locations of the five collection sites sampled in this study.



specimens according to their molecular operational taxonomic units. The clustering respected the boundaries at a sequence divergence of 2%, which found high concordance with the final species delimitation in previous studies (Smith *et al.* 2008; Fernandez-Triana *et al.* 2014; van Achterberg *et al.* 2014; Sharkey *et al.* 2015, 2018; Tucker *et al.* 2015; Sharkey and Chapman 2016; Kang *et al.* 2017; Meierotto *et al.* 2019). Sequences are deposited at the National Center for Biotechnology Information, with the following GenBank accession numbers: MT784201–MT784659, and at the Barcode of Life Database, with process ID DEBE001-21–DEBE459-21.

Sample-based species rarefaction curves and estimates of species richness (Chao 1, Jackknife 2, and ACE) were calculated using EstimateS 9.1 for Windows (Gotelli and Colwell 2011; Colwell 2013). Trends in species richness were analysed using the molecular operational taxonomic units randomly pooled from the Orthocentrinae (459 specimens from 1566 total). Sampling sites were grouped into six 300-m elevation bands (700–1000, 1000–1300, 1300–1600, 1600–1900, 1900–2200, and 2200–2500). To evaluate Rapoport's rule, the method developed by Stevens (1992) was applied using the elevational range of each species to calculate the mean elevational range of all species present in each band. The mean elevational range for each band was regressed against elevation – that is, the midpoint for each of the six elevation bands. Steven's method suffers from spatial nonindependence, as pointed out by Colwell and Hurr (1994), but the overall performance is similar to the quartile test designed to correct for these errors (McCain and Knight 2013). The midpoint method (Rohde *et al.* 1993; Colwell and Hurr 1994), which also corrects for spatial nonindependence but depends on large samples, was not used because our samples are restricted to one week.

## Results

### Taxonomy

#### *Aperileptus* Förster, 1869

Type species: *Plectiscus albipalpus* Gravenhorst, 1829.

**Material examined.** One specimen from Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (960 m).

**Biology.** *Aperileptus* species have been reported to parasitise *Mycetophila alea* Laffoon, 1965, *Mycetophila evanida* Lastovka, 1972, and *Exechia bicincta* (Staeger, 1840) (Diptera: Mycetophilidae) (Šedivý and Ševčík 2003).

**Distribution.** Eighteen species are described from the Nearctic, Palearctic, and Afrotropical regions (Yu *et al.* 2016). The genus is recorded for the first time in Brazil and for the second time in the Neotropics (Veijalainen *et al.* 2012), although no Neotropical species have yet been described.

#### *Batakamacrus* Kolarov, 1986

Type species: *Batakamacrus crassicaudatus* Kolarov, 1986.

**Material examined.** Thirty-eight specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–2149 m); 15 specimens from Reserva Biológica da Serra do Japi, Jundiá, São Paulo state, Brazil (895–1134 m); nine specimens from Parque Nacional da Serra da Bocaina, São José do Barreiro, São Paulo state, Brazil (1446–1969 m); four specimens from Parque Nacional de Itatiaia, Rio de Janeiro state, Brazil (786–1652 m).

**Biology.** Unknown

**Distribution.** Eight species are described, of which seven are Palearctic (Yu *et al.* 2016). The only Neotropical species, *Batakamacrus hidalgo* Humala, 2010, is from Mexico (Humala 2010). This is the first record of the genus in Brazil despite the apparent commonness of the genus.

**Comments.** The identification of the genus is difficult, and no autapomorphic characters define *Batakamacrus* (Broad 2010). Male specimens previously identified as *Picrostigeus* Förster, 1869 based on the parameres (apex level with the apex of the metasomal tergites, following Broad (2010)) were clustered by DNA barcoding with females identified as *Batakamacrus*, based mainly on the ovipositors, which are very distinctive in both genera.

This result indicates that males from the Neotropics have more variable genitalia than are reported from the Palearctic fauna and could not be reliably identified using morphology, based on current knowledge. Therefore, a revision including the Neotropical fauna is recommended.

### **Catstenus Förster, 1869**

Type species: *Catstenus femoralis* Förster, 1871.

**Material examined.** One specimen from Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (960 m).

**Biology.** Unknown

**Distribution.** Four species are described in this genus, recorded from the Palearctic, Nearctic, Afrotropical, and Neotropical regions (Yu *et al.* 2016; Watanabe 2019). The only Neotropical species is *Catstenus rufithoracicus* (Cresson, 1868), from Cuba (Yu *et al.* 2016). This is the first record of the genus in Brazil.

### **Chilocyrtus Townes, 1971**

Type species: *Chilocyrtus carinatus* Townes, 1971.

**Material examined.** Four specimens from Reserva Biológica da Serra do Japi, Jundiaí, São Paulo state, Brazil (895–1134 m); 37 specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–2149 m); five specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1149–1969 m); two specimens from Parque Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–1652 m); 75 specimens from Reserva Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (906–1004 m).

**Biology.** Unknown.

**Distribution.** Two species are described: *C. carinatus* Townes, 1971 from the Neotropical region, including Brazil, and *C. hortorum* (Seyrig, 1934) from the Afrotropical region (Yu *et al.* 2016).

### **Eusterinx Förster, 1869**

Type species: *Eusterinx oligomera* Förster, 1871.

**Material examined.** Thirteen specimens from Parque Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–934 m); 15 specimens from Parque Nacional da Serra da Bocaina, São José do Barreiro, São Paulo state, Brazil (1149–1969 m); 30 specimens from Reserva Biológica da Serra do Japi, Jundiaí, São Paulo state, Brazil (890–1190 m); one specimen from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1443 m); five specimens from Parque Estadual Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (960–1002 m).

**Biology.** *Eusterinx* species are known to parasitise Mycetophilidae (Diptera) (Dasch 1992).

**Distribution.** The genus is distributed in the Palearctic, Nearctic, Oriental, and Neotropical regions (Yu *et al.* 2016). There are three Neotropical species: *Eusterinx (Eusterinx) solida* Dasch, 1992, *Eusterinx (Divinatrix) longipes* Humala and Ruiz-Cancino, 2017, and *Eusterinx (Ischyracis) tenuiventris* Humala and Ruiz-Cancino, 2017 (Humala and Ruiz-Cancino 2017). This is the first record of the genus in Brazil.

### **Gnathochorisis Förster, 1869**

Type species: *Gnathochorisis flavipes* Förster, 1871.

**Material examined.** Seven specimens from Reserva Biológica da Serra do Japi, Jundiaí, São Paulo state, Brazil (895–1134 m); 71 specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–1652 m); 10 specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1149–1969 m); 10 specimens from Parque Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–2451 m); six specimens from Reserva Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil.

**Biology.** *Gnathochoris flavipes* is a parasitoid of *Neoempheria striata* (Meigen, 1818) (Diptera: Mycetophilidae) (Humala 2003, 2017).

**Distribution.** The genus is known to occur in the Palearctic, Nearctic, Afrotropical, and Neotropical regions, with nine Neotropical species, none of which are recorded in Brazil (Yu *et al.* 2016; Humala 2017; Watanabe 2020; Varga 2021).

### ***Megastylus* Schiødte, 1838**

Type species: *Megastylus cruentator* Schiødte, 1838.

**Material examined.** Eighty-six specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–2149 m); 15 specimens from Reserva Biológica da Serra do Japi, Jundiaí, São Paulo state, Brazil (895–1190 m); 13 specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1149–1969 m); five specimens from Parque Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–1652 m); eight specimens from Reserva Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (940–1002 m).

**Biology.** The species with known biology are solitary koinobiont endoparasitoids of Keroplatidae (Diptera) larvae (Humala *et al.* 2017). *Megastylus woelkei* Humala, 2017 was observed parasitising Keroplatidae (Diptera) larvae in orchid roots (Humala *et al.* 2017).

**Distribution.** Worldwide, 41 species are described, and three of these are from the Neotropics: *Megastylus (Megastylus) panamensis* Wahl, 1997, *Megastylus (Dicolus) kaspanyani* Humala, 2014, and *Megastylus (Dicolus) pectoralis* (Förster, 1871) (Yu *et al.* 2016; Zhang *et al.* 2022). This is the first record of the genus from Brazil.

### ***Orthocentrus* Gravenhorst, 1829**

Type species: *Orthocentrus anomalus* Gravenhorst, 1829.

**Material examined.** Fifty-six specimens from Reserva Biológica da Serra do Japi, Jundiaí, São Paulo state, Brazil (895–1190 m); 260 specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–2149 m); 31 specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1149–1969 m); 40 specimens from Parque Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–2451 m); 124 specimens from Reserva Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (906–1004 m).

**Biology.** An *Orthocentrus* species was recorded emerging from the host cocoon of *Sciophila* Meigen, 1818 (Diptera: Mycetophilidae) (Šedivý and Ševčík 2003). Mukai and Kitajima (2019) reported *Orthocentrus* sp. parasitising *Neoempheria* Osten Sacken, 1878 (Diptera: Mycetophilidae) on shiitake mushrooms, providing an effective biological control of *Neoempheria carinata* Sueyoshi, 2014.

**Distribution.** There are 154 described species for all regions. In the Neotropical region, 79 species are reported, but only two of these are recorded from Brazil (Veijalainen *et al.* 2014a; Zwakhals and Diller 2015; Humala 2019).

### ***Pantisarthrus* Förster, 1871**

Type species *Pantisarthrus inaequalis* Förster, 1871.

**Material examined.** Thirty specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1412–1605 m); two specimens from Reserva Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (961–1004 m).

**Biology.** *Pantisarthrus lubricus* (Förster, 1871) is a parasitoid of *Phronia strenua* (Diptera: Mycetophilidae) (Roman, 1923), and *P. luridus* Förster, 1871 is a parasitoid of *Cordyla nitidula* Edwards, 1925 and *Cordyla fusca* Meigen, 1804 (Diptera: Mycetophilidae) (Šedivý and Ševčík 2003).

**Distribution.** The genus occurs in the eastern and western Palearctic, Nearctic, Oceanic (Mascarenes Island), and Neotropical regions (Yu *et al.* 2016). Three species are known in Brazil: *P. communis* Camargo and Pentead-Dias, 2020, *P. flavocingulatus* Camargo and Pentead-Dias, 2020, and *P. paraitinga* Camargo and Pentead-Dias, 2020 (Camargo *et al.* 2020).



**Comments.** The *Pantisarthrus* molecular operational taxonomic unit was identified as *Pantisarthrus flavocingulatus* Camargo and Pentead-Dias, 2020 (Camargo *et al.* 2020).

### ***Plectiscus* Gravenhorst, 1829**

Type species: *Plectiscus impurator* Gravenhorst, 1829.

**Material examined.** Twenty-eight specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–2149 m); one specimen from Reserva Biológica da Serra do Japi, Jundiá, São Paulo state, Brazil (1036–1134 m); six specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1446–1969 m); four specimens from Reserva Nacional do Itatiaia, Rio de Janeiro state, Brazil (1443–2451 m); 51 specimens from Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (940–1003 m).

**Biology.** Two *Plectiscus* species were recorded emerging from the pupae of *Bradysia giraudii* (Egger, 1862) (Diptera: Sciaridae) (Broad *et al.* 2018).

**Distribution.** Eleven species are described from the Afrotropical, Nearctic, and Palearctic regions (Yu *et al.* 2016). Even though this genus is known to occur in the Neotropics (Veijalainen *et al.* 2012), to date, no species are described from this region. This is the first record from Brazil.

### ***Proclitus* Förster, 1869**

Type species: *Proclitus grandis* Förster, 1871.

**Material examined.** Nine specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–1606 m); one specimen from Reserva Biológica da Serra do Japi, Jundiá, São Paulo state, Brazil (1183–1190 m); six specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1446–1528 m); seven specimens from Reserva Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–2451 m); 95 specimens from Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (934–1002 m).

**Biology.** *Proclitus* species are known to parasitise *Cordyla fusca* Meigen, 1804, *Mycetophila finlandica* Edwards, 1913, and *Allodia zaitzevi* Kurina, 1998 (Diptera: Mycetophilidae) (Šedivý and Ševčík 2003).

**Distribution.** The genus is found worldwide. Two species are described from the Neotropical region: *Proclitus floridanus* Dasch, 1992 and *P. fulvicornis* Förster, 1871, neither of which occurs in Brazil (Yu *et al.* 2016).

### ***Stenomacrus* Förster, 1869**

Type species: *Orthocentrus silvaticus* (Holmgren, 1858).

**Material examined.** There are 220 specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–2149 m); 54 specimens from Reserva Biológica da Serra do Japi, Jundiá, São Paulo state, Brazil (895–1190 m); 32 specimens from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1149–1969 m); 19 specimens from Reserva Nacional do Itatiaia, Rio de Janeiro state, Brazil (786–1652 m); six specimens from Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (934–1002 m).

**Biology.** *Stenomacrus meijeri* Humala, 2020 was reported rearing from *Bradysia impatiens* (Johannsen, 1912) (Diptera: Sciaridae) (Woelke *et al.* 2020), and *Stenomacrus curvulus* (Thomson, 1897) was reported from *Trichosia sinuata* Menzel and Mohrig, 1997 (Diptera: Sciaridae) (Vilkamaa and Komonen 2001).

**Distribution.** The genus has 74 described species distributed in the Palearctic, Nearctic, Oceanic, Australasian, and Neotropical regions (Yu *et al.* 2016; Woelke *et al.* 2020). The single Neotropical species, *Stenomacrus meridionalis* Ashmead, 1900, is known only from the

type locality in the Caribbean (Yu *et al.* 2016). This is one of the most common genera found in our samples. Nevertheless, this is the first record of *Stenomacrus* in Brazil.

### **Symplecis Förster, 1869**

Type species: *Symplecis alpicola* Förster, 1871.

**Material examined.** Fourteen specimens from Reserva Privada do Patrimônio Cultural de Itamonte, Minas Gerais state, Brazil (1399–1606 m); one specimen from Reserva Nacional da Serra Bocaina, São José do Barreiro, São Paulo state, Brazil (1446 m); four specimens from Parque Estadual da Serra do Mar, São Luiz do Paraitinga, São Paulo state, Brazil (934–1002 m).

**Biology.** *Symplecis breviscula* Roman, 1923 is a parasitoid of *Diadocidia ferruginosa* Meigen (Diptera: Diadocidiidae), and *Symplecis matilei* Delobel, 1975 parasitises *Neoempheria ombrofila*, Matile, 1975 (Diptera: Mycetophilidae) (Delobel and Matile 1975; Humala *et al.* 2016).

**Distribution.** The genus has a worldwide distribution, with 16 described species. There is only one Neotropical species, *Symplecis laticinta* (Cresson, 1868), from Mexico (Yu *et al.* 2016). This is the first record from Brazil.

### **Key to Brazilian genera of Orthocentrinae (based on Townes 1971, Dasch 1992, and Broad 2010)**

1. Clypeus not separated from face by a groove, making the face uniformly convex (Fig. 2A).... 2
  - Face not uniformly convex, clypeus evident, separated from face by a groove (Fig. 2B)..... 6
2. Mandibles hardly overlapping, labrum never exposed; vein cu-a on hind wing mostly reclined, sometimes vertical; ovipositor short, never surpassing the metasoma apex (Fig. 2A, C) ..... **Orthocentrus**
  - Mandibles overlapping when closed, labrum exposed; vein cu-a on hind wing inclivous or vertical; ovipositor variable ..... 3
3. Epicnemial carina absent; ovipositor short and straight (Fig. 2D) ..... **Plectiscus**
  - Epicnemial carina present, ovipositor variable (Fig. 2E) ..... 4
4. Mandibles with lower tooth present and visible in antero-ventral view; with the following combination of characters: vein cu1 on hind wing present; female with ovipositor not so long, upcurved, ovipositor sheaths, and metasoma capable of rotational movements; male with apex of parameres usually before metasoma apex, metasoma elongate, T3 usually longer than T2 (Figs. 3A, 4A, B) ..... **Batakmacrus**
  - Mandibles rotated, if lower tooth present, it is located on the inner face of the mandible; without the combination of characters above (Fig. 3B, C) ..... 5
5. Clypeus with a transverse convexity on ridge and with apex incurved in a concave way, propodeum without median longitudinal carinae (Figs. 3C, 4C) ..... **Chilocyrtus**
  - Clypeus without a transverse convexity on ridge and with apex incurved but not concave, flat; propodeum usually with median longitudinal carinae (Figs. 3B, 4D) ..... **Stenomacrus**
6. Sternite of first metasomal segment separated from tergite, usually with posterior end at or in front of spiracle; glymma present (Fig. 4E) ..... 7
  - Sternite of first metasomal segment fused to tergite, usually with posterior end at or behind spiracle; glymma absent (Fig. 4F) ..... 8
7. Propodeum only with posterior portion of lateral longitudinal carinae; ovipositor long, 1.45–2.55 × hind tibia length (Fig. 4G, H) ..... **Aperileptus**
  - Propodeum with more complete carination, only anterior transverse carina absent, and median longitudinal is present only in some species; ovipositor short, 0.16–0.5 × length of hind tibia (Fig. 5A, B) ..... **Pantisarthrus**

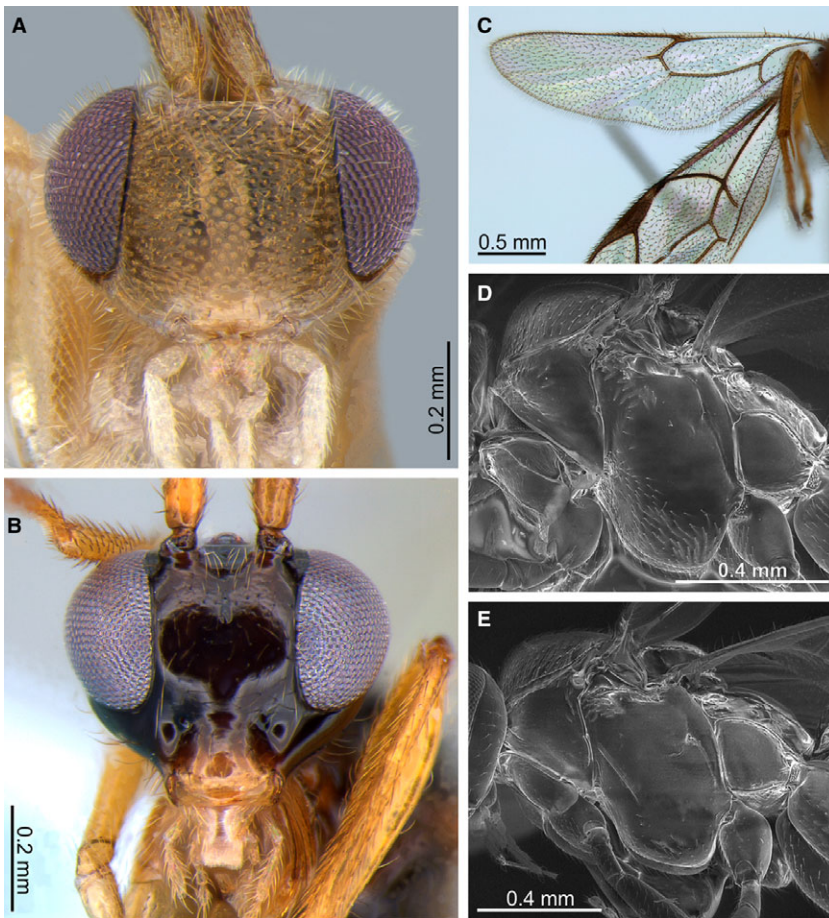


Fig. 2. **A**, *Orthocentrus* sp. head, anterior view; **B**, *Pantisarthrus communis* (Camargo et al. 2020), head, anterior view; **C**, *Orthocentrus* sp. posterior wing; **D**, *Plectiscus* sp. mesosoma, lateral view; **E**, *Chilocyrtus* sp. mesosoma lateral view.

- 8. Epipleura of second and third metasomal tergites not separated from terga by a crease; with the following combination of characters: areolet absent; vein cu1 of hind wing absent; antenna with very long flagellum; clypeus foveae as impressed areas, not open; propodeum with a deep impression towards the anterior and a prolonged anterior section

**Sphingozona**

- Epipleura of second and in some cases third metasomal tergites separated from terga by a crease; without the combination of characters above (Fig. 5C) ..... 9
- 9. Scape inflated, hind margin membranous; areolet never present; ovipositor short (Fig. 5D, E)..... **Megastylus**
- Scape variable, but not inflated and without hind margin membranous; areolet present or absent; ovipositor variable..... 10
- 10. Mandibles twisted, only one tooth visible; with the following combination of characters: antennae with 14–24 segments; vein cu1 of hind wing absent; ovipositor 0.5–1.25 × hind tibia length (Fig. 6A)..... **Eusterinx**
- Mandibles not twisted, both teeth visible; without the combination above..... 11
- 11. Lateral margins of face subparallel (Fig. 6B)..... 12

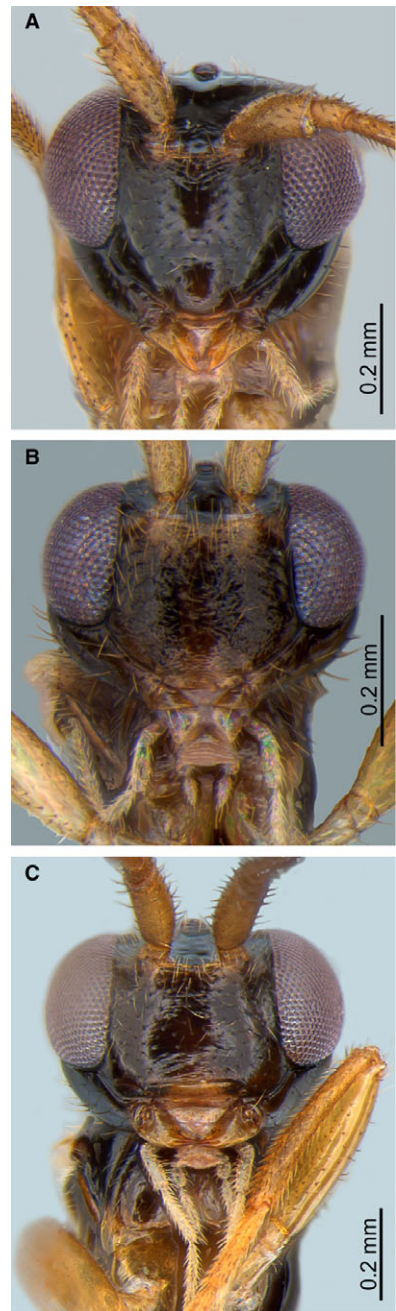


Fig. 3. **A**, *Batakomacrus* sp. face, anterior view; **B**, *Stenomacrus* sp. face, anterior view; **C**, *Chilocyrtus* sp. face, anterior view.

- Lateral margins of face convergent ventrally (Fig. 6C) ..... 13
- 12. Areolet absent; vein cu1 of hind wing weak; clypeus narrow to moderately long, usually wide; ovipositor  $1.2\text{--}3.7 \times$  hind tibia length, not upcurved (Fig. 7A) ..... **Proclitus**
- Areolet present; vein cu1 of hind wing strong; clypeus small, width  $1.3\text{--}2.25 \times$  height; ovipositor  $0.65\text{--}1.10 \times$  hind tibia length, upcurved with a subapical notch (Fig. 7B) ..... **Gnathochoris**



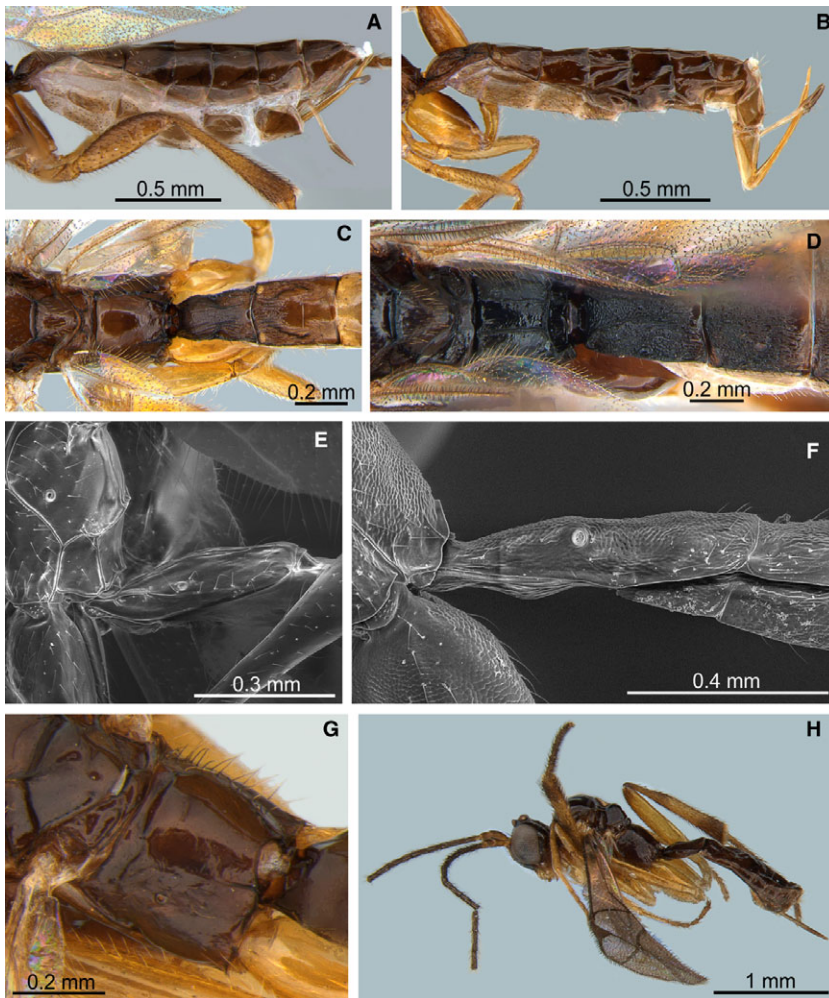


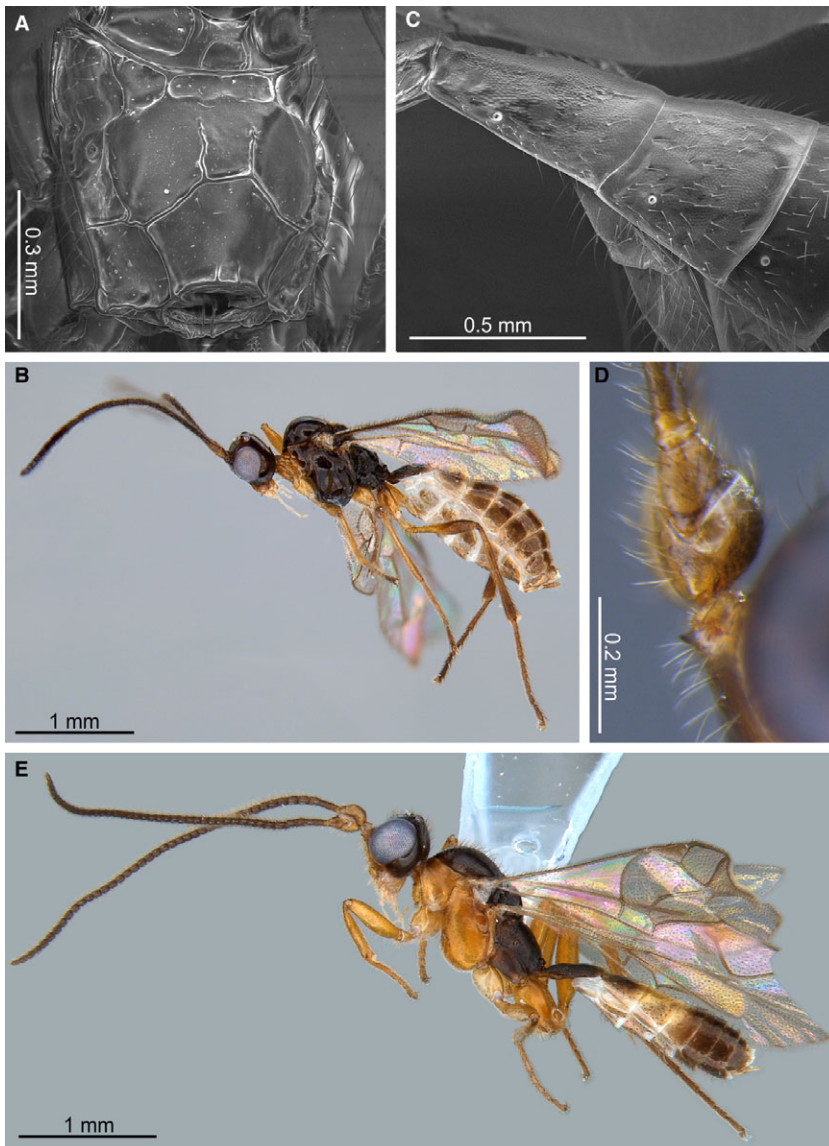
Fig. 4. A, B, *Batakomacrus* sp. metasoma, lateral view; C, *Chilocyrtus* sp. propodeum, dorsal view; D, *Stenomacrus* sp. propodeum dorsal view; E, *Pantisarthrus communis* Camargo et al., 2020, first metasomal tergite, lateral view; F, *Megastylus* sp. first metasomal tergite, lateral view; G, *Aperileptus* sp. propodeum, dorsal view; H, *Aperileptus* sp. female habitus.

- 13. Ovipositor  $0.65\text{--}1.75 \times$  hind tibia length; hind tarsal claws small; hind femur length  $4.0\text{--}6.10 \times$  height (Fig. 7C)..... **Symplecis**
- . Ovipositor  $0.4\text{--}0.6 \times$  hind tibia length; hind tarsal claws exceptionally large; hind femur length  $3.45\text{--}4.0 \times$  height (Fig. 7D, E) ..... **Catastenus**

**Faunistic analyses**

The 1566 specimens of Orthocentrinae collected in our samples were identified to 13 genera (Fig. 8), of which only three were previously recorded in Brazil. These are *Chilocyrtus*, *Orthocentrus*, and *Pantisarthrus* (Townes 1971; Veijalainen et al. 2014a; Camargo et al. 2020). The genera *Aperileptus*, *Batakomacrus*, *Catastenus*, *Eusterinx*, *Gnathochoris*, *Megastylus*, *Plectiscus*, *Proclitus*, *Stenomacrus*, and *Symplecis* are reported here for the first time from





**Fig. 5.** **A**, *Pantisarthus paraitinga* (Camargo *et al.* 2020), propodeum, dorsal view; **B**, *Pantisarthus communis* (Camargo *et al.*, 2020), female habitus; **C**, *Megastylus* sp. metasomal tergites 2 and 3, dorsal view; **D**, *Megastylus* sp. scape; **E**, *Megastylus* sp. female habitus.

Brazil. The most common genera sampled in this survey were *Orthocentrus* and *Stenomacrus*. Most of the Neotropical genera were represented in our samples, except for *Apoclima* Förster, *Helictes* Haliday, *Plectiscidea* Viereck, and *Sphingozona* Townes. To date, only *Sphingozona* has been previously recorded in Brazil.

Our samples covered an elevational range of 1665 m, ranging from 786 to 2451 m above sea level. The abundance of each genus, divided into six 300-m elevational bands, is shown in Figure 8. Most genera occurred throughout the entire elevational range of the study, except for the highest elevational band, where only nine specimens in four genera were collected and the most common genera were *Gnathochoris* and *Plectiscus*. The genera *Aperileptus* and *Catastenus* were restricted



Fig. 6. **A**, *Eusterinx* sp. head, anterior view; **B**, *Proclitus* sp. face, anterior view; **C**, *Symplecis* sp. face, anterior view.

to the lowest band (700–1000 m), and another two genera – *Pantisarthrus* and *Symplecis* – were not observed above 1900 m.

In the molecular analysis, 459 specimens from 10 genera were included and were found to be clustered in 127 molecular operational taxonomic units (Fig. 9). Because the molecularly analysed specimens represent roughly one-third of the collected specimens, the actual diversity should be much higher. Our results represent a subsample of the local fauna, and estimations of species richness resulted in nearly twice the observed richness for the 54 samplings (Fig. 10), meaning at least 197 species might occur in the studied area.

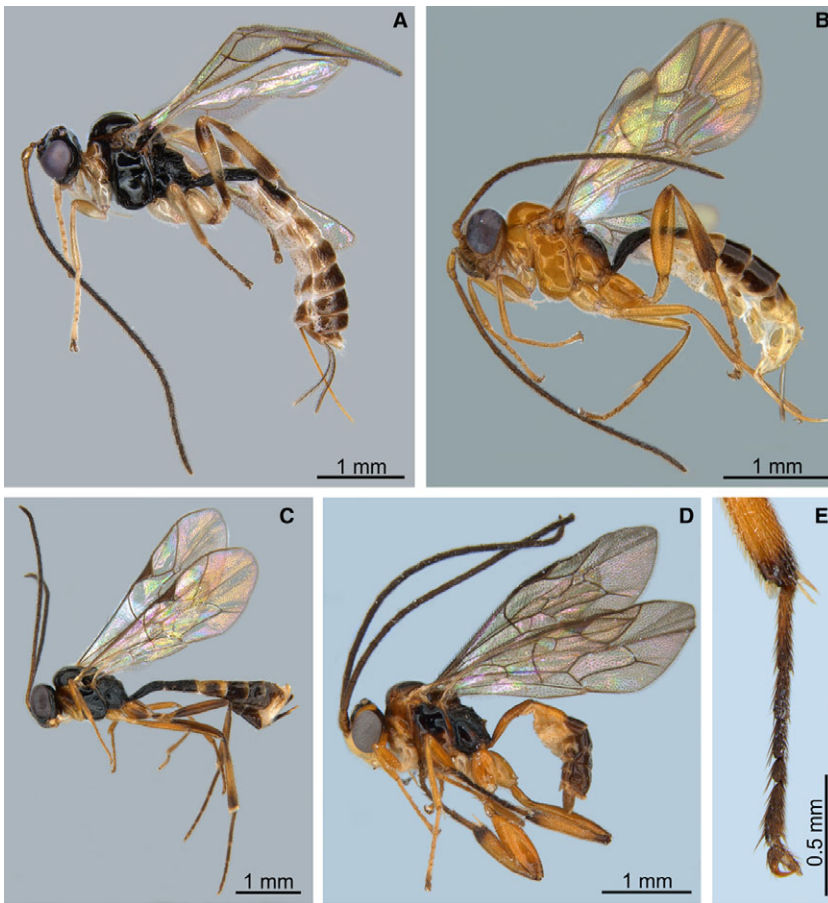


Fig. 7. **A**, *Proclitus* sp. female habitus; **B**, *Gnathochoris* sp. female habitus; **C**, *Symplecis* sp. female habitus; **D**, *Catastenus* sp. male habitus; **E**, *Catastenus* sp. hind tarsal claw.

The elevational range for the molecular operational taxonomic units varied from sea level to 1240 m, with a mean elevational range of  $241.61 \pm 79.87$  m ( $1 - \alpha = 95\%$ ). As observed in the elevational distribution for genera, diversity dropped steeply at the highest elevational band (2200–2500 m; Fig. 11), in part due to a reduced sampling effort (only one Malaise trap; Table 1). The absence of *Eusterinx* above 1300 m and of *Plectiscus* below 1300 m is clearly an artefact of subsampling because both genera are recorded from the lowest band up to at least 2200 m. The elevational range size of the species was positively correlated with elevation, according to our linear regression using the Stevens's (1992) method ( $n = 06$ ,  $r^2 = 0.78$ ,  $P = 0.02$ ; Fig. 12).

## Discussion

The observed species richness of Orthocentrinae is based on molecular operational taxonomic units inferred from less than one-third of the 1566 specimens collected during a one-week survey in a small region of Brazil. We found at least 55 new species in 10 genera, meaning a potential increase of 50% in species richness for the Neotropical Orthocentrinae, compared with the 109 described species, and nearly eight times the number of species in Brazil (Fig. 9). Estimates confirm that this number represents only a fraction of the local fauna (Fig. 10) and, thus,

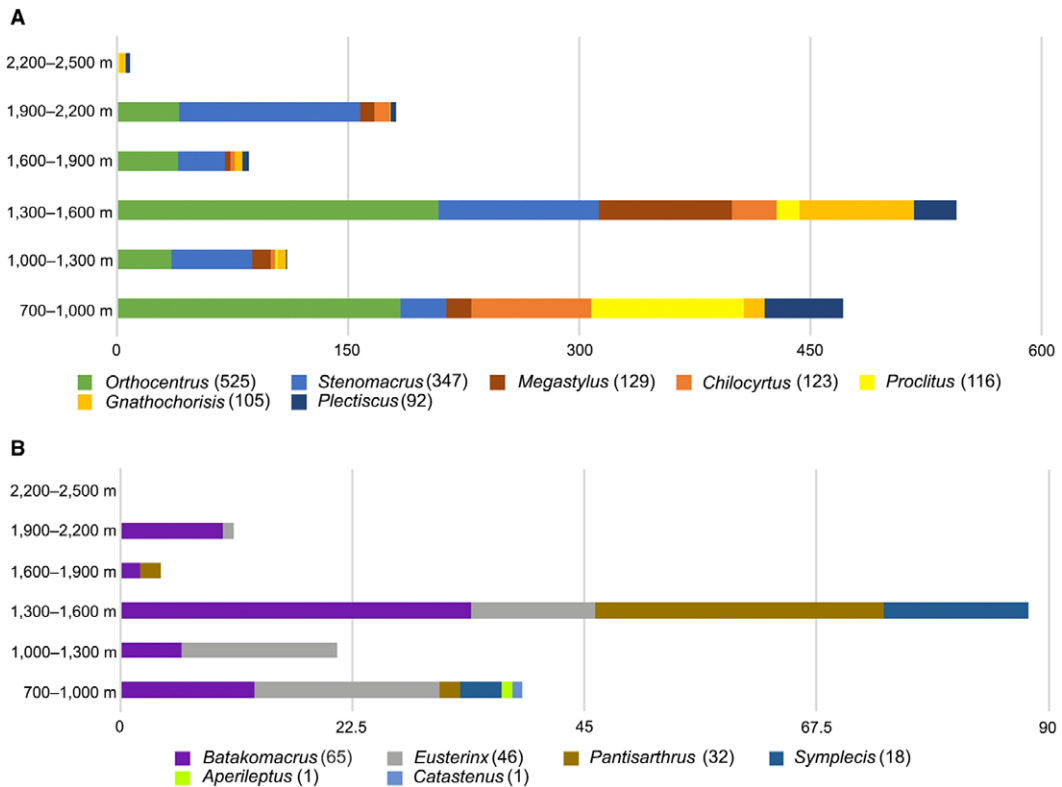
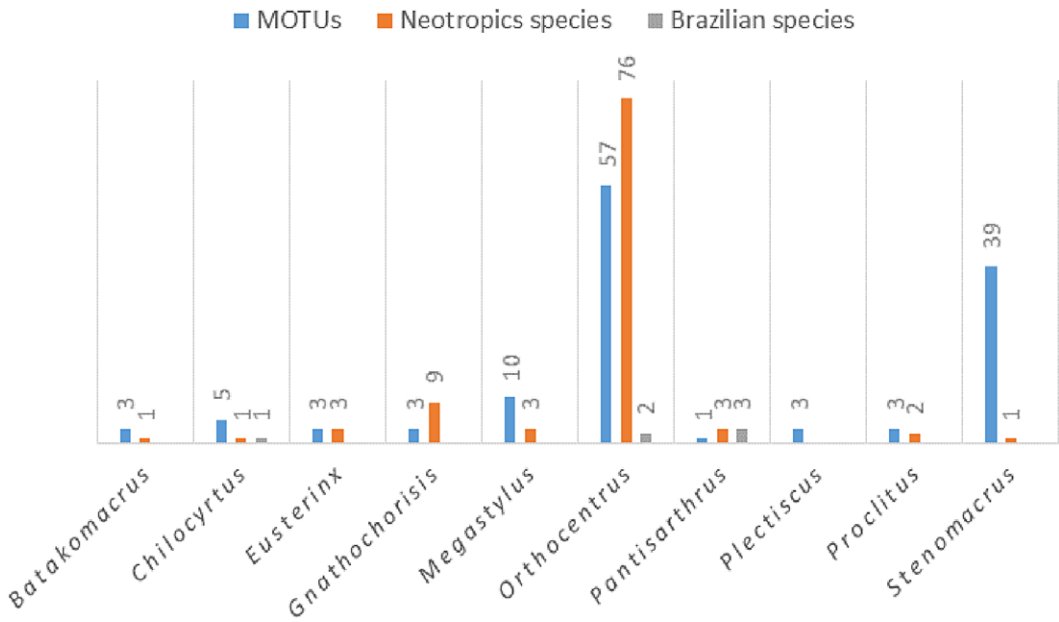


Fig. 8. Abundance of Orthocentrinae genera in the 300-m elevational bands in Brazilian mountains: **A**, most abundant genera; **B**, least abundant genera.

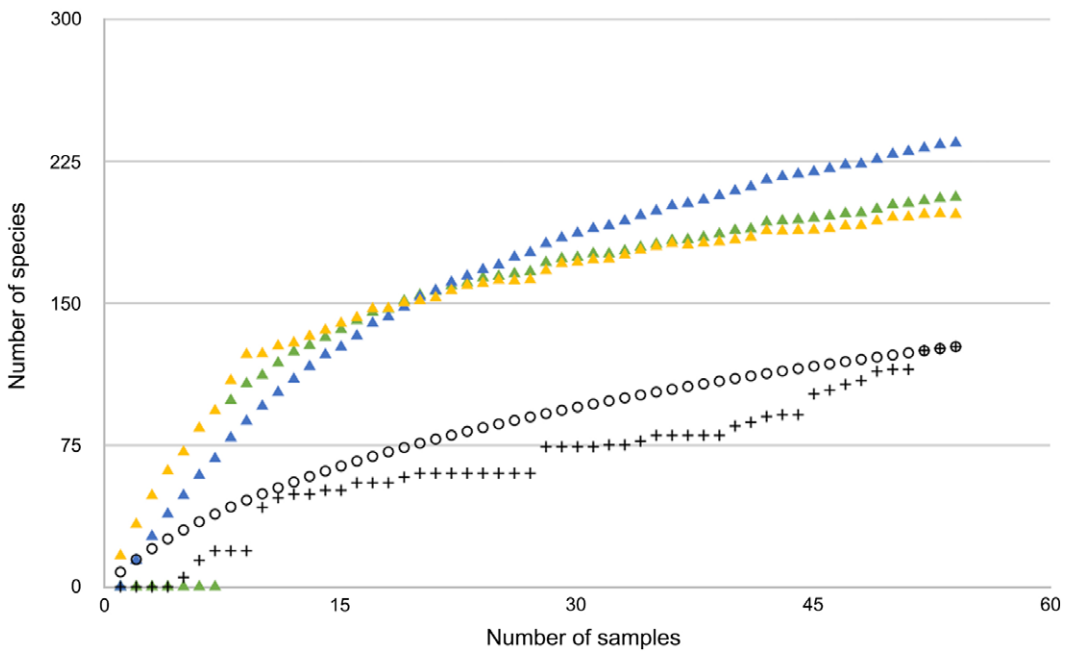
probably a very small fraction of the Neotropical diversity. The significance of this result alone, in terms of the enormous taxonomic gap, is remarkable, and the result corroborates the works of Veijalainen *et al.* (2012, 2013, 2014b) and Timms *et al.* (2015), indicating a huge and largely unknown diversity in the tropics, especially considering that this study covered only a small area of the rainy mountains in southeastern Brazil.

Smith *et al.*'s (2009) study of Orthocentrinae based on molecular operational taxonomic units from a temperate region (latitude: 58° N) also showed a high diversity and several cryptic species, suggesting that the global knowledge of the group is still incipient. The problem of cryptic species was also observed for the Neotropical Orthocentrinae (Veijalainen *et al.* 2012), corroborating the importance of integrating molecular data in species identification. Our results demonstrate the adequacy of the DNA barcoding *COI* region as a taxonomic tool for the separation of species in understudied groups. For example, barcoding allowed us to identify some taxonomic problems at the genus level and to better understand the morphological diversity of the Neotropical genera (*e.g.*, see comments for the genus *Batakocomacrus* in the taxonomy section). On the other hand, the costs of this method are usually prohibitive, limiting the number of specimens analysed. The aforementioned reasons emphasise the need for taxonomic revisions using integrative taxonomy.

When comparing our results with Veijalainen *et al.*'s (2012) faunistic survey of Neotropical Orthocentrinae from Amazonian Peru, Ecuador, and Central America, we found most genera to be common to both surveys, with only three genera absent in the present survey (*i.e.*, *Helictes*, *Plectiscidea*, and *Sphingozona*) and two not found in the earlier study (*i.e.*, *Batakocomacrus* and *Catastenus*). *Orthocentrus* was the most common and species-rich



**Fig. 9.** Number of molecular operational taxonomic units in the total data set (blue bars), described Orthocentrinae species in the Neotropics (orange bars) and described Orthocentrinae species in Brazil (grey bars).



**Fig. 10.** Species accumulation curve (+), and sample-based rarefaction (o) and estimations of species richness curves (▲): Chao 1 (▲); ACE (▲); Jackknife 2 (▲).



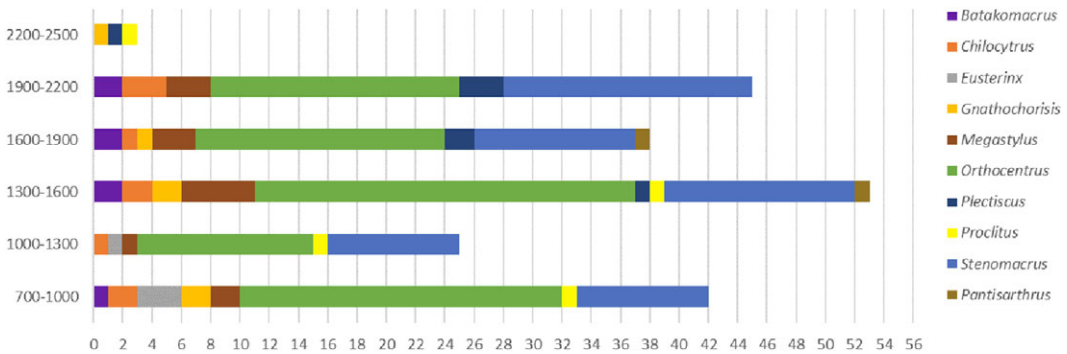


Fig. 11. Species (molecular operational taxonomic units) richness for each genus in six 300-m elevational bands in the Brazilian mountains (legend colour for genera is the same for all figures).

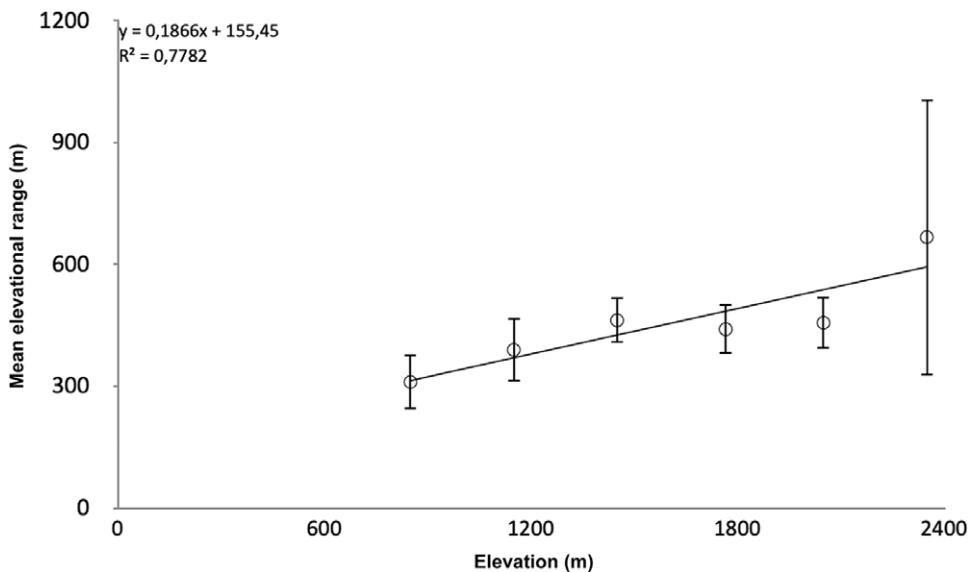


Fig. 12. Linear regression analysis based on Stevens's (1992) method, of the relationship between elevation (midpoint for each 300-m band) and the mean elevational range of the species of Orthocentrinae present in each of the six elevational 300-m bands in Brazilian mountains ( $P = 0.02$ ). The increase in range with increasing elevations supports prediction of the Rapoport's rule.

genus in both surveys. Interestingly, *Stenomacrus* was the second-most common and speciose genus in Brazil but is relatively rare in Peru, Ecuador, and Central America.

All Neotropical studies on Orthocentrinae fauna were conducted in moist environments, highlighting the need of studies in different biomes such as dry forests and savannahs, especially in a huge and megadiverse country like Brazil. For example, Shapiro and Pickering (2000) observed that aridity negatively affects abundance of ichneumonids. They observed that Orthocentrinae are also affected by forest type, being most abundant in primary old-growth forests, which is interesting, considering their hosts are fungus gnats. Therefore, we expect to find lower diversity in drier areas, and studies in the Brazilian Cerrado and Caatinga would be important to analyse this hypothesis.

Trends in species richness inferred from Figure 11 should not be considered for comparison with previous studies nor be interpreted as representative of actual local trends, because the sampling efforts were not standardised and no samples at low elevations (below 780 m) were taken in the present survey. The elevational distribution most commonly found for ichneumonids is a mid-elevation peak in species richness (van Noort 2004; Peck *et al.* 2008; Veijalainen *et al.* 2014b). In apparent disagreement with this general trend for elevational gradients, latitudinal trends of higher diversity in the tropics, even in the lowlands, seem to be consistently corroborated by recent publications (Veijalainen *et al.* 2012, 2013; Timms *et al.* 2015; Shimizu *et al.* 2020), indicating that the anomalous pattern therewith observed and theorised for ichneumonids (Owen and Owen 1974; Askew and Shaw 1986; Gauld 1986, 1987) might be caused by biased data, especially for small-bodied koinobiont species that are harder to identify (Sääksjärvi *et al.* 2004; Santos and Quicke 2011; Quicke 2012), such as the Orthocentrinae.

### Conclusions

Here, we evaluated Rapoport's rule by testing range sizes against elevation and observed a significant correlation supporting the rule, with species at higher elevations having larger ranges. Support for Rapoport's rule, even for invertebrates, and using Stevens's (1992) method, which is less rigorous, was found in less than 41% of the literature (McCain and Knight 2013). Although criticisms have been made of Rapoport's rule and on the methods for its evaluation, the underlying mechanism seems to be valid and received good support recently (*e.g.*, Chan *et al.* 2016), even when species ranges do not follow predictions due to complex climate gradients (Pintor *et al.* 2015). This and the fact that mountain passes are higher in the tropics (Janzen 1967; Sheldon *et al.* 2018) may be important factors that could lead to observations confirming larger elevational ranges at higher elevations in tropical mountains, as observed for braconids (Aguirre *et al.* 2018). This is an extremely relevant consideration for conservation because tropical lowlands could harbour the highest diversity and concurrently suffer the greatest impacts.

The need for taxonomic studies on Neotropical Orthocentrinae, especially revisions of most genera, is indisputable. The taxonomic problems, aggravated by the fact that the subfamily has been considered a "trash basket" for many years, have prevented the advancement of biological and ecological knowledge and the subfamily's application as biological control agents. In the present study, we presented the first illustrated key for Brazilian Orthocentrinae genera, including both genus groups (*Orthocentrus* and *Helictes*). By providing this taxonomic synopsis, we hope to facilitate future taxonomic reviews and to encourage research on this group.

**Acknowledgements.** The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), and INCT-Hympar for financial support; the managements of the Reserva Privada do Patrimônio Cultural de Itamonte, Reserva Biológica da Serra do Japi, Parque Nacional da Serra da Bocaina, Parque Nacional do Itatiaia, Parque Estadual da Serra do Mar, and Airton Santos Soares and the collection team for the collections of material; Luciana Bueno dos Reis Fernandes for taking the images; and all the technicians from INCT-Hympar.

### References

Aguirre, H., Shaw, S.R., and Rodríguez-Jiménez, A. 2018. Contrasting patterns of altitudinal distribution between parasitoid wasps of the subfamilies Braconinae and Doryctinae (Hymenoptera: Braconidae). *Insect Conservation and Diversity*, **11**: 219–229.

- Askew, R.R. and Shaw, M.R. 1986. Parasitoid communities: their size, structure and development. *In* *Insect Parasitoids*. Edited by J. Waage and D. Greathead. Academic Press, London, United Kingdom. Pp. 225–264.
- Broad, G.R. 2010. Status of *Batakomacrus* Kolarov (Hymenoptera: Ichneumonidae: Orthocentrinae), with new generic combinations and description of a new species. *Zootaxa*, **2394**: 51–68. <https://dx.doi.org/10.11646/zootaxa.2394.1.4>.
- Broad, G.R., Shaw M.R., and Fitton M.G. 2018 The ichneumonid wasps of Britain and Ireland (Hymenoptera: Ichneumonidae): their classification and biology. Volume 7, Part 12. Handbooks for the identification of British insects. Royal Entomological Society and Field Studies Council, Telford, United Kingdom.
- Camargo, L.F., Shimbori, E.M., and Pentead-Dias A.M. 2020. New Neotropical species of *Pantisarthrus* Forster, 1871 (Hymenoptera: Ichneumonidae). *Zootaxa*, **4728**: 443–452. <https://doi.org/10.11646/zootaxa.4728.4.2>.
- Chan, W.P., Chen, I.C., Colwell, R.K., Liu, W.C., Huang, C.Y., and Shen, S.F. 2016. Seasonal and daily climate variation have opposite effects on species elevational range size. *Science*, **351**: 1437–1439.
- Colwell, R.K. 2013. EstimateS, version 9.1: statistical estimation of species richness and shared species from samples (software and user's guide). Freeware for Windows and Mac OS. Available from: <http://viceroy.eeb.uconn.edu/estimates/index.html> [accessed 22 July 2020].
- Colwell, R.K. and Hurtt, G.C. 1994. Nonbiological gradients in species richness and a spurious Rapoport effect. *The American Naturalist*, **144**: 570–595.
- Colwell, R.K. and Lees, D.C. 2000. The mid-domain effect: geometric constraints on the geography of species richness. *Trends in Ecology and Evolution*, **15**: 70–76.
- Dasch, C. 1992. The ichneumon flies of America north of Mexico. Part 12. Subfamilies Microleptinae, Helictinae, Cylloceriinae and Oxytorinae (Hymenoptera: Ichneumonidae). *Memoirs of the American Entomological Institute*, **52**: 1–470.
- Delobel, A. and Matile, S. 1975. Un nouveau Microleptinae (Hym. Ichneumonidae) parasite de *Neoempheria ombrophila*, n.sp. (Dipt. Mycetophilidae) en République Centrafricaine. *Bulletin de l'Institut Fondamental d'Afrique Noire*, **37**: 385–394.
- Fernandez-Triana, J.L., Whitfield, J.B., Rodriguez, J.J., Smith, M.A., Janzen, D.H., Hallwachs, W.D., *et al.* 2014. Review of *Apanteles sensu strictu* (Hymenoptera, Braconidae, Microgastrinae) from Area de Conservación Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. *ZooKeys*, **383**: 1–565. <https://doi.org/10.3897/zookeys.383.6418>.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.
- Gauld, I.D. 1984. An introduction to the Ichneumonidae of Australia. British Museum (Natural History), London, United Kingdom.
- Gauld, I.D. 1986. Latitudinal gradients in ichneumonid species richness in Australia. *Ecological Entomology*, **11**: 155–161.
- Gauld, I.D. 1987. Some factors affecting the composition of tropical ichneumonid faunas. *Biological Journal of the Linnean Society*, **30**: 299–312.
- Gauld, I.D. 1988. Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biological Journal of the Linnean Society*, **35**: 351–377. <https://doi.org/10.1111/j.1095-8312.1988.tb00476.x>.
- Gauld, I.D. 1991. The Ichneumonidae of Costa Rica. Volume 1. *Memoirs of the American Entomological Institute*, **47**: 1–589.
- Gauld, I.D. 1997. The Ichneumonidae of Costa Rica. Volume 2. *Memoirs of the American Entomological Institute*, **57**: 1–485.

- Gauld, I.D. 2000. The Ichneumonidae of Costa Rica. Volume 3. Memoirs of the American Entomological Institute, **63**: 1–453.
- Gauld, I.D., Godoy, C., Sithole, R., and Ugalde Gomez, J. 2002. The Ichneumonidae of Costa Rica. Volume 4. Memoirs of the American Entomological Institute, **66**: 1–768.
- Gotelli, N.J. and Colwell, R.K. 2011. Estimating species richness. *In* Biological diversity: frontiers in measurement and assessment. *Edited by* A.E. Magurran and B.J. McGill. Oxford University Press, New York, New York, United States of America. Pp. 39–54.
- Gupta, V.K. 1988. The Ichneumonidae of the Indo–Australian area (Hymenoptera): a synoptic catalogue of the taxa described through 1985 together with bibliography, 1960–1985. *Memories of American Entomological Institute*, **41**: 1–121.
- Humala, A.E. 2003. The ichneumonid wasps in the fauna of Russia and adjacent countries. Subfamilies Microleptinae and Oxytorinae (Hymenoptera, Ichneumonidae). Nauka, Moscow, Russia. 175 pp. [In Russian.]
- Humala, A.E. 2010. Review of the genus *Batakocomacrus* Kolarov, 1986 (Hymenoptera: Ichneumonidae, Orthocentrinae) with description of new species. *Proceedings of the Russian Entomological Society*, **81**: 29–38. [In Russian with English summary.]
- Humala, A.E. 2014. Mexican species of the subgenus *Dicolus* Förster, genus *Megastylus* Schiødte (Hymenoptera: Ichneumonidae: Orthocentrinae), with descriptions of a new species. *Proceedings of the Russian Entomological Society*, **85**: 98–102.
- Humala, A.E. 2017. New species of the genus *Gnathochorisis* Förster (Hymenoptera: Ichneumonidae: Orthocentrinae) from the Neotropical Region. *Zootaxa*, **4250**: 201–218.
- Humala, A.E. 2019. Mexican species of the genus *Orthocentrus* (Hymenoptera, Ichneumonidae: Orthocentrinae). *Zootaxa*. **4709**: 1–83. <https://doi.org/10.11646/zootaxa.4709.1.1>.
- Humala, A.E., Choi, J.K., and Lee, J.W. 2016. A review of the genera *Gnathochorisis* Förster and *Symplecis* Förster of South Korea, with notes on Korean orthocentrines (Hymenoptera, Ichneumonidae, Orthocentrinae). *ZooKeys*, **56**: 85–104. <https://doi.org/10.3897/zookeys.562.7303>.
- Humala, A.E., Kruidhofb, H.M., and Woelke, J.B. 2017. New species of *Megastylus* (Hymenoptera: Ichneumonidae: Orthocentrinae) reared from larvae of Keroplatidae fungus gnats (Diptera) in a Dutch orchid greenhouse. *Journal of Natural History*, **51**: 83–95. <https://dx.doi.org/10.1080/00222933.2016.1257074>.
- Humala, A.E. and Ruíz-Cancino, E. 2017. Two new species of the genus *Eusterinx* Förster (Hymenoptera: Ichneumonidae: Orthocentrinae) from Mexico. *Zoosystematica Rossica*, **26**: 161–166.
- Janzen, D.H. 1967. Why mountain passes are higher in the tropics. *American Naturalist*, **101**: 233–249.
- Janzen, D.H. 1981. The peak in North American ichneumonid species richness lies between 38° and 42° N. *Ecology*, **62**: 532–537.
- Kang, I., Chapman, E.G., Janzen, D.H., Hallwachs, W., Dapkey, T., Smith, M.A., and Sharkey, M.J. 2017. Revision of the species of *Lytopylus* from Area de Conservación Guanacaste, northwestern Costa Rica (Hymenoptera, Braconidae, Agathidinae). *ZooKeys*, **721**: 93–158, <https://doi.org/10.3897/zookeys.721.20287>.
- Klopfstein, S., Langille, B., Spasojevic, T., Broad, G.R., Cooper, S.J.B., Austin, A.D., and Niehuis, O. 2019. Hybrid capture data unravels a rapid radiation in pimpliform parasitoid wasps (Hymenoptera: Ichneumonidae: Pimpliformes). *Systematic Entomology*, **44**: 361–383. <https://doi.org/10.1111/syen.12333>.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**: 1547–1549.
- Lis, J.T. and Schleif, R. 1975. Size fractionation of double-stranded DNA by precipitation with polyethylene glycol. *Nucleic Acids Research*, **2**: 383–390. <https://dx.doi.org/10.1093/nar/2.3.383>.

- McCain, C.M. and Knight, K.B. 2013. Elevational Rapoport's rule is not pervasive on mountains. *Global Ecology and Bio-geography*, **22**: 750–759.
- Meierotto, S., Sharkey, M.J., Janzen, D.H., Hallwachs, W., Hebert, P.D.N., Chapman, E.G., and Smith, M.A. 2019. A revolutionary protocol to describe understudied hyperdiverse taxa and overcome the taxonomic impediment. *Deutsche Entomologische Zeitschrift*, **66**: 119–145. <https://doi.org/10.3897/dez.66.34683>.
- Mukai, H. and Kitajima, H. 2019 Parasitoid wasps regulate population growth of fungus gnats genus *Neoempheria* Osten Sacken (Diptera: Mycetophilidae) in shiitake mushroom cultivation. *Biological Control*, **134**: 15–22.
- Owen, D.F. and Owen, J. 1974. Species diversity in temperate and tropical Ichneumonidae. *Nature*, **249**: 583–584.
- Peck, R.W., Banko, P.C., Schwarzfeld, M., Euaparadorn, M., and Brinck, K.W. 2008. Alien dominance of the parasitoid wasp community along an elevation gradient on Hawai'i Island. *Biological Invasions*, **10**: 1441–1455.
- Pintor, A.F., Schwarzkopf, L., and Krockenberger, A.K. 2015. Rapoport's Rule: do climatic variability gradients shape range extent? *Ecological Monographs*, **85**: 643–659.
- Quicke, D.L.J. 2012. We know too little about parasitoid wasp distributions to draw any conclusions about latitudinal trends in species richness body size, and biology. *PLOS One*, **7**: e32101. <https://doi.org/10.1371/journal.pone.0032101>.
- Quicke, D.L.J. 2015. *The braconid and ichneumonid parasitoid wasps*. Wiley-Blackwell, London, United Kingdom.
- Rohde, K., Heap, M., and Heap, D. 1993. Rapoport's rule does not apply to marine teleosts and cannot explain latitudinal gradients in species richness. *The American Naturalist*, **142**: 1–16.
- Sääksjärvi, I.E., Haataja, S., Neuvonen, S., Gauld, I.D., Jussila, R., Salo, J., and Burgos, A.M. 2004. High local species richness of parasitic wasps (Hymenoptera: Ichneumonidae; Pimplinae and Rhyssinae) from the lowland rainforests of Peruvian Amazonia. *Ecological Entomology*, **29**: 735–743.
- Sambrook, J. and Russell, D.W. 2001. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, New York, New York, United States of America.
- Santos, A.M.C. and Quicke, D.L.J. 2011. Large-scale diversity patterns of parasitoid insects. *Entomological Science*, **14**: 371–382.
- Šedivý, J. and Ševčík, J. 2003. Ichneumonid (Hymenoptera: Ichneumonidae) parasitoids of fungus gnats (Diptera: Mycetophilidae): rearing records from the Czech Republic. *Studia Dipterologica*, **10**: 153–158.
- Shapiro, B.A. and Pickering, J. 2000. Rainfall and parasitic wasp (Hymenoptera: Ichneumonidae) activity in successional forest stages at Barro Colorado Nature Monument, Panama, and La Selva Biological Station, Costa Rica. *Agricultural and Forest Entomology*, **2**: 39–47.
- Sharkey, M.J. and Chapman, E.G. 2016. Revision of *Aerophilus* Szépligeti (Hymenoptera, Braconidae, Agathidinae) from eastern North America, with a key to the Nearctic species. *Contributions in Science*, **524**: 51–110.
- Sharkey, M.J., Chapman, E.G., Janzen, D.H., Hallwachs, W., and Smith, M.A. 2015. Revision of *Aphelagathis* (Hymenoptera, Braconidae, Agathidinae, Agathidini). *Zootaxa*, **4000**: 73–89. <https://doi.org/10.11646/zootaxa.4000.1.3>.
- Sharkey, M.J., Meierotto, S., Chapman, E., Janzen, D.H., Hallwachs, W., Dapkey, T., and Solis, M.A. 2018. *Alabagrus* Enderlein (Hymenoptera, Braconidae, Agathidinae) species of Costa Rica, with an emphasis on specimens reared from caterpillars in Area de Conservación Guanacaste. *Contributions in Science*, **526**: 31–180. <https://doi.org/10.3897/zookeys.130.1569>.



- Sheldon, K.S., Huey, R.B., Kaspari, M., and Sanders, N.J. 2018. Fifty years of mountain passes: a perspective on Dan Janzen's classic article. *The American Naturalist*, **191**: 553–565.
- Shimizu, S., Broad, G.R., and Maeto, K. 2020. Integrative taxonomy and analysis of species richness patterns of nocturnal Darwin wasps of the genus *Encospilus* Stephens (Hymenoptera, Ichneumonidae, Ophioninae) in Japan. *ZooKeys*, **990**: 1–144. <https://doi.org/10.3897/zookeys.990.55542>.
- Shorthouse, D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. Available from: <http://www.simplemappr.net> [accessed 20 February 2020].
- Smith, A.S., Fernandez-Triana, J., Roughley, R., and Hebert, P.D.N. 2009. DNA barcode accumulation curves for under studied taxa and areas. *Molecular Ecology Resources*, **9**: 208–216.
- Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W., and Hebert, P.D. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences of the United States of America*, **105**: 12359–12364. <https://doi.org/10.1073/pnas.0805319105>.
- Stevens, G.C. 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. *The American Naturalist*, **133**: 240–256.
- Stevens, G.C. 1992. The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. *The American Naturalist*, **140**: 893–911.
- Timms, L.L., Schwarzfeld, M., and Sääksjärvi, I.E. 2015. Extending understanding of latitudinal patterns in parasitoid wasp diversity. *Insect Conservation and Diversity*, **9**: 74–86. <https://doi.org/10.1111/icad.12144>.
- Townes, H. 1971. Genera of Ichneumonidae, 4. *Memoirs of the American Entomological Institute*, **17**: 1–372.
- Tucker, E.M., Chapman, E.G., and Sharkey, M.J. 2015. A revision of the New World species of *Cremnops* Förster (Hymenoptera: Braconidae: Agathidinae). *Zootaxa*, **3916**: 1–83. <https://doi.org/10.11646/zootaxa.3916.1.1>.
- van Achterberg, C., Sharkey, M.J., and Chapman, E.G. 2014. Revision of the genus *Euagathis* Szépligeti (Hymenoptera, Braconidae, Agathidinae) from Thailand, with description of three new species. *Journal of Hymenoptera Research*, **36**: 1–25. <https://doi.org/10.3897/jhr.36.5658>.
- van Noort, S. 2004. Ichneumonid (Hymenoptera: Ichneumonoidea) diversity across an elevational gradient on Monts Doudou in Southwestern Gabon. *California Academy of Sciences Memoir*, **28**: 187–216.
- Varga, O. 2021. First record of the genus *Gnathochoris* Förster, 1869 (Hymenoptera: Ichneumonidae: Orthocentrinae) from the Afrotropical region, with descriptions of two new species from Kenya. *Zootaxa*, **5052**: 441–446. <https://doi.org/10.11646/zootaxa.5052.3.9>.
- Veijalainen, A., Broad, G.R., and Sääksjärvi, I.E. 2014a. Twenty-seven new species of *Orthocentrus* (Hymenoptera: Ichneumonidae; Orthocentrinae), with a key to the Neotropical species of the genus. *Zootaxa*, **3768**: 201–252.
- Veijalainen, A., Sääksjärvi, I.E., Erwin, T.L., Gómez, I.C., and Longino, J.T. 2013. Subfamily composition of Ichneumonidae (Hymenoptera) from western Amazonia: insights into diversity of tropical parasitoid wasps. *Insect Conservation and Diversity*, **6**: 28–37. <https://doi.org/10.1111/j.1752-4598.2012.00185.x>.
- Veijalainen, A., Sääksjärvi, I.E., Tuomisto, H., Broad, G.R., Bordera, S., and Jussila, R. 2014b. Altitudinal trends in species richness and diversity of Mesoamerican parasitoid wasps (Hymenoptera: Ichneumonidae). *Insect Conservation and Diversity*, **7**: 496–507. <https://doi.org/10.1111/icad.12073>.
- Veijalainen, A., Wahlberg, N., Broad, G.R., Erwin, T.L., Longino, J.T., and Sääksjärvi, I.E. 2012. Unprecedented ichneumonid parasitoid wasp diversity in tropical forests. *Proceedings of Royal Society of London Biological Science*, **279**: 4694–4698.

- Vilkamaa, P. and Komonen, A. 2001. Redescription and biology of *Trichosia* (*Baeosciara*) *sinuata* Menzel & Mohrig (Diptera: Sciaridae). *Entomologica Fennica*, **12**: 46–49. <https://doi.org/10.33338/ef.84095>.
- Villemant, C., Jingxian, L., and Rouse, P. 2016. Deep into the head of a remarkable new genus of Orthocentrinae (Hymenoptera: Ichneumonidae) from the highest peak of Papua New Guinea. *In* *Insects of Mount Wilhelm, Papua New Guinea. Edited by* T.F.L. Robillard, C. Villemant, and M. Leponce. Mémoires du Muséum national d'histoire naturelle. Muséum national d'histoire naturelle, Paris, France. Pp. 375–392.
- Wahl, D.B. 1990. A review of the mature larvae of Diplazontinae, with notes on larvae of Acaenitinae and Orthocentrinae and proposal of two new subfamilies (Insecta: Hymenoptera, Ichneumonidae). *Journal of Natural History*, **24**: 27–52.
- Watanabe, K. 2019. Review of the genera *Aniseres* Förster, 1871 and *Catastenus* Förster, 1868 (Hymenoptera: Ichneumonidae: Orthocentrinae) from Japan. *Japanese Journal of Systematic Entomology*, **25**: 81–85.
- Watanabe, K. 2020. Review of *Gnathochoris* Förster, 1869 (Hymenoptera: Ichneumonidae: Orthocentrinae) from Japan, with description a new species. *Japanese Journal of Systematic Entomology*, **26**: 48–52.
- Woelke, J.B., Pham, K., and Humala, A.E. 2020. New species of *Stenomacrus* (Hymenoptera: Ichneumonidae: Orthocentrinae) reared from *Bradysia impatiens* (Diptera: Sciaridae) in the Netherlands. *Journal of Natural History*, **54**: 1603–1616. <https://doi.org/10.1080/00222933.2020.1814890>.
- Yu, D.S.K., van Achterberg, C., and Horstmann, K. 2016. Taxapad 2016, Ichneumonoidea. Database on flash-drive, Nepean, Ottawa. Available from: <http://www.taxapad.com> [accessed 17 April 2020].
- Zhang, S.J., Zhou, Q., Li, T., and Sheng, M.L. 2022. The genus *Megastylus* Schiödte (Hymenoptera, Ichneumonidae, Orthocentrinae) in China with a key to the species of China and Oriental region. *Zootaxa*, **5091**: 63–74.
- Zwakhals, C.J. and Diller, E. 2015. Eight new *Orthocentrus* species from South America (Hymenoptera; Ichneumonidae, Orthocentrinae). *Mitteilungen der Münchner Entomologischen Gesellschaft*, **105**: 65–78.