RESEARCH ARTICLE



# Two new species of Lobellini from Tianmu Mountain, China (Collembola, Neanuridae)

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#### Abstract

Three species of the subfamily Neanurinae (Collembola: Neanuridae) are recorded from Tianmu Mountain, Zhejiang Province, east China. Two of them, *Lobellina fusa* **sp. n.** and *Paralobella tianmuna* **sp. n.**, are new to science and described in this paper. *Lobellina fusa* **sp. n.** can be recognized by the presence of six teeth on mandible and the fusion of dorsointernal tubercles on the head. *Paralobella tianmuna* **sp. n.** is characterized by a mandible with seven teeth, the lateral tubercle of Abd. II–III respectively with 7 (6+s) chaetae. *Crossodonthina bidentata* Luo & Chen, 2009 is widely distributed in the mountain from 300 to 1500 m a.s.l.

#### Keywords

Crossodonthina bidentata, Lobellina fusa sp. n., Paralobella tianmuna sp. n., Neanurinae, taxonomy

# Introduction

To date, on a worldwide scale, the tribe Lobellini consists of 17 genera (including two subgenera) and approximately 157 species mainly from South-East Asia and the Australian-Oceania region (Bellinger et al. 2017). Up to now, only six genera and 12 species were reported from mainland China (Denis 1929; Stach 1964; Yue and Yin 1999;

Wang 2003; Xiong et al. 2005; Ma and Chen 2008; Luo and Chen 2009; Jiang and Zhang 2012; Jiang et al. 2012; Luo and Palacios-Vargas 2016; Wang et al. 2016). The tribe is diversified in all regions sampled so far, but huge areas have never been sampled and the knowledge of Chinese fauna of Lobellini can be considered as very incomplete.

Tianmu Mountain, located in Lingan City, Zhejiang Province, east China, covers an area of 4300 hectares. The elevation of the highest peak of the mountain is more than 1500 meters. It belongs to the subtropical humid monsoon climate zone. The flora is a typical subtropical evergreen broad-leaved forest. One of the main targets of the Zhejiang Tianmu Mountain National Nature Reserve is the protection of rare and endangered plants, such as *Ginkgo biloba*, *Cercidiphyllum japonicum*, and *Liriodendron chinensis*. Till now, more than 4000 species (including 657 type species) of insects were reported from the mountain (Wu and Pan 2001). However, the Collembolan fauna of the mountain is poorly known, and only very few neanurid species were reported from it (Luo and Chen 2009, Jiang et al. 2012). In 2011, organized by The Management Bureau of Zhejiang Tianmu Mountain National Nature Reserve, we carried out field work in this mountain. Three species of the tribe Lobellini were identified and two of them are described as new.

# Terminology

The terminology and layout of the tables used in this paper follow Deharveng (1983), Deharveng and Weiner (1984), and Smolis and Deharveng (2006).

#### Abbreviations used

Fu

Ve

Vi

VI

### General morphology:

furcal.

ventroexternal,

ventrointernal,

ventrolateral.

Abd	abdomen,	Ti	tibiotarsus,
Ant	antenna,	Th	thorax,
Cx	coxa,	Tr	trochanter,
Fe	femur,	VT	ventral tube.
Scx2	subcoxa 2,		
Grouj	ps of Chaetae:		
Ag	antegenital,	De	dorsoexternal,
An	anal	Di	dorsointernal.

Dl	doi	rsolateral
-		

- L lateral,
- **Oc** ocular,
- **So** subocular.

# **Tubercles:**

An Fr	antennal, frontal,	Cl	clypeal,
Types	of chaetae:		
Ml	long macrochaeta,	s	s-chaeta,
Mc	short macrochaeta,	or	organite of Ant. IV,
Mcc	very short macrochaeta,	i	small ordinary chaeta on Ant. IV,
me	mesochaeta,	mou	thin cylindrical chaetae on Ant.
mi	microchaeta,		IV ("soies mousses"),
i	microchaeta,	x	labial papilla x.
ms	s-microchaeta,		* *

# Materials and methods

All specimens were collected with the aid of Tullgren funnels or aspirators, and preserved in 95% ethanol. They were cleared in Nesbitt's fluid and mounted on slides in Hoyer's medium. Preparations were dried for 7–10 days in oven at 55 °C, and then ringed with lacquer. The morphological characters were observed and figures were drawn using a phase contrast microscope Nikon 80i. Material is deposited in the Key Laboratory of Zoology, Hunan University of Arts and Science, Changde, Hunan Province, China.

# Taxonomy

Tribe Lobellini Cassagnau, 1983 Genus *Crossodonthina* Yosii, 1954

#### Crossodonthina bidentata Luo & Chen, 2009

**Material.** Three males and two females, on the path from Qili Pavilion to Longfengjian, Tianmu Mountain, Zhejiang Province, China. Coordinates: 30°20'36"N, 119°26'17"E, 800–1050 m a.s.l., 26 July 2011, leg. Ji-Gang Jiang (collection number: J2011072601). Ten males and eight females, Zhonglie Temple, Tianmu Mountain, Zhejiang Province. Coordinates: 30°23'03"N, 119°26'20"E, 300–400 m a.s.l., 24 July 2011, leg. Ji-Gang Jiang (collection number: J2011072401). Four males and two females, in bamboo forest, Hengwu, Tianmu Mountain, Zhejiang Province. Coordinates: 30°19'01"N, 119°26'15"E, 400–500 m a.s.l., 27 July 2011, leg. Ji-Gang Jiang (collection number: J2011072703.

**Short redescription.** Eyes 2+2. Labrum truncate and chaetal formula as 2/5, 2. Mandible consisting of two basal teeth and three rami. Chaeta O of cephalic tubercle Fr present. Body macrochaeta acuminate and ciliate. Formula of dorsal sensilla on tho-

rax and abdomen as 0, 2+ms, 2/1, 1, 1, 1, 1, 0. Unguis with one inner tooth and no lateral teeth. Unguiculus absent. Di tubercles on Abd. V fused on the axis.

**Remarks.** *Crossodonthina bidentata* is widely distributed in the mountain from 300 to 1500 m, living in decayed leaves of woody plants as well as bamboo.

#### Genus Lobellina Yosii, 1956

#### Lobellina fusa sp. n.

http://zoobank.org/0D0D9A9D-5159-48B6-BEC0-AE0976E274FB Figs 1–7, Appendix 1, Tables 1a–1b

**Type material.** Holotype male on slide, on the path from Longfengjian to Fairy Peak, Tianmu Mountain, Zhejiang Province, China. Coordinates: 30°23'11"N, 119°26'07"E, 1100–1500 m a.s.l., 25 July 2011, leg. Ji-Gang Jiang (Housed in Hunan University of Arts and Science, J2011072501). Paratypes: three females and two males, same data as holotype (Housed in Hunan University of Arts and Science, J2011072501).

Etymology. The species name refers to the fused Di tubercles on head.

**Diagnosis.** Habitus typical of the genus *Lobellina*. Body dorsal tubercles well developed. Cephalic Di tubercles fused. Chaeta O of tubercle Fr present. 3+3 black eyes. Labrum chaetotaxy as 0/2, 2. Mandible with six teeth. Maxilla consisting of two crochet-like lamellae and two teeth. Chaetae formula of tubercle Di on Th. I–Abd. V as 1, 3, 3/2, 2, 2, 2, 3. S-chaetae and s-microchaeta formula on Th. I–Abd. V as 0, 2+ms, 2/1, 1, 1, 1. Ventral tube with 4+4 chaetae, furcular vestige with 3 chaetae. Unguis with an inner tooth and without lateral tooth.

Description. Body length 2.2–3.7 mm. Body color red while alive and white in alcohol.

*Head*: eyes 3+3, black (Fig. 1). Antenna 4-segmented. Ant. I and II with seven (two with swollen apex) (Fig. 2) and eleven chaetae respectively. Ant. III dorsally fused to Ant. IV. Ant. III organ consists of two short rods and two long guard chaetae, two short rods exposed in separate pits. Ant. IV with trilobed apical bulb, dorsally with eight sensilla, slender *i* chaeta, 12 slender cylindrical chaetae (mou) and minute organite (or) (Fig. 2). Labrum chaetotaxy as 0/2, 2. Labium with eleven chaetae and two minute distal x sensilla (Fig. 5). Mandible with three main teeth, the apical one subdivided in four minute toothlets (Fig. 3). Maxilla consists of two fused lamellae, stylet-like and one with two small apical teeth (Fig. 4). Cephalic tubercles and chaetotaxy shown on Fig. 1 and Appendix 1, Table 1a. Macrochaetae and mesochaetae on body weakly serrate (observe under lens of 100x), and sheathed on distal half, ending in a swollen and blunt apex (Fig. 1). Microchaetae smooth and pointed. All dorsal cephalic tubercles (except Di) independent.

*Thoracic and abdominal tubercles and chaetotaxy* shown in Figs. 1, 7 and in Appendix 1, Table 1b. Sensory chaetae and s-microchaetae formula on Th. I–Abd. V as 0, 2+ms, 2/1, 1, 1, 1, 1. Chaetae Di3 free on Th. II and III. Each tubercle on Abd. VI with 7 chaetae (3 Ml and 4 Mc or me).

*Appendages*: Unguis with an inner tooth and without lateral tooth (Fig. 6). Unguiculus absent. Ventral tube with 4+4 chaetae (Fig. 7), furcular vestige with three chaetae



**Figures 1–4.** *Lobellina fusa* sp. n. **I** Dorsum of body **2** Antenna **3** Mandible **4** Maxilla. Scale bars: 100 µm (**1**); 20 µm (**2–4**).

and no microchaetae (Fig. 7). Chaetotaxy of legs, ventral tube, and furcular remnant shown in Appendix 1, Table 1b.

Ecology. Under leaves in forest.

**Remarks.** The taxon *Lobellina* was erected by Yosii in 1956 as a subgenus of the genus *Lobella* Börner, 1906. It was raised to generic status by Cassagnau (1983) and redefined by Deharveng and Weiner (1984): body without blue pigment, 3+3 black



**Figures 5–7.** *Lobellina fusa* sp. n. **5** Labium **6** Tibiotarsus and claw of hind leg **7** Ventral side of Abdomen. Scale bars: 20 µm (**5–6**); 100 µm (**7**).

eyes. Tubercles on the head and the tergites well developed, marked by a bump of the tegument and /or by differentiated tertiary grain, or some more strong secondary grains. Body without reticulation. Chaetotaxy of labrum as 0/2, 2. Maxilla styliform, mandible tridentate to multidentate. The sgd of Ant. III organ not shifted to ant. IV. Dorsal macrochaetae thickened and double lined, rounded to the apex. Chaetotaxy of type s normal (2+ms, 2/1, 1, 1, 1, 1). Abd. I without supplemental s-chaeta on lateral tubercle. Posterior chaetotaxy of head of cross-type. Chaetotaxy of tubercles Di of Th. II and III characteristic, with two macrochaeta (Di1 and Di2) and a small microchaeta, sometimes indistinct (Di3). Abd. V with 2+2 or 3+3 tubercle, tubercle De is isolated from the tubercle Dl, or fused to tubercle Dl.

To date, 12 valid species are known in the genus *Lobellina* (Deharveng and Weiner 1984, Ma and Chen 2008). The new species can be distinguished from others by having six teeth on the mandible and fused tubercle Di on head. A key for all species of the genus is given below.

# Key to species of genus Lobellina

1	Cephalic chaeta O present
_	Cephalic chaeta O absent
2	Body macrochaetae smooth
_	Body macrochaetae serrate
3	Maxilla with 2 separate lamella and 3 teeth
_	Maxilla with 2 fused lamella and 2 teeth
4	Body color yellow, mandible with 7 teeth, tubercle Oc with 2 chaetae, ventral
	tube with 5+5 chaetae, cephalic tubercle Di separate
_	Body color red, mandible with 6 teeth, tubercle Oc with 3 chaetae, ventral
	tube with 4+4 chaetae, cephalic tubercle Di fused
5	Body macrochaetae smooth
_	Body macrochaetae serrate
6	Cephalic tubercle Oc with 3 chaetae7
_	Cephalic tubercle Oc with 2 chaetae
7	Abd. V with 2+2 dorsal tubercles
_	Abd. V with 3+3 dorsal tubercles
8	Tubercle Di on Abd. V with 2 chaetae9
_	Tubercle Di on Abd. V with 3 chaetae L. minuta (Lee, 1980) (Korea)
9	Mandible with 3 teeth L. ipohensis (Yosii, 1976) (Malaysia)
_	Mandible with 8 teeth
10	Cephalic tubercle Oc with 2 chaetae11
_	Cephalic tubercle Oc with 3 chaetae12
11	Abd. V with 2+2 dorsal tubercles L. ionescui (Massoud & Gruia, 1974) (Cuba)
_	Abd. V with 3+3 dorsal tubercles L. perfusionides (Stach, 1965) (Vietnam)
12	Abd. V with 3+3 dorsal tubercles L. kitazawai (Yosii, 1969) (Japan)
_	Abd. V with 2+2 dorsal tubercles L. roseola (Yosii, 1954) (Japan)

## Genus Paralobella Cassagnau & Deharveng, 1984

#### Paralobella tianmuna sp. n.

http://zoobank.org/CF024D84-DF1F-449E-ADBC-25E025FC585E Figs 8–15, Appendix 1, Tables 2a–2b

**Material.** Holotype, female, 3.0 mm. Chanyuan Temple, Tianmu Mountain, Zhejiang Province. Coordinates: 30°19'40"N, 119°26'15"E, ca. 390 m a.s.l., 30 July 2011, leg. Ji-Gang Jiang (Housed in Hunan University of Arts and Science, J2011073001). Paratypes: two males, three females and two juveniles, same data as holotype (Housed in Hunan University of Arts and Science, J2011073001); one female, Hengwu, Tianmu Mountain, Zhejiang Province. 30°19'41"N, 119°26'14"E, 400–500 m a.s.l., 27 July 2011, leg. Ji-Gang Jiang (Housed in Hunan University of Arts and Science, J2011072704).



Figures 8–9. Paralobella tianmuna sp. n. 8 left half of head and thorax 9 left half of abdomen. Scale bar: 100  $\mu$ m.



**Figures 10–15.** *Paralobella tianmuna* sp. n. **10** Antenna **11** Mandible **12** Maxilla **13** Labium **14** Labrum **15** Tibiotarsus and claw of hind leg. Scale bars: **10–14** 20 μm; **15** 50μm.

**Etymology.** The new species is named after the type locality, Tianmu Mountain.

**Diagnosis.** Habitus typical of the genus *Paralobella*. Dorsal tubercles round or oval and well developed. 3+3 unpigmented eyes. Labrum round, chaetotaxy as 0/ 2, 2. Mandible with seven teeth. Maxilla nearly styliform, apex with two hook-like teeth. Cephalic tubercle Fr with chaeta O. Tubercle An with four chaetae, chaetae C and D free from the tubercle. Tubercle Oc with three chaetae. Cephalic tubercle Di, De, Dl respectively with 1, 3, 4 chaetae. Tubercle De of Th. II-III each with four (3+s) chaetae. Unguis with an inner tooth, and without lateral tooth. VT with 4+4 chaetae. Furcular vestige with three chaetae and no microchaetae.

**Description.** Body length: male 2.0–2.5 mm; female 2.1–3.5 mm. Body entirely red while alive and white in alcohol.

*Head*: Eyes 3+3 without pigment (Fig. 8). Ant. IV with trilobed apical bulb, and dorsal chaetotaxy as 8 sensilla, slender chaeta *i*, 12 sender, cylindrical chaetae and minute organite (Fig. 10). Labrum chaetotaxy as 0/2, 2 (Fig. 14). Labium with two minute distal x sensillum (Fig. 13). Mandible with seven teeth (Fig. 11). Maxilla nearly styliform, apex with two hook-like teeth (Fig. 12). Cephalic

tubercles and chaetotaxy see Fig. 8 and Appendix 1, Table 2a. Macrochaetae on body weakly serrate, acuminate or with blunt apex. Mesochaetae and microchaetae smooth and pointed.

*Body tubercles* round or oval (Fig. 8–9). Chaetae Di3 free on the tubercles Di on Th. II-III. Abd. I-IV each with four tubercles. Abd. V with three tubercles, tubercle De only with an S-chaeta. The tubercles of Abd. VI with seven chaetae each. Body tubercles and chaetotaxy as in Appendix 1, table 2a and 2b.

*Appendages*: Unguis with an inner tooth, and without lateral tooth (fig. 15). VT with 4+4 chaetae. Furcular vestige with three chaetae and no microchaetae.

**Remarks.** At present, 12 species belong to the genus *Paralobella*, all from Asia (Jiang et al. 2012, Luo and Palacios-Vargas 2016). In general appearance, *Paralobella tianmuna* sp. n. strongly resembles *P. perfusa* (Denis, 1934) from Indochina in the structure of maxilla, the number of mandible teeth, the arrangement of body tubercles, the presence of chaeta O of tubercle Fr, the tubercle Di of Th. I with one chaeta, and the tubercle De and Dl of Abd. V separate. However, *Paralobella tianmuna* sp. n. can be distinguished from the latter by number of chaetae on body, the former has 3 chaetae on each tubercle Di of Th. II–III, 7 (6+s) chaetae on each tubercle L of Abd. II-III; the latter has 2 chaetae on each tubercle Di of Th. II–III, 4 (3+s) chaetae on each tubercle L of Abd. II-III. The new species is also similar to Chinese species *P. breviseta* Luo & Palacios-Vargas, 2016 and *P. palustris* Jiang, Luan & Yin, 2012 in the arrangement of body tubercles, the presence of chaeta O of tubercle De and Dl of Abd. V. The new species can be separated from its congeners by the following key.

#### Key to species of genus Paralobella Cassagnau & Deharveng, 1984

1	Chaeta O of tubercle Fr absent
_	Chaeta O of tubercle Fr present6
2	Tubercle Dl of Th. II-III with 5 (4+s) chaetae (besides of ms)
_	Tubercle Dl of Th. II–III with 4 (3+s) chaetae (besides of ms)
3	Tubercle Di of Th. II-III only with 1 chaeta, tubercle Oc on head only with
	Ocm chaeta
_	Tubercle Di of Th. II-III with 2-3 chaetae, tubercle Oc on head with 2-3
	chaetae4
4	Tubercle Di of Th. II-III with 2 chaetae
_	Tubercle Di of Th. II–III with 3 chaetae5
5	Abd. I–III: tubercle De with 5 (4+s) chaetae, tubercle Dl with 3 chaetae; man-
	dible complicated, totally with 16 teeth in two rowsP. apsala (Thailand)
_	Abd. I-III: tubercle De with 4 (3+s) chaetae, tubercle Dl with 2 chaetae
	mandible relatively simple, with 5 or 6 teeth in one row

6	Tubercle De of Th. II–III with 4 (s+3) chaetae, VT with 3+3 chaetae
_	Tubercle De of Th. II–III with 5 (s+4) chaetae, VT with 4(5)+4(5) chaetae 7
7	Tubercle Di, De, DL of Th. I respectively with 2, 2, 1 chaetae8
_	Tubercle Di, De, DL of Th. I respectively with 1, 2, 1 chaetae
8	Tubercle Dl of Th. II–III with 4 (3+s) chaetae, tubercle Dl of Abd. IV with
	6 (5+s) chaetae
_	Tubercle Dl of Th. II-III with 5 (4+s) chaetae, tubercle Dl of Abd. IV
	with 3 (2+s) chaetae P. paraperfusa (Philippines)
9	Tubercle De and Dl of Abd. V fused, body tricolour
_	Tubercle De and Dl of Abd. V separate, body red color10
10	Mandible with 20 teeth P. palustris (China)
_	Mandible with less than 8 teeth11
11	Mandible with 6 teeth, tubercle L of Abd. II-III with 6 (5+s) chaetae
_	Mandible with 7 teeth, tubercle L of Abd. II-III with 4 (3+s) or 7 (6+s)
	chaetae12
12	Tubercle Di of Th. II–III with 2 chaetae, tubercle L of Abd. II-III with 4 (3+s)
	chaetae, mandibular basal tooth much larger than the second tooth
_	Tubercle Di of Th. II-III with 3 chaetae, tubercle L of Abd. II-III with 7
	(6+s) chaetae, mandibular basal tooth slightly larger than the second one
	*

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# Appendix I

Tubercle	Number and type of chaeta	Names of chaeta
Cl	2 Ml	F
CI	2 Mcc	G
4	2 Ml	В, Е
All	2 mi	C, D
E.	2 Ml	А
FI FI	1 mc	О
Oc	2 Ml	Oca, Ocm
00	1 mi	Оср
Di+Di	1+1 Ml	Dil
D.	1 Ml+1 Mc	De1, De2
De	1 mi	De3
DI	2 Ml	Dl1, Dl5
DI	3 mi	Dl2, Dl3, Dl6
т	1 Ml	L1
L	1 Mc	Dl4
S -	2 Ml+6 me	
30	5 me	uncertain

Table 1a. Cephalic tubercles and chaetotaxy of *Lobellina fusa* sp. n.

Table 1b. Body tubercles and chaetotaxy of Lobellina fusa sp. n.

Terga				Legs					
	Di	De	Dl	L	Scx2	Cx	Tr	Fe	Т
Th.I	1	2	1	_	0	3	6	12	19
Th.II	3	4+s	3+s+ms	3	2	6	7	9	19
Th.III	3	4+s	3+s	3	2	8	6	10	18
Terga			sterna						
Abd. I	2	3+ s	2	4	VT	4			
Abd. II	2	3+ s	2	5	Ve	2(4)	Vl	-	
Abd. III	2	3+ s	2	5	Ve	3	Fu	3	
Abd. IV	2	2+ s	3	7	Ve	7	Vl	5	
Abd. V	3	s	4		Ag	3	Vl	1	
Abd. VI	7				Ve	14	Ve	5	

Tubercle	Number and type of chaeta	Names of chaeta
	2 Mc	F
CI	2 Mcc	G
	1 Mc	В
An	1 Mcc	E
	2 me	C, D
E.	2 Mc	А
Fr	1 Mcc	О
0	1Ml	Ocm
Üc	2 me	Oca, Ocp
Di	1 Ml	Di1
D	1 Ml	Del
De	2 me	De2, De3
DI	1 Mc	Dl1,
DI	3 me	Dl3,Dl5, Dl6
T	2 Mc	L1, L4
L	1 me	L3
C	2 Mc	
30	8 me	uncertain

Table 2a. Cephalic tubercles and chaetotaxy of Paralobella tianmuna sp. n.

Table 2b. Body tubercles and chaetotaxy of Paralobella tianmuna sp. n.

Terga						Le	gs			
	Di	De	Dl	L	Scx2	Cx	Г	Îr 🛛	Fe	Т
Th.I	1	2	1	-	0	3	(	<u>5</u>	13	19
Th.II	3	4+s	4+s+ms	3	2	7	(	<u>ó</u>	12	19
Th.III	3	4+s	4+s	3	2	8	(	<u>5</u>	11	18
Terga			sterna							
Abd. I	2	3+ s	2	6+s	VT	4	4			
Abd. II	2	3+ s	2	6+s	Ve	4	4	Vl	_	
Abd. III	2	3+ s	2	6+s	Ve	4	4	Fu	3	
Abd. IV	2	2+ s	3	7+s	Ve	7(	(8)	Vl	5	
Abd. V	3	s	4		Ag	1	3	Vl	2	
Abd. VI	7				Ve	1	4	Ve	6	

RESEARCH ARTICLE



# A missing piece in the puzzle: the presence of Euglossa viridissima in the Baja California Peninsula (Hymenoptera, Apidae)

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#### Abstract

Orchid bees are a conspicuous component of the neotropical bee fauna, with a few species reaching the northernmost natural distribution for the group in northwestern continental Mexico. Among them, *Euglossa viridissima* Friese is here reported for the first time in the Cape Region of the Baja California peninsula, Mexico, where no species of the group have been found previously. These records are presented, their biogeographical implications discussed, and some interpretations of the local factors that influence the bees is presented.

#### Keywords

Biogeography, Cape Region, oases, orchid bees, neotropics

### Introduction

Under the recent documentation of the decline of local pollinator populations (Biesmeijer et al. 2006, Burkle et al. 2013), it is important to monitor the bee fauna composition at local levels (Potts et al. 2010, Goulson et al. 2015). The discovery of species not previously found in particular areas is part of such endeavors. Isolated new records of species are noteworthy particularly in cases involving taxa of exotic origin, from distant or unrelated biogeographic areas, or when they represent a considerable expansion of their known native range. Orchid bees are well known for their external morphological features such as metallic body coloration and long mouthparts, and also for the peculiar perfume collecting behavior of the males (Dressler 1982, Roubik and Hanson 2004). The Euglossini are the only bees within the corbiculate clade (Apini, Bombini, Euglossini, and Meliponini) that are restricted to the neotropics (Cardinal and Packer 2007, Engel et al. 2009), reaching their northernmost distribution in northwestern Mexico, where at least three species of the around 35 found in the country have been recorded (Búrguez 1997, Gonzalez et al. 2017). Orchid bees are powerful long distance flyers, such that females have been found to fly several kilometers while foraging (Janzen 1971, López-Uribe et al. 2008), and males have been recaptured nearly 100 km away (Pokorny et al. 2015). Some euglossine species have been recently discovered in areas that expand considerably their known native range (Anjos-Silva et al. 2006, Anjos-Silva 2007, 2008, Silva and Rebêlo 2009). A notable example of an introduction to a distant area is Euglossa dilemma Bembé & Eltz which was discovered in 2003 in southern Florida, and is now naturalized (Skov and Wiley 2005, Pemberton and Wheeler 2006). This species is a cryptic sibling species of *E. viridissima* Friese from which it was recently split (Eltz et al. 2011). Euglossa viridissima occurs from Guatemala throughout southern and central Mexico, being one of the few euglossine species that reach the northwestern continental areas of Mexico (Búrguez 1997, Hinojosa-Díaz et al. 2009) with no previous records (before this work) in the Baja California Peninsula. During the development of a wider faunistic bee survey in the state of Baja California Sur, euglossine bees were first observed in the Cape Region. Here the confirmation of these observations is presented, with first records of E. viridissima from the Cape Region of Baja California which represent the first for any euglossine species in the area. The biogeographical implications of these records and local factors that could influence its distribution is briefly discussed.

#### Materials and methods

The Cape Region of Baja California Sur (BCS), Mexico, is a biogeographic province with distinctive floristic and faunistic elements (Morrone 2005, Halffter et al. 2008). From a paleogeographic approach, it is considered a big island (200 km from continental Mexico) as it has undergone isolation processes (last vicariance event around 3 MYA) since the peninsula's origins 5-10 MYA (Brusca and Moore 2013, González-

Locality	Coordinates	Elevation (m)
Cabo Pulmo	23°26'06.70"N, 109°25'58.00"W	24
El Triunfo	23°48'12.90"N, 110°06'31.60"W	482
Las Cuevas	23°30'52.50"N, 109°41'23.50"W	125
La Ribera	23°33'21.80"N, 109°33'00.90"W	35
Los Planes	23°58'41.70"N, 109°58'18.20"W	24
Melitón Albáñez	23°38'26.80"N, 110°17'02.00"W	163
Santiago	23°28'37.90"N, 109°42'36.70"W	113
San Antonio de la Sierra	23°40'22.40"N, 109°55'51.10"W	947
San Bartolo	23°44'18.07"N, 109°50'48.85"W	389
San Dionisio	23°32'16.80"N, 109°47'53.60"W	371
Santuario de los cactus	23°44'45.10"N, 110°06'43.60"W	435
Sierra de la Laguna	23°33'06.60"N, 109°59'07.00"W	1752
San Pedrito	23°23'23.40"N, 110°12'26.90"W	17
Todos Santos	23°26'57.53"N, 110°13'35.35"W	32

Table 1. Sampled localities in the Cape Region, Baja California Sur State, Mexico.

Trujillo et al. 2016), favoring high rates of endemism in the region (Wiggins 1980, Roberts 1989). The vegetation of the region includes low deciduous tropical dry forest communities, xeric scrublands, and ecotones between both. The ecotones mark the delimitation of the Cape Region within the subdivision of the Sonoran desert (Axelrod 1978, Rzedowski 2006). An important component of the vegetation of the area are the oases, which are patches of vegetation associated with fresh water springs, which provide water, food and shelter in the middle of arid conditions of the peninsula (Arriaga and Rodríguez-Estrella 1997).

Sampling was carried out from May to November 2016 at 14 localities in the Cape Region (Table 1). Using insect nets the sampling of blooming areas was emphasized, specifically those of *Tecoma stans*. Additionally, chemical attractants (eugenol and eucalyptus oil) were tested intending to collect male bees in those localities where activity of the orchid bees was thought to be more likely, that is, San Bartolo, Santiago, and Todos Santos. The baited traps consisted of 600 ml plastic bottles following protocols used in South America (Sydney and Gonçalves 2015) arranged in 100 m lineal transects (ten traps per transect, 10m separation among individual traps). On each locality mentioned above one transect was set, with the traps staying for 24 h in every case.

# Results

Of the 14 Cape Region localities sampled, *E. viridissima* (Figs 1–4) was found only in Todos Santos on the Pacific slope, Santiago and San Bartolo on the Gulf of California slope (Fig. 5).

In total, 33 specimens (19 males, 14 females) of *E. viridissima* were collected. Per locality, 30 specimens (17 males, 13 females) came from Todos Santos; all were



Figures 1–4. Male details of *E. viridissima* found in Baja California Sur State. I Habitus (lateral view) 2 Facial aspect 3 Ventral view of metasoma 4 Mesotibia.

collected in August, a single female from San Bartolo collected in April, and two males from Santiago collected in October. Most of the specimens were caught in oases vegetation (96.7%). All the bees were captured while visiting flowers of *Tecoma stans*. The male specimens of *E. viridissima* were not attracted to the traps baited with chemical attractants.

Voucher specimens are deposited into in the entomological collection at the CIBNOR (La Paz, Mexico).

# Discussion

The finding of *Euglossa viridissima* as the first record of an orchid bee species on the Baja California peninsula has several implications. Biogeographically, *E. viridissima* has the northernmost natural distribution within Euglosines (Búrquez 1999, Roubik and Hanson 2004, Hinojosa-Díaz et al. 2009, Ramírez et al. 2010). In addition, this finding represents both a new and distinctive biogeographic area to the distribution of the



**Figure 5.** Localities in which was registered the presence of *E. viridissima* in the Cape Region. Biogeographic Provinces map from CONABIO (1997).

species, and a new Neotropical bee record to the mainly Nearctic peninsula (Morrone 2005). Before our records of *E. viridissima* in the Cape Region of the peninsula, no other euglossines had been reported from there (Ayala et al. 1996, Moure et al. 2007, Ascher and Pickering 2017).

The Cape Region of the Baja California Peninsula is separated from the nearest Mexican mainland by the Gulf of California by around 200 km, much further than the nearly 100 km that a male *E. viridissima* was registered to fly when attracted to a bait in the Yucatán Peninsula (Pokorny et al. 2015). While most of the South American expansion records are likely due to the bees own dispersal capabilities (Anjos-Silva et al. 2006, Anjos-Silva 2007, 2008, Silva and Rebêlo 2009, Silva et al. 2013, Martins et al. 2016), the *E. viridissima* records from Baja California are unlikely to have gotten there by long distance migration. Alternatively, these bees are cavity nesters (May-Itzá et al. 2014), making it possible that occupied nests would survive the carrying from the continental lands to the peninsula. Also possible is that they were brought over accidentally along with normal commerce. The morphological conspicuousness of these bees (Figs 1–4) makes it hard to think that they have been in the area for long with no one noticing them before, so our best guess is that as these bees are a relatively recent arrival.

*Euglossa viridissima* appears to be well-established on Baja California, since the three sampled points (Fig. 5) are rather spread over the Cape Region and both sexes were relatively abundant at Todos Santos. However, the potential distribution mod-

eled by Hinojosa-Díaz et al. (2009) predicted there was not suitable habitat anywhere in the peninsula of Baja California for *E. viridissima*, as understood then, but for *Eulaema polychroma*, one of the other orchid bee species reaching the northwestern continental areas of Mexico.

Our floristic observations of the host plant differ from Arriaga and León de la Luz (1989), who found *T. stans* as a predominant species in some patches at the Pacific hills compared to the Gulf slope. Weather conditions are complex when comparing these two slopes in the Cape Region. Roberts (1989) mentioned that climatic and physiographic conditions make the Gulf slope more humid and hotter than the west side of the peninsula. However, Díaz and Troyo (1997) found drier and hotter oases in the east slope influenced by local phenomena. Arriaga and León de la Luz (1989) explained higher plant richness at the Pacific slope because its cooler and more humid conditions compared to the Gulf slope.

It is possible that abiotic factors (e.g. moisture, temperature) have more of an effect on populations than food availability. We do not discard the possibility that natural enemies also play an important role on these boundaries (e.g. more humid places increase likelihood to fungus attacks on immature stages). The new finding of *E. viridissima* at the Cape Region highlights its biological relevance as an important element of the Neotropical area. In addition, since the oases of the Baja California peninsula are shaped by different factors such as water availability, type of soil, geographical position, and degree of anthropogenic disturbance, the biological communities may respond to such insular-like conditions, presenting variation in structure and abundance (Jiménez et al. 2015, Arriaga and Rodríguez-Estrella 1997). Furthermore, considering the about 21 species of orchids restricted to some deep valleys or higher elevations (>600 m) in Sierra de La Laguna, BCS (Medel-Narváez pers. com. Jan. 20<sup>th</sup> 2017), it makes conceivable to think of possible euglossines-plant interactions, but also to find specific relationships with endemic orchid species. However, further research on these subjects is encouraged.

Overall, the records of *E. viridissima* in the Cape Region of the Baja California peninsula represent an important piece of information regarding these bee's distribution and likely dispersal or ecological capabilities. They also bring the opportunity to stress the need to sample the local bee faunas, in a time when pollinators are known to be declining in different parts of the world.

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RESEARCH ARTICLE



# Species limits within the widespread Amazonian treefrog Dendropsophus parviceps with descriptions of two new species (Anura, Hylidae)

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# Abstract

The genus *Dendropsophus* is one of the most speciose among Neotropical anurans and its number of described species is increasing. Herein, molecular, morphological, and bioacoustic evidence are combined to assess species limits within *D. parviceps*, a widely distributed species in the Amazon Basin. Phylogenetic relationships were assessed using 3040 bp sequences of mitochondrial DNA, genes 12S, ND1, and CO1. The phylogeny shows three well-supported clades. Bioacoustic and morphological divergence is congruent with those clades demonstrating that *Dendropsophus parviceps* is a species complex. *Dendropsophus parviceps sensu stricto* occurs in the Amazon basin of Ecuador, northern Peru, southern Colombia and northwestern Brazil. It is sister to two previously undescribed species, *D. kubricki* **sp. n.** from central Peru and *D. kamagarini* **sp. n.** from southern Peru, northeastern Bolivia, and northwestern Brazil. Genetic distances (uncorrected *p*, gene 12S) between *D. parviceps* and the new species is 3 to 4%. *Dendropsophus kamagarini* **sp. n.** can be distinguished from *D. parviceps*). *Dendropsophus kubricki* **sp. n.** differs from *D. parviceps* by having scattered low tubercles on the upper eyelids (smooth in *D. parviceps*). *Dendropsophus parviceps* and both new species differ from all their congeners by their small size (adult maximum SVL = 28.39 mm in females, 22.73 mm in males) and by having a bright orange blotch on the hidden

areas of the shanks and under arms. The advertisement call of the two new species has lower dominant frequency relative to *D. parviceps*. Probable speciation modes are discussed. Available evidence indicates that ecological speciation along an elevation gradient is unlikely in this species complex.

#### **Keywords**

Advertisement call, Amazon Basin, cryptic species, integrative taxonomy, morphology, phylogeny

#### Introduction

The upper Amazon Basin harbors the highest diversity of amphibian species in the world (Bass et al. 2010; Duellman 1999). In the last decade, the use of genetic characters in amphibian taxonomy has helped to discover a large number of cryptic species through the upper and lower Amazon Basin (e.g., Almendáriz et al. 2014; Brown et al. 2008; Brown and Twomey 2009; Caminer and Ron 2014; Elmer and Cannatella 2008; Fouquet et al. 2015; Moravec et al. 2014; Páez-Vacas et al. 2010; Rivera-Correa and Orrico 2013; Rojas et al. 2015; Rojas et al. 2016; Ron et al. 2012; Twomey and Brown 2008). The use of genetic characters in combination with morphological and bioacoustic evidence allows unambiguous delimitation of species under the evolutionary species concept (de Queiroz 1998; de Queiroz 2007; Padial et al. 2009).

*Dendropsophus* Fitzinger 1843 is the most speciose genus of hylid frogs in the Neotropics. Currently it has 102 formally described species (Frost 2017). The few systematics studies of *Dendropsophus* that have included genetic evidence have resulted in the discovery of a large number of undescribed species (e.g., Fouquet et al. 2015; Gehara et al. 2014; Motta et al. 2012; Rivera-Correa and Orrico 2013). These studies underscore the need of genetics-based taxonomic reviews in the genus *Dendropsophus*.

Within *Dendropsophus*, some species groups are not monophyletic and relationships among their species are unclear (Faivovich et al. 2005; Fouquet et al. 2011; Fouquet et al. 2015; Motta et al. 2012; Pyron and Wiens 2011; Wiens et al. 2010). One of them is the *Dendropsophus parviceps* species group (*sensu* Faivovich et al. 2005). It contains 15 species (Frost 2017): *D. bokermanni* (Goin, 1960), *D. brevifrons* (Duellman and Crump 1974), *D. counani* Fouquet et al., 2015, *D. frosti* Motta et al., 2012, *D. giesleri* (Mertens, 1950), *D. grandisonae* (Goin, 1966), *D. koechlini* (Duellman & Trueb, 1989), *D. luteoocellatus* (Roux, 1927), *D. microps* (Peters, 1872), *D. parviceps* (Boulenger, 1882), *D. pauiniensis* (Heyer, 1977), *D. ruschii* (Weygoldt & Peixoto, 1987), *D. schubarti* (Bokermann, 1963), *D. subocularis* (Dunn, 1934), and *D. timbeba* (Martins & Cardoso, 1987). Eleven species of the group occur in the Amazon basin: *D. bokermanni*, *D. brevifrons*, *D. counani*, *D. frosti*, *D. grandisonae*, *D. koechlini*, *D. luteoocellatus*, *D. pauiniensis*, *D. schubarti*, and *D. timbeba*. Of the remaining, three occur in the Brazilian Atlantic Forest (*D. giesleri*, *D. microps*, and *D. ruschii*) and one in the lowlands of eastern Darien (Panama) and northwestern Colombia (*D. subocularis*). A recent phylogeny by Fouquet et al. (2015) recovered the "*D. parviceps* clade" with strong support. The clade included *D. bokermanni*, *D. brevi-frons*, *D. counani*, *D. frosti*, *D. koechlini*, and *D. parviceps*, where *D. koechlini* is sister to a clade of the remaining species.

Dendropsophus parviceps is a small treefrog described by Boulenger (1882) from "Sarayacu" (= Sarayaku), Pastaza Province, Ecuador. Dendropsophus parviceps is characterized by having a short and truncate snout, one bar below the orbit, dark brown markings on dorsum, and a bright orange blotch on the proximal ventral surface of the shanks (Duellman and Crump 1974; Duellman 1978). Dendropsophus parviceps is widely distributed in the Amazon Basin of Brazil, Venezuela, Colombia, Ecuador, Peru, and Bolivia (Frost 2017). Its elevation range is 186–1600 m (Ron and Read 2012). Duellman (2005) suggested that D. parviceps from southern Peru might not be conspecific with D. parviceps sensu stricto because Ecuadorian populations are smaller and lack a prominent tubercle on the edge of the upper eyelid. Until now a comprehensive taxonomic review of D. parviceps has been missing. Herein we assess the species limits within "D. parviceps" with genetic, morphological, and bioacoustic data. Our results reveal the existence of two new species that we describe here.

### Materials and methods

#### Morphological analyses

Frogs were fixed in 10% formalin and preserved in 70% ethanol. Examined specimens, listed in Appendix 1, are housed at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ), and the División de Herpetología, Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru. We also examined the holotype of *Dendropsophus parviceps* at the Natural History Museum (NHM), London, UK.

The following measurements were made with digital calipers (nearest 0.01 mm) for adult specimens, following Cisneros-Heredia and McDiarmid (2007), Duellman (1970), and Motta et al. (2012):

**SVL** snout-vent length; **HW** head width; **HL** head length; **END** eye to nostril distance; **IN** internarial distance; **FL** femur length; **TL** tibia length; **FL** foot length.

A total of 159 specimens from Ecuador and Peru was measured. Webbing formulae are described following Cisneros-Heredia and McDiarmid (2007). Sex was determined by size differences (mean male SVL = 16.4 mm and mean female SVL = 22.5 mm) and by gonadal inspection. Description of coloration in life is based on field notes and digital color photographs.

Principal Components Analysis (PCA) was used to assess morphometric differentiation between species. Prior to analysis, all morphometric variables were logtransformed to achieve a normal distribution. To remove the effect of body size, the PCA was applied to the residuals of the linear regressions between the SVL and the morphometric variables, for males and females separately. Only principal components with eigenvalues > 1 were retained. We compared morphometric variables between species with Student's t-test. All analyses were performed using JMP<sup>®</sup> 9.0.1 (SAS Institute 2010).

#### **Bioacoustic analyses**

Recordings were made with two digital recorders Olympus LS-10 and Marantz professional PMD620MKII Handheld Solid State Recorder attached to a directional microphone Sennheiser K6–ME67. We also included published recordings from Peru, Tambopata (Cocroft et al. 2001), and Bolivia, Cobija (Márquez et al. 2002). Recordings are deposited at the on QCAZ collection.

Calls were analyzed using software Raven 1.3 (Charif et al. 2004) at a sampling rate of 44100 Hz and a resolution of 16 bits. Spectral parameters were obtained using a Fast Fourier Transformation (FFT) of 4096 points, a frequency resolution of 10.8 Hz, window type Hann and filter bandwidth of 52.2 Hz.

Terminology for call parameters follows Köhler et al. (2017) and Toledo et al. (2015). We measured the following variables: (1) call duration: time from the beginning to the end of the call; (2) note duration: time from beginning to end of the note; (3) rise time: time from the beginning of the note to the point of maximum amplitude; (4) number of pulses: number of pulses in the note; (5) pulse rate: number of pulses per note duration; (6) interval between notes: time from the end of one note to the beginning of the next; (7) dominant frequency: frequency with the most energy, measured along the entire call; (8) initial frequency: frequency at the beginning of the note; and (9) final frequency: frequency at the end of the note. If available, several calls or notes were analyzed per individual to calculate an individual average.

The calls of members of the *Dendropsophus parviceps* group (*sensu* Fouquet et al. 2015) consist of one high-pitched pulsed trill followed or not by a series of clicks: *D. counani* (Fouquet et al. 2015), *D. bokermanni* (Duellman and Crump 1974; Fouquet et al. 2015; Read and Ron 2012), *D. brevifrons* (Fouquet et al. 2015; Read and Ron 2012), *D. brevifrons* (Fouquet et al. 2015; Read and Ron 2011), and *D. koechlini* (Duellman and Trueb 1989; Fouquet et al. 2015). Therefore, we used a note-centered approach to define what is considered a call and a note (*sensu* Köhler et al. 2017).

A Principal Components Analysis (PCA) was conducted to evaluate call differentiation between species. We also performed Student's t-test to assess differences between species in the acoustic variables. For the PCA, only components with eigenvalues > 1 were retained. All statistical analyses were performed using JMP<sup>®</sup> 9.0.1 (SAS Institute 2010). Some recordings did not have temperature registered but temperature variation in equatorial rainforests at night is low (Duellman 1978) and therefore unlikely to severely influence the analyses.

#### Phylogenetic analyses

#### DNA extraction, amplification, and sequencing

Total DNA was extracted from muscle and liver preserved in 95% ethanol or tissue storage buffer using guanidine–thiocyanate extraction protocol of M. Fujita (unpublished). Polymerase chain reaction (PCR) was used to amplify the mitochondrial genes 12S rRNA (12S), Cytochrome Oxidase 1 (CO1), and a continuous fragment of 16S (partial sequence), tRNA<sup>Leu</sup>, NADH dehydrogenase subunit 1 (ND1), tRNA<sup>le</sup>, and tRNA<sup>Gln</sup>. PCRs were performed in 25  $\mu$ l reactions using 2.5  $\mu$ l of PCR buffer, 1.5  $\mu$ l MgCl<sub>2</sub>, 0.5  $\mu$ l of each primer, 0.5  $\mu$ l of each dNTP, 0.25  $\mu$ l of Taq polymerase, 1 U of DNA, and 18.25  $\mu$ l dH<sub>2</sub>O. Primers are listed in Table 1. PCR amplification was carried under standard protocols. PCR products were visualized in 1% agarose gel, and primers residues and dNTPs were removed from PCR products using ExoSAP-It purification. Amplified products were sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea).

New sequences were obtained from 61 specimens from the upper Amazon Basin of Ecuador and Peru. A sequence of *Dendropsophus parviceps* available in GenBank published by Faivovich et al. (2005) from Brazil (Acre) was also downloaded. Sequences of three closely related species, *D. brevifrons*, *D. frosti*, and *D. koechlini* were also included. *Dendropsophus marmoratus* and *Xenohyla truncata* were used as outgroups. Sequences of *D. brevifrons*, *D. frosti*, and *X. truncata* were published by Fouquet et al. (2015), Motta et al. (2012), and Faivovich et al. (2005), respectively.

Sequences were assembled and aligned in Geneiuos Pro v5.4.6 (Kearse et al. 2012) using the MAFFT plugin under the L-INS-i algorithm (Katoh et al. 2002). Manual adjustments to the alignment were made using Mesquite v3.04 (Maddison and Maddison 2015). ND1 and CO1 gene sequences were translated into amino acids in Mesquite to confirm the alignment and verify the absence of stop codons.

#### Phylogeny

Phylogenetic relationships were inferred using Maximum likelihood (ML) with software GARLI v2.0 (Zwickl 2006) and Bayesian inference with MrBayes v3.1.2 (Ronquist et al. 2012). The best partition strategy and the best-fit substitution model of DNA evolution for each partition were selected using PartitionFinder v1.1.0 (Lanfear et al. 2012) according to the Bayesian Information Criterion (BIC). We defined nine *a priori* partitions: 12S, 16S, tRNAs, and one partition for each codon position of ND1 and CO1.

Maximum likelihood analyses were performed with ten replicates starting from stepwise addition trees (streefname = stepwise). Other GARLI settings were set to default values (Zwickl 2006). Bootstrap support was evaluated through 500 replicates. The 50% majority rule consensus for the bootstrap trees was obtained with Mesquite v3.04 (Maddison and Maddison 2015). Bayesian analyses were performed with two

Gene	Primer	Primer sequence (5'-3')	Source	
125	tPhe-frog	ATAGCRCTGAARAYGCTRAGATG	Wiens et al. (2005)	
	tVal-frog	TGTAAGCGARAGGCTTTKGTTAAGCT	Wiens et al. (2005)	
ND1	16S-frog	TTACCCTRGGGATAACAGCGCAA	Wiens et al. (2005)	
	WL384	384 GAGATWGTTTGWGCAACTGCTCG Moen and V		
	WL379b	GCACTAGCAATAATTATYTGAACBCC	This study	
	tMet-frog	TTGGGGTATGGGCCCAAAAGCT	Wiens et al. (2005)	
CO1	COI-BirdF1	TTCTCCAACCACAAAGACATTGGCAC	Hebert et al. (2004)	
	COI-BirdR2	ACGTGGGAGATAATTCCAAATCCTGG	Hebert et al. (2004)	
	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)	
	dgHCO2198	TAAACTTCAGGGTGACCAAARAAYCA	Folmer et al. (1994)	

Table 1. Primers used in this study.

searches of  $35 \times 10^7$  generations each with four Markov chains and trees sampled every 5000 generations; stationarity and convergence were assessed in Tracer v1.6 (Rambaut et al. 2014) examining the standard deviation of split frequencies and plotting the –lnL per generation. Trees generated before stationarity were discarded as burn-in. Additionally, pairwise genetic distances (uncorrected *p*) were calculated for 12S using MEGA 6.0 (Tamura et al. 2013).

# Results

#### Phylogenetic relationships

The total alignment of concatenated DNA sequences had 3040 base pairs from mitochondrial markers 12S rRNA (~895 bp), small fragment of 16S rRNA (~282 bp), portions of tRNA (~215 bp), ND1 (~961 bp) and CO1 (~687 bp) from 70 individuals. Genes sequenced and GenBank accession numbers are listed in Appendix 2. The best partition strategy and the best-fit model for each partition are shown in Table 2.

The phylogenetic relationships strongly support *Dendropsophus parviceps* as monophyletic (posterior probability, pp = 1 and bootstrap = 99) (Fig. 1). There are three clades within *D. parviceps*, each strongly supported. One clade is distributed in southern Peru (e.g., Madre de Dios and Cusco regions) and northwest Brazil (Acre); we refer to this clade as the "Southern Clade" hereafter. The second clade is distributed in northern and central Peru (e.g., Sierra del Divisor, Río Tapiche, and Chambira) ("Central Clade" hereafter). The third clade is distributed in eastern Ecuador (called "Northern Clade" hereafter). Maximum pairwise uncorrected genetic distance for 12S between the Central Clade and the Southern Clade is 2.8%, between the Northern Clade and the Central Clade is 3.2% and between the Northern Clade and the Southern Clade is 3.7%.

Mean *p* genetic distance within the Central Clade is 1.3% (range 0-1.3%) while within the Southern Clade is 0.07% (range 0-0.15%). The Northern Clade is divided

Partition Best Model		Partition blocks		
1	GTR + G	12S, tRNA, ND1, 1 <sup>st</sup> position		
2	K80 + I + G	165		
3	HKY + I	ND1, 2 <sup>nd</sup> position		
4	GTR + I	ND1, 3 <sup>rd</sup> position, CO1, 3 <sup>rd</sup> position		
5	K80 + I	CO1, 1 <sup>st</sup> position		
6	F81	CO1, 2 <sup>nd</sup> position		

**Table 2.** Partition strategy and the best-fit model of substitution for each partition block used in phylogenetic analyses.

in two subclades also with high support (pp = 1 and bootstrap = 99%). One subclade includes populations in the northern Amazon of Ecuador on the Napo River while the other includes populations in the central and southern Amazon of Ecuador (Fig. 1). Mean genetic divergence between these two subclades is 0.8% (range 0.4–1.2%) suggesting that they are deep conspecific lineages.

### Morphological comparisons

Morphometric variables from adults are summarized in Table 3. The Northern Clade has smaller size than the Southern and Central clades (Fig. 2; Table 3; Student's t test Northern Clade vs. Southern Clade, t = 16.18, df = 98, p < 0.001 for males and t = 6.85, df = 35, p < 0.001 for females; Student's t test Northern Clade vs. Central Clade, t = -12.86, df = 77, p < 0.001 for males and t = -6.08, df = 36, p < 0.001 for females).

Two components with eigenvalues > 1.0 were extracted from the PCA. Both PCs account for 52.1% of the total variation for males (Table 4). Principal Component I has high positive loadings for femur length and tibia length and PC II for head width and internarial distance (Table 4). The morphometric space shows high overlap between clades (Fig. 3).

Two PCs with eigenvalues > 1.0 explain the 58% of total variation among females (Table 4). The highest loadings for PC I were head width, femur length, and tibia length; PC II has high loadings for eye to nostril distance and is negatively correlated with head length and foot length (Table 4). As in the PCA for males, there is high overlap between clades in morphometric space (Fig. 3).

#### **Bioacoustic comparisons**

The call of the *Dendropsophus parviceps* species complex consists of one pulsed trill (Fig. 4A, C, E). The pulsed trill is facultatively followed by one or more click notes (Fig. 4B, D, F). The pulsed trill appears to function as advertisement call because males produce these calls repeatedly and antiphonally. Acoustic parameters for the advertisement calls and click notes are shown in Table 5–7.



**Figure 1.** Bayesian consensus phylogeny of *Dendropsophus parviceps* species complex based on 3040 bp of mtDNA. Node support is indicated with Bayesian posterior probabilities (pp) above branches and non-parametric bootstrap support below. Asteriks denote nodes with pp = 1 and bootstrap values = 100%. Outgroups, bootstrap values < 60%, and pp < 0.8 are not shown. Museum number and locality are provided for each sample. Abbreviations: BR = Brazil, PE = Peru, and EC = Ecuador.

**Table 3.** Descriptive statistics for morphometric measurements of adult *Dendropsophus parviceps* (Northern Clade), *D. kamagarini* sp. n. (Southern Clade), and *D. kubricki* sp. n. (Central Clade). Mean  $\pm$  SD is given with range below. Abbreviations are: SVL = snout-vent length; HW = head width; HL = head length; END = eye to nostril distance; IN = internarial distance between the nostrils; FL = femur length; TL = tibia length; FL = foot length. All measurements are in mm.

	Dendropsop	bus parviceps	Dendropsophus kamagarini sp. n.		Dendropsophus kubricki sp. n.	
	Males	Females	Males	Females	Males	Females
	<i>n</i> = 65	<i>n</i> = 30	<i>n</i> = 35	<i>n</i> = 7	n = 14	n = 8
SVL	$16.4 \pm 0.84$	22.5 ± 1.17	19.9 ± 1.33	26.1 ± 1.67	$19.4 \pm 0.48$	26.0 ± 2.33
	(14.3-18.7)	(20.3-24.4)	(17.6-22.7)	(24.0-28.1)	(18.3-20.1)	(22.0-28.4)
HW	$5.2 \pm 0.30$	6.8 ± 0.32	$6.3 \pm 0.40$	8.2 ± 0.50	$6.4 \pm 0.24$	8.2 ± 0.85
	(4.6-5.9)	(6.2–7.4)	(5.5-7.0)	(7.3-8.8)	(6.0-6.7)	(6.8-9.3)
HL	4.9 ± 0.36	6.1 ± 0.54	$6.2 \pm 0.34$	7.7 ± 0.41	6.3 ± 0.29	7.5 ± 0.40
	(4.2–5.8)	(5.3–7.5)	(5.4-6.8)	(6.9-8.1)	(5.9–7.0)	(7.0-8.2)
END	$1.7 \pm 0.14$	$2.1 \pm 0.17$	$2.0 \pm 0.16$	$2.4 \pm 0.17$	2.1 ± 0.26	2.7 ± 0.33
	(1.4-2.2)	(1.9-2.4)	(1.7-2.3)	(2.4-2.6)	(1.8-2.7)	(2.3-3.3)
IN	$1.6 \pm 0.14$	$2.0 \pm 0.18$	$1.8 \pm 0.16$	$2.2 \pm 0.12$	$1.8 \pm 0.11$	$2.3 \pm 0.22$
	(1.3-2.0)	(1.7-2.4)	(1.5-2.2)	(2.0-2.4)	(1.5-2.0)	(2.0-2.7)
FL	7.8 ± 0.48	$11.2 \pm 0.67$	9.8 ± 0.67	$13.1 \pm 0.74$	9.7 ± 0.52	12.7 ± 0.69
	(6.6-8.9)	(9.9–12.6)	(8.5-11.3)	(12.1-14.0)	(8.9–10.7)	(11.9–13.6)
TL	8.6 ± 0.49	$12.2 \pm 0.65$	$10.6 \pm 0.74$	14.1 ± 0.56	$10.4 \pm 0.41$	$13.8 \pm 1.08$
	(7.2–9.8)	(10.7–13.5)	(9.0-11.8)	(13.3-15.0)	(9.8-11.1)	(12.3–15.5)
FI	$6.5 \pm 0.49$	9.1 ± 0.88	8.3 ± 0.65	$11.3 \pm 0.81$	7.9 ± 0.38	$10.4 \pm 0.55$
<b>FL</b>	(5.4-7.7)	(7.3–10.6)	(7.0-9.4)	(10.3-12.6)	(7.3-8.8)	(9.6-11.5)



Figure 2. Boxplots for snout-vent length of adults of *Dendropsophus parviceps* (Northern Clade), *D. kamagarini* sp. n. (Southern Clade), and *D. kubricki* sp. n. (Central Clade). The line in the middle of the box represents the median, and the lower and upper ends of the box are the 25% and 75% quartiles, respectively; whiskers represent the minimum and maximum values. Each specimen is shown with a symbol.

The dominant frequency of the advertisement call of the Northern Clade is higher (range 5081.8–6869.1 Hz) than that of the Southern Clade (range 3164.1–4306.6 Hz) and Central Clade (range 3542.2–4394.5 Hz). There are significant differences in dominant frequency for advertisement calls between the Northern Clade and the

**Table 4.** Character loadings and eigenvalues for Principal Components (PC) I–II. The analysis was based on seven size-corrected morphometric variables of adult *Dendropsophus parviceps* (Northern Clade), *D. kamagarini* sp. n. (Southern Clade), and *D. kubricki* sp. n. (Central Clade). Bold numbers indicate highest loadings.

V	PCA f	emales	PCA males		
variables	PCI	PCII	PCI	PCII	
Head width	0.44	-0.08	0.37	0.44	
Head length	0.33	-0.47	0.35	0.08	
Eye to nostril distance	0.24	0.66	0.34	0.14	
Internarial distance	0.35	-0.12	0.13	0.76	
Femur length	0.44	0.22	0.51	-0.16	
Tibia length	0.43	0.32	0.47	-0.33	
Foot length	0.39	-0.41	0.36	-0.26	
Eingevalue	2.84	1.22	2.50	1.15	
% of variation	40.6	17.4	35.7	16.4	

**Table 5.** Acoustic parameters of *Dendropsophus parviceps* (Northern Clade). Mean ± SD is given with range below. Sample sizes are number of calls. All frequencies are in Hz and durations in s.

	Dendropsophus parviceps				
	Sarayaku	Canelos Río Verde		Yasuní	Combined
	(n = 3)	(n = 3)	(n = 2)	(n = 3)	(n = 11)
Advertisement call	$0.14 \pm 0.03$	$0.13 \pm 0.02$	$0.19 \pm 0.04$	0.11 ± 0.03	$0.14 \pm 0.04$
duration	(0.06-0.18)	(0.10 - 0.17)	(0.11 - 0.24)	(0.06-0.18)	(0.06 - 0.24)
Advertisement call	6523.1 ± 184.18	6454.1 ± 146.63	5364.7 ± 167	6490.4 ± 300.7	6278.8 ± 503.75
dominant frequency	(6115.4-6836.8)	(6169.3-6686.1)	(5081.8-5824.7)	(5953.9-6869.1)	(5081.8-6869.1)
Advertisement call	5997.4 ± 223.5	6020.5 ± 253.51	5074.7 ± 260.71	6130.3 ± 227.74	5870.1 ± 459.02
initial frequency	(5674-6546.1)	(5630.9-6352.3)	(4758.8-5835.5)	(5717.1-6729.1)	(4758.8-6729.1)
Advertisement call	6602.4 ± 202.04	6565.7 ± 186.92	5419.8 ± 178.33	6567.64 ± 261.07	6356.5 ± 510
final frequency	(6126.2-6836.8)	(6147.7-6750.7)	(5103.4-5835.5)	(6007.8-6966)	(5103.4-6966)
Advertisement call	$0.07 \pm 0.01$	$0.07 \pm 0.01$	$0.10 \pm 0.02$	$0.06 \pm 0.01$	$0.07 \pm 0.02$
rise time	(0.03-0.09)	(0.05 - 0.01)	(0.05 - 0.12)	(0.04 - 0.09)	(0.03 - 0.12)
Number of pulses of	17.53 ± 3.22	17 ± 1.10	16.61 ± 3.53	$14.2 \pm 4.45$	16.1 ± 3.87
advertisement call	(8-22)	(16-19)	(8-20)	(9–25)	(8-25)
Advertisement call	126.76 ± 10.6	133.43 ± 13.60	86.1 ± 11.79	126.57 ± 19.35	119.61 ± 22.2
pulse rate	(97.83-146.34)	(111.76-152.38)	(65.57-125.9)	(77.92-171.88)	(65.57-171.88)
Call duration	$0.45 \pm 0.21$	$0.44 \pm 0.20$	$0.61 \pm 0.30$	$0.72 \pm 0.4$	$0.6 \pm 0.3$
	(0.27 - 0.90)	(0.26 - 0.77)	(0.37 - 1.22)	(0.26 - 2.12)	(0.26 - 2.12)
Inter note interval	$0.08 \pm 0.021$	$0.07 \pm 0.02$	$0.12 \pm 0.017$	$0.08 \pm 0.012$	$0.09 \pm 0.02$
	(0.03-0.10)	(0.05-0.11)	(0.10-0.15)	(0.06-0.12)	(0.03-0.15)
Click pate dynation	$0.05 \pm 0.014$	$0.052 \pm 0.015$	$0.051 \pm 0.015$	$0.042 \pm 0.011$	$0.05 \pm 0.013$
	(0.03-0.076)	(0.028-0.075)	(0.03-0.082)	(0.028 - 0.078)	(0.028 - 0.082)
Click note dominant	6334.2 ± 151.8	6471.9 ± 194.80	5284.8 ± 109.5	6544.9 ± 207.7	6334.6 ± 460.3
frequency	(5964.7-6567.6)	(6190.8-6761.4)	(4866.5-5415.6)	(5997-6922.9)	(4866.5-6922.9)
Number of pulses of	$2.2 \pm 1.3$	$3.3 \pm 1.7$	$1.65 \pm 0.92$	$2.1 \pm 0.84$	$2.1 \pm 1.08$
click note	(1-5)	(1-6)	(1-5)	(1-4)	(1-6)
Cli I a stati	$0.024 \pm 0.007$	$0.024 \pm 0.008$	$0.025 \pm 0.008$	$0.021 \pm 0.006$	$0.023 \pm 0.007$
	(0.015-0.038)	(0.014-0.038)	(0.015-0.042)	(0.013-0.039)	(0.013-0.042)
Click note pulse rate	47.96 ± 31.29	59.10 ± 22.22	31.37 ± 9.95	48.54 ± 15.3	46.89 ± 20.06
	(14.08-138.89)	(20.83-100)	(18.52-60.98)	(20-103.45)	(14.08-138.89)
Inter click notes	0.064 ±0.016	0.066 ± 0.019	$0.12 \pm 0.03$	$0.075 \pm 0.012$	$0.08 \pm 0.023$
interval	(0.031-0.088)	(0.017-0.088)	(0.08 - 0.18)	(0.038-0.098)	(0.017 - 0.18)



**Figure 3.** Principal components from analysis of seven size-corrected morphological variables of adults of *Dendropsophus parviceps* (Northern Clade), *D. kamagarini* sp. n. (Southern Clade), and *D. kubricki* sp. n. (Central Clade). The contribution of each axis to total variation is indicated in parenthesis.

Southern Clade (Student's t test, t = 13.68, df = 17, p < 0.001), and between the Northern Clade and the Central Clade (Student's t test, t = 9.94, df = 13, p < 0.001). The number of pulses of the advertisement calls of Southern Clade is larger (12–32) than that of the Northern Clade (8–25; differences are significant: Student's t test, t = -2.48, df = 17, p = 0.02).

The PCA for advertisement calls shows that the Northern Clade is acoustically distinct from the Southern and Central clades (Fig. 5). Two components with eigenvalues > 1.0 account for 87.7% of the acoustic variation (Table 8). The highest loadings for PC I were dominant frequency, initial frequency, final frequency, and number of pulses; the highest loadings for PC II were note duration, rise time, and pulse rate. The northern clade differs from the southern and central clades mostly along PC I, which mainly represents variation in call frequency (Fig. 5).

#### Species limits

The integrative analyses presented in this work show congruent differences in genetic, morphological, and bioacoustic characters that demonstrate the existence of three confirmed candidate species within "*Dendropsophus parviceps*": Northern, Central, and Southern clades. Because the type locality of *Dendropsophus parviceps* is in Amazonian Ecuador (Sarayaku), we consider that the Northern Clade is *Dendropsophus parviceps sensu stricto*. This assignment is confirmed by the lack of tubercles in the eyelid of the holotype, a character state unique to the Northern Clade. Therefore, the two Peruvian species are new and we describe them in the following section.



**Figure 4.** Advertisement calls of the *Dendropsophus parviceps* species complex. On each species, the oscillograms are shown above and spectrograms . *Dendropsophus parviceps* (Northern Clade): **A** Advertisement call from Sarayaku (QCAZ 52753) **B** Advertisement call + clicks from Canelos (QCAZ 52837). *Dendropsophus kamagarini* sp. n. (Southern Clade): **C** Advertisement call from Tambopata **D** Advertisement call + clicks from Tambopata. *Dendropsophus kubricki* sp. n. (Central Clade): **E** Advertisement call from Río Tapiche **F** Advertisement call + clicks from Río Tapiche. Calls from Peru lack specimen vouchers. A note-centered approach was used to define what is considered a call and a note (*sensu* Köhler et al. 2017).


**Figure 5.** Principal components from analysis of seven acoustic variables of advertisement calls of *Dendropsophus parviceps* (Northern Clade), *D. kamagarini* sp. n. (Southern Clade), and *D. kubricki* sp. n. (Central Clade). The contribution of each principal component to explain total variation is indicated in parenthesis.

#### Systematic accounts

#### Dendropsophus parviceps (Boulenger, 1882)

Figs 1, 6, 7A, 8

Hyla parviceps Boulenger, 1882: 393. Holotype BMNH 1947.2.13.51, an adult female from "Sarayacu", Pastaza Province, Ecuador.
Hyla parviceps – Duellman and Crump 1974: 19; Duellman 1978: 156.
Dendropsophus parviceps – Faivovich et al. 2005: 93.

**Diagnosis.** Throughout the species account, coloration refers to preserved specimens unless otherwise noted. *Dendropsophus parviceps* is characterized by: (1) small size, mean SVL 16.4 mm in males (range 14.3–18.7; n = 65), 22.5 mm in females (range 20.3–24.4; n = 30); (2) throat sexually dimorphic, dark flecks posteriorly in males vs. white blotch with two or three longitudinal stripes or without stripes posteriorly in females (Fig. 8); (3) snout truncate in dorsal and lateral views, slightly inclined posteroventrally in lateral view; (4) nostrils slightly prominent; (5) tympanum visible,

	Dendropsophus kamagarini sp. n.							
	Tambopata ( <i>n</i> = 4)	Amarakaeri (n = 1)	Chontachaka ( <i>n</i> = 1)	Cobija ( <i>n</i> = 1)	Rio Madeira (n = 1)	Combined ( <i>n</i> = 8)		
Advertisement call duration	0.14 ± 0.03 (0.09-0.20)	0.12 ± 0.01 (0.10-0.14)	0.12 ± 0.01 (0.11-0.14)	0.15 ± 0.005 (0.14-0.16)	0.13 ± 0.02 (0.1-0.17)	0.14 ± 0.02 (0.09-0.2)		
Advertisement call dominant frequency	3669.6 ± 277.16 (3164.1-4112.8)	3639.1 ± 79.5 (3542.2-3703.7)	4208.19 ± 66.5 (4091.3-4306.6)	3948.1 ± 184.8 (3779.1–4263.6)	3983.6 ± 64.1 (3811.4-4059)	3782.3 ± 286.92 (3164.1-4306.6)		
Advertisement call initial frequency	3442.5 ± 246.8 (2964.8-3854.4)	3397.9 ± 59.27 (3316.1-3456.1)	4011.3 ± 153.4 (3886.7-4274.3)	3800.6 ± 51.8 (3671.4-3876)	3746.8 ± 73.5 (3639.1–3854.4)	3562.2 ± 274.4 (2964.8-4274.3)		
Advertise- ment call final frequency	3685.8 ± 277.9 (3175.8-4123.6)	3636.9 ± 69.53 (3542.2-3703.7)	4205.1 ± 69.2 (4080.5-4306.6)	3982.6 ± 175.2 (3779.1-4252.8)	4000.3 ± 45.11 (3929.8–4059)	3798.2 ± 285.91 (3175.8-4306.6)		
Advertisement call rise time	0.07 ± 0.01 (0.04-0.1)	0.06 ± 0.01 (0.05-0.07)	0.06 ± 0.004 (0.05-0.07)	0.08 ± 0.002 (0.072-0.079)	0.06 ± 0.01 (0.05-0.08)	0.07 ± 0.01 (0.04-0.1)		
Number of pulses of adver- tisement call	22.8 ± 4.14 (14-32)	16.6 ± 1.52 (15-19)	14.1 ± 1.07 (12-15)	23 ± 1.4 (20-24)	17 ± 2.88 (13-21)	21 ± 4.62 (12-32)		
Advertisement call pulse rate	161.91 ± 9.66 (107.69-178.95)	140 ± 5.47 (131.15-145.63)	114.94 ± 7.11 (103.44–125)	151.4 ± 6.4 (140.8–155.8)	132.32 ± 5.23 (123.9–143.9)	151.84 ± 17.18 (103.45-178.94)		
Call duration	0.31 ± 0.048 (0.26-0.41)	0.46 ± 0.062 (0.40-0.55)	0.69 ± 0.093 (0.54-0.80)	NA	0.53 ± 0.13 (0.45-0.81)	0.46 ± 0.17 (0.26-0.81)		
Inter note interval	0.10 ± 0.011 (0.09-0.13)	0.09 ± 0.014 (0.08-0.11)	0.08 ± 0.009 (0.07-0.09)	NA	$0.09 \pm 0.006$ (0.08-0.01)	0.09 ± 0.01 (0.07-0.13)		
Click note duration	0.051 ± 0.009 (0.03-0.067)	0.052 ± 0.017 (0.03-0.082)	0.063 ± 0.02 (0.035-0.10)	NA	0.07 ± 0.008 (0.05-0.08)	0.06 ± 0.01 (0.03-0.10)		
Click note dominant frequency	3610.3 ± 267.3 (3164.1-4048.2)	3563.7 ± 62.11 (3445.3-3649.9)	4351.9 ± 75.3 (4242-4532.7)	NA	4024.9 ± 41.86 (3962.1-4102.1)	3981.2 ± 341.9 (3164.1-4532.7)		
Number of pulses of click note	6.3 ± 2.2 (1-9)	3.75 ± 2.7 (1-8)	4.5 ± 2.3 (1-10)	NA	7.83 ± 0.91 (6-10)	5.87 ± 2.53 (1-10)		
Click note rise time	$\begin{array}{c} 0.025 \pm 0.005 \\ (0.015 - 0.034) \end{array}$	0.026 ± 0.008 (0.014-0.04)	0.032 ± 0.010 (0.017-0.05)	NA	0.03 ± 0.004 (0.02-0.04)	0.03 ± 0.007 (0.01-0.05)		
Click note pulse rate	124.01 ± 34.53 (23.81–151.52)	64.11 ± 33.80 (25.64–112.9)	67.82 ± 19.72 (18.52–111.11)	NA	117 ± 8.11 (91–140.35)	95.6 ± 35.28 (18.52–151.52)		
Inter click notes interval	$0.08 \pm 0.007$ (0.07-0.084)	$0.085 \pm 0.015$ (0.069-0.11)	$0.068 \pm 0.008$ (0.054-0.084)	NA	$0.07 \pm 0.005$ (0.06-0.08)	$0.07 \pm 0.01$ (0.05-0.1)		

**Table 6.** Acoustic parameters of *Dendropsophus kamagarini* sp. n. (Southern Clade). Mean ± SD is given with range below. Sample sizes are number of calls. All frequencies are in Hz and durations in s.

concealed posterodorsally, tympanic membrane differentiated and annulus evident; (6) conical tubercles on upper eyelid absent; (7) thoracic fold absent; (8) ulnar tubercles and outer tarsal tubercles indistinct; (9) axillary membrane present; (10) skin on dorsal surfaces smooth with scattered small tubercles; skin on chest areolate; skin on belly, posterior surfaces of thighs, and subcloacal area coarsely areolate; skin on throat and other surfaces smooth; (11) dark brown markings on dorsum (Fig. 8); (12) thenar tubercle is distinct; (13) hand webbing formula II1<sup>1/2</sup>–2III2<sup>-</sup>–2<sup>-</sup>IV, feet webbing formula II1<sup>-</sup>–2<sup>-</sup>III1<sup>-</sup>–2<sup>-</sup>III1<sup>-</sup>–2<sup>-</sup>III1<sup>-</sup>–2<sup>-</sup>IV2–1<sup>-</sup>V; (14) in life, dorsal surfaces brown, tan or grayish tan; (15) orange to amber blotch on the proximal ventral surface of shanks and under arms, from the axillae to near the elbow, in life (white to creamy white in preservative);

	Dendropsophus kubricki sp. n.			
	Río Tapiche	Cordillera Azul	Combined	
	(n = 3)	( <i>n</i> = 1)	(n = 4)	
A. J	$0.13 \pm 0.02$	$0.23 \pm 0.04$	$0.19 \pm 0.06$	
Advertisement call duration	(0.1-0.16)	(0.13-0.3)	(0.1-0.3)	
	4062.6 ± 248.78	3998.9 ± 137.88	4024.7 ± 191.88	
Advertisement can dominant frequency	(3691.4-4394.5)	(3542.2-4242)	(3542.2-4394.5)	
Advortisoment call initial frequency	3722 ± 261.11	3664.9 ± 182	3688.1 ± 217.86	
Advertisement can initial frequency	(3222.7-4066.4)	(3380.7-4015.9)	(3222.7-4066.4)	
Advertisement call final frequency	4066.7 ± 250.85	4026.7 ± 105.17	4042.9 ± 178.72	
Advertisement can final frequency	(3691.4-4394.5)	(3703.7-4242)	(3691.4-4394.5)	
Advertisement call rise time	$0.06 \pm 0.008$	$0.12 \pm 0.02$	$0.09 \pm 0.03$	
	(0.05-0.08)	(0.06-0.15)	(0.05-0.15)	
Number of pulses of advertisement call	17.76 ± 2.47	27 ± 6.16	$23.26 \pm 6.24$	
Number of pulses of advertisement can	(14-22)	(14-27)	(14-34)	
Advertisement call pulse rate	140.76 ± 5.27	129.05 ± 34.18	133.79 ± 27.1	
Advertisement can pulse late	(133.33-158.27)	(110.6-228.57)	(110.6-228.57)	
Call duration	$0.42 \pm 0.11$	$0.54 \pm 0.06$	$0.44 \pm 0.12$	
	(0.23-0.63)	(0.45-0.59)	(0.23-0.63)	
Inter note interval	$0.076 \pm 0.013$	$0.08 \pm 0.006$	$0.08 \pm 0.011$	
	(0.05-0.10)	(0.075-0.088)	(0.05-0.10)	
Click note duration	$0.051 \pm 0.011$	$0.073 \pm 0.009$	$0.06 \pm 0.014$	
	(0.03-0.07)	(0.051-0.09)	(0.03-0.09)	
Click note dominant frequency	4069.2 ± 269.6	4023.3 ± 32.82	4057.4 ± 233.03	
Click note dominant nequency	(3703.1-4500)	(3962.1-4080.5)	(3703.1-4500)	
Number of pulses of click note	$4.5 \pm 1.71$	$6.4 \pm 1.3$	5 ± 1.8	
Number of pulses of click note	(1-7)	(2-7)	(1-7)	
Click note rise time	$0.026 \pm 0.005$	$0.04 \pm 0.005$	$0.028 \pm 0.007$	
	(0.014-0.03)	(0.026-0.04)	(0.014-0.04)	
Click note pulse rate	85.84 ± 21.27	87.66 ± 15.0	86.3 ± 19.7	
	(27.03-117.6)	(39.22-111.11)	(27.03-117.6)	
Inter click notes interval	$0.083 \pm 0.012$	0.080 ± 0.0092	$0.082 \pm 0.011$	
	(0.058-0.10)	(0.066-0.095)	(0.06-0.10)	

**Table 7.** Acoustic parameters of *Dendropsophus kubricki* sp. n. (Central Clade). Mean ± SD is given with range below. Sample sizes are number of calls. All frequencies are in Hz and durations in s.

**Table 8.** Character loadings and eigenvalues for Principal Components (PC) I–II. The analysis was based on seven acoustic variables from advertisement calls of *Dendropsophus parviceps* (Northern Clade), *D. kamagarini* sp. n. (Southern Clade), and *D. kubricki* sp. n. (Central Clade). Bold numbers indicate highest loadings.

37 .• 11	PCA Advertisement call			
Variables	PCI	PCII		
Note duration	-0.24	0.58		
Dominant frequency	0.48	0.17		
Initial frequency	0.48	0.16		
Final frequency	0.48	0.17		
Rise time	-0.21	0.59		
Number of pulses	-0.40	0.20		
Pulse rate	-0.23	-0.43		
Eingevalue	3.80	2.34		
% of variation	54.2	33.5		



**Figure 6.** Dorsolateral and ventral views of *Dendropsophus parviceps* in life: **A, B** Adult male, from type locality Sarayaku, Pastaza, Ecuador (QCAZ 52752) **C, D** Adult male, from Canelos, Pastaza, Ecuador (QCAZ 52816) **E** Adult male, from Yasuní, Orellana, Ecuador (QCAZ 51073) **F** Amplectant pair from Nuevo Rocafuerte, Río Napo, Orellana, Ecuador (QCAZ 44773–74) **G, H** Adult female, from Chiroisla, Río Napo, Orellana, Ecuador (QCAZ 44440). Photographs by S. Ron.



**Figure 7.** Dorsal and ventral views of the holotypes of the *Dendropsophus parviceps* species complex. **A** *Dendropsophus parviceps*, adult female, SVL = 26.55 mm (BMNH 1947.2.13.51) **B** *D. kamagarini* sp. n. adult male, SVL = 19.65 mm (CORBIDI 5246) **C** *D. kubricki* sp. n. adult male, SVL = 19.05 mm (CORBIDI 15778). Scale bar 10 mm.



**Figure 8.** Adults of *Dendropsophus parviceps* showing variation in dorsal and ventral coloration of preserved specimens. From left to right, first and second rows: QCAZ 52017, 52026, 52755, 51230 (males); third and fourth rows: CORBIDI 1040, 1059, QCAZ 48929, 52816 (males); fifth and sixth rows: QCAZ 44440–41, 27983, 44736 (females). See Appendix 1 for locality data. Scale bar 10 mm.

(16) one suborbital white bar present both in life and preservative; (17) thighs are black to dark brown with two or three white spots on the anterodorsal surfaces both in life and preservative; (18) iris in life is creamy white to reddish brown with brow or dark brown reticulations.

**Comparisons with other species.** *Dendropsophus parviceps* is most similar to *D. kamagarini* sp. n. and *D. kubricki* sp. n. The three species differ from other species of the *D. parviceps* group *sensu* Fouquet et al. 2015 (characters of other species of the group in parenthesis) by lacking dorsolateral light stripes [present in *D. bokermanni* (from Goin 1960) and in *D. brevifrons* (see Duellman and Crump 1974 and Read and Ron 2011)] and having, in life, an orange or amber blotch on the proximal ventral surface of shanks and under arms, from the axillae to near the elbow [absent in *D. bokermanni* (Goin 1960; Duellman and Crump 1974), in *D. brevifrons* (Duellman and Crump 1974), in *D. counani* (Fouquet et al. 2015), in *D. frosti* (Motta et al. 2012) and in *D. koechlini* (Duellman and Trueb 1989)]. *Dendropsophus parviceps* is also similar to *D. pauiniensis* (Heyer, 1977), but it can be distinguished by the presence of an orange or amber blotch on the proximal ventral surfaces of shanks in life (absent in *D. pauiniensis*).

Dendropsophus parviceps, D. kamagarini sp. n., and D. kubricki sp. n. further differ from species of the D. parviceps group (traits of other species of the D. parviceps group in parenthesis) as follows: from D. koechlini by having a white chest both in life and preserved [white with black flecks both in life and preserved (see Duellman and Trueb 1989)]; from D. bokermanni, D. brevifrons, D. counani, and D. frosti by having a mottled ventral coloration both in life and preserved [plain coloration both in life and preserved in D. bokermanni (from Goin 1960), in D. brevifrons (from Duellman and Crump 1974), in D. counani (from Fouquet et al. 2015), and in D. frosti (from Motta et al. 2012)]; from D. bokermanni, D. brevifrons, and D. counani by having a single suborbital bar [two suborbital bars (data of D. bokermanni and D. brevifrons from Duellman and Crump 1974, and of D. counani from Fouquet et al. 2015)] and two or three white spots on the anterior dorsal surfaces of the black thighs in life [cream or yellow spots in life (data of *D. bokermanni* and *D. brevifrons* from Duellman and Crump 1974, and of *D. counani* from Fouquet et al. 2015)]. The absence of canthal and rostral stripes also differentiates D. parviceps, D. kamagarini sp. n., and D. kubricki sp. n. from D. bokermanni, D. brevifrons, D. frosti, and D. koechlini [both stripes present in D. bokermanni and D. brevifrons (data of both species from Duellman and Crump 1974), canthal stripes in D. frosti (see Motta et al. 2012), and rostral stripes in D. koechlini (see Duellman and Trueb 1989)].

Dendropsophus parviceps differs from both new species by the absence of tubercles on the upper of eyelid (present). Dendropsophus parviceps also differs from D. kamagarini sp. n. and D. kubricki sp. n. by having translucent gray on the ventral surface of the thighs with dark brown flecks posteriorly in males, in life (black posteriorly in males, in life, in D. kamagarini sp. n. and in D. kubricki sp. n.).

**Variation.** Morphometric variation is shown in Table 3. Variation in dorsal and ventral coloration of preserved specimens is depicted on Figure 8. Dorsal coloration

varies from brown (e.g., QCAZ 52026, 52816) to dark brown (e.g., QCAZ 52755, CORBIDI 1059), gray (e.g., QCAZ 52017), grayish tan (e.g., QCAZ 44441, 51230), or grayish brown (e.g., QCAZ 44736, 52026) with dark brown markings with varying shapes (Fig. 8). The specimens with gray, grayish tan, and grayish brown coloration have scattered iridophores. The dorsum is smooth (e.g., QCAZ 51108, 52755), but some specimens have scattered small tubercles (e.g., QCAZ 53181, CORBIDI 1046).

The chest is white to cream (Fig. 8) with throat and belly varying from creamy white (e.g., QCAZ 51230), grayish brown (e.g., QCAZ 48929, 52017) to dark brown (e.g., QCAZ 44440) with dark brown or black flecks. The subcloacal area is areolate, its coloration is white (e.g., QCAZ 44441, 48929), but in some specimens is dark brown (e.g., QCAZ 52755).

**Color in life.** Based on digital photographs (Fig. 6): dorsum varies from brown, tan, grayish tan to reddish brown, some individuals have few scattered dorsolateral dark brown flecks; dorsal markings are dark brown; flanks are white or creamy yellow with black or dark brown diagonal bars; dorsal surfaces of forelimbs and shanks have dark brown transversal bars; anterodorsally, thighs are black or dark brown with two or three white spots. The single suborbital bar is white. The venter is translucent gray mottled with black or dark brown; in some females venter is black; chest is white; in adult males, throat is olive tan mottled with dark brown flecks anteriorly and translucent gray posteriorly; in adult females, throat is grayish tan or olive brown, dark brown, or black anteriorly with a white blotch with stripes posteriorly; the ventral surfaces of the limbs are translucent gray or translucent white, thighs are mottled with dark brown posteriorly; there is one bright orange or amber blotch in ventral surface of shank next to the knee, and in the posterior arm, from the axillae to near the elbow. The iris is creamy white to reddish brown with brown or dark brown reticulations.

**Calls (Fig. 4A–B).** Descriptive statistics of acoustic variables are provided in Table 5. Calls from ten individuals were analyzed. Three individuals (two of them unvouchered specimens and QCAZ 52753) were recorded at the type locality, Sarayaku, Pastaza Province, at night, on 6 April 2012 (QCAZ 52753 was recorded at 01:00h, temperature 22.4°C). Three individuals (QCAZ 52820, 52837 and one individual not collected) were recorded at Canelos, Pastaza Province, on 11 April 2012 (QCAZ 52820 recorded at 01:00h, 23.4°C). Two individuals (QCAZ 52017, 52918) were recorded at Río Verde, Tungurahua Province, on 19 September 2011 (QCAZ 52018, air temperature = 15.6°C). Finally, two individuals, not collected, were recorded at PUCE's Yasuní Research Station, Orellana Province, on 1 June 2011. We obtained one recording (unvouchered specimen) from the sound archives of Museo de Zoología, Pontificia Universidad Católica del Ecuador, made by Morley Read, at Pompeya-Iro road, km. 38, Yasuní National Park, Orellana Province.

The advertisement call is a pulsed note (Fig. 4A–B). The amplitude increases gradually at the beginning and falls sharply towards the end. The advertisement call may be emitted alone or followed by one or more click notes. However, the click notes occasionally are emitted alone. The click notes may be non-pulsed or pulsed. *Call comparisons between populations.* The advertisement calls from Río Verde separate along PC II from the calls of other populations (Fig. 5; Table 8). Mean dominant frequency is 5364.7 Hz (SD = 167) at Río Verde and 6498.1 Hz (SD = 239) at the other populations. Mean pulse rate is 86.1 pulses/s (SD = 11.79) at Río Verde and 127.6 pulses/s (SD = 15.5) at others. Mean rise time is 0.1 (SD = 0.02) and mean advertisement call duration is 0.19 s (SD = 0.04) at Río Verde, while mean rise time is 0.06 (SD = 0.01) and mean advertisement call duration is 0.12 s (SD = 0.03) at the other populations.

Distribution and ecology. Dendropsophus parviceps is known from 39 localities in the Ecuadorian Amazon basin (Napo, Orellana, Pastaza, Sucumbíos, and Tungurahua provinces; Fig. 9), few localities in the Peruvian Amazon basin at northwest Loreto (Andoas and San Jacinto; Fig. 9), the Colombian Amazon (Río Apaporis, Vaupés Department, and Ceilán, Caquetá Department; Cochran and Goin 1970; Fig. 9), and northern Brazil ("Taracuá" [= Taracuacá], Río Uaupés, Amazonas State; Melin 1941; see Remarks section). Elevation range is 151 m (Andoas) to 1600 m above sea level (Río Verde). Our Colombian records are unverified and are based on Cochran and Goin (1970) who examined three specimens (MLS 54 and MCZ 28058-59) and explicitly mention the absence of tubercles on the upper eyelids. Moreover, the SVL for a gravid female from Ceilan (MLS 54, 21.8 mm) falls outside the known size range of D. kubricki sp. n. and D. kamagarini sp. n. (Table 3). Ecuadorian localities from Sucumbíos province are close to the Colombian border further suggesting the presence of D. parviceps in Colombia. In addition, there is an unconfirmed register of D. parviceps from Ramal do Purupuru, km 34 on the BR-319 highway (3.3535°S, 59.8557°W, 35 m, Amazonas State, Brazil; Fig. 9).

*Dendropsophus parviceps* inhabits Amazonian lower montane forest, Amazonian foothill forest, and Amazonian evergreen lowland rainforest (habitat types based on Ron et al. 2017). *Dendropsophus parviceps* is an opportunistic breeder and can be found in primary and secondary forest, temporary ponds, flooded areas, swamps, and artificial open areas. Calling activity starts at dusk (17–18h), but it is mainly nocturnal. According to Lynch (2005), *D. parviceps* is a canopy species that visits the lower forest strata for breeding.

**Conservation status.** Its extent of occurrence is 256,944 km<sup>2</sup>. There is habitat degradation and fragmentation within its distribution as result of human activities, especially cattle rising, agriculture, and oil exploitation. Its presence in artificial open areas suggests that is tolerant of at least some level of habitat modification (Azevedo-Ramos et al. 2004). Its distribution range is large and includes extensive undisturbed areas (Ministerio de Ambiente Ecuador 2013). Therefore, we propose that *D. parviceps* should be assigned to the Least Concern category, following IUCN (2001) criteria.

**Remarks.** The advertisement call from Río Verde differs from other population calls (Fig. 5; Table 5). However, low genetic and morphological differences between Río Verde and the other populations indicate that they are conspecific. The Brazilian record from Tarauacá, Río Uaupés (Amazonas State) is based on Melin (1941) who reported a juvenile specimen with SVL = 21 mm. This specimen could be an adult



**Figure 9.** Distribution of *Dendropsophus parviceps* species complex. *Dendropsophus parviceps* (Northern Clade, blue crosses), *D. kubricki* sp. n. (Central Clade, green circles), *D. kamagarini* sp. n. (Southern Clade, orange rhombi). Stars = type locality, figures with a small black dot at the center = referred specimens, and hollow figures = unconfirmed records.

male because the throat is mottled with brown, characteristic of all adult males of *Dendropsophus parviceps*. Nevertheless, the SVL of the male from Taracuá falls above the range of variation of males of *D. parviceps* (14.3–18.7 mm) and it has a thoracic

fold (fold absent in *D. parviceps*; see above in Diagnosis section). Therefore, the record from Tarauacá requires verification.

The holotype has SVL = 26.5 mm (adult female; Fig. 7A). This value is above the range of variation of females of *D. parviceps* reported in Table 3 (20.3–24.4 mm). To confirm that the holotype falls within the range of variation of *D. parviceps* from Ecuador, we measured the SVL of the largest adult females from the QCAZ collection. We found three specimens with size close to the holotype: QCAZ 4340 (SVL = 26.13 mm) from La Selva (Sucumbíos Province), QCAZ 27028 (SVL = 26.03 mm) from Ahuano (Napo Province), and QCAZ 59772 (SVL 26.26 mm) from Comunidad Zarentza (Pastaza Province; Appendix 1). Although the holotype is the largest specimen known for *D. parviceps*, other specimens are smaller by just ~1% of SVL. Other characteristics of the external morphology of the holotype fall within the known variation of the Ecuadorian populations confirming that they are conspecific (Figs 7A, 8).

### Dendropsophus kamagarini sp. n.

http://zoobank.org/85BACA9D-07C6-4C1C-A818-B83DDD1510CA Figs 1, 7B, 10–11

**Holotype.** CORBIDI 5246, an adult male from Peru, Madre de Dios Department, Tambopata Province, Inotawa Lodge (12.8092°S, 69.3182°W), 192 m above sea level, collected on 9 October 2009 by P. J. Venegas.

Paratypes. CORBIDI 5259, an adult male from Peru, Madre de Dios Department, Tambopata Province, La Habana (12.6537°S, 69.1796°W), 192 m above sea level, collected on 18 October 2009 by V. Durán and M. Cuyos. Thirty-three adult males and seven adult females from Peru, Cusco Department, La Convención Province: Comunidad Ochigoteni (12.5758°S, 73.0900°W), 1696 m above sea level, CORBIDI 5392, adult female, collected on 19 October 2009 by G. Chávez; Pongo de Mainique (12.2581°S, 72.8425°W), 670 m above sea level, CORBIDI 5471, 5473, 5480, 5484, adult males, collected on 23 April 2010 by G. Chávez; Megantoni (12.2581°S, 72.8425°W), 670 m above sea level, CORBIDI 6659, 6664, 6679, 6685, 6687-88, 6698, adult males, CORBIDI 6692, 6694, adult females; Comunidad Nativa Chokoriari (11.9569°S; 72.9409°W), 434 m above sea level, CORBIDI 8067–68, 8070, adult males, CORBIDI 8069, adult female, collected on 8 December 2010 by D. Vásquez; Comunidad Nativa Poyentimari (12.1885°S, 73.0009°W), 725 m above sea level, CORBIDI 8150-51, 8153, 8228-36, 8285-86, 8305, 8476, adult males, CORBIDI 8152, 8463, adult females, collected on 28 November 2010 by G. Chávez; Puyantimari (12.1861°S, 73.0004°W), 710 m above sea level, CORBIDI 9762, adult male, collected on 8 September 2011 by D. Vásquez and K. García; Pagoreni norte (11.7115°S, 73.8967°W), 402 m above sea level, CORBIDI 10018, adult female, CORBIDI 10019, adult male, collected on 22 November 2011 by V. Durán; Palmeiras-Alto Shimá (12.5453°S, 73.1350°W), 1500 m above sea level, CORBIDI 10585,

adult female, collected on 7 February 2012 by G. Chávez and D. Vásquez; Chokoriari (11.9569°S, 72.9409°W), 413 m above sea level, CORBIDI 10628, adult male, collected on 19 February 2012 by G. Chávez and D. Vásquez.

**Referred specimens.** Two adults from Bolivia, Cochabamba Department, Ayopaya Province: confluence of the Altamachi and Ipiri rivers (16.0543°S, 66.6667°W), 600 m above sea level, MHNC-A 427, 429, collected on 15 September 2004 by A. Muñoz and G. Rey. An adult from Bolivia, Cochabamba Department, Carrasco Province: Valle del Sacta (17.118°S, 64.767°W), 230 m above sea level, MHNC-A 2116, collected on 18 April 2014 by G. Callapa, A. Muñoz, D. Ercken, S. Barron, and M. Careaga.

**Etymology.** The specific name *kamagarini* is a noun derived from the Matsigenka language, which means demon or devil (Snell et al. 2011). The Matsigenka language is spoken by the Matsigenka people who inhabit the highlands and lowlands of southeastern Peru, in the departments of Cusco and Madre de Dios. Judeo-Christian religions depict the demon as a human figure with horns. The species name is in allusion to the prominent horn-like tubercles on the upper eyelid of *D. kamagarini*.

Diagnosis. Throughout the species description, coloration refers to preserved specimens unless otherwise noted. The new species is assigned to the genus Dendropsophus based on our phylogenetic results (Fig. 1) and the overall similarity with *D. parviceps* and other species of the genus (Figs 10-11). Dendropsophus kamagarini is a mediumsized species, relative to other species in the D. parviceps group and is characterized by the following combination of traits: (1) size sexually dimorphic; mean SVL 19.9 mm in males (range 17.6–22.7; n = 35), 26.1 mm in females (range 24.0–28.1; n = 7); (2) throat brown mottled with white flecks posteriorly in males vs. white blotch with flecks or with stripes posteriorly in females (Fig. 11); (3) snout is short and truncate in dorsal and lateral views; (4) nostrils slightly protuberant; (5) tympanum visible, tympanic membrane non-differentiated, annulus distinct; (6) one prominent conical tubercle on the distal edge of the upper eyelid; (7) thoracic fold indistinct to barely evident; (8) ulnar tubercles and outer tarsal tubercles distinct; (9) axillary membrane present; (10) skin on dorsal surfaces smooth with scattered tubercles; skin on chest, belly, posterior surfaces of thighs, and subcloacal area coarsely areolate; skin on throat grooved with scattered tubercles; (11) dark brown markings on dorsum (Fig. 11); (12) thenar tubercle distinct; (13) hand webbing formula II1-2+III1-1 IV, feet webbing formula  $I1^{1/2}-2$ +III--1III1--2-IV2-1V; (14) in life, dorsum tan, brown or reddish brown; (15) orange to amber blotch on the proximal ventral surface of shanks and under arms, from the axillae to near the elbow, in life (white to creamy white in preserved); (16) one suborbital white bar present both in life and preserved; (17) thighs black to dark brown with two or three spots on the anterodorsal surfaces both in life and preserved; (18) iris in life creamy white with brown to reddish brown reticulations and a cream ring around pupil.

**Comparisons with other species.** *Dendropsophus kamagarini* is most similar to *D. parviceps* and *D. kubricki* sp. n. It can be distinguished from *D. parviceps* by having a prominent conical tubercle on the distal edge of the upper eyelid (tubercle absent in *D. parviceps*; Fig. 12) and a blunt and short snout in lateral view (slightly inclined

posteroventrally in profile in *D. parviceps*; Fig. 12). *Dendropsophus kamagarini* is larger than *D. parviceps* (Fig. 2; see Morphological comparisons) and has a throat with white flecks posteriorly in males both in life and preserved (dark flecks posteriorly in males both in life and preserved in *D. parviceps*). Advertisement calls of *D. kamagarini* also have lower dominant frequency than those of *D. parviceps* (Fig. 4A–D; see Bioacoustic comparisons) and more pulses in the advertisement call (less pulses in *D. parviceps*; Fig. 4A–D; see Bioacoustic comparisons). *Dendropsophus kamagarini* differs from *D. kubricki* sp. n. by having a prominent conical tubercle on the distal edge of the upper eyelid (scattered low tubercles in *D. kubricki*; Fig. 12).

Dendropsophus kamagarini differs from other species of the D. parviceps group (sensu Fouquet et al. 2015) by having an orange or amber blotch on the proximal ventral surface of shanks and arms in life and a prominent conical tubercle on the distal edge of the upper eyelid (orange blotches and tubercle absent in D. bokermanni [Goin 1960; Duellman and Crump 1974], in D. brevifrons [Duellman and Crump 1974], in D. counani [Fouquet et al. 2015], in D. frosti [Motta et al. 2012] and in D. koechlini [Duellman and Trueb 1989]). Dendropsophus kamagarini also resembles D. pauiniensis, but it can be distinguished by the presence of an orange or amber blotch on the proximal ventral surface of shanks and a prominent conical tubercle on the distal edge of the upper eyelid (blotch and tubercle are absent in D. pauiniensis; Heyer 1977).

Description of holotype. Adult male (Fig. 7B), SVL 19.6 mm. Head as wide as body, wider than long, widest below eyes; snout truncate and short in dorsal view, slightly inclined posteroventrally in lateral view; loreal region flat; lips thin; internarial region slightly concave; nostrils slightly protuberant dorsally and laterally; interorbital area flat; tympanum rounded distinct, tympanic annulus evident, tympanic membrane non-differentiated, supratympanic fold thin, restricted to upper edge of tympanum. Arms slender, not hypertrophied; axillary membrane extending to one third of upper arm; ulnar fold distinct, low ulnar tubercles present; fingers short, bearing small, round discs; relative length of fingers I < II < IV < III; subarticular tubercles small, round on fingers I and II, bifid on finger III, and divided on finger IV; supernumerary tubercles small, slightly evident; thenar tubercle distinct; palmar tubercle flat, round; webbing basal between fingers I and II; webbing formula of fingers II1<sup>-</sup>-2III1<sup>1/2</sup>-1<sup>-</sup>IV. Hindlimbs long, slender; tarsal fold absent, outer tarsal tubercles present, low; calcar and heel tubercles absent; toes bearing round discs, smaller than those of fingers; relative length of toes I < II < III < V < IV; subarticular tubercles small, round; supernumerary tubercles indistinct; inner metatarsal tubercle small, flat, elliptical; outer metatarsal tubercle absent; webbing formula of toes I11/2-1-III1-1III1-2IV2-1-V. Skin on dorsum, dorsal surfaces of limbs, flanks, and groin smooth; skin on head smooth with scattered tubercles and one prominent conical tubercle on the distal edge of the upper eyelid; skin on venter, chest, posterior surfaces of thighs, and subcloacal area coarsely areolate; skin on throat and ventral surfaces of limbs smooth. Cloacal opening directed posteriorly at upper level of thighs; cloacal sheath short; cloacal folds and tubercles absent. Tongue cordiform, barely free posteriorly; dentigerous



**Figure 10.** Dorsolateral and ventral views of *Dendropsophus kamagarini* sp. n. in life: **A, B** Adult male, from La Habana, Tambopata, Peru (CORBIDI 5259) **C, D** Adult male, from Bahuaja, Puno, Peru (CORBIDI 13148) **E–H** Adult females, from Pagoreni norte, La Convención, Peru **E, F** not collected.

process of vomers small, prominent, narrowly separated, each bearing three and two vomerine teeth (left/right), positioned obliquely to choanae; choanae small, rounded; vocal slits long, extending from midlateral base of tongue to angle of jaws; vocal sac single, median, subgular.

**Color of holotype in preservative.** Figure 7B. Dorsal surfaces of head, body, and limbs brown, grayish tan dorsolaterally with dark brown markings on dorsum consisting of median blotch anteriorly, transverse bars posteriorly; dark brown broad transverse bars on the forelimbs and shanks; anterodorsal surfaces of thighs black with three white spots; white suborbital bar. Ventral surface of belly white anteriorly, creamy mottled posteriorly with dark brown scattered flecks; chest white, throat brown anteriorly and white with brown flecks posteriorly; ventral surfaces of limbs creamy.



**Figure 10.** Continue. Dorsolateral and ventral views of *Dendropsophus kamagarini* sp. n. in life: **G**, **H** (CORBIDI 10018) **I**, **J** Adult male, from Tahuamanu, Nicolás Suárez, Bolivia (11.4074°S, 69.0180°W, 260 m, not collected) **K**, **L** Adult male, from El Negro, Manuripi, Bolivia (12.3134°S, 68.6689°W, 187 m, not collected) **N** Adult male, from Rio Branco, Acre, Brazil (10.0387°S, 67.7957°W, 160 m, not collected) **M** Adult male, from Rio Madeira, Rondônia, Brazil (8.8482°S, 64.0689°W, 110 m, not collected). Photos **A**, **B**, **E**–**H** by V. Duran, **C**, **D** by P. J. Venegas I–L by A. Muñoz, **N** by P.R. Melo-Sampaio, and **M** by A.P. Lima.



**Figure 11.** Adults of *Dendropsophus kamagarini* sp. n. showing variation in dorsal and ventral coloration of preserved specimens. From left to right, first and second rows: CORBIDI 8232, 8229, 8151, 8234 (males); third and fourth rows: CORBIDI 5480, 10019, 5471, 8305 (males); fifth and sixth rows: CORBIDI 8463, 10018, 6694 (females). See Appendix 1 for locality data. Scale bar 10 mm.



**Figure 12.** Frontal and lateral views of the head of adults of *Dendropsophus parviceps* species complex. *Dendropsophus parviceps*: **A** Male (QCAZ 52752) and **D** Female (QCAZ 44440) without tubercles (indicated by arrow); *D. kamagarini*: **B** Male (CORBIDI 8151) and **E** Female (CORBIDI 8152) with a conspicuous tubercle (arrow); *D. kubricki*: **C** Male (CORBIDI 15776) and **F** Female (CORBIDI 15785) with small tubercles (arrow). Note the snout shape in lateral view, truncate to slightly inclined posteroventrally in *D. parviceps*, truncate in *D. kamagarini*, and rounded and inclined posteroventrally in *D. kubricki*. Scale bar 5 mm.

**Measurements of holotype (in mm).** SVL 19.6, HW 6.3, HL 5.9, END 2.1, IN 2.0, FL 10.4, TL 10.7, FL 8.6.

**Variation.** Morphometric variation in the paratype series is given in Table 3. Variation in dorsal and ventral coloration of preserved specimens is shown in Figure 11.

Dorsal coloration in preservative varies from gray (e.g., CORBIDI 8305, 10019) to grayish tan (e.g., CORBIDI 8232, 8234), brown (e.g., CORBIDI 5471, 5480), dark brown (e.g., CORBIDI 6694, 8229), reddish brown (e.g., CORBIDI 8463) or creamy tan (e.g., CORBIDI 8151) with dark brown markings (Fig. 11); some specimens have a blotch in occipital region, a blotch in scapular region, and a transverse blotch extending onto flanks in sacral region (e.g., CORBIDI 8234) or two ")(" shaped stripes beginning on the upper eyelids, extending onto the flanks, and reaching the sacral region; an indistinct creamy middorsal line extends from the occipital region to the sacral region (e.g., CORBIDI 6694, 8151); some specimens have brown, creamy or grayish tan stripes around the dark brown markings (e.g., CORBIDI 10018). The dorsum has scattered tubercles, mainly on head and upper eyelid (e.g., CORBIDI 5471, 6694), but in some specimens the dorsum is smooth (e.g., CORBIDI 8069, 10018). The prominent conical tubercle on the upper eyelid in live individuals becomes smaller in preserved specimens (based on comparisons between photographs and their specimens; Fig. 10).

The venter of preserved specimens (Fig. 11) varies from grayish tan (e.g., COR-BIDI 8234), to dark brown (e.g., CORBIDI 5471, 8305), and black (e.g., CORBIDI 8463, 10018) with white scattered flecks. The throat (Fig. 11) varies from gray (e.g., CORBIDI 8305), grayish tan (e.g., CORBIDI 8229), brown (e.g., CORBIDI 5480, 6694), to dark brown (e.g., CORBIDI 5471, 10018) anteriorly, with white flecks (e.g., CORBIDI 5471, 10019), one white blotch (e.g., CORBIDI 10018) or stripes (e.g., CORBIDI 6694) posteriorly. The subcloacal area is white in most specimens (e.g., CORBIDI 6694, 8229, 8232, 10018), but in some specimens the subcloacal area is black (e.g., CORBIDI 8463).

Color in life. Based on digital photographs (Fig. 10): dorsum varies from tan to brown or reddish brown; creamy tan or mustard brown dorsolaterally; dorsal markings are dark brown, some individuals have brown, creamy, or grayish tan stripes around markings; some individuals also have scattered dark brown flecks dorsolaterally; the flanks are white with black vertical bars: dorsal surfaces of forelimbs and shanks have dark brown transversal bars; the thighs are black with two or three spots on the anterodorsal surfaces. The single suborbital bar is white. The venter is white anteriorly and dark brown or black mottled with translucent gray posteriorly, with white scattered flecks; chest is white and mottled with brown anteriorly; throat is brown or dark brown anteriorly and spotted with white flecks posteriorly in males (posterior part of throat with white blotch with or without stripes in females); the ventral surfaces of the limbs are translucent gray, thighs are mottled with black or dark brown anteriorly and posteriorly the thighs are black with white flecks; the ventral surface of shanks, from the knee to one third or on half the length of the shank, and arms, from the axillae to near the elbow, have a bright amber or orange blotch. Vocal sac in males is olive tan. The iris is creamy white with brown to reddish brown reticulations or reddish brown with creamy white reticulations and a cream ring around pupil.

**Calls (Fig. 4C-D).** Descriptive statistics of acoustic variables are provided in Table 6. We analyzed recordings from: (1) three males from Tambopata (13.1343°S,

69.6090°W, 233 m) on 5 March 2016, at 19:00h, 21:40h and 24:47h; (2) one male from Reserva Comunal Amarakaeri (12.7834°S, 70.9548°W, 365 m, Madre de Dios Department, Manu Province, Peru) recorded on 5 February 2015; (3) one male from Chontachaka (12.0405°S, 71.7230°W, 630 m, Cusco Department, Paucartambo Province, Peru); and (4) one male from Río Madeira (8.8482°S, 64.0689°W, 110 m, Rondônia State, Brazil) (Lima et al. 2012). Recorded males were not collected.

The advertisement call is a pulsed note (Fig. 4C–D). The amplitude of the advertisement call gradually increases until three-quarters of the note duration to decrease abruptly until the end. The advertisement call may be followed or not by secondary click notes. Nonetheless, the click notes are occasionally vocalized alone. The click notes are pulsed except for the last note.

One recording from Cobija, Bolivia (Pando Department, Nicolás Suárez Province) by Márquez et al. (2002) falls within the range of variation of advertisement calls of *Dendropsophus kamagarini* from Peru (Table 6). In addition, the number of pulses (15) and the dominant frequency (4150 Hz) of the call described by Duellman (2005) fall within the range for calls of *D. kamagarini* (Table 6).

**Distribution and ecology.** *Dendropsophus kamagarini* occurs in the Amazon basin of southeastern Peru (Cusco and Madre de Dios regions; Fig. 9), northwestern Brazil (Acre and Rondônia states; Fig. 9), and northeastern Bolivia, from the Andean slopes to lowland tropical rainforest (Fig. 9). Localities with known elevation range from 150 m (Acre) to 1696 m (Ochigoteni) above sea level.

Bolivian records are partly based on De la Riva et al. (2000) report of "*Dendrop-sophus parviceps*" in central northeastern Bolivia, Departments of Beni, Cochabamba, La Paz, Pando, and Santa Cruz. One photograph from Puerto Almacén (Santa Cruz Department; pp. 102 in De la Riva et al. 2000) and two photographs from Tahuamanu and El Negro (both from Pando Department; Fig. 10) show the conical tubercle on the upper eyelid characteristic of *D. kamagarini. Dendropsophus parviceps* distribution range is at a distance of over 1500 km, which make very unlikely that Bolivian records are conspecific. Thus, we propose that all historic records of "*Dendropsophus parviceps*" from Bolivia are assigned to *D. kamagarini*.

The call from Cobija (Pando Department) falls within the range of advertisement call of *D. kamagarini* (Table 6). The localities of El Negro, Tahuamanu, and Cobija are at a distance of 89 km, 158 km, and 203 km, respectively, to the type locality of *D. kamagarini* (Inotawa). In addition, specimens from Museo de Historia Natural Alcide d'Orbigny, Cochabamba, Bolivia, also have a prominent conical tubercle on the distal edge of the upper eyelid. These specimens are from Valle del Sacta and the confluence of the Altamachi and Ipiri rivers (both from Cochabamba Department; see Appendix 1). There is also one record from Santa Elena (16.6791°S, 66.6791°W, 600 m, Cochabamba Department, Ayopaya Province; Fig. 9) based on a locality record from Museo de Historia Natural Alcide d'Orbigny, Cochabamba, Bolivia, are also supported by Cochran and Goin (1970) who examined one specimen (WCAB 2511) and report the presence of the conical tubercle on the upper eyelid.

Dendropsophus kamagarini congregates for breeding at temporary and permanent ponds in flooded forest and Terra Firme forest; it is an opportunistic breeder (Duellman 2005). Adults of both sexes were found at night perching on leaves of bushes and trees, on branches and on palm fronds. Males were calling perched from 2–3 m above the water.

**Conservation status.** Extent of occurrence (B1) is 637,800 km<sup>2</sup>. *Dendropsophus kamagarini* occurs in the following protected areas from Peru: Otishi National Park, Megantoni National Sanctuary, Amarakaeri Communal Reserve, Manu National Park, Tambopata National Reserve and Bahuaja-Sonene National Park, and protected areas from Bolivia: Manuripi-Heath Amazonian Wildlife National Reserve and Isiboro Sécure National Park and Indigenous Territory. Because its distribution range is large and occurs in several protected areas we suggest that *D. kamagarini* is assigned to the Least Concern category, following IUCN (2001) criteria.

Remarks. The specimens from Cochabamba Department (Appendix 1) are assigned as referred specimens because we lack genetic data. Márquez et al. (1993) report a maximum SVL = 24.6 for males from Puerto Almacén. This value is slightly above the maximum SVL of males of D. kamagarini (see Table 3). Márquez et al. (1993) also report the dominant frequency range of the advertisement calls (2476-3144 Hz), which is lower than the dominant frequency range of D. kamagarini (3164.1-4306.6 Hz). Therefore, further data is needed to determine the status of that population. We tentatively assign those specimens to D. kamagarini as referred material. Schlüter (1979) described the advertisement calls of males from Panguana (Huanáco Department, Peru; Fig. 9), where he reported a dominant frequency range of 3200-4700 Hz and a call duration less than 0.2 s. The frequency range from Panguana is relatively closer to the range of D. kamagarini (3164.1-4306.6 Hz) and D. kubricki sp. n. (3542.2-4394.5 Hz); however, the call duration (less than 0.2 s) is within the range of calls of *D. kamagarini* (0.09–0.2 s) while the maximum value (0.3 s) of call duration of D. kubricki sp. n. exceeds the call duration reported by Schlüter (1979). Thus, we consider the population from Panguana as an unconfirmed register of D. kamagarini unless specimen examination demonstrates otherwise. The population from Rio Madeira (Rondônia State, Brazil) is also unconfirmed until specimens are examined.

#### Dendropsophus kubricki sp. n.

http://zoobank.org/C3677ED1-80E2-412C-A6EE-2949E6C1BB8B Figs 1, 7C, 13–14

Holotype. CORBIDI 15778, an adult male from Peru, Loreto Department, Requena Province, Campamento Wishuincho-Río Tapiche (7.1914°S, 73.9781°W), 120 m above sea level, collected on 11 October 2014 by P. J. Venegas.

**Paratypes.** Nine adult males and an adult female from Peru, Loreto Department, Requena Province, Campamento Wishuincho-Río Tapiche (7.1914°S, 73.9781°W),

120 m above sea level: CORBIDI 15775–77, adult males, collected on 9 October 2014 by P. J. Venegas; CORBIDI 15779–84, adult males, collected on 12 October 2014 by P. J. Venegas; CORBIDI 15785, adult female, collected on 12 October 2014 by P. J. Venegas. Three adult males and four adult females from Peru, Loreto Department, Requena Province, Sierra del Divisor (6.9187°S, 73.8461°W), 500 m above sea level: CORBIDI 3762, 3970, 3983, adult males, collected in January 2009 by R. Santa Cruz; CORBIDI 2281, adult female, collected on 1 November 2008 by D. Vásquez; CORBIDI 3743–44, 3780, adult females, collected in January 2009 by R. Santa Cruz.

**Referred specimens.** An adult male and three adult females from Peru, San Martin Department, Picota Province, Área de Conservación Municipal Chambira (7.0375°S, 76.0900°W), 679 m above sea level: CORBIDI 8863, adult male, collected on 27 November 2010 by P. J. Venegas; CORBIDI 8861–62, 8864, adult females, collected on 27 November 2010 by P. J. Venegas.

**Etymology.** The specific name *kubricki* is a noun in the genitive case and is a patronym for Stanley Kubrick, an American filmmaker who is one of the most brilliant and influential film directors of all time. We dedicate this species to him for his legacy to film culture and science fiction.

Diagnosis. Throughout the species description, coloration refers to preserved specimens unless otherwise noted. The new species is assigned to the genus Dendropsophus based on our phylogenetic results (Fig. 1) and the overall similarity with D. parviceps and other species of the genus (Figs 13-14). Dendropsophus kubricki is a medium-sized species, relative to other species in the D. parviceps group and is characterized by the following combination of traits: (1) size sexually dimorphic; mean SVL 19.4 mm in males (range 18.3–20.1; n = 14), 26.0 mm in females (range 22.0–28.4; n = 8; (2) throat with white flecks posteriorly in males and white blotch with stripes posteriorly in females (Fig. 14); (3) snout truncate in dorsal view, rounded and inclined posteroventrally in lateral view; (4) nostrils slightly prominent; (5) tympanum distinct, rounded, concealed posterodorsally, tympanic membrane non-differentiated and annulus evident; (6) low tubercles on upper eyelid can be distinct or ill-defined; (7) thoracic fold slightly evident or indistinct; (8) ulnar tubercles and outer tarsal tubercles low; (9) axillary membrane present; (10) skin on dorsal surfaces smooth with scattered tubercles mainly on head; skin on throat areolate, skin on chest, belly, posterior surfaces of thighs, and subcloacal area coarsely areolate; (11) dark brown markings on dorsum consisting of chevrons and transverse blotches in variable arrangements (Fig. 14); (12) thenar tubercle distinct; (13) hand webbing formula II1<sup>-</sup>-2<sup>+</sup>III1<sup>-</sup>-1<sup>-</sup>IV, foot webbing formula I1-2-III1-2-III1-2IV2-1-V; (14) in life, dorsal surfaces reddish brown, brown, or grayish tan; (15) orange to amber blotch on the proximal ventral surface of shanks and under arms, from the axillae to near elbow, in life (white to creamy white in preserved); (16) one suborbital white bar present both in life and preserved; (17) anterodorsal surfaces of thighs are black to dark brown with two or three white spots, both in life and preserved; (18) iris in life is reddish brown, brown or silver gray.

**Comparisons with other species.** *Dendropsophus kubricki* is most similar to *D. kamagarini* and *D. parviceps*. It is distinguished from *D. parviceps* by its larger size



**Figure 13.** Dorsolateral and ventral views of *Dendropsophus kubricki* sp. n. in life: **A**, **B** Holotype, adult male, from Río Tapiche, Requena, Peru (CORBIDI 15778) **C**, **D** Adult male from Río Tapiche, Requena, Peru (CORBIDI 15782) **E** Adult male from Jenaro Herrera, Requena, Peru (not collected) **F** Adults, pair in amplexus from Jenaro Herrera, Requena, Peru (not collected).

(Fig. 2; see Morphological comparisons), lower dominant frequency in advertisement call (Fig. 4A–B, E–F; see Bioacoustic comparisons), throat with white flecks or white medial spot posteriorly in males, both in life and preservative (dark flecks posteriorly in males both in life and preservative in *D. parviceps*), and scattered low tubercles on the upper eyelids (smooth in *D. parviceps*). *Dendropsophus kubricki* differs from *D. kamagarini* by lacking a prominent conical tubercle on the distal edge of the upper eyelid (present in *D. kamagarini*; Fig. 12). *Dendropsophus kubricki* also differs from *D. parviceps* and *D. kamagarini* by having a more rounded snout in profile (Fig. 12). *Dendropsophus kubricki* differs from quet et al. 2015) by having, in life, an orange blotch on the proximal ventral surface of



**Figure 13. Continued.** Dorsolateral and ventral views of *Dendropsophus kubricki* sp. n. in life: **G**, **H** Adult female from Jenaro Herrera, Requena, Peru (not collected) **I**, **J** Adult female from Area de Conservación Municipal Chambira, Picota, Peru (CORBIDI 8864) **K** Adult female from Tarapoto, San Martín, Peru (6.4306°S, 76.2903°W, 600 m, not collected) **L** Adults, pair in amplexus from Area de Conservación Municipal Chambira, Picota, Peru (CORBIDI 8864–63). Photographs by P. J. Venegas.

shanks and arms [orange blotches are absent in *D. bokermanni* (Goin 1960; Duellman and Crump 1974), in *D. brevifrons* (Duellman and Crump 1974), in *D. counani* (Fouquet et al. 2015), in *D. frosti* (Motta et al. 2012) and in *D. koechlini* (Duellman and Trueb 1989)]. *Dendropsophus kubricki* also resembles *D. pauiniensis*, but it differs by the presence of an orange blotch on the proximal ventral surface of shanks (absent in *D. pauiniensis*; Heyer 1977).

**Description of holotype.** Adult male (Fig. 7C), SVL 19.0 mm. Head as wide as body, slightly wider than long, widest below eyes; snout truncate and short in dorsal view, moderately rounded and slightly inclined posteroventrally in lateral view; loreal

region concave; lips thin; internarial region slightly concave; nostrils slightly protuberant dorsally and laterally; interorbital area flat; tympanum rounded and distinct, tympanic annulus evident, tympanic membrane non-differentiated, supratympanic fold thin, covering tympanum posterodorsally. Arms slender, not hypertrophied; axillary membrane extending along proximal one third of arm; ulnar fold distinct, low ulnar tubercles present; fingers short, bearing small, round discs; relative length of fingers I < II < IV < III; subarticular tubercles small, round on fingers I and II, bifid on finger III, and divided on finger IV; supernumerary tubercles small, slightly evident; thenar tubercle distinct; palmar tubercle flat, round; webbing basal between fingers I and II; hand webbing formula II1-2III1-1IV. Hindlimbs long, slender; tarsal fold absent, low outer tarsal tubercles present; calcar and heel tubercles absent; toes moderately long, bearing round discs, smaller than those of fingers; relative length of toes I < II < III < V < IV; subarticular tubercles small, round; supernumerary tubercles indistinct; inner metatarsal tubercle small, flat, elliptical; outer metatarsal tubercle absent; foot webbing formula I1-2II1-2III1-2IV2-1 V. Skin on dorsum and head smooth with scattered tubercles, skin on dorsal surfaces of limbs, flanks and groins smooth; skin on venter, posterior surfaces of thighs and subcloacal area coarsely areolate; skin on chest and throat areolate; skin on other surfaces smooth. Cloacal opening directed posteriorly at upper level of thighs; cloacal sheath short; cloacal folds and tubercles absent. Tongue cordiform, barely free posteriorly; dentigerous process of vomers small, prominent, adjacent medially, each bearing three and five vomerine teeth (left/right), positioned obliquely to choanae; choanae small, rounded; vocal slits long, extending from midlateral base of tongue to angle of jaws; vocal sac single, median, subgular.

**Color of holotype in preservative (Fig. 7C).** Dorsal surfaces of head, body, and limbs brownish gray with scattered reddish brown flecks with melanophores and leucophores on dorsum, dark brown markings on dorsum consisting of a median blotch anteriorly and transverse bar posteriorly; dark brown broad transverse bars on the forelimbs and shanks; snout brown dorsally; scapular region gray; thighs black with three white spots on the anterodorsal surfaces; one small white suborbital bar. Ventral surface of belly dark brown mottled with white anteriorly and with translucent white posteriorly and laterally; chest white; throat dark brown anteriorly and white with dark brown flecks posteriorly; ventral surfaces of the forelimbs translucent white; thighs translucent white anteriorly and dark brown posteriorly.

**Measurements of holotype (in mm).** SVL 19.0, HW 6.6, HL 6.3, END 1.8, IN 1.5, FL 9.1, TL 10.0, FL 7.3.

**Variation.** Morphometric variation of the paratype series is summarized in Table 3. Variation in dorsal and ventral coloration of preserved specimens is shown in Figure 14. Dorsal coloration in alcohol varies from gray (e.g., CORBIDI 15777) to grayish tan (e.g., CORBIDI 2281, 15775), reddish brown (e.g., CORDIBI 15779), brown (e.g., CORBIDI 8864), grayish brown (e.g., CORBIDI 15780), dark brown (e.g., CORBIDI 8862), or pinkish gray (e.g., CORBIDI 15785); some specimens have scattered reddish brown low tubercles and slightly black flecks (e.g., CORBIDI 15775, 15777, 15784). Occipital region with dark brown median blotch, one blotch in scapular region



**Figure 14.** Adults of *Dendropsophus kubricki* sp. n. showing variation in dorsal and ventral coloration of preserved specimens. From left to right, first and second rows: CORBIDI 15780, 15775, 15784, 15777 (males); third and fourth rows: CORBIDI 15779, 8863 (males), 2281, 15785 (females); fifth and sixth rows: CORBIDI 8862, 8864 (females). See Appendix 1 for locality data. Scale bar 10 mm.

and dark brown transverse bar in sacral region extending onto flanks (e.g., CORBIDI 15779, 15780, 15784); some specimens have two ")(" shaped dorsolateral stripes beginning on the upper eyelids and ending on the posterior flanks (e.g., CORBIDI 8862, 8864). The dorsum has tubercles mainly on head and upper eyelid (e.g., CORBIDI 8864, 8861, 15776, 15785), but some specimens have a smooth dorsum without tubercles (e.g., CORBIDI 2281, 3744; Fig. 14). Some specimens have postrictal tubercles, posteroventrally to tympanic annulus (e.g., CORBIDI 15775, 15780).

The venter of preserved specimens (Fig. 14) varies from black (e.g., CORBIDI 15780, 15785) to dark brown (e.g., CORBIDI 8864) with scattered white or creamy flecks. The throat anteriorly (Fig. 14) varies from brown (e.g., CORBIDI 8863, 15784), dark brown (e.g., CORBIDI 8862), grayish tan (e.g., CORBIDI 2281, 15775) to black (e.g., CORBIDI 15779, 15780) with white flecks (e.g., CORBIDI 15779), one irregular white stain (e.g., CORBIDI 2281, 8862) or stripes (e.g., CORBIDI 8864, 15785) posteriorly. The subcloacal area is white in most specimens (e.g., CORBIDI 15775, 15780, 15785), but it is black in some (e.g., CORBIDI 8862, 8863, 8864).

Color in life. Based on digital photographs (Fig. 13): dorsum varies from reddish brown, brownish gray, brown to creamy tan suffused with gray and brown with scattered white flecks, with or without reddish brown or brown low tubercles; some individuals have few scattered dark brown flecks on the dorsum; flanks are white with black or dark brown vertical bars; dorsal markings are dark brown, some individuals are mustard brown dorsolaterally; dorsal surfaces of forelimbs and shanks have dark brown transversal bars; anterodorsal surfaces of the thighs are black to dark brown with two or three spots. The single suborbital bar is white. The venter is black to dark brown mottled with translucent gray, and with white flecks anteriorly; some individuals have scattered white flecks on venter; chest is white; throat is brown, reddish brown, or dark brown with white flecks posteriorly in males (posterior part of throat with white blotch with or without stripes in females), some individuals have a white medial spot adjacent to the chest; the ventral surfaces of the limbs are translucent gray, the thighs are mottled with black or dark brown anteriorly; posteriorly the thighs are black with white flecks; the proximal ventral surface of shanks and arms, from the axillae to near the elbow, have an orange or amber blotch. The iris is reddish brown, brown, or silver gray.

**Calls (Fig. 4E–F).** Descriptive statistics of acoustic variables are provided in Table 7. We recorded three males (two not collected and CORBIDI 15778) at Campamento Wishuincho-Río Tapiche (Loreto Department, Requena Province, Peru) on 11 October 2014 at 12:53h and 12 October 2014 at 21:53h. We also recorded one male (not collected) at Cordillera Azul (7.8103°S, 75.9928°W, 725 m, San Martín Department, Picota Province, Peru).

The advertisement call is a pulsed note (Fig. 4E–F). The amplitude of the advertisement call increases gradually until three-quarters of the note duration to decrease abruptly until the end. This call may be followed by one or more click notes. These clicks sometimes are emitted alone. Moreover, the click notes are pulsed, except for the last one which is unpulsed. **Distribution and ecology.** *Dendropsophus kubricki* is distributed in the Amazon basin in northeastern and central Peru (Fig. 9), at elevations between 106 (Jenaro Herrera) and 725 m (Cordillera Azul). *Dendropsophus kubricki* was found in flooded forest. Specimens from Chambira were collected in a small pond in a Terra Firme forest. Males call at night while perching on leaves of bushes and trees. They were observed between 0.3 and 0.4 m above the water.

**Conservation status.** Extent of occurrence (B1) is 53,548 km<sup>2</sup>. *Dendropsophus kubricki* occurs in the following protected areas: Sierra del Divisor National Park, Cordillera Azul National Park, and Cordillera Escalera Regional Conservation Area. Because its distribution range is large and occurs in protected areas, we recommend that *D. kubricki* is assigned to the Least Concern category, following IUCN (2001) criteria.

**Remarks.** Specimens from Chambira (Picota Province) are closely related to Río Tapiche and Jenaro Herrera specimens (both localities from Requena Province) (Fig. 1); genetic distances between these populations are low (mean p-distances 1.3% in mitochondrial gene 12S). Therefore, we include them tentatively in *Dendropsophus kubricki*. However, individuals from Chambira and Tarapoto (Picota and San Martin provinces, respectively) show differences in coloration because their dorsal tubercles have the same color as the background, white flecks and reddish brown low tubercles on the dorsum and dorsal surfaces are absent both in life and preservative (present in Río Tapiche and Jenaro Herrera individuals; Figs 13–14). In addition, there is segregation in acoustic space between advertisement calls from Cordillera Azul (Picota Province) and the type locality (Fig. 5; Table 7). Therefore, it is conceivable that they represent a separate species.

## Discussion

Our genetic, morphologic, and bioacoustic data demonstrated that *Dendropsophus parviceps*, as previously defined, was a complex of three cryptic species. Duellman (2005) noted the morphological distinctiveness of populations from Peru and suggested that they may represent separate species. However, without genetic information the definition of species limits within "*D. parviceps*" was difficult because the morphology of the three species is highly conserved. The dark brown markings on dorsum, the suborbital bar, spots on the anterior surfaces of thighs, and orange or amber blotches on shanks and arms are shared between the three species. Differences between *D. parviceps*, *D. kamagarini*, and *D. kubricki* are limited to body size, skin texture, and advertisement calls. This pattern of highly conserved morphology has also been reported in other species complexes in Amazonian amphibians (e.g., Caminer and Ron 2014; Elmer et al. 2007; Fouquet et al. 2012; Funk et al. 2012; Kieswetter and Schneider 2013; Moravec et al. 2014; Rojas et al. 2016).

The pattern of variation in bioacoustic, and quantitative and qualitative morphological characters found in the *D. parviceps* species complex is not unusual among closely related species of Amazonian amphibians. Genetic divergence usually covaries with size, bioacoustic, and qualitative morphological characters (e.g., skin ornamentation and coloration) while it has low covariation with size-corrected morphometric variables (Caminer et al. 2017; dos Santos et al. 2015; Fouquet et al. 2012; Funk et al. 2012; Ortega-Andrade et al. 2015; Ron et al. 2016, but see Acevedo et al. 2016). This suggests that advertisement calls and qualitative morphological characters are among the first components of the phenotype to diverge during speciation. In contrast, body shape, as quantified in linear morphometric analyses, is highly conserved and of limited value to assess limits among closely related species.

#### Speciation modes

Several authors have discussed the role of niche evolution in the speciation of vertebrates in tropical mountains (e.g., Cadena et al. 2012; Kozak and Wiens 2007). Some studies have shown that sister species tend to segregate along environmental gradients suggesting ecological speciation and niche lability (Arteaga et al. 2016; Graham et al. 2004; Ron et al. 2012). Other studies have shown that sister species tend to be allopatric but with similar environmental niches (Cadena et al. 2012; Ortega-Andrade et al. 2015). Those results imply vicariant speciation and niche conservativism. Clearly, both speciation mechanisms have contributed to the high diversity of the Andes and adjacent Amazon lowlands. Our results with the *Dendropsophus parviceps* complex suggest niche conservativism and vicariance speciation. We base this conjecture in the elevational distribution of the three species and in the intraspecfic genetic variation among populations of *D. parviceps*.

*Dendropsophus parviceps* and *D. kamagarini* show wide and overlapping elevation ranges: 151 m to 1600 m in *D. parviceps* and 150 m to 1696 m in *D. kamagarini*. Fewer localities are known for *D. kubricki* but its known range (106–725 m) overlaps with the ranges of the other two species. Because elevation is the most influential variable defining the environmental niche in tropical regions, overlapping elevation ranges suggest conserved environmental niches. The allopatric distribution of the three species also indicates that vicariant speciation with latitudinal replacement is more likely than ecological speciation with elevational replacement.

The lack of importance of elevation in promoting genetic differentiation is also suggested by interpopulation genetic differentiation in *D. parviceps*. We sampled 55 populations encompassing an elevation range of 186–1600 m. If disruptive selection across the elevation gradient were generating genetic isolation, we would expect to find parapatric clades segregating by elevation. Instead, two parapatric clades were found that segregate latitudinally, each occurring across a wide range of elevations. Both clades have a contact zone in central Amazonia, Ecuador (Fig. 1). A northern subclade occurs in Napo, Orellana, Sucumbíos provinces reaching marginally Pastaza Province; a southern subclade occurs in Pastaza, Tungurahua, and Amazonian Peru. Both clades have a narrow zone of contact near the limit between Napo and Pastaza provinces at an elevation of 900 m. Genetic distances between both clades are moderate (range for gene

12S is 0.4–1.2%), but there is strong support for each clade indicating structured genetic differentiation. Currently, there are not conspicuous geographic barriers between both clades leaving as an open question the processes that promoted genetic divergence within *D. parviceps*. Overall, the available evidence suggests that species of the *D. parviceps* complex speciated allopatrically instead of ecologically along an elevation gradient.

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# Appendix I

### Specimens examined

## Dendropsophus parviceps

ECUADOR: PROVINCIA SUCUMBIOS: Puerto Bolívar (0.0886°S, 76.1420°W), 240 m (QCAZ 28247, 28249); Playas Cuyabeno (0.2415°S, 75.9305°W), 230 m (QCAZ 28269, 28399); Rey de los Andes (0.2082°S, 76.2369°W), 270 m (QCAZ 28455, 28492); Reserva Limoncocha (0.4062°S, 76.6194°W), 261 m (QCAZ 43140, 44915); La Selva Lodge (0.4982°S, 76.3738°W), 229 m (QCAZ 4340, 44055, 47736); Río Napo, Pañacocha (0.4712°S, 76.0667°W), 225 m (QCAZ 44140); Zábalo (0.3181°S, 75.7662°W), 220 m (QCAZ 27983); Garza Cocha (0.4816°S, 75.3442°W), 195 m (CORBIDI 15). PROVINCIA NAPO: Río Hollín (0.6958°S, 77.7303°W), 1068 m (QCAZ 17942, 22208); Sumaco (0.6866°S, 77.6013°W), 1430 m (QCAZ 48919, 48929, 48931); Estación Biológica Jatun Sacha (1.0650°S, 77.6142°W), 397 m (OCAZ 18177, 18184); Ahuano (1.0546°S, 77.5484°W), 385 m (QCAZ 27028). PROVINCIA ORELLANA: Río Napo, Chiroisla, Banco sur (0.5799°S, 75.9177°W), 203 m (QCAZ 44355-57); Río Napo, Santa Teresita (0.9009°S, 75.4136°W), 186 m (QCAZ 44734-36); Río Napo, Huiririma, (0.7116°S, 75.6239°W), 194 m (QCAZ 44599-601); Río Napo, Nuevo Rocafuerte, (0.9193°S, 75.4010°W), 187 m (QCAZ 44775, 44778); Río Napo, Chiroisla, (0.5756°S, 75.8998°W), 203 m (QCAZ 44440-42); Río Napo, Eden, Banco sur (0.4983°S, 76.0711°W), 216 m (QCAZ 44227-29); Río Napo, San Vicente (0.6790°S, 75.6511°W), 196 m (QCAZ 44492-94); Río Napo, Áñangu (0.5249°S, 76.3844°W), 255 m (QCAZ 43968-69); Coca, Río Napo (0.4778°S, 76.9898°W), 267 m (QCAZ 43680, 43704); Coca, Banco sur Río Napo (0.4989°S, 77.0075°W), 264 m (QCAZ 43775); Río Napo, Primavera (0.4310°S, 76.7865°W), 244 m (QCAZ 43892); Parque Nacional Yasuní, Estación Científica Yasuní, Pontificia Universidad Católica del Ecuador (0.6744°S, 76.3970°W), 250 m (QCAZ 35720, 51075, 51229-30); Parque Nacional Yasuní, Pompeya-Iro road, 80-75 km (0.8401°S, 76.3024°W), 250 m (QCAZ 43066-67); Parque Nacional Yasuní. Pompeya-Iro, 96 km (0.9065°S, 76.2214°W), 233 m (QCAZ 51195); Parque Nacional Yasuní, Pozo SPF, 8 km (0.6916°S, 75.9196°W), 250 m (QCAZ 31267). PRO-VINCIA PASTAZA: Bobonaza (1.4981°S, 77.8793°W), 660 m (QCAZ 32491); Cononaco (1.2083°S, 76.7167°W), 220 m (QCAZ 38948); Fátima (1.4114°S, 78.0000°W), 1023 m (QCAZ 15424, 49334); Bobonaza, Tuculí (1.4945°S, 77.8696°W), 620 m (QCAZ 32543); Zanjarajuno (1.3572°S, 77.8705°W), 977 m (QCAZ 49314, 49340); Arajuno (1.3243°S, 77.6890°W), 580 m (QCAZ 38332); Canelos (1.6065°S, 77.7576°W), 465 m (QCAZ 52816, 52820); Canelos-Puyo road (1.6016°S, 77.7576°W), 465 (QCAZ 52837-38); Sarayaku, Río Palandayaku
(1.7355°S, 77.4902°W), 325 m (QCAZ 52751–53, 52755); Montalvo, Comunidad Campus (1.9924°S, 76.9168°W), 392 m (QCAZ 53161, 53165–67, 53181–82, 53184); Killu Allpa (2.1871°S, 76.8577°W), 335 m (QCAZ 52892); Parque Nacional Llanganates, Comunidad Zarentza (1.3587°S, 78.0511°W), 1338 m (QCAZ 59772). PROVINCIA TUNGURAHUA: Río Verde (1.4001°S, 78.3006°W), 1600 m (QCAZ 52017–19); Río Negro (1.4125°S, 78.2042°W), 1244 m (QCAZ 52023–24, 52026). PERU: REGION LORETO: PROVINCIA DATEM DEL MARAŃÓN: Andoas (2.6516°S, 76.5137°W), 151 m (CORBIDI 1039–41, 1044, 1046, 1059, 5029). PROVINCIA LORETO: San Jacinto (2.3308°S, 75.8637°W), 160 m (CORBIDI 1144, 1151–52).

## Dendropsophus kamagarini

PERU: DEPARTAMENTO MADRE DE DIOS: PROVINCIA TAMBOPA-TA: Inotawa (12.8092°S, 69.3182°W), 192 m (CORBIDI 5246); La Habana (12.6537°S, 69.1796°W), 192 m (CORBIDI 5259). DEPARTAMENTO CUSCO: PROVINCIA LA CONVENCIÓN: Comunidad Nativa Poyentimari (12.1885°S, 73.0009°W), 725 m (CORBIDI 8150-53, 8228-36, 8285-86, 8305, 8463, 8476); Megantoni (12.2581°S, 72.8425°W), 670 m (CORBIDI 6659, 6664, 6679, 6685, 6687-88, 6692, 6694, 6698); Comunidad Nativa Chokoriari (11.9569°S, 72.9409°W), 434 m (CORBIDI 8067-70); Puyantimari (12.1861°S, 73.0004°W), 710 m (CORBIDI 9762); Pongo de Mainique (12.2581°S, 72.8425°W), 670 m (CORBIDI 5471, 5473, 5480, 5484); Pagoreni norte (11.7115°S, 73.8967°W), 402 m (CORBIDI 10018–19); Comunidad Ochigoteni (12.5758°S, 73.09°W), 1696 m (CORBIDI 5392); Palmeiras-Alto Shimá (12.5453°S, 73.1350°W), 500 m (COR-BIDI 10585); Chokoriari (11.9569°S, 72.9409°W), 413 m (CORBIDI 10628). BOLIVIA: DEPARTAMENTO COCHABAMBA: PROVINCIA AYOPAYA: Confluence of the Altamachi and Ipiri rivers (16.0543°S, 66.6667°W), 600 m (MHNC-A 427, 429). PROVINCIA CARRASCO: Valle del Sacta (17.118°S, 64.767°W), 230 m (MHNC-A 2116).

### Dendropsophus kubricki

PERU: DEPARTAMENTO LORETO: PROVINCIA REQUENA: Sierra del Divisor (6.9187°S, 73.8461°W), 500 m (CORBIDI 2281, 3743–44, 3762, 3780, 3970, 3983); Campamento Wishuincho-Río Tapiche (7.1914°S, 73.9781°W), 120 m (CORBIDI 15775–85). DEPARTAMENTO SAN MARTÍN: PROVINCIA PI-COTA: Área de Conservación Municipal Chambira (7.0375°S, 76.0900°W), 679 m (CORBIDI 8861–64).

# Appendix 2

GenBank accession numbers for DNA sequences used in the phylogenetic analyses. Abbreviations: BRA = Brazil, CO = Colombia, EC = Ecuador, PE = Peru.

N			41.		Genbank Accession numbers			
Museum number	Species	Locality	Alt. (m)	Latitude	Longitude	125	16S-ND1- tRNA	CO1
QCAZ 28247	D. parviceps	Sucumbíos, Puerto Bolívar. EC	240	-0.0886	-76.1420	MG041769	MG041894	MG041832
QCAZ 28492	D. parviceps	Sucumbíos, Rey de los Andes. EC	270	-0.2082	-76.2369	MG041770	MG041895	MG041831
QCAZ 28455	D. parviceps	Sucumbíos, Rey de los Andes. EC	270	-0.2082	-76.2369	MG041771	MG041896	MG041830
QCAZ 28399	D. parviceps	Sucumbíos, Cuyabeno. EC	230	-0.2415	-75.9305	MG041768	MG041892	MG041833
QCAZ 28269	D. parviceps	Sucumbíos, Cuyabeno. EC	230	-0.2415	-75.9305	MG041767	MG041893	MG041834
QCAZ 27983	D. parviceps	Sucumbíos, Zábalo. EC	220	-0.3181	-75.7662	MG041772	MG041897	MG041835
QCAZ 28249	D. parviceps	Sucumbíos, Puerto Bolívar. EC	240	-0.0886	-76.1420	MG041773	MG041898	MG041836
QCAZ 44915	D. parviceps	Sucumbíos, Limoncocha. EC	261	-0.4062	-76.6195	MG041774	MG041899	MG041837
QCAZ 47736	D. parviceps	Sucumbíos, La Selva. EC	229	-0.4982	-76.3738	MG041775	MG041900	MG041838
QCAZ 31267	D. parviceps	Orellana, PNY, Pozo SPF, 8 km. EC	250	-0.6916	-75.9196	MG041776	MG041901	MG041839
QCAZ 43066	D. parviceps	Orellana, PNY, Pompeya-Iro road, 80–75 km. EC	250	-0.8401	-76.3024	MG041777	MG041902	MG041840
QCAZ 43067	D. parviceps	Orellana, PNY, Pompeya-Iro road, 80–75 km. EC	250	-0.8401	-76.3024	MG041778	MG041903	MG041841
QCAZ 43680	D. parviceps	Orellana, Coca, northern Río Napo. EC	267	-0.4778	-76.9898	MG041779	MG041904	MG041842
QCAZ 43892	D. parviceps	Orellana, Primavera. EC	244	-0.431	-76.7865	MG041780	MG041905	MG041843
QCAZ 43969	D. parviceps	Orellana, Áñangu. EC	255	-0.5249	-76.3844	MG041781	MG041906	MG041844
QCAZ 44734	D. parviceps	Orellana, Santa Teresita. EC	186	-0.9009	-75.4136	MG041782	MG041907	MG041845
QCAZ 43775	D. parviceps	Orellana, Coca, southern Río Napo. EC	264	-0.4989	-77.0075	MG041783	MG041908	MG041846
QCAZ 51229	D. parviceps	Orellana, PNY, Estación Científica Yasuní, PUCE. EC	250	-0.6744	-76.3970	MG041784	MG041909	MG041847

Marrie			A1.			Genbank Accession numbers			
number	Species	Locality	Alt. (m)	Latitude	Longitude	125	16S-ND1- tRNA	CO1	
QCAZ 44355	D. parviceps	Orellana, Chiroisla, southern Río Napo. EC	207	-0.5799	-75.9177	MG041785	MG041910	MG041848	
QCAZ 44492	D. parviceps	Orellana, San Vicente. EC	196	-0.6790	-75.6511	MG041786	MG041911	MG041849	
QCAZ 44600	D. parviceps	Orellana, Huiririma. EC	194	-0.7116	-75.6239	MG041787	MG041912	MG041850	
QCAZ 44735	D. parviceps	Orellana, Santa Teresita. EC	186	-0.9009	-75.4136	MG041788	MG041913	MG041851	
QCAZ 44228	D. parviceps	Orellana, Edén. EC	216	-0.4983	-76.0711	MG041789	MG041914	MG041852	
QCAZ 44227	D. parviceps	Orellana, Edén. EC	216	-0.4983	-76.0711	MG041790	MG041915	MG041853	
QCAZ 44442	D. parviceps	Orellana, Chiroisla, northern Río Napo. EC	203	-0.5756	-75.8998	MG041791	MG041916	MG041854	
QCAZ 51230	D. parviceps	Orellana, PNY, Estación Científica Yasuní, PUCE. EC	250	-0.6744	-76.3970	MG041792	MG041917	MG041855	
QCAZ 51195	D. parviceps	Orellana, PNY, Pompeya-Iro, 96 km. EC	233	-0.9065	-76.2214	MG041793	MG041918	MG041856	
QCAZ 44778	D. parviceps	Orellana, Nuevo Rocafuerte. EC	187	-0.9193	-75.4010	MG041794	MG041919	MG041857	
QCAZ 18184	D. parviceps	Napo, Estación Biológica Jatun Sacha. EC	397	-1.0650	-77.6142	MG041795	NA	NA	
QCAZ 48931	D. parviceps	Napo, Sumaco. EC	1430	-0.6866	-77.6013	MG041796	MG041920	MG041858	
QCAZ 18177	D. parviceps	Napo, Estación Biológica Jatun Sacha. EC	397	-1.0650	-77.6142	MG041797	MG041921	MG041859	
QCAZ 22208	D. parviceps	Napo, Río Hollín. EC	1068	-0.6958	-77.7303	MG041798	MG041922	MG041860	
QCAZ 17942	D. parviceps	Napo, Río Hollín. EC	1068	-0.6958	-77.7303	MG041799	MG041923	MG041861	
QCAZ 48919	D. parviceps	Napo, Sumaco. EC	1430	-0.68659	-77.60133	MG041800	MG041924	MG041862	
QCAZ 49340	D. parviceps	Pastaza, Zanjarajuno. EC	977	-1.3572	-77.8706	MG041801	MG041925	MG041863	
QCAZ 52024	D. parviceps	Tungurahua, Río Negro. EC	1244	-1.4125	-78.2042	MG041802	NA	MG041864	
QCAZ 52026	D. parviceps	Tungurahua, Río Negro. EC	1244	-1.4125	-78.2042	MG041803	MG041926	MG041865	
QCAZ 52017	D. parviceps	Tungurahua, Río Verde. EC	1600	-1.4001	-78.3006	MG041804	MG041927	MG041866	
QCAZ 52018	D. parviceps	Tungurahua, Río Verde. EC	1600	-1.4001	-78.3006	MG041805	MG041928	MG041867	

M			41.			Genbank Accession numbers		
number	Species	Locality	Alt. (m)	Latitude	Longitude	125	16S-ND1- tRNA	CO1
QCAZ 52753	D. parviceps	Pastaza, Sarayaku, Río Palandayaku. EC	325	-1.7355	-77.4902	MG041806	NA	MG041868
QCAZ 32543	D. parviceps	Pastaza, Tuculí. EC	620	-1.4945	-77.8696	MG041807	MG041929	MG041869
QCAZ 32555	D. parviceps	Pastaza, Tuculí. EC	620	-1.4945	-77.8696	MG041808	MG041930	MG041870
QCAZ 52755	D. parviceps	Pastaza, Sarayaku, Río Palandayaku. EC	325	-1.7355	-77.4902	MG041809	MG041931	MG041871
QCAZ 49334	D. parviceps	Pastaza, Fátima. EC	1023	-1.4114	-78	MG041810	MG041932	MG041872
QCAZ 52838	D. parviceps	Pastaza, Canelos- Puyo road. EC	465	-1.6016	-77.7576	MG041811	MG041933	MG041873
QCAZ 52816	D. parviceps	Pastaza, Canelos. EC	465	-1.6065	-77.7536	MG041812	MG041934	MG041874
QCAZ 52752	D. parviceps	Pastaza, Sarayaku, Río Palandayaku. EC	325	-1.7355	-77.4902	MG041813	MG041935	MG041875
QCAZ 52751	D. parviceps	Pastaza, Sarayaku, Río Palandayaku. EC	325	-1.7355	-77.4902	MG041814	MG041936	MG041876
QCAZ 53161	D. parviceps	Pastaza, Montalvo. EC	392	-1.9924	-76.9168	MG041815	MG041937	MG041877
QCAZ 52892	D. parviceps	Pastaza, Killu Allpa. EC	335	-2.1871	-76.8577	MG041816	MG041938	MG041878
QCAZ 32491	D. parviceps	Pastaza, Bobonaza. EC	660	-1.4981	-77.8793	MG041817	MG041939	MG041879
QCAZ 53167	D. parviceps	Pastaza, Montalvo. EC	392	-1.9924	-76.9168	MG041818	MG041940	MG041880
QCAZ 53183	D. parviceps	Pastaza, Montalvo. EC	392	-1.9924	-76.9168	MG041819	MG041941	MG041881
QCAZ 38332	D. parviceps	Pastaza, Arajuno. EC	580	-1.3243	-77.689	MG041820	MG041942	MG041882
QCAZ 39515	D. parviceps	Pastaza, Cononaco. EC	220	-1.2083	-76.7167	MG041821	MG041943	MG041883
CORBIDI 8150	D. kamaga- rini	La Convencion, Poyentimari. PE	725	-12.1885	-73.0009	MG041822	NA	MG041884
CORBIDI 5471	D. kamaga- rini	La Convencion. Pongo de Mainique. PE	670	-12.2581	-72.8425	MG041823	MG041944	MG041885
CORBIDI 5246	D. kamaga- rini	Tambopata, Inotawa. PE	192	-12.8092	-69.3182	MG041824	MG041945	MG041886
AMNHA 139315	D. kamaga- rini	Acre, Rio Branco- Porto Velho. BRA	160	-9.9556	-67.8648	AY843652	NA	NA
CORBIDI 8861	D. kubricki	Picota, Chambira. PE	679	-7.0375	-76.0900	MG041825	MG041946	MG041887
CORBIDI 6235	D. kubricki	Requena, Jenaro Herrera. PE	500	-4.9080	-73.6669	MG041826	MG041947	MG041888

M			41.			Genbank Accession numbers			
number	Species	Locality	Alt. (m)	Latitude	Longitude	125	16S-ND1- tRNA	CO1	
CORBIDI 15778	D. kubricki	Requena, Río Tapiche. PE	120	-7.1914	-73.9781	NA	MG041948	NA	
QCAZ 43835	D. brevifrons	Orellana, Primavera. EC	244	-0.4443	-76.7868	KT721809	NA	NA	
QCAZ 28273	D. brevifrons	Sucumbíos, Cuyabeno. EC	230	-0.2415	-75.9305	KT721806	NA	NA	
QCAZ 17826	D. brevifrons	Orellana, Yasuní. EC	245	-0.6782	-76.396	KT721824	NA	NA	
ANDES A1025	D. frosti	Amazonas, Leticia-Tarapacá km 11. CO	103	-4.1067	-69.9493	JQ088283	NA	NA	
CORBIDI 5217	D. koechlini	Tambopata, Tambopata. PE	233	-13.1343	-69.6090	MG041827	MG041949	MG041889	
CORBIDI 5235	D. koechlini	Tambopata, Inotawa. PE	192	-12.6537	-69.1796	MG041828	MG041950	MG041890	
QCAZ 10594	D. marmo- ratus	Orellana, Yasuní. EC	250	-0.6744	-76.3971	MG041829	MG041951	MG041891	
CFBH 7600	Xenohyla truncata	Rio de Janeiro, Restinga de Maricá. BRA	9	-22.9419	-42.8266	AY843775	NA	NA	

SHORT COMMUNICATION



# Effects of habitat and time of day on flock size of Turkey Vultures in Cuba (*Cathartes aura*)

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#### Abstract

In agricultural landscapes, the Turkey Vulture *Cathartes aura* feeds mainly on carcases of domestic animals. In spring 2017, data on 214 flocks of Turkey vultures were collected in a road survey in Cuba (in total 2384 km). Turkey Vultures were found to be common accross Cuba, but flock size varied between habitats, reaching a maximum of 43 in valleys and 31 in agricultural landscapes with domestic animal farms. Vultures were active throughout the day, but the time of day did not significantly affect flock size. This study corroborates previous studies which suggested that carrion resources located in agricultural habitats and river valleys is crucial for the continued survival of this still abundant species. Changes in Cuba's socio-political system in the near future will likely impact agricultural practices, and this in turn will likely affect Turkey Vultures. Our study may serve as a baseline against which future population changes and flocking behaviour of Turkey Vultures can be compared.

#### Keywords

Cuba, large-scale survey, roadside, scavenger, vulture

#### Introduction

Vultures are globally endangered birds (Ogada et al. 2012). Vultures play significant roles in the environment and have interacted with humans over long periods of time (Morelli et al. 2015). Recently, several species of vultures have shown steep declines in population size. However, some species of vultures still have seemingly healthy populations. One such species is the Turkey vulture Cathartes aura (Linnaeus, 1758), which is found in North, Central and South America, including the Caribbean islands (Kirk and Mossman 1998). The species is common in Cuba, where it is the most abundant raptor species recorded (Wotzkow and Wiley 1988, Ferrer-Sánchez and Rodriguez-Estrella 2014). This presents an opportunity to investigate the reasons for the success of this species in comparison to other vultures which are in danger of extinction. One strategy to understand vulture declines is to study access to healthy food and behaviour in relation to foraging capability, including the formation of flocks, habitat use, and time-activity pattern (Ogada et al. 2012). However, all these parameters are susceptible to rapid environmental changes caused by changing agricultural and urban policies, including use of medical products, such as the anti-inflammatory drug diclofenac (Camiña and Yosef 2012, Balmford 2013). Cuba is just beginning to transform its urban and rural landscapes, which is associated with the socio-political changes of recent vears (Graddy-Lovelace 2017).

The aims of this study were to (1) assess the influence of habitat variation on flock size, (2) describe perches preferred by Turkey Vultures, and (3) assess how the time of the day affects flocking behaviour of the vultures. Results are discussed in the light of the changes expected to occur in the agricultural systems of Cuba due to recent socio-political changes, and which may affect bird populations in a way similar to what occurred in post-communist Europe (Tryjanowski et al. 2011, Batáry et al. 2017).

#### Methods

Surveys were conducted from March to April 2017 along roads throughout Cuba (in total 2384 km; Figure 1) using methods described by Wuczyński (2001) and Watson and Simpson (2014). One or two observers, passenger(s), scanned power lines, utility poles, trees, fences, buildings, open fields, and the skyline for vultures, and identified the birds using binoculars. Species, the number of individuals, location, time, and habitat for all vultures were recorded. Groups of vultures flying or sitting together were defined as a flock (cf. Kirk and Mossman 1998, Jackson et al. 2008).

Search effort was determined by habitat availability from the roads. Habitats were classified according to Ferrer-Sánchez and Rodríguez-Estrella (2015): (1) open fields and meadows; (2) valleys in mountains connected mainly with rivers; (3) waters, including also coastlines, lagoons, and swamp marsh; (4) villages, and (5) urban habitats including hotels and their infrastructure.



Figure 1. The map of Cuba with indicated (grey line) map of the transect.

The associations among flock size, habitat type and time of day were assessed by means of a linear model procedure. Flock size was entered as response variable while time and type of habitat were used as predictors. The model was fitted assuming a log-normal distribution of response variables after having explored the distribution of these variable as suggested by Box and Cox (1964) using the packages 'MASS' (Venables and Ripley 2002), and 'glmmADMB' in R (Fournier et al. 2012, Skaug et al. 2013). The full model was based on 214 observations. Confidence intervals for the significant variables were calculated by the Wald method from the package 'MASS' (Venables and Ripley 2002). All statistical tests were performed in R (R Development Core Team 2017).

#### Results

#### **Flock sizes**

In total, 1231 individuals in 214 flocks were observed during the study period. Habitat influenced average flock size, with the largest flocks recorded in valleys (43 individuals) and in the vicinity of domestic animal farms (31 individuals) in an agricultural landscape (Figures 2 and 3). Differences in flock size between habitats were statistically significant (Table 1). The flock size of vultures was significantly smaller in urbanised areas (urban areas and villages) than in agricultural landscapes (Figure 4, Table 1). Although flock size strongly varied with time of day, there was no significant relationship between flock size and time of day, nor between flock size and the interaction of time with habitat (Table 1, Figures 4 and 5).



**Figure 2.** Turkey vulture foraging on a road-kill dog, in a village on the north from Moron, province Ciego de Ávila (photograph P. Tryjanowski).



**Figure 3.** Perched Turkey vultures on a horse farm, Sierra del Chorrillo, province Camagüey (photograph P. Tryjanowski).

Variable	Estimate	2.5%	97.5%	SE	t	р
(Intercept)	2.304	1.780	2.828	0.266	8.669	< 0.05
Habitat (open field)	-0.900	-1.250	-0.549	0.177	-5.064	< 0.05
Habitat (urban)	-1.002	-1.354	-0.649	0.178	-5.609	< 0.05
Habitat (valley)	0.774	0.284	1.263	0.248	3.116	< 0.05
Habitat (village)	-1.457	-1.779	-1.136	0.163	-8.924	< 0.05
Habitat (water)	-0.582	-1.091	-0.074	0.258	-2.260	< 0.05
Time	-0.014	-0.049	0.017	0.016	-0.886	n.s.

**Table 1.** Relationship between flock size of Turkey Vultures to habitat type and time of day (N = 214). Abbreviations: 2.5%, lower level of confidence interval; 97.5%, upper level of confidence interval; SE, standard error. The  $R^2$  for the model multiple was 0.419.



**Figure 4.** Flock size of Turkey Vultures in relation to habitat type. The y-axis represents the estimated variable. The boxplots show the median (bar in middle of rectangles), mean (black rhombus), upper and lower quartiles, and extreme values. The horizontal dashed line is the average values of flock sizes considering all cases.

#### Perch sites

A total of 57 perched birds was observed: 32 (56.1%) on trees, eleven (19.3%) sitting on the ground, eight (14.0%) on electric pylons, and six (10.5%) on fences (Figure 3).



**Figure 5.** Flock size of Turkey Vultures in relation to time of day. The y-axis represents the estimated variable. The boxplots show the median (bar in middle of rectangles), mean (black rhombus), upper and lower quartiles, and extreme values.

#### Discussion

Turkey vultures feed heavily on carrion of domestic animals in landscapes dominated by agriculture (Kirk and Mossman 1998, Ogada et al. 2012). On the other hand, Turkey vultures are also recognised as foraging opportunists, making use of various food sources, including vegetables (Kelly et al. 2007, Platt and Rainwater 2009).

In our study, we found the largest flocks near domestic animal farms and in river valleys, where probably carrion of large animals, both wild and domestic, is frequently available. In agreement with previous studies, relatively small numbers of vultures were observed in non-agricultural landscapes. This pattern of flock size related to habitat suggests that the availability carrion of domestic animals is crucial for the vultures' presence and abundance (Camiña and Yosef 2012, Ogada et al. 2012, Balmford 2013). Perched vultures were noted less often than flying birds (only 4.6% of all recorded individuals). This can be partially attributed to survey methods and differences in detectability of perched and flying vultures (Watson and Simpson 2014).

Flying vultures are often noted throughout the day (Mandel and Bildstein 2007); however, no strong pattern emerged from the data. There was a tendency towards larger flock in the afternoon when thermals were stronger and vultures could extend their daily activity period, especially in urban habitats, as was suggested also by Mandel and Bildstein (2007).

During this study, we confirmed previous findings that the Turkey Vulture is still very common in Cuba (Wotzkow and Wiley 1988, Ferrer-Sánchez and Rodríguez-Estrella 2015). However, due to expected socio-economical changes (Ferrer-Sánchez and Rodríguez-Estrella 2015, Graddy-Lovelace 2017) changes in bird populations can be very rapid (Balmford 2013), as were shown, for example, in farmland birds in eastern Europe since the early 1990s (Tryjanowski et al. 2011). Therefore, to conserve the best patches of habitat for vultures in Cuba, it is crucial to identify the appropriate habitats today and implement protection measures in order to ensure healthy populations in the future.

#### Acknowledgements

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RESEARCH ARTICLE



# Seven new species of the spider genus Ochyrocera from caves in Floresta Nacional de Carajás, PA, Brazil (Araneae, Ochyroceratidae)

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#### Abstract

Seven new species of the spider genus *Ochyrocera* from cave areas in Floresta Nacional de Carajás (state of Pará, northern Brazil) are described: *Ochyrocera varys* **sp. n.**, *Ochyrocera atlachnacha* **sp. n.**, *Ochyrocera laracna* **sp. n.**, *Ochyrocera aragogue* **sp. n.**, *Ochyrocera misspider* **sp. n.**, *Ochyrocera charlotte* **sp. n.**, and *Ochyrocera ungoliant* **sp. n.** Two groups of the species are discussed, the *quinquivittata* group that include specimens with an apparently bifid retrolateral apophysis in the cymbium of the male palp and the *arietina* group, here proposed, that include those specimens with an entire cymbium, with no retrolateral apophysis, in the male palp. Although these species were abundant inside caves, the examined specimens do not have troglomorphic characteristics and can be classified as edaphic troglophile species, capable of completing its life cycle in soil, shallow subterranean habitats, or caves.

#### **Keywords**

Amazonian region, Haplogynae, taxonomy

#### Introduction

Ochyroceratidae is a small spider family from the tropical areas in Neotropical, African, and Indo-Pacific regions. Its members live among litter or in caves, do not exceed 2 mm in total size, and have six eyes (Jocqué and Dippenaar-Schoeman 2006). The group currently includes 175 species distributed in 15 genera (World Spider Catalog 2017). No generic revision has been carried out to date; therefore, the family's diversity is poorly understood.

Species from the genus *Ochyrocera* Simon, 1891 are characterized in having modifications on the cymbial apophysis, which can be triangular or conical, with an elongated base, projected forward, and often with an apical cuspule (Dupérré 2015; Pérez-González et al. 2016). The genus comprises 35 species, with the type species, *O. arietina* from Island of Saint Vincente, Antilles region (Simon 1892). From Brazil were described only six species: *Ochyrocera coerulea* Keyserling, 1891 and *O. viridissima* Brignoli, 1974, both described from the state of Santa Catarina, *Ochyrocera cornuta* Mello-Leitão, 1944 from the state of Mato Grosso, *Ochyrocera ibitipoca* Batista, González & Tourinho, 2008 from the state of Minas Gerais and *Ochyrocera hamadryas* Brignoli, 1978 from state of Amazonas (World Spider Catalog 2017).

In this paper, seven new species are described from iron caves in Floresta Nacional de Carajás, state of Pará, northern Brazil. This area has iron formations such as itabirites, ferruginous dolomites, hematite, phyllite, jaspillite, and hematite (Carmo and Jacobi 2013). Two of the new species herein described were also collected outside the caves, and none of the seven species have troglomorphic traits, being classified as edaphic troglophile specimens.

#### Materials and methods

Morphological terms follow Dupérré (2015) and Pérez-González et al. (2016). Descriptions and measurements were performed using a Nikon SMZ 745T stereomicroscope. Photographs were taken with a Leica DFC 500 digital camera on a Leica MZ16A stereomicroscope. Focal range images were made using Leica Application Suite software, version 2.5.0.

The following abbreviations were used in the description:

ALE	anterior lateral eyes;
AME	anterior median eyes;
PME	posterior median eyes.

Clypeus height was represented in relation to the size of the posterior median eyes (PME). Total and femur lengths were measured in lateral view without detaching any part from the specimen. All measurements are in millimeters. Female genitalia were excised with a sharp needle and photographs were taken using Hoyer's microscope slides,

following Krantz and Walter (2009). For scanning electron microscopy (SEM) images, body parts were dehydrated in a series of graded ethanol washes (80% to 100%), dried by critical point, mounted on metal stubs using adhesive copper tape and nail polish for fixation and covered with gold. SEM photographs were taken with a FEI Quanta 250 scanning electron microscope at the Laboratório de Biologia Celular of Instituto Butantan, São Paulo, Brasil.

The examined specimens are deposited in the following collections (abbreviation and curator in parentheses): Instituto Butantan, São Paulo (IBSP, A.D. Brescovit); Coleção de Invertebrados Subterrâneos da Universidade Federal de Lavras, Lavras (ISLA, R.L. Ferreira), Museu Paraense Emílio Goeldi, Belém (MPEG, A.B. Bonaldo), Museu de Zoologia da Universidade de São Paulo (MZSP, R. Pinto da Rocha).

#### Taxonomy

#### Ochyrocera Simon, 1892

Ochyrocera Simon, 1892: 565 (Type species, O. arietina Simon)
Ceruleocera Marples, 1955: 462 (Type species by original designation C. ransfordi Marples); Brignoli 1979: 598 (Syn.)

**Diagnosis.** Species of the genus *Ochyrocera* can be distinguished by having a tracheal spiracle between the epigastric fold and spinnerets (see Pérez-González et al. 2016: fig. 8A); clypeus sloping (Fig. 1A–B); tip of labium notched (Fig. 8E); long legs; male palp without tibial apophysis; cymbium conical and with prolateral extension, with or without apical cuspule; and flexed embolus projecting forward (Fig. 1C–D).

**Composition.** Thirty-nine species (World Spider Catalog 2017).

**Distribution.** With the exception of *Ochyrocera ransfordi*, described by Marples (1955) from Samoa, all described species are from Mexico, Cuba, Hispaniola, Lesser Antilles, Saint Vincent, Guatemala, Venezuela, French Guiana, Ecuador, Peru, Argentina, and Brazil.

#### Ochyrocera varys sp. n.

http://zoobank.org/A3F555D5-02E7-4C30-8B5F-3C8CEE3672F7 Figures 1, 2, 3, 19A, 21A, D

**Types.** Holotype male from Cave N5S–0021 (6°5'15"S, 50°7'34"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 25/VIII–03/IX/2009, R. Andrade & I. Cizauskas (IBSP 177662). Paratypes: female from Cave N4E–0033 (6°2'25"S, 50°9'36"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 15–22/IX/2009, R. Andrade & I. Cizauskas (IBSP 176843); male and female from N5S–0085 (6°5'12"S, 50°7'35"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 25/VIII–03/IX/2009, R. Andrade & I. Cizauskas (MPEG 34434, ex IBSP 177644); 2 males, 2 females, N5S–0085 (6°5'12"S, 50°7'35"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 14/III–04/IV/2010, R. Andrade & I. Cizauskas (MZSP 72854, ex IBSP 177735).

Other material examined. BRAZIL. Pará: HYPOGEAN SAMPLES: Canaá dos Carajás, Floresta Nacional de Carajás, Serra Sul, Cave CAV 0017 (6°24'23"S; 50°22'9"W), 1Q, 22-31/V/2010 (IBSP 175566); Cave CAV\_0034 (6°24'9"S, 50°22'56"W), 13, 22-31/V/2010 (IBSP 175316); Cave CAV 0018 (6°29'50"S, 51°9'31"W), 3♀, 08–15/III/2012 (IBSP 175979) all collected by R. Andrade & I. Cizauskas et al.; Cave S11A-03 (6°20'60"S, 50°27'2"W), 2379, 23/VIII-02/IX/2007 (IBSP 174480); Cave S11A-05 (6°21'7"S, 50°27'3"W), 3Å11Q, 23/VIII-02/IX/2007 (IBSP 174405); 1339, 06/IV/2017, R. Zampaulo & X. Prous (IBSP 194762); Cave S11A-07 (6°21'6"S, 50°26'36"W), 2∂3♀, 23/VIII-02/IX/2007 (IBSP 174410); Cave S11A-12 (6°19'53"S; 50°27'4"W), 1∂5♀, 23/VIII-02/IX/2007 (IBSP 174413); Cave S11A-20 (6°19'4"S, 50°26'23"W), 1∂5♀, 23/VIII-02/IX/2007 (IBSP 174416, IBSP 174417); Cave S11A-26 (6°18'26"S, 50°26'55"W),  $3^{\circ}_{\circ}6^{\circ}_{\circ}$ , 23/VIII-02/IX/2007 (IBSP 174423, IBSP 174418); Cave S11A-36 (6°19'1"S, 50°27'17"W), 4∂4♀, 23/ VIII-02/IX/2007 (IBSP 174429); Cave S11B-09 (6°21'19"S, 50°23'24"W), 1∂1♀, 23/VIII-02/IX/2007 (IBSP 174433); Cave S11B-11 (21'27"S; 50°23'22"W), 32, 23/ VIII-02/IX/2007 (IBSP 174435); Cave S11B-13 (6°21'16"S, 50°26'51"W), 6♀, 23/ VIII-02/IX/2007 (IBSP 174439); Cave S11B-14 (6°20'59"S, 50°24'10"W), 1♀, 23/ VIII-02/IX/2007 (IBSP 174441); Cave S11B-23 (6°20'43"S, 50°24'35"W), 2♀, 23/ VIII-02/IX/2007 (IBSP 174443); Cave S11B-24 (6°20'43"S, 50°24'34"W), 3♀, 23/ VIII-02/IX/2007 (IBSP 174446); Cave S11B-49 (6°21'31"S, 50°23'21"W), 23, 23/ VIII-02/IX/2007 (IBSP 174448); Cave S11C-14 (6°24'10"S, 50°22'57"W), 3∂5♀, 23/VIII-02/IX/2007 (IBSP 174451); Cave S11C-27 (6°23'51"S, 50°23'21"W), 22, 23/VIII-02/IX/2007 (IBSP 174453) all collected by R. Andrade & I. Arnori et al.; Cave S11D-06 (6°24'4"S, 50°21'1"W), 1319, 19–22/II/2010 (IBSP 175534); Cave S11D-11 (6°23'51"S, 50°21'30"W), 1♀, 13–30/I/2010 (IBSP 175512); Cave S11D-43 (6°24'48"S, 50°19'17"W), 1∂2♀, 13–30/I/2010 (IBSP 175521); Cave S11D-64 (6°23'31"S, 50°18'48"W), 1∂1♀, 01–14/VII/2010 (IBSP 175653); Cave S11D-78 (6°23'32"S, 50°18'58"W), 1♀, 13–30/I/2010 (IBSP 175423) all collected by R. Andrade & I. Cizauskas et al.; Parauapebas, Cave CRIS-28 (6°27'31"S, 49°42'33"W),  $2^{\circ}$ , 29/VII-06/VIII/2008, R. Andrade (IBSP 174624); Floresta Nacional de Carajás. Serra Norte, Cave GEM-1564, 10♀, 17-24/X/2008 (IBSP 174508); Cave GEM-1590, 5<sup>♀</sup>, 17-24/X/2008, R. Andrade (IBSP 174521); Cave N1\_0002 (6°2'24"S, 50°16'12"W), 1♀, 28/IX-03/X/2007, R. Andrade & I. Arnori et al. (IBSP 174652); Cave N1 0004 (6°2'23"S, 50°16'12"W), 1∂8♀, 28/IX-03/X/2007, R. Andrade & I. Arnori et al. (IBSP 174655); 39, 16/VII-06/VIII/2014, Equipe Carste et al. (IBSP 188836, IBSP 188842); Cave N1\_0008 (6°2'20"S, 50°16'13"W),  $6^{\circ}$ , 28/ IX-03/X/2007 (IBSP 174657, IBSP 174656); Cave N1 0014 (6°2'2"S, 50°16'20"W), 4♀, 28/IX-03/X/2007 (IBSP 174662); Cave N1\_0015 (6°2'2"S, 50°16'16"W), 1∂7♀, 28/IX-03/X/2007 (IBSP 174665, IBSP 174664); Cave N1\_0018 (6°2'1"S,

50°16'17"W), 2Å12, 28/IX-03/X/2007 (IBSP 174669) all collected by R. Andrade & I. Arnori et al.; Cave N1 0019 (6°2'1"S, 50°16'17"W), 19, 16/VII-06/VIII/2014, Equipe Carste et al. (IBSP 188834); Cave N1 0020 (6°1'53"S, 50°18'1"W), 19, 28/ IX-03/X/2007 (IBSP 174674); Cave N1 0022 (6°1'57"S, 50°16'19"W), 2∂ 5♀, 28/ IX-03/X/2007 (IBSP 174679, IBSP 174683); Cave N1 0025 (6°1'53"S, 50°16'20"W), 6∂17♀, 28/IX-03/X/2007 (IBSP 174685) all collected by R. Andrade & I. Arnori et al.; 1<sup>(2)</sup>, 02–29/IV/2015, Equipe Carste et al. (IBSP 188847); Cave N1 0037 (6°1'50"S, 50°16'28"W), 2♀, 28/IX-03/X/2007, R. Andrade & I. Arnori et al. (IBSP 174688); Cave N1\_0055 (6°1'12"S, 50°16'43"W), 1♀, 16/VII-06/VIII/2014, Equipe Carste et al. (IBSP 188831); Cave N1\_0064 (6°1'7"S; 50°16'45"W), 3<sup>Q</sup>, 28/IX-03/X/2007 (IBSP 174692); Cave N1 0072 (6°1'13"S; 50°17'18"W), 4♀, 28/IX-03/X/2007 (IBSP 174698) all collected by R. Andrade & I. Arnori et al.; Cave N1\_0073 (6°1'13"S; 50°17'17"W), 1♂5♀, 16/VII-06/VIII/2014 (IBSP 188845, IBSP 188833, IBSP 188838); 19, 02-29/IV/2015, Equipe Carste et al. (IBSP 188843); Cave N1 0075 (6°1'14"S, 50°16'49"W), 2♀, 28/IX-03/X/2007 (IBSP 174699, IBSP 174700); Cave N1 0098 (6°1'9"S, 50°17'5"W), 2♀, 28/IX-03/X/2007 (IBSP 174706); Cave N1 0103 (6°0'13"S, 50°17'55"W), 3∂10♀, 28/IX-03/X/2007 (IBSP 174707, IBSP 174708) all collected by R. Andrade & I. Arnori et al.; Cave N1 0109 (6°0'41"S, 50°18'41"W), 432, 16/VII-06/VIII/2014, Equipe Carste et al. (IBSP 188848, IBSP 188846); Cave N1\_0116 (6°0'39"S, 50°18'50"W) 1332, 28/IX-03/X/2007 (IBSP 174711), collected by R. Andrade & I. Arnori et al.; Cave N1 0141 (6°2'34"S, 50°16'32"W), 1∂10♀, 16/VII-06/VIII/2014 (IBSP 188840) collected by Equipe Carste et al.; Cave N1 0143 (6°1'36"S, 50°17'27"W), 3∂8♀, 28/IX-03/X/2007 (IBSP 174718, IBSP 174714); Cave N1\_0170 (6°1'23"S, 50°17'58"W), 3<sup>o</sup>, 28/ IX-03/X/2007 (IBSP 174724); Cave N1 0173 (6°1'27"S, 50°17'55"W), 3♀, 28/ IX-03/X/2007 (IBSP 174726) all collected by R. Andrade & I. Arnori et al.: Cave N1–0162 (6°0'55"S, 50°18'46"W), 1♀, 02–29/IV/2015 (IBSP 188841), collected by Equipe Carste et al.; Cave N2-026 (6°3'16"S, 50°14'23"W), 1Å12, 26/IX-17/X/2012 (IBSP 178499); 2Å32, 03–17/IV/2013 (IBSP 178502, IBSP 178501); Cave N3 0003 (6°1'44"S, 50°12'3"W), 1♂, 26/IX-17/X/2012 (IBSP 178481), 1♂1♀, 05-17/ III/2013 (IBSP 178504, IBSP 178505); Cave N3 0004 (6°1'45"S, 50°12'2"W),  $2 \stackrel{?}{_{\sim}} 0 \stackrel{\circ}{_{\sim}}$ , 26/IX-17/X/2012 (IBSP 178482); Cave N3 0006 (6°1'45"S, 50°12'3"W), 1∂1♀, 26/IX-17/X/2012 (IBSP 178486, IBSP 178485) 1∂1♀, 05-17/III/2013 (IBSP 178506); Cave N3 0023 (6°2'35"S, 50°13'10"W),  $4\sqrt[3]{2}$ , 05–17/III/2013 (IBSP 178508, IBSP 178507) 1∂1♀, 02–23/VIII/2013 (IBSP 178539); Cave N3\_0026 (6°2'39"S, 50°13'9"W), 2Å, 26/IX-17/X/2012 (IBSP 178489, IBSP 178491); 4∂1♀, 05–17/III/2013 (IBSP 178509, IBSP 178512, IBSP 178510); Cave N3 0033 (6°2'42"S, 50°13'12"W), 2♂, 26/IX-17/X/2012 (IBSP 178492); 2♂3♀, 05-17/III/2013 (IBSP 178514, IBSP 178515); Cave N3 0036 (6°2'46"S, 50°13'13"W), 1∂0♀, 26/IX-17/X/2012 (IBSP 178494); Cave N3\_0037 (6°2'45"S, 50°13'14"W), 1♀, 05–17/III/2013 (IBSP 178516); 1∂1♀, 26/IX–17/X/2012 (IBSP 178495); Cave N3\_0039 (6°2'24"S, 50°13'21"W), 1002, 26/IX-17/X/2012 (IBSP 178497); Cave N3\_0047 (6°2'27"S, 50°13'40"W), 3∂9♀, 03-17/IV/2013 (IBSP

178527, IBSP 178531, IBSP 178564, IBSP 178529, IBSP 178525, IBSP 178532); 2∂2♀, 02–23/VIII/2013 (IBSP 178541, IBSP 178540); Cave N3 0072 (6°2'36"S, 50°13'50"W), 2♀, 03-17/IV/2013 (IBSP 178533, IBSP 178534); Cave N3 0074 (6°2'35"S; 50°13'49"W), 6♂5♀, 05–17/III/2013 (IBSP 178518, IBSP 178517, IBSP 178521, IBSP 178519, IBSP 178524); 3♂, 02–23/VIII/2013 (IBSP 178546, IBSP 178548, IBSP 178549); Cave N3 0076 (6°2'28"S; 50°13'36"W), 1Å12, 03–17/ IV/2013 (IBSP 178537); 2∂2♀, 02–23/VIII/2013 (IBSP 178552, IBSP 178553); all collected by Equipe Carste et al.; Cave N4E 0003 (6°2'25"S; 50°9'38"W), 3♀, 20/X-01/XI/2006 (IBSP 174942); Cave N4E\_0007 (6°2'21"S; 50°9'36"W), 1♀, 20/X-01/ XI/2006 (IBSP 174952); Cave N4E\_0008 (6°2'21"S; 50°9'36"W), 9♀, 20/X-01/ XI/2006 (IBSP 174954); Cave N4E 0010 (6°2'20"S; 50°9'38"W), 1399, 20/X-01/ XI/2006 (IBSP 174968); 3♂5♀, 07–12/X/2008 (IBSP 174970, IBSP 174969); 4♂7♀, 20/IV-04/V/2010 (IBSP 176878, IBSP 176877, IBSP 176879, IBSP 176881, IBSP 176880); all collected by R. Andrade & I. Cizauskas et al.; Cave N4E 0011 (6°2'20"S; 50°9'38"W), 1∂6♀, 20/X-01/XI/2006, R. Andrade et al. (IBSP 174974); 3<sup>Q</sup>, 20/IV-04/V/2010 R. Andrade & I.Cizauskas et al. (IBSP 176883, IBSP 176882); Cave N4E 0012 (6°2'16"S; 50°9'37"W), 1∂0♀, 20/X-01/XI/2006 (IBSP 174976); Cave N4E 0013 (6°2'18"S; 50°9'38"W), 1∂7♀, 20/X-01/XI/2006 (IBSP 174979); all collected by R. Andrade et al.; 83/162, 20/IV-04/V/2010 (IBSP 176886, IBSP 176888, IBSP 174981, IBSP 176885, IBSP 176884, IBSP 176889, IBSP 176887, IBSP 176890, IBSP 174060), collected by R. Andrade & I.Cizauskas et al.; Cave N4E\_0014 (6°2'17"S; 50°9'37"W), 3Å49, 20/X-01/XI/2006 (IBSP 174987) 2Å69, 07-12/X/2008 (IBSP 174989, IBSP 174986, IBSP 174985); all collected by R. Andrade et al.; 3Å11Q, 20/IV-04/V/2010 (IBSP 176893, IBSP 176894, IBSP 176892, IBSP 176895, IBSP 176891); all collected by R. Andrade & I. Cizauskas et al.; Cave N4E 0015 (6°2'10"S; 50°9'35"W), 1∂3♀, 20/X-01/XI/2006 (IBSP 174991, IBSP 176876); Cave N4E\_0016 (6°2'6"S; 50°9'37"W), 4<sup>o</sup>, 20/X-01/XI/2006 (IBSP 174993); all collected by R. Andrade et al.; 1Å29, 20/IV-04/V/2010 (IBSP 176896, IBSP 176897); Cave N4E 0019 (6°2'4"S; 50°9'37"W), 2♀, 20/X-01/XI/2006 R. Andrade et al. (IBSP 175003); 3/82, 20/IV-04/V/2010, R. Andrade & I. Cizauskas et al. (IBSP 176898, IBSP 176900, IBSP 176899); Cave N4E\_0021 (6°2'2"S;  $50^{\circ}9'37''W$ ),  $6^{\circ}5^{\circ}2$ , 20/X-01/XI/2006, R. Andrade et al. (IBSP 175010);  $3^{\circ}2$ , 20/2IV-04/V/2010 (IBSP 176901, IBSP 176902, IBSP 176905); 3∂3♀, 20/IV-04/V/2010 (IBSP 176903, IBSP 176904); all collected by R. Andrade & I. Cizauskas et al.; Cave N4E 0022 (6°2'2"S; 50°10'4"W), 3♂7♀, 20/X-01/XI/2006 (IBSP 175012); 2♂3♀, 07–12/X/2008 (IBSP 175015); collected by R. Andrade et al.; 2Å72, 20/IV-04/V/2010 (IBSP 176906, IBSP 176908, IBSP 176907, IBSP 174061); all collected by R. Andrade & I. Cizauskas et al.; Cave N4E 0023 (6°2'1"S; 50°10'7"W), 1319, 20/ IV-04/V/2010, R. Andrade & I. Cizauskas et al. (IBSP 176909); Cave N4E 0025 (6°2'1"S; 50°10'8"W), 1♀, 20/X-01/XI/2006, R. Andrade et al. IBSP 175024); Cave N4E 0026 (6°2'14"S; 50°10'3"W), 4∂10♀, 08–12/II/2007 R. Andrade & I. Arnori et al. (IBSP 175025); 4∂10♀, 08–12/II/2007 (IBSP 17502) 2∂4♀, 07–12/X/2008 R. Andrade et al. (IBSP 175028, IBSP 175033); 2372, 18/VIII-03/IX/2009, R. Andrade & I. Cizauskas et al. (IBSP 176829, IBSP 176830, IBSP 176832, IBSP 176833, IBSP 176831); Cave N4E 0033 (6°2'25"S; 50°9'36"W) 4♂5♀, 08–12/II/2007, R. Andrade & I. Arnori et al. (IBSP 175060, IBSP 175053, IBSP 175055); 1∂7♀, 07– 12/X/2008, R. Andrade & I. Cizauskas et al. (IBSP 175058, IBSP 175062, IBSP 175057); 2♂3♀, 18/VIII-03/IX/2009 (IBSP 176835) 8♂22♀, 15–22/IX/2009 (IBSP 176842, IBSP 176843, IBSP 176845, IBSP 176847, (IBSP 176849, IBSP 176836, IBSP 176846, IBSP 176844, IBSP 176848, IBSP 176837, IBSP 176841, IBSP 176838, IBSP 176839, IBSP 176840); all collected by R. Andrade & I. Cizauskas et al.; Cave N4E\_0039 (6°1'58"S; 50°9'39"W) 2<sup>Q</sup>, 24-30/VII/2009 (IBSP 176850, IBSP 176851) 5<sup>Q</sup>, 19/II-04/III/2010 (IBSP 176866) collected by R. Andrade & I. Cizauskas et al.: Cave N4E 0041 (6°1'59"S: 50°9'42"W), 1312, 08–12/II/2007, R. Andrade & I. Arnori et al. (IBSP 175075); 122, 24–30/VII/2009 R. Andrade & I. Cizauskas et al. (IBSP 176853, IBSP 176852); Cave N4E 0043 (6°1'55"S; 50°9'50"W) 1♀, 24–30/VII/2009 (IBSP 176854); Cave N4E 0044 (6°1'55"S; 50°9'50"W) 3♀, 24–30/VII/2009 (IBSP 176855, IBSP 176856) 3♀, 19/II-04/III/2010 (IBSP 176863, IBSP 176864); Cave N4E 0046 (6°2'16"S; 50°9'36"W) 1∂1♀, 19/II-04/III/2010 (IBSP 176868, IBSP 176867); Cave N4E 0051 (6°2'22"S; 50°9'38"W) 1♀, 24–30/ VII/2009 (IBSP 176857); Cave N4E 0054 (6°2'1"S; 50°10'8"W) 1312, 19/II-04/ III/2010 (IBSP 176865); all collected by R. Andrade & I. Cizauskas et al.; Cave N4E\_0061 (6°2'21"S; 50°10'3"W) 432, 08–12/II/2007, R. Andrade & I. Arnori et al. (IBSP 175080); 2∂9♀, 07–12/X/2008 (IBSP 175077, IBSP 175079); 2♀, 24–30/ VII/2009 (IBSP 176861) 3∂7♀, 24–30/VII/2009 (IBSP 176858, IBSP 176860, IBSP 176859), all collected by R. Andrade et al.; Cave N4E 0062 (6°2'1"S; 50°9'12"W), 1♀, 24–30/VII/2009 (IBSP 176823); Cave N4E\_0070 (6°1'56"S; 50°9'10"W), 1♀, 19/II-04/III/2010 (IBSP 176916); Cave N4E 0072 (6°1'56"S; 50°9'13"W), 6♀, 24-30/VII/2009 (IBSP 176826, IBSP 176827, IBSP 176828) 4♀, 19/II-04/III/2010 (IBSP 176874, IBSP 176875, IBSP 176872); Cave N4E\_0080 (6°1'58"S; 50°9'4"W), 3♀, 19/II-04/III/2010 (IBSP 176869); Cave N4E 0089 (6°1'59"S; 50°9'6"W), 2♀, 24-30/VII/2009 (IBSP 176824, IBSP 176825); Cave N4WS-07 (6°5'22"S; 50°11'41"W), 13, 23/VIII/2010 (IBSP 176990); all collected by R. Andrade & I. Cizauskas et al.; Cave N4WS-08 (6°5'22"S; 50°11'41"W) 19, 07–12/X/2008, R. Andrade et al. (IBSP 174801); Cave N4WS-13 (6°3'59"S; 50°11'23"W), 2∂42, 20/X-01/XI/2006 (IBSP 174780); 1∂1♀, 20/IV-04/V/2010 (IBSP 176384, IBSP 176383); Cave N4WS-15 (6°3'59"S; 50°11'22"W), 14∂18♀, 20/X-01/XI/2006 (IBSP 174788, IBSP 174930, IBSP 174787, IBSP 174793, IBSP 174795, IBSP 174789), 3∂4♀, 07-12/X/2008 (IBSP 174812, IBSP 174808); all collected by R. Andrade et al.; 7∂24♀, 20/IV-04/V/2010 (IBSP 176386, IBSP 176394, IBSP 176395, IBSP 176396, IBSP 176398, IBSP 176399, IBSP 176388, IBSP 176389, IBSP 176393, IBSP 176392, IBSP 176387, IBSP 176391, IBSP 176385, IBSP 176397); all collected by R. Andrade & I. Cizauskas et al.; Cave N4WS-67 (6°4′22″S; 50°11′30″W), 1∂2♀, 18/ XI-01/XII/2010 (IBSP 174070, IBSP 174069); all collected by C.R.A Souza & F.P. Franco et al.; Cave N5S-01 (6°5'27"S; 50°7'31"W), 19, 03/IV/2017, R. Zampaulo & X. Prous (IBSP 194763); Cave N5S-03 (6°6'18"S; 50°8'4"W) 1Å, 22/III-03/IV/2005,



**Figure 1.** *Ochyrocera varys* sp. n., male holotype (**A**, **C–D**), female paratype, IBSP 176843 (**B**, **E–F**) **A–B** habitus, dorsal view **C** left male palp, retrolateral view **D** same, prolateral view **E** genitalia, enzyme cleared, dorsal view **F** same, dorsal view. Abbreviations: CUE = columnar uterus externus, NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae, UE, uterus externus. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 µm (**E**, **F**).

R. Andrade & I. Arnori et al. (IBSP 55367); 2312, 14–23/X/2009, R. Andrade & I. Cizauskas et al. (IBSP 177683, IBSP 177684, IBSP 177685); Cave N5S-05 (6°6'21"S; 50°8'1"W), 1♀, 22/III-03/IV/2005 (IBSP 55338); Cave N5S\_06 (6°6'21"S; 50°8'2"W), 2∂1♀, 22/III-03/IV/2005 (IBSP 55344); Cave N5S-08 (6°6'21"S; 50°7'57"W), 1∂2♀, 22/III-03/IV/2005 (IBSP 55343); all collected by R. Andrade & I. Arnori et al.; 1Å, 07–12/X/2008 (IBSP 174524); 4Å59, 14–23/X/2009 (IBSP 177687, IBSP 177689, IBSP 177690, IBSP 177688); Cave N5S-09 (6°6'21"S; 50°7′53″W) 4∂12♀, 14–23/X/2009 (IBSP 177712, IBSP 177716, IBSP 177713, IBSP 177715, IBSP 177717, IBSP 177714); collected by R. Andrade & I. Cizauskas et al.; Cave N5S-10 (6°6'20"S; 50°7'53"W), 1Å, 22/III-03/IV/2005 (IBSP 55342), collected by R. Andrade & I. Arnori et al., 4, 07-12/X/2008 (IBSP 174526, IBSP 174528); 12∂23♀, 14–23/X/2009 (IBSP 177693, IBSP 177699, IBSP 177695, IBSP 177702, IBSP 177700, IBSP 177694, IBSP 177696, IBSP 177697, IBSP 177692, IBSP 177698, IBSP 177701, IBSP 177706); Cave N5S-11 (6°6'18"S; 50°7'47"W), 12∂25♀, 14–23/X/2009 (IBSP 177711, IBSP 177707, IBSP 177705, IBSP 177704, IBSP 177709, IBSP 177710, IBSP 177708, IBSP 177703); Cave N5S-14 (6°6'19"S;

 $50^{\circ}8'1''W$ ,  $1^{\circ}$ , 14-23/X/2009 (IBSP 177691); Cave N5S-15/16 (6°6'20''S; 50°7'60"W), 13, 14–23/X/2009 (IBSP 177686); Cave N5S-17 (6°5'15"S; 50°7'11"W), 1Å2Q, 25/VIII-03/IX/2009 (IBSP 177667, IBSP 177668, IBSP 177669); Cave N5S-18 (6°5'11"S; 50°7'39"W), 4♀, 25/VIII-03/IX/2009 (IBSP 177632, IBSP 177672, IBSP 177673, IBSP 177674); Cave N5S-19 (6°5'13"S; 50°7'37"W), 1♀, 25/VIII-03/ IX/2009 (IBSP 177671); Cave N5S-20 (6°5'15"S; 50°7'35"W), 2∂7♀, 25/VIII-03/ IX/2009 (IBSP 177651, IBSP 177652, IBSP 177653, IBSP 177654, IBSP 177655); Cave N5S-21 (6°5'15"S; 50°7'34"W), 2∂10♀, 07–12/X/2008 (IBSP 174533, IBSP 174537, IBSP 174541, IBSP 174534, IBSP 174536, IBSP 174538), 5∂11♀, 25/VIII-03/IX/2009 (IBSP 177656-IBSP 177658, IBSP 177660-IBSP 177666); Cave N5S-25 (6°5'12"S; 50°7'38"W)  $1\overset{?}{\sim}6^{\circ}$ , 14–16/XII/2010 (IBSP 188854, IBSP 178176); Cave N5S-30 (6°5'18"S; 50°7'10"W), 3∂8♀, 14–16/XII/2010 (IBSP 188855–IBSP 188859 4 (39, 10-19/V/2011 (IBSP 188860-IBSP 188867); collected by R. Andrade & I. Cizauskas et al.; Cave N5S-37 (6°6'22"S; 50°7'57"W), 1∂6♀, 07–12/X/2008, R. Andrade & et al. (IBSP 174543, IBSP 174545, IBSP 174542); 2∂20♀, 15–21/ IX/2009 (IBSP 177679, IBSP 177718, IBSP 177722, IBSP 177681, IBSP 177723, IBSP 177680, IBSP 177678); 3∂4♀, 14/III-04/IV/2010 (IBSP 177721, IBSP 177719, IBSP 177720); Cave N5S-40 (6°6'19"S; 50°8'0"W), 12, 15–21/IX/2009 (IBSP 177724); Cave N5S-42 (6°6'21"S; 50°8'2"W), 1∂0♀, 25/VIII-03/IX/2009 (IBSP 177659); Cave N5S-52/53 (6°6'28"S; 50°7'59"W), 6♀, 25/VIII-03/IX/2009 (IBSP 177676, IBSP 177675, IBSP 177677); 1352, 14/III-04/IV/2010 (IBSP 177633, IBSP 177725, IBSP 177726, IBSP 177634); Cave N5S-57 (6°6'31"S; 50°7'57"W), 2Å6♀, 25/VIII-03/IX/2009 (IBSP 177635, IBSP 177636); 1Å3♀, 14/ III-04/IV/2010 (IBSP 177727, IBSP 177728); Cave N5S-61 (6°6'19"S; 50°8'4"W), 1♀, 15–21/IX/2009 (IBSP 177729); Cave N5S-63/64/65 (6°6'12"S; 50°8'7"W) 1♀, 15–21/IX/2009 (IBSP 177682); 2♀, 14/III-04/IV/2010 (IBSP 177730, IBSP 177731); Cave N5S-84 (6°5'13"S; 50°8'12"W) 1♀, 25/VIII-03/IX/2009 (IBSP 177650); Cave N5S-85 (6°5'12"S; 50°7'35"W), 163202, 25/VIII-03/IX/2009 (IBSP 177637–IBSP 177649, IBSP 174066), 5Å7♀, 14/III–04/IV/2010 (IBSP 177732– IBSP 177735; IBSP 174065), all collected by R. Andrade & I. Cizauskas et al.; Cave N5SM1\_0008 (-6,108887; -50,134387), 2352, 31/XIII/2010 (ISLA 14614) Cave N5SM1-05 (6°6'41"S; 50°8'7"W), 2♀, 01/IX/2010 (IBSP 176989); Cave N5SM1-13 (6°6'22"S; 50°8'6"W), 1∂2♀, 29/VIII/2010 (IBSP 176992); Cave N5SM1-21 (6°6'19"S; 50°8'16"W), 4♀, 02/IX/2010 (IBSP 176993); Cave N5SM1-38 (6°6'22"S; 50°8'12"W),  $133^{\circ}$ , 25/II/2011 (IBSP 176972); Cave N5SM1-42 (6°6'26"S;  $50^{\circ}8'6''W$ ),  $10^{\circ}$ , 19/II/2011 (IBSP 176999); Cave N5SM2 0003 (6°8'31"S; 50°8'6"W),  $1\sqrt[3]{19}$ , 2010–11 (ISLA 14624); Cave N5SM2 0008 (6°8'27"S; 50°8'9"W), 1♀ (ISLA 14646); Cave N5SM2 0015 (6°8'17"S; 50°8'1"W), 1♂ (ISLA 14642); Cave N5SM2 0016 (6°8'17"S; 50°7'59"W), 1 $3^{\circ}$  (ISLA 14643); Cave N5SM2\_0018 (6°8'18"S; 50°8'2"W), 2∂1♀ (ISLA 14638); Cave N5SM2\_0022 (6°8'8"S; 50°8'7"W), 2♀ (ISLA 14627); Cave N5SM2 0023 (-6,135119; -50,13496),  $1^{\circ}$  (ISLA 14639); Cave N5SM2\_0024 (6°8'8"S; 50°8'6"W),  $1^{\circ}_{\circ}1^{\circ}_{\circ}$  (ISLA 14644); Cave N5SM2\_0025 (6°8'9"S; 50°8'6"W), 1∂, (ISLA 14632); Cave N5SM2\_0026



**Figure 2.** SEM images of *Ochyrocera varys* sp. n., male IBSP 174714 (**A–F**) **A** chelicerae, frontal view **B** epiandrous area, abdomen, ventral view **C–F** male palp **C** prolateral view (inset, cuspule) **D** retrolateral view (inset, base of long hair and tarsal organ) **E** ventral view (inset, embolus tip) **F** embolar tip, detail, retrolateral view. Scale bars: 100 μm.

(6°8'9"S; 50°8'6"W), 3 $\bigcirc$  (ISLA 14621); Cave N5SM2\_0037 (6°7'59"S; 50°8'5"W), 1 $\circlearrowright$ 3 $\bigcirc$  (ISLA 14636); Cave N5SM2\_0038 (6°7'58"S; 50°8'5"W), 1 $\circlearrowright$ 1 $\circlearrowright$ 1 $\bigcirc$  (ISLA 14647); Cave N5SM2\_0042 (6°7'57"S; 50°8'11"W), 1 $\circlearrowright$ 5 $\circlearrowright$  (ISLA 14635, ISLA

14648); Cave N5SM2\_0043 (6°7'56"S; 50°8'10"W), 1∂1♀ (ISLA 14628); Cave N5SM2 0044 (6°7'56"S; 50°8'6"W), 1∂1♀ (ISLA 14633); Cave N5SM2 0045  $(6^{\circ}7'55''S; 50^{\circ}8'6''W)$ ,  $1\sqrt[3]{2}$  (ISLA 14641); Cave N5SM2 0053 ( $6^{\circ}7'49''S$ ; 50°8'5"W),  $1 \stackrel{?}{\oslash} 1 \stackrel{?}{\subsetneq} 1$  (ISLA 14629); Cave N5SM2 0054 (6°7'48"S; 50°8'4"W),  $1 \stackrel{?}{\ominus} 1$ (ISLA 14637); Cave N5SM2 0056 (6°7'47"S; 50°8'5"W), 3♀ (ISLA 14640); Cave N5SM2 0057 (6°7'47"S; 50°8'5"W), 1∂3♀ (ISLA 14620); Cave N5SM2 0058  $(6^{\circ}7'46''S; 50^{\circ}8'5''W)$ ,  $1^{\circ}_{\circ}4^{\circ}_{\circ}$  (ISLA 14616); Cave N5SM2 0064 ( $6^{\circ}7'43''S$ ; 50°8'7"W), 1♀ (ISLA 14625); Cave N5SM2 0074 (6°7'32"S; 50°7'56"W), 2∂7♀ (ISLA 14623); Cave N5SM2\_0076 (6°7'31"S; 50°7'54"W), 1♀ (ISLA 14645); Cave N5SM2\_0078 (6°7'23"S; 50°7'49"W), 3∂5♀ (ISLA 14630, ISLA 14631); Cave N5SM2 0086 (6°7'16"S; 50°7'47"W), 1♂6♀ (ISLA 14618); Cave N5SM2 0087 (6°7'16"S; 50°7'43"W), 1∂1♀ (ISLA 14626); Cave N5SM2\_0093 (6°7'17"S; 50°7'56"W), 6♂8♀ (ISLA 14619); Cave N5SM2 0096 (6°8'6"S; 50°8'12"W), 1♂1♀ (ISLA 14634), 2010–2011, all collected by Equipe UFLA; Cave N5W-03 (6°4'53"S; 50°8'4"W), 6∂2♀, 02–23/VIII/2013 (IBSP 178554, IBSP 178557, IBSP 178559, IBSP 178560, IBSP 178555, IBSP 178558); Cave N8-0024 (6°10'24"S; 50°9'8"W), 2∂59, 16/VII-06/VIII/2014 (IBSP 188830, IBSP 188832, IBSP 188835, IBSP 188839, IBSP 188844), 1Å, 02–29/IV/2015 (IBSP 188849); Cave N8-0026 (6°10'14"S; 50°9'28"W), 1♀, 16/VII-06/VIII/2014 (IBSP 188837), all collected by Equipe Carste et al.; Cave N5E-006 (6°05'05"S, 50°07'50"W), 1∂ 1♀, 22/III-03/V/2005, R. Andrade & I. Armoni (IBSP 55344); Cave N5E 0008 (6°04'54"S, 50°07′50″W), 1♂ 2♀, 22/III–03/V/2005, R. Andrade & I. Arnoni (IBSP 55343); Cave N5E 0005 (6°05'10"S, 50°07'48"W), 22/III–03/V/2005, 1∂ 1♀, R. Andrade & I. Arnoni (IBSP 55338); Cave N5E\_0001 6°04'25"S, 50°07'05"W), 1Å, 22/III-03/V/2005, R. Andrade & I. Arnoni (IBSP 55342); EPIGEAN SAMPLES: Parauapebas, Floresta Nacional de Carajás, Serra Norte (6°5'19"S; 50°7'13"W), 29, 25/ IV-03/V/2012 (IBSP 191339, IBSP 191357); (6°5'31"S; 50°7'35"W), 1♀, 25/ IV-03/V/2012 (IBSP 191351); (6°6'12"S; 50°7'54"W), 7♂7♀, 27/I/2012 (IBSP 191340–IBSP 191348); (6°6'8"S; 50°7'54"W), 1♀, 26/IV-03/V/2012 (IBSP 191349); (6°6'17"S; 50°7'48"W), 1∂2♀, 26/IV-03/V/2012 (IBSP 191381, IBSP 191350), all collected by I. Cizauskas & R. Andrade et al.; (6°3'2"S; 50°14'56"W), 13, 10/X/2012 (IBSP 191393); (6°2'33"S; 50°13'6"W), 1♀, 28/IX/2012 (IBSP 191394); (6°3'9"S; 50°14'31"W), 1♀, 14/III/2013 (IBSP 191395), all collected by Equipe Carste et al.

**Etymology.** The specific name refers to Varys, a fictional character in George R. R. Martin's book, "A Song of Ice and Fire". Lorde Varys is a character with a venomous spirit, known as a spider in the plot.

**Diagnosis.** Ochyrocera varys resembles Ochyrocera atlachnacha in its carapace, which is yellow and bright lime (Figs 1A–B; 4A–B) and palp with conical, elongated cymbial apophysis, and have a distal cuspule on the cymbial apophysis (Figs 1C–D, 4C–D). This species can be distinguished by the male palp having a cymbial apophysis without an accentuated lateral projection (present in *O. atlachnacha*) and by the curved distal area of embolus (Figs 1C–D, 2C–F); females have a thick spermathecae enveloping large pore-plates (Fig. 1E–F).



**Figure 3.** SEM images of *Ochyrocera varys* sp. n., female IBSP 174714 (**A–D**) **A** colulus, ventral view **B** chelicerae, frontal view **C** pedipalp, distal, prolateral view **D** pedipalp, tarsal organ. Scale bars: 50 μm.

**Description. Male** (holotype). Total length 2.3. Carapace length 0.7, ovoid; narrowing gradually anteriorly with yellow and bright lime pattern, fovea flattened and inconspicuous (Fig. 1A). Clypeus length 0.7. Eyes: PME oval; ALE and PLE rounded. Chelicerae light yellow; promargin with eight teeth, attached to long lamina (Fig. 2A); retromargin without teeth. Sternum yellow. Endites yellowish. Legs: light yellow; formula 1423; total length: I 7.0; II 5.9; III 4.1; IV 6.5. Male palp: palpal femur length 0.4; palpal tibia enlarged basally with several trichobothria; cymbial apophysis slightly curved distally, short apical cuspule, retrolateral paired long hair on non-projected base, a single tarsal organ, with two basal setae (Fig. 2C–D), cymbial prolateral extension subtriangular (Fig. 2C); bulb oval; embolus elongated, wide at base and projecting upward, with sinuous tip (Fig. 2C–F). Abdomen length 1.3, oval; uniformly grayishgreen; six epiandrous spigots, with short base (Fig. 2B).

**Female** (paratype IBSP 176843). Total length: 2.0; carapace length: 0.74; Carapace pattern as in male (Fig. 1B). Pedipalp without claw, with conical tip and subdistal tarsal organ (Fig. 3C–D). Clypeus: 0.67 diameter; Eyes, chelicerae, sternum, endites and labium as in male (Fig. 3B). Legs as in male; formula 4123, total length: I 6.3; II

4.7; III 3.6 IV4.3. Abdomen length 0.97. Colulus triangular with long bristles (Fig. 3A). Internal genitalia with well-developed medial columnar uterus externus, shorter than spermathecae length and internally with inconspicuous chambers. Uterus externus ending in a narrow neck. Rounded pore-plates covered by spermathecae, with approximately 15 glandular ducts (Fig. 1E–F).

**Distribution.** Recorded from caves and epigean areas of Carajás, state of Pará, northern Brazil (Fig. 19A).

#### Ochyrocera atlachnacha sp. n.

http://zoobank.org/BA66C49B-BA97-4B0E-8C18-5EBB21D0EB1C Figures 4, 5, 6, 19B, 21B

**Types.** Male holotype from Cave CAV\_0034 (50°22'56"W, 6°24'9"S), Serra Sul, Floresta Nacional de Carajás, Canaã dos Carajás, Pará, Brazil, 22–31/V/2010, R. Andrade & I. Cizauskas et al., deposited in IBSP 175570. Paratypes: female from Cave S11D-64 (50°18'48"W, 6°23'31"S), Serra Sul, Floresta Nacional de Carajás, Canaã dos Carajás, Pará, Brazil, 13–30/I/2010, R. Andrade & I. Cizauskas et al. (IBSP 188899);  $2^{\circ}_{\circ}$  2 $^{\circ}_{\circ}$  from Cave S11D-64, as above (MPEG 34435, ex IBSP 175494);  $1^{\circ}_{\circ}$  1 $^{\circ}_{\circ}$  from S11-0011 (6°26'11"S; 50°17'40"W), Serra Sul, Floresta Nacional de Carajás, Canaã dos Carajás, Pará, Brazil, 03–19/VIII/2010, R. Andrade & I. Cizauskas et al. (MZSP 72855, ex IBSP 175664).

Other material examined. BRAZIL. Pará: HYPOGEAN SAMPLES: Canaá dos Carajás, Floresta Nacional de Carajás, Serra Sul, Cave CAV\_0001 (6°24'42"S; 50°20'5"W), 1♂2♀, 22–31/V/2010 (IBSP 175562, IBSP 175563); 1♂, 22–28/ IX/2010 (IBSP 175659); Cave CAV 0003 (6°24'41"S; 50°20'5"W), 2♀, 22-31/ V/2010 (IBSP 175564, IBSP 175565); Cave CAV\_0006 (6°24'40"S; 50°19'57"W), 1∂, 22–28/IX/2010 (IBSP 175660); Cave CAV 0018 (6°24'23"S; 50°22'9"W), 1♀, 22-31/V/2010 (IBSP 175567); Cave CAV 0020 (6°24'21"S; 50°22'8"W), 19, 22-28/IX/2010 (IBSP 175661) 13, 22-31/V/2010 (IBSP 175568); Cave CAV\_0021 (6°24'20"S; 50°22'10"W), 1♀, 22–31/V/2010 (IBSP 175569); 1♂2♀, 22–28/ IX/2010 (IBSP 175663, IBSP 175662); Cave CAV\_0034 (6°24'9"S; 50°22'56"W), 1<sup>Q</sup>, 17/X-23/X/2014 (IBSP 188882); Cave CAV\_0034 (6°24'9"S; 50°22'56"W), 3∂6♀, 22–31/V/2010 (IBSP 175573, IBSP 175574, IBSP 175575, IBSP 175570, IBSP 175571, IBSP 175572) 1∂1♀, 17/X–23/X/2014 (IBSP 188881); Cave S11–03 (6°26'12"S; 50°17'38"W), 1∂, 19–22/III/2010 (IBSP 175556); Cave S11–05 (6°26'19"S; 50°17'35"W), 1♀, 19–22/III/2010 (IBSP 175557); Cave S11–11  $(6^{\circ}26'11''S; 50^{\circ}17'40''W), 1^{\circ}, 24/II-04/III/2010$  (IBSP 175555);  $2^{\circ}_{\circ}2^{\circ}_{\circ}, 03-19/$ VIII/2010 (IBSP 175664); Cave S11-19 (6°26'36"S; 50°17'30"W) 3♀, 19-22/ III/2010 (IBSP 175558, IBSP 175559); Cave S11–20 (6°26'37"S; 50°17'30"W), 1♀, 03–19/VIII/2010 (IBSP 175665); 1<sup>Q</sup>, 19–22/III/2010 (IBSP 175560); Cave S11–21 (6°26'40"S; 50°17'28"W), 1♀, 19-22/III/2010 (IBSP 175561); Cave S11D-01 (6°23'54"S; 50°21'25"W), 4♀, 13–30/I/2010 (IBSP 175462, IBSP 175461); 3♂4♀,

17/X–23/X/2014 (IBSP 188889, IBSP 188884, IBSP 188885, IBSP 188886); 3∂1♀, 01-14/VII/2010 (IBSP 175595, IBSP 175596, IBSP 175594); Cave S11D-05 (6°24'3"S; 50°20'60"W), 1∂3♀, 01–14/VII/2010 (IBSP 175597, IBSP 175598) 1∂1♀, 19–22/II/2010 (IBSP 175523, IBSP 175524); Cave S11D–06 (6°24'4"S; 50°21'1"W), 1♂1♀, 01–14/VII/2010 (IBSP 175599, IBSP 175600); 3♂6♀, 19–22/ II/2010 (IBSP 175532, IBSP 175533, IBSP 175535, IBSP 175536), all collected by R. Andrade & I. Cizauskas et al.; Cave S11D–10 (6°23'54"S; 50°21'25"W), 3♀, 23/ VIII-02/IX/2007, R. Andrade & I. Arnori et al. (IBSP 174462); 2∂2♀, 01-14/ VII/2010 (IBSP 175601, IBSP 175602, IBSP 175603) 2♀, 13–30/I/2010 (IBSP 175443, IBSP 175444) 1∂2♀, 17/X–23/X/2014 (IBSP 188888, IBSP 188891, IBSP 188893); Cave S11D-104 (6°23'50"S; 50°21'59"W), 52, 14-19/XII/2011 (IBSP 175884); 1<sup>(2</sup>2<sup>(2)</sup>, 30/VII–02/IX/2011 (IBSP 175883); Cave S11D–108 (6°23'58"S; 50°21'18"W), 1∂1♀, 14–19/XII/2011 (IBSP 175885); Cave S11D–11 (6°23'51"S; 50°21'30"W), 2<sup>Q</sup>, 17/X-23/X/2014 (IBSP 188887, IBSP 188890); Cave S11D-110 (6°23'49"S; 50°20'27"W), 1<sup>3</sup>, 30/VII–02/IX/2011 (IBSP 175879); Cave S11D–111 (6°23'48"S; 50°20'27"W), 1∂3♀, 30/VII–02/IX/2011 (IBSP 175881, IBSP 175880), 2∂3♀, 14–19/XII/2011 (IBSP 175886, IBSP 175887); Cave S11D–112 (6°24'46"S; 50°21'14"W), 1♀, 30/VII–02/IX/2011 (IBSP 175882); 1♂3♀, 14–19/XII/2011 (IBSP 175888); Cave S11D-114 (6°25'15"S; 50°18'53"W), 2∂, 14-19/XII/2011 (IBSP 175889, IBSP 175890); Cave S11D–12 (6°23'46"S; 50°21'34"W), 3♀, 01–14/ VII/2010 (IBSP 175604, IBSP 175608, IBSP 175609), all collected by R. Andrade & I. Cizauskas et al.; Cave S11D-12 (6°23'46"S; 50°21'34"W), 2<sup>♀</sup>, 23/VIII-02/ IX/2007, R. Andrade & I. Arnori et al. (IBSP 174465, IBSP 174466); 3∂30♀, 13– 30/I/2010 (IBSP 175445, IBSP 175446, IBSP 175454, IBSP 175456, IBSP 175458, IBSP 175459, IBSP 175447, IBSP 175455, IBSP 175448, IBSP 175450, IBSP 175451, IBSP 175452, IBSP 175449, IBSP 175453, IBSP 175457); 1♀, 17/X-23/X/2014 (IBSP 188892) 1♂9♀, 01-14/VII/2010 (IBSP 175605, IBSP 175607, IBSP 175606); Cave S11D–13 (6°24'6"S; 50°21'8"W), 1♂9♀, 13–30/I/2010 (IBSP 175499, IBSP 175500, IBSP 175501, IBSP 175502); 4♀, 01–14/VII/2010 (IBSP 175610, IBSP 175611, IBSP 175612); Cave S11D-14 (6°24'6"S; 50°21'10"W), 2∂4♀, 13–30/I/2010 (IBSP 175473, IBSP 175474, IBSP 175475, IBSP 175476); 2∂39, 01–14/VII/2010 (IBSP 175614, IBSP 175615, IBSP 175616); Cave S11D– 15 (6°23'45"S; 50°21'25"W), 1∂1♀, 01–14/VII/2010 (IBSP 175618, IBSP 175617); Cave S11D-16 (6°23'47"S; 50°21'37"W), 1Å, 13-30/I/2010 (IBSP 175471); Cave S11D-17 (6°23'56"S; 50°21'23"W), 2∂1♀, 13-30/I/2010 (IBSP 175441, IBSP 175442); 3<sup>♀</sup>, 01–14/VII/2010 (IBSP 175619, IBSP 178177); Cave S11D–22 (6°24'46"S; 50°21'33"W), 3∂4♀, 19–22/II/2010 (IBSP 175541, IBSP 175542, IBSP 175543); 1<sup>Q</sup>, 03–19/VIII/2010 (IBSP 175620); Cave S11D–23 (6°24'46"S;  $50^{\circ}21'32''W$ ),  $1^{\circ}_{\circ}$ , 19-22/II/2010 (IBSP 175540); Cave S11D-24 (6°24'46''S; 50°21'15"W), 1∂2♀, 19–22/II/2010 (IBSP 175537, IBSP 175538, IBSP 175539); Cave S11D-26 (6°24'49"S; 50°21'17"W), 2∂1♀, 19-22/II/2010 (IBSP 175544, IBSP 175545); 132, 03–19/VIII/2010 (IBSP 175621, IBSP 175622); Cave S11D– 27 (6°24'43"S; 50°21'9"W); 1♂, 19–22/II/2010 (IBSP 175546), 2♂1♀, 03–19/



**Figure 4.** *Ochyrocera atlachnacha* sp. n., male holotype (**A**, **C**, **D**), female paratype, IBSP 188899 (**B**, **E**, **F**) **A–B** habitus, dorsal view **C** left male palp, retrolateral view **D** same, prolateral view (arrow = cymbial lateral projection) **E** genitalia, enzyme cleared, dorsal view **F** same, dorsal view. Abbreviations: CUE = columnar uterus externus, NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae, UE, uterus externus. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 μm (**E**, **F**).

VIII/2010 (IBSP 175623, IBSP 175624); Cave S11D–28 (6°24'40"S; 50°21'6"W), 1 $\bigcirc$ , 03–19/VIII/2010 (IBSP 175625); Cave S11D–29 (6°24'40"S; 50°20'44"W), 2 $\circlearrowright$ 5 $\bigcirc$ , 19–22/II/2010 (IBSP 175547, IBSP 175548, IBSP 175549, IBSP 175550); 1 $\bigcirc$ , 03–19/VIII/2010 (IBSP 175626); Cave S11D–31 (6°24'41"S; 50°20'43"W) 2 $\bigcirc$ , 19–22/II/2010 (IBSP 175551, IBSP 175552); Cave S11D–32 (6°24'40"S; 50°20'38"W), 2 $\bigcirc$ , 13–30/I/2010 (IBSP 175460), all collected by R. Andrade & I. Cizauskas et al.; Cave S11D–33 (6°24'40"S; 50°20'37"W), 4 $\circlearrowright$ 5 $\bigcirc$ , 23/VIII–02/ IX/2007 (IBSP 174467); collected by R. Andrade & I. Arnori et al.; 3 $\circlearrowright$ 4 $\bigcirc$ , 13–30/ I/2010 (IBSP 175506, IBSP 175507, IBSP 175508, IBSP 175509, IBSP 175510, IBSP 175511); 3 $\circlearrowright$ 6 $\bigcirc$ , 03–19/VIII/2010 (IBSP 175627, IBSP 175628, IBSP 175629, IBSP 175630, IBSP 175631); Cave S11D–34 (6°24'41"S; 50°20'36"W), 1 $\circlearrowright$ , 13–30/ I/2010 (IBSP 175515); Cave S11D–35 (6°24'40"S; 50°20'35"W), 1 $\circlearrowright$ 1 $\bigcirc$ , 13–30/ I/2010 (IBSP 175515); Cave S11D–35 (6°24'40"S; 50°20'35"W), 1 $\circlearrowright$ 1 $\bigcirc$ 1 $\bigcirc$ , 13–30/ I/2010 (IBSP 175517, IBSP 175518); 2 $\circlearrowright$ 1 $\bigcirc$ , 03–19/VIII/2010 (IBSP 174072, IBSP

175632); 2Å19, 17/X–23/X/2014 (IBSP 188883, IBSP 188894); Cave S11D–36 (6°24'40"S; 50°20'34"W), 1♀, 13–30/I/2010 (IBSP 175513); Cave S11D–37 (6°24'46"S; 50°21'31"W), 2♀, 13–30/I/2010 (IBSP 175553, IBSP 175554), all collected by R. Andrade & I. Cizauskas et al.; Cave S11D–39 (6°23'46"S; 50°20'27"W), 1Å59, 23/VIII-02/IX/2007 (IBSP 174472, IBSP 174473, IBSP 174471), R. Andrade & I. Arnori et al.; 2♀, 03–19/VIII/2010 (IBSP 175633, IBSP 175634); 6♀, 13– 30/I/2010 (IBSP 175525, IBSP 175527, IBSP 175528, IBSP 175529, IBSP 175530, IBSP 175531); 1, 13–30/I/2010 (IBSP 175526); 03–19/VIII/2010 (IBSP 175635); all collected by R. Andrade & I. Cizauskas et al.; Cave S11D-40 (6°24'38"S; 50°19'29"W), 1♀, 23/VIII-02/IX/2007 (IBSP 174476); collected by R. Andrade & I. Arnori et al.; 1 (12, 13–30/I/2010 (IBSP 175516); 1 (124), 03–19/VIII/2010 (IBSP 175636, IBSP 175637, IBSP 175638); all collected by R. Andrade & I. Cizauskas et al.; Cave S11D-41 (6°24'38"S; 50°19'29"W), 1Å1Q, 13-30/I/2010 (IBSP 175472) 19, 03–19/VIII/2010 (IBSP 175639); Cave S11D–43 (6°24'48"S; 50°19'17"W), 1♀, 23/VIII–02/IX/2007 (IBSP 174478) R. Andrade & I. Arnori et al., 2♂4♀, 03– 19/VIII/2010 (IBSP 175641, IBSP 175520, IBSP 175519, IBSP 175522), 2∂2♀, 03–19/VIII/2010 (IBSP 175640); Cave S11D–46 (6°24'53"S; 50°18'59"W), 29, 13–30/I/2010 (IBSP 175514); 3♀, 03–19/VIII/2010 (IBSP 175642, IBSP 175643); Cave S11D-50 (6°24'25"S; 50°19'14"W), 1♀, 13-30/I/2010 (IBSP 175470); 1♀, 01–14/VII/2010 (IBSP 175644); Cave S11D–51 (6°24'25"S; 50°19'14"W), 2♀, 13– 30/I/2010 (IBSP 175463); Cave S11D-52 (6°24'25"S; 50°19'15"W), 1<sup>o</sup>, 13-30/ I/2010 (IBSP 175464); Cave S11D-53 (6°24'25"S; 50°19'15"W), 5∂7♀, 13-30/ I/2010 (IBSP 175465, IBSP 175466, IBSP 175467, IBSP 175468); 1∂4♀, 01–14/ VII/2010 (IBSP 175645, IBSP 175646, IBSP 175647); all collected by R. Andrade & I. Cizauskas et al.; Cave S11D-55 (6°24'23"S; 50°19'12"W), 1Å39, 23/VIII-02/ IX/2007 (IBSP 174485, IBSP 174487); R. Andrade & I. Arnori et al.; 3∂3♀, 13–30/ I/2010 (IBSP 175479, IBSP 175480, IBSP 175481, IBSP 175482); 3♀, 01–14/ VII/2010 (IBSP 175648, IBSP 175649); Cave S11D-56 (6°24'24"S; 50°19'12"W), 2♀, 13-30/I/2010 (IBSP 175469); Cave S11D-58 (6°24'21"S; 50°19'13"W), 1♀, 13-30/I/2010 (IBSP 175477); 1<sup>Q</sup>, 01-14/VII/2010 (IBSP 175650); Cave S11D-61 (6°23'33"S; 50°18'47"W), 1∂4♀, 13–30/I/2010 (IBSP 175503, IBSP 175504, IBSP 175505); 1<sup>Q</sup>, 01–14/VII/2010 (IBSP 175651); Cave S11D–62 (6°23'32"S; 50°18'47"W), 1∂, 13–30/I/2010 (IBSP 175496) all collected by R. Andrade & I. Cizauskas et al.; Cave S11D-64 (6°23'31"S; 50°18'48"W), 1∂7♀, 23/VIII-02/ IX/2007, R. Andrade & I. Arnori et al. (IBSP 174489); 13∂289, 13–30/I/2010 (IBSP 175484, IBSP 175493, IBSP 175495, IBSP 175492, IBSP 175486, IBSP 175491, IBSP 175489, IBSP 175494, IBSP 175485, IBSP 175483, IBSP 175487, IBSP 175490) 3∂4♀, 01–14/VII/2010 (IBSP 175655, IBSP 175657, IBSP 175658, IBSP 175652, IBSP 175654, IBSP 175656); Cave S11D-66 (6°23'34"S; 50°18'53"W), 1♀, 13-30/I/2010 (IBSP 175498); Cave S11D-67 (6°23'34"S; 50°18'53"W), 1♀, 13-30/I/2010 (IBSP 175497); Cave S11D-71 (6°23'34"S; 50°19'8"W), 1♀, 13-30/ I/2010 (IBSP 175418); Cave S11D−77 (6°23'33"S; 50°18'59"W), 2♀, 13–30/I/2010 (IBSP 175422); 1♂8♀, 01–14/VII/2010 (IBSP 175576, IBSP 175577, IBSP 175579,



**Figure 5.** SEM images of *Ochyrocera atlachnacha* sp. n. male IBSP 175516 (**A–F**) **A** chelicerae, frontal view, **B** epiandrous, abdomen, ventral view **C–F** male palp **C** prolateral view **D** retrolateral view (inset, base of long hair and tarsal organ) **E** subapical view **F** dorsal view. Scale bars: 0.3 mm.

IBSP 175580, IBSP 175578); all collected by R. Andrade & I. Cizauskas et al.; Cave S11D–78 (6°23'32"S; 50°18'58"W) 1♀, 23/VIII–02/IX/2007 R. Andrade & I. Arnori et al., (IBSP 174490); 1♂5♀, 13–30/I/2010 (IBSP 175425, IBSP 175427, IBSP

175424); 1♀, 13–30/I/2010 (IBSP 175426); 1♀, 01–14/VII/2010 (IBSP 175581); Cave S11D-79 (6°23'33"S; 50°18'56"W), 1♀, 13-30/I/2010 (IBSP 175437) 3♀, 01-14/VII/2010 (IBSP 175582, IBSP 175583); Cave S11D-80 (6°23'34"S; 50°18'57"W), 1♀, 13–30/I/2010 (IBSP 175433); 2♀, 01–14/VII/2010 (IBSP 175584); Cave S11D-81 (6°23'34"S; 50°18'53"W); 2∂7♀, 13-30/I/2010 (, IBSP 175428, IBSP 175429, IBSP 175430, IBSP 175431, IBSP 175432) 3∂8♀, 01–14/ VII/2010 (IBSP 175585, IBSP 175587, IBSP 175586, IBSP 175588, IBSP 175589); Cave S11D-83 (6°23'48"S; 50°19'25"W), 1♀, 13-30/I/2010 (IBSP 175438); Cave S11D–86 (6°23'47"S; 50°19'24"W), 1♂, 13–30/I/2010 (IBSP 175439); 1♂1♀, 01– 14/VII/2010 (IBSP 175590); Cave S11D-88 (6°23'45"S; 50°19'22"W), 3<sup>Q</sup>, 13-30/ I/2010 (IBSP 175434, IBSP 175435, IBSP 175436); Cave S11D-94 (6°23'40"S; 50°19'17"W), 3♀, 13–30/I/2010 (IBSP 175419, IBSP 175420, IBSP 175421), all collected by R. Andrade & I. Cizauskas et al.; Cave S11D–96 (6°23'37"S; 50°19'27"W), 3♀, 23/VIII–02/IX/2007 (IBSP 174498) R. Andrade & I. Arnori et al., 1∂5♀, 13– 30/I/2010 (IBSP 175440, IBSP 175592, IBSP 175593) 1♀, 01–14/VII/2010 (IBSP 175591), all collected by R. Andrade & I. Cizauskas et al.

**Etymology.** The specific name refers to Atlach-Nacha, a supernatural entity from Cthulhu mythology that resembles a huge spider with an almost human face.

**Diagnosis.** Ochyrocera atlachnacha resembles O. varys by its carapace yellow and bright lime (Figs 1A–B, 4A–B) and palp with conical, elongated and distal cuspule in the cymbial apophysis (Figs 1C–D, 4C–D). It can be distinguished from the latter and other Neotropical species by the male palpal cymbium with accentuated cymbial prolateral projection (Figs 4D, 5C, E–F); females have enlarged and projected pore-plates on the inconspicuous spermathecae (Fig. 4E–F).

**Description. Male** (holotype). Total length 2.2 Carapace length 0.7, ovoid; narrowing gradually anteriorly; yellow and bright lime, flat pars cephalic, fovea not visible (Fig. 4A). Clypeus length 0.7, with a pair of long bristles (Fig. 4A). Eyes: PME oval; ALE and PLE rounded. Chelicerae light yellow; promargin with 6 teeth, attached to a very long lamina (Fig. 5A); retromargin without teeth. Sternum light gray to darker. Endites dark yellow to green. Legs: gray; formula 1423; total length: I 7.0; II 5.9; III 4.1; IV 6.5. Male palp: palpal femur length 0.4; palpal tibia enlarged basally; cymbium slightly curved distally, bearing short apical cuspule; retrolateral paired long setae on non-projected base and an elongated tarsal organ (Fig. 5D) with three basal setae on the rounded cymbial prolateral extension (Fig. 5C); bulb oval; embolus flattened, short, with sinuous tip (Figs 4C–D, 5C–F). Abdomen length 1.3, oval; uniformly grayish-green. Six epiandrous spigots, with short base (Fig. 5B)

**Female** (paratype IBSP 188899). Total length: 2.0; carapace length: 0.74; Carapace as in male, light yellow pattern (Fig. 4B). Pedipalp without claw, with conical tip and subdistal trichobothrium (Fig. 6A–B). Clypeus: 0.68 diameter. Eyes, chelicerae, sternum, endites (Fig. 6C) and labium as in male. Legs as in male; formula 4123, total length: I 6.3; II 4.7; III 3.6 IV4.3. Abdomen length 0.96. Colulus triangular with approximately 10 bristles (Fig. 6D). Internal genitalia with inconspicuous and very narrow spermathecae, under the conspicuous pore-plate; short medial columnar



**Figure 6.** SEM images of *Ochyrocera atlachnacha* sp. n., female IBSP 175516 (**A–D**) **A** trichobothria, dorsal view **B** pedipalp, distal, prolateral view **C** chelicerae, frontal view **D** colulus, ventral view. Scale bars: 0.3 mm.

uterus externus, internally with no visible chambers. Uterus externus ending in a narrow neck. Oval pore-plates on the spermathecae, with approximately 25–30 glandular ducts (Fig. 4E–F).

**Distribution.** Recorded exclusively from caves in Carajás, state of Pará, northern Brazil (Fig. 20B).

*Ochyrocera laracna* sp. n. http://zoobank.org/9DA06567-8A56-4C0D-A84E-864BC55E38A5 Figures 7, 8, 9, 20A

**Types.** Holotype male from Cave N5S-15/16 (50°7'60"W, 6°6'20"S), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 14–23/X/2009, R. Andrade & I. Cizauskas et al. (IBSP 177631). Paratypes: Cave N4E\_13 (6°2'18"S; 50°9'38"W),



**Figure 7.** *Ochyrocera laracna* sp. n. male holotype (**A**, **C–D**), female paratype, IBSP 191335 (**B**, **E–F**) **A–B** habitus, dorsal view **C** left male palp, retrolateral view **D** same, prolateral view **E** genitalia, enzyme cleared, dorsal view **F** same, dorsal view. Abbreviations: NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 μm (**E**, **F**).

Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 20/X-01/XI/2006, R. Andrade (IBSP 188898).

**Other examined material.** BRAZIL. Pará: **HYPOGEAN SAMPLES**: Canaã dos Carajás, Floresta Nacional de Carajás, Serra Sul, Cave S11D-64 (6°23'31"S; 50°18'48"W),  $2\overset{\circ}{\partial}4^{\circ}$ , 13–30/I/2010 (IBSP 174071); Cave CAV\_0014 (6°24'59"S; 50°19'15"W),  $1^{\circ}$ , 22–28/IX/2010 (IBSP 174075); Cave CAV-19 (6°29'52"S; 51°9'55"W),  $1^{\circ}$ , 08–15/III/2012 (IBSP 175981);  $1\overset{\circ}{\partial}$ , 20–29/VI/2012 (IBSP 175999); Cave CAV\_06 (6°29'51"S; 51°9'44"W),  $1^{\circ}$ , 08–15/III/2012 (IBSP 176047); Cave CAV\_18 (6°29'50"S; 51°9'31"W),  $1^{\circ}$ , 08–15/III/2012 (IBSP 176065); Cave CAV\_03 (6°29'39"S; 51°9'31"W),  $1^{\circ}$ , 20–29/VI/2012 (IBSP 176071); Cave CAV\_06 (6°29'51"S; 51°9'44"W),  $1^{\circ}$ , 20–29/VI/2012 (IBSP 176071); Cave CAV\_06 (6°29'51"S; 51°9'44"W),  $1^{\circ}$ , 20–29/VI/2012 (IBSP 176071); Cave CAV\_06 (6°29'51"S; 51°9'44"W),  $1^{\circ}$ , 20–29/VI/2012 (IBSP 176073), all collected by R. Andrade & I. Cizauskas et al.; Parauapebas, Cave CRIS\_38 (6°27'32"S; 49°42'13"W),  $1^{\circ}$ , 29/VII-06/VIII/2008 (IBSP 174645); collected by R. Andrade et al.; Floresta Nacional de Carajás, Serra Norte, Cave N5S\_no number (6°5'16"S; 50°7'11"W),  $1^{\circ}$ , 28/IX-03/X/2007, collected by R. Andrade & I. Arnori et al. (IBSP 191356); Cave



**Figure 8.** SEM images of *Ochyrocera laracna* sp. n., male IBSP 191335 (**A–F**) **A** left palp, retroapical view **B** same, prolateroapical view **C** cymbium, distal view, double cuspules, long hair and tarsal organ **D** cymbium, dorsal view **E** same, basal setae **F** embolus, distal area. Scale bars: 0.3 mm.

N5S\_15/16 (6°6'20"S; 50°7'60"W), 1 $\stackrel{\circ}{\sim}$ , 14–23/X/2009 (IBSP 177631), collected by R. Andrade & I. Cizauskas et al.; Cave N3\_0070 (6°2'39"S; 50°13'48"W), 1 $\stackrel{\circ}{\sim}$ , 03–17/IV/2013 (IBSP 178566); Cave N3\_0078 (6°2'36"S; 50°13'43"W), 1 $\stackrel{\circ}{\sim}$ , 03–17/

IV/2013 (IBSP 178567), all collected by Equipe Carste et al.; Cave N5SM1\_0004 (-6,112018; -50,135539), 1Å, 04/IX-06/X/2014 (ISLA 14615); Cave N5SM2 0058 (6°7'46"S; 50°8'5"W), 1Å, 24/II-13/III/2015 (ISLA 14617), all collected by Bioespeleo et al.; EPIGEAN SAMPLES: Parauapebas, Floresta Nacional de Carajás, Serra Norte (6°5'15"S; 50°7'12"W), 1♀, 25/I/2012 (IBSP 191368); (6°5'16"S; 50°7'36"W), 2♀, 19/XI/2012 (IBSP 191352, IBSP 191356); (6°5'18"S; 50°7'11"W), 5♂, 25/ IV-03/V/2012 (IBSP 191366, IBSP 191367, IBSP 191380, IBSP 191385), 2∂3♀, 08-14/XI/2012 (IBSP 191354, IBSP 191353, IBSP 191365); (6°5'18"S; 50°7'12"W) 1♂, 25/I/2012 (IBSP 191371); (6°5'20"S; 50°7'10"W), 2♂1♀, 08–14/XI/2012 (IBSP 191369, IBSP 191384); (6°5'20"S; 50°7'10"W), 12Å, 25/IV-03/V/2012 (IBSP 191360, IBSP 191362, IBSP 191383, IBSP 191387, IBSP 191391, IBSP 191392, IBSP 191364, IBSP 191372, IBSP 191376); (6°6'12"S; 50°7'45"W), 1∂, 26/IV-03/V/2012 (IBSP 191382) 1♀, 27/I/2012 (IBSP 191355); (6°6'17"S; 50°7'48"W), 1♂, 26/IV--03/V/2012 (IBSP 191388); (6°6'20"S; 50°7'41"W), 1♂1♀, 16-20/ XI/2012 (IBSP 191361, IBSP 191378); (6°6′20″S; 50°7′55″W), 1♀, 27/I/2012 (IBSP 191370); (6°6'22"S; 50°7'47"W), 9Å1\$, 25/IV-03/V/2012 (IBSP 191379, IBSP 191363, IBSP 191377, IBSP 191389, IBSP 191390, IBSP 191373, IBSP 191358) 2∂1♀, 16–20/XI/2012 (IBSP 191386, IBSP 191374, IBSP 191375); (6°6'24"S; 50°7'55"W), 1♀, 25/IV-03/V/2012 (IBSP 191359); all collected by I. Cizauskas & R. Andrade et al.; (6°0'59"S; 50°4'43"W), 1∂2♀, 9/III/2013 (IBSP 191396, IBSP 191402); (6°1'46"S; 50°12'4"W), 2♀, 13/X/2012 (IBSP 191400, IBSP 191401); (6°2'33"S; 50°13'23"W), 2<sup>3</sup>, 11/III/2013 (IBSP 191397); (6°2'33"S; 50°13'6"W), 1♀, 5/III/2013 (IBSP 191399); (6°3'24"S; 50°4'49"W), 1♂, 6/III/2013 (IBSP 191403); (6°3'9"S; 50°14'31"W); 1Å, 14/III/2013 (IBSP 191398); 1Å, 12/X/2012 (IBSP 191332); all collected by Equipe Carste et al.

**Etymology.** The specific name refers to Laracna, a giant and very old spider created by J. R. R. Tolkien in the classic book "The Lord of the Rings".

**Diagnosis.** *Ochyrocera laracna* resembles *O. aragogue* by the yellowish-green body pattern (Figs 7A–B; 8A–B) and by the short cymbial apophysis with two distal spurs on projections (Fig. 7C–D), a unique character for both these Neotropical species. The male of the former species can be distinguished from the latter due to the palp having a flap at the distal area of embolus (Figs 7C–D; 8A–B) and a laminar spur in the curved area 8F). The female is distinguished from *O. aragogue* by the small distal area of the spermathecae and pore plates adjacent to the spermathecae base (Fig. 7E–F).

**Description. Male** (holotype). Total length 2.2. Carapace length 0.7, ovoid; narrowing gradually anteriorly, with yellowish-green body pattern, pars cephalica flat, fovea not visible (Fig. 7A). Clypeus length 0.7, curved forward. Eyes: PME oval; ALE and PLE rounded. Chelicerae light yellow, promargin with seven teeth, attached to long lamina (Fig. 9C); retromargin without teeth. Sternum light yellow to slightly darker. Endites dark yellow suffused. Legs light yellow; formula 1423; total length: I 7.0; II 5.9; III 4.1; IV 6.5. Male palp: palpal femur length 0.4; palpal tibia enlarged medially with dorsal trichobothrium (Fig. 9A); cymbial apophysis short, bearing parallel double short cuspules at tip, retrolateral long hair next to the tarsal organ (Fig. 8C); with four basal setae (Fig. 8E) and cymbial prolateral extension squared (8A); bulb oval; embolus


**Figure 9.** SEM images of *Ochyrocera laracna* sp. n., male IBSP 191335 (**A–C**), female IBSP 191335 (**D–F**) **A** trichobothria, palp, dorsal view **B** epiandrous area, abdomen, ventral view **C** chelicerae, frontal view **D** same, frontal view **E** endites and labium, ventral view **F** serrula, ventral view. Scale bars: 300 μm.

enlarged at base (Figs 7C–D, 8A–F). Abdomen length 1.3, oval; uniformly greenpurplish with black spots. Six epiandrous spigots, with short base (Fig. 9B).

**Female** (paratype IBSP 188898). Total length 2.0; carapace length: 0.74; Carapace as in male (Fig. 7B). Pedipalp without claw, with conical tip and subdistal trichobothria. Clypeus: 0.68 diameter. Eyes, chelicerae, sternum, endites and labium as in male,

serrula with more than 50 teeth (Fig. 9E–F). Legs as in male; leg formula 4123, total length: I 6.3; II 4.7; III 3.6 IV 4.3. Abdomen length 0.96. Colulus triangular, with 8–10 bristles. Internal genitalia weakly sclerotized, spermathecae tubular, thicker at basal area and curved at middle; elongated medial columnar uterus externus, shorter than spermathecae, internally with inconspicuous chambers. Uterus externus ending in a narrow neck. Oval pore-plates at the spermathecae base, with approximately 40–50 glandular ducts (Figs 7E–F).

**Distribution.** Recorded from caves and epigean areas in the Carajás region, state of Pará, northern Brazil (Fig. 20A).

#### Ochyrocera aragogue sp. n.

http://zoobank.org/E9FA58F0-3F9B-4468-AB05-BD26723FCBDB Figures 10, 11, 12, 19B

**Types.** Holotype male from Cave N4E\_0008 (50°9'36"W, 6°2'21"S), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 07–12/X/2008, R. Andrade et al. (IBSP 174962). Paratype female from Cave N4E\_0013 (6°2'18"S; 50°9'38"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Brazil, 20/X\_01/XI/2006, R. Andrade & I. Arnori et al. (IBSP 174983).

**Other examined material.** BRAZIL. Pará: **HYPOGEAN SAMPLES**: Curionópolis, Serra Leste, Cave SL\_no number (5°58'35"S; 49°37'55"W), 1 $\bigcirc$ , 17–24/X/2008, R. Andrade et al. (IBSP 188853); Parauapebas, Floresta Nacional de Carajás, Serra Norte, Cave N4E\_0008 (6°2'21"S; 50°9'36"W), 1 $\bigcirc$ , 07–12/X/2008, R. Andrade et al. (IBSP 174962); Cave N3\_0070 (6°2'39"S; 50°13'48"W), 1 $\bigcirc$ , 03–17/IV/2013 (IBSP 174076); Cave N3\_0078 (6°2'36"S; 50°13'43"W), 1 $\bigcirc$ , 03–17/IV/2013 (IBSP 174076); Cave N3\_0078 (6°2'36"S; 50°13'43"W), 1 $\bigcirc$ , 03–17/IV/2013 (IBSP 174077); Cave N1\_0125 (6°0'15"S; 50°17'15"W), 1 $\bigcirc$ , 07–28/I/2015 (IBSP 188850); Cave N1\_0170 (6°1'23"S; 50°17'58"W), 1 $\bigcirc$ , 03–17/XII/2014 (IBSP 188851); Cave N1\_0038 (6°1'49"S; 50°16'17"W), 1 $\bigcirc$ , 04/IX–06/X/2014 (IBSP 188852), all collected by Equipe Carste et al.

**Etymology.** The specific name refers to Aragog, a spider capable of communicating with humans and a lover of human flesh, from the literary classic "Harry Potter and the Chamber of Secrets", by J.K. Rowling.

**Diagnosis.** *Ochyrocera aragogue* resembles *O. laracna* by the yellowish green body color pattern (Fig. 10A–B) and by the short cymbial apophysis with two distal cuspules on projections (Fig. 10C–D, 11F), a unique character for both these Neotropical species. The male can be distinguished from *O. laracna* by the palp with a sinuous distal area of embolus without laminar spur (Figs 10C–D, 11A–B, D). The female has an enlarged distal area of spermathecae and pore plates at the spermathecae base (Fig. 10E–F).

**Description. Male** (holotype). Total length 2.3. Carapace length 0.7, ovoid; narrowing gradually anteriorly, with yellowish-green pattern, pars cephalica flat, fovea not visible (Fig. 10A). Clypeus length 0.7, curved foward. Eyes: PME elongated oval; ALE and PLE rounded. Chelicerae light yellow, promargin with seven teeth, attached to



**Figure 10.** *Ochyrocera aragogue* sp. n., male holotype (**A**, **C**, **D**), female paratype, IBSP 188850 (**B**, **E**, **F**) **A**, **B** habitus, dorsal view **C** left male palp, retrolateral view **D** same, prolateral view **E** genitalia, enzyme cleared, dorsal view **F** same, dorsal view. Abbreviations: NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 μm (**E**, **F**).

long lamina (Fig. 12A); retromargin without teeth. Sternum light yellow gray. Endites dark yellow suffused. Legs light yellow, formula 1423, total length I 7.0; II 5.9; III 4.1; IV 6.5. Male palp: palpal femur length 0.4; palpal tibia short, as long as cymbium; cymbial apophysis short, bearing two short distal cuspules at tip, in different heights, retrolateral long hair on non-projected base, next to the tarsal organ; with four basal setae (Fig. 11C, E–F); cymbial prolateral extension almost squared; embolus elongated, enlarged at base and subapically twisted (Figs 10C–D, 11A). Abdomen length 1.3, oval, uniformly green-purplish color (Fig. 9A). Six epiandrous spigots, with short base (Fig. 12B).

**Female** (paratype IBSP 174983). Total length 2.0; carapace length 0.74, pattern light yellowish (Fig. 10B). Pedipalp without claw, with conical tip and subdistal tarsal organ (Fig. 12E–F). Clypeus 0.68 diameter. Eyes, chelicerae, sternum, endites (Fig. 12D) and labium as in male. Legs as in male, formula 4123, total length I 6.3; II 4.7; III 3.6 IV 4.3. Abdomen length 0.96, globular (Fig. 10B). Colulus triangular, with approximately 10 bristles (Fig. 12C). Internal genitalia weakly sclerotized, spermathecae tubular, slender in basal area and curved and thickened at distal area. Uterus



**Figure 11.** SEM images of *Ochyrocera aragogue* sp. n., male IBSP 188851 (**A–F**) **A** left palp, retrolateral view **B** same, prolateral view **C** cymbium, basal setae **D** embolus, distal area **E** cymbium, tarsal organ **F** same, cuspules, long hair and tarsal organ, distal tip. Scale bars: 0.3 mm (**A–D**); 5 μm (**E**); 30 μm (**F**).



**Figure 12.** SEM images of *Ochyrocera aragogue* sp. n., male IBSP 188851 (**A–B**), female, IBSP 188850 (**C–F**) **A** chelicerae, frontal view, **B** epiandrous area, abdomen, ventral view **C** colulus, ventral view **D** chelicerae, frontal view **E** pedipalp, distal, prolateral view **F** same, tarsal organ. Scale bars: 0.3 mm (**A–D**); 20  $\mu$ m (**F**).

externus shorter than spermathecae, internally with no visible chambers, ending in truncated neck. Oval pore-plates at the base of spermathecae, with approximately 25–30 glandular ducts (Fig. 10E–F).

**Distribution.** Recorded exclusively from caves in the Carajás region, state of Pará, northern Brazil (Fig. 19B).

#### Ochyrocera misspider sp. n.

http://zoobank.org/A4A64C11-B4B6-4B3B-9E95-323239941030 Figures 13, 14, 15, 16, 19B, 21C

**Types.** Holotype male from Cave N4E\_0070 (6°1'56"S, 50°9'10"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 24–30/VII/2009, R. Andrade & I. Cizauskas et al. (IBSP 176910). Paratype: female from Cave N4E\_0070 (6°1'56"S, 50°9'10"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 19/II-04/III/2010, R. Andrade & I. Cizauskas et al. (IBSP 176870).

Other material examined. BRAZIL. Pará: HYPOGEAN SAMPLES: Canaã dos Carajás, Floresta Nacional de Carajás, Serra Sul, Cave CAV 0024 (6°24'20"S; 50°21′57″W), 1♀, 22–31/V/2010 (IBSP 175310), 2♀, 22–28/IX/2010 (IBSP 175314); Cave CAV\_0032 (6°25'35"S; 50°19'25"W), 1♀, 22–28/IX/2010 (IBSP 175315); Cave S11D-101 (6°23'22"S; 50°21'48"W), 1♀, 01–14/VII/2010 (IBSP 175311); Cave S11D-26 (6°24'49"S; 50°21'17"W), 1♀, 19–22/II/2010 (IBSP 175309); Cave S11D-31 (6°24'41"S; 50°20'43"W), 3♀, 03–19/VIII/2010 (IBSP 175312); Cave S11D-39 (6°23'46"S; 50°20'27"W), 1♀, 03–19/VIII/2010 (IBSP 175313); 5♀, 13–30/I/2010 (IBSP 175307, IBSP 175306, IBSP 175308); Cave S11D-89 (6°23'45"S; 50°19'20"W), 3♀, 13–30/I/2010 (IBSP 175305); Cave S11D 94 (6°23'40"S; 50°19'17"W), 1♀, 13– 30/I/2010 (IBSP 175304), all collected by R. Andrade & I. Cizauskas et al.; Parauapebas, Cave CRIS\_20 (6°25'35"S; 49°41'18"W), 6<sup>Q</sup>, 29/VII-06/VIII/2008, R. Andrade (IBSP 174597); Floresta Nacional de Carajás, Serra Norte, Cave GEM 1570, 39, 17-24/X/2008, R. Andrade (IBSP 174516); Cave N1\_0025 (6°1'54"S; 50°16'21"W), 1♀, 02–29/IV/2015 (IBSP 188874); Cave N1\_0039 (6°1'46"S; 50°16'13"W), 1<sup>♀</sup>, 02–29/IV/2015 (IBSP 188873); Cave N1\_0169 (6°1'23"S; 50°17'59"W), 1♀, 03–17/XII/2014 (IBSP 188870); Cave N1\_0176 (6°1'29"S; 50°18'2"W), 1♀, 02-23/IV/2015 (IBSP 188872); Cave N1 0180 (6°2'33"S; 50°16'25"W), 29, 28/ IX-03/X/2007 (IBSP 174731); Cave N1\_0221 (6°1'48"S; 50°18'2"W), 1♀, 04/IX--06/X/2014 (IBSP 188869); Cave N1-0106 (6°0'46"S; 50°18'22"W), 1♀, 07-28/ I/2015 (IBSP 188871); Cave N1-0226 (6°2'16"S; 50°16'2"W), 1♀, 02–29/IV/2015 (IBSP 188875); Cave N2 026 (6°3'16"S; 50°14'23"W), 3♀, 26/IX-17/X/2012 (IBSP 178500, IBSP 178498); Cave N3 0002 (6°1'43"S; 50°12'2"W), 1♀, 05–17/III/2013 (IBSP 178503); Cave N3\_0003 (6°1'44"S; 50°12'3"W), 1♀, 26/IX-17/X/2012 (IBSP 178480); Cave N3 0004 (6°1'45"S; 50°12'2"W), 1♀, 26/IX-17/X/2012 (IBSP 178483); Cave N3\_0006 (6°1'45"S; 50°12'3"W), 1♀, 26/IX-17/X/2012 (IBSP 178484); Cave N3\_0023 (6°2'35"S; 50°13'10"W), 1♀, 02-23/VIII/2013



**Figure 13.A–F** *Ochyrocera misspider* sp. n., male holotype (**A**, **C**, **D**), female paratype, IBSP 176870 (**B**, **E**, **F**) **A**, **B** habitus, dorsal view **C** left male palp, retrolateral view **D** same, prolateral view **E** genitalia, enzyme cleared, dorsal view **F** same, dorsal view. Abbreviations: NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500  $\mu$ m (**E**, **F**).

(IBSP 178538); Cave N3 0026 (6°2'39"S; 50°13'9"W),  $4^{\circ}$ , 26/IX-17/X/2012 (IBSP 178487, IBSP 178488, IBSP 178490), 1♀, 05–17/III/2013 (IBSP 178511); Cave N3 0028 (6°2'32"S; 50°13'5"W), 1♀, 05–17/III/2013 (IBSP 178513); Cave N3\_0033 (6°2'42"S; 50°13'12"W), 1Q, 26/IX-17/X/2012 (IBSP 178493); Cave N3\_0037 (6°2'45"S; 50°13'14"W), 1Q, 26/IX-17/X/2012 (IBSP 178496); Cave N3 0047 (6°2'27"S; 50°13'40"W), 5♀, 03–17/IV/2013 (IBSP 178526, IBSP 178528, IBSP 178530); 1♀, 02–23/VIII/2013 (IBSP 178542); Cave N3\_0054 (6°2'25"S; 50°13'42"W), 3♀, 02–23/VIII/2013 (IBSP 178543, IBSP 178544, IBSP 178545); Cave N3\_0074 (6°2'35"S; 50°13'49"W), 4♀, 05-17/III/2013 (IBSP 178520, IBSP 178522, IBSP 178523); 2♀, 02–23/VIII/2013 (IBSP 178547, IBSP 178550); Cave N3\_0076 (6°2'28"S; 50°13'36"W), 1∂1♀, 02–23/VIII/2013 (IBSP 178551); 2♀, 03-17/IV/2013 (IBSP 178535, IBSP 178536); Cave N5SM2 0081 (6°7'19"S; 50°7'44"W) 2♀, 2010–11 (ISLA 14622); Cave N5W-03 (64'53"S; 50°8'4"W) 2♀, 02– 23/VIII/2013 (IBSP 178556, IBSP 178561); Cave N8\_0002 (6°10'5"S; 50°9'35"W),  $4^{\circ}$ , 02–29/IV/2015 (IBSP 188876); Cave N8 0003 (6°10'6"S; 50°9'32"W), 2 $^{\circ}$ , 02–29/IV/2015 (IBSP 188877); Cave N8\_0018 (6°10'8"S; 50°9'28"W), 1♀, 02–29/ IV/2015 (IBSP 188878); Cave N8\_0019 (6°10'11"S; 50°9'27"W), 1♀, 02–29/IV/2015



**Figure 14.** SEM images of *Ochyrocera misspider* sp. n., male IBSP176911 (**A–F**) **A** palp, prolateral view **B** same, retrolateral view **C** same, cymbium, distal area, prolateral view **D** same, cymbium, retrolateral view **E** cymbium, spur with long hairs and tarsal organ **F** same, tarsal organ, detail. Scale bars: 0.3 mm (**A–D**); 20  $\mu$ m (**E**); 5  $\mu$ m (**F**).

(IBSP 188879); Cave N8\_0022 (6°10'6"S; 50°9'30"W), 1♀, 02–29/IV/2015 (IBSP 188895); Cave N8–0025 (6°10'29"S; 50°9'4"W), 1♀, 02–29/IV/2015 (IBSP 188880); all collected by Equipe Carste et al.; Cave N4E\_0002 (6°2'25"S; 50°9'39"W), 1♀, 20/



**Figure 15.** SEM images of *Ochyrocera misspider* sp. n., male palp, IBSP 176911(**A–D**) **A** cymbium, basal setae (**B–D**) embolus, distal tip **B** prolateral view **C** retrolateral view **D** ventral view Scale bars: 0.5 mm (**A, B**); 0.7 mm (**C, D**); 500 μm (**E, F**).

IV-04/V/2010 (IBSP 176940); Cave N4E 0003 (6°2'25"S; 50°9'38"W), 2♀, 20/IV--04/V/2010 (IBSP 176941, IBSP 176942); Cave N4E\_0007 (6°2'21"S; 50°9'36"W), 29, 20/IV-04/V/2010 (IBSP 176943, IBSP 176944); Cave N4E 0008 (6°2'21"S; 50°9'36"W), 10♀, 20/IV-04/V/2010 (IBSP 176948, IBSP 176949, IBSP 176950, IBSP 176951, IBSP 176946, IBSP 176947); Cave N4E 0010 (6°2'20"S; 50°9'38"W),  $1^{\circ}$ , 20/IV-04/V/2010 (IBSP 176952); Cave N4E 0011 (6°2'20"S; 50°9'38"W), 3♀, 20/IV-04/V/2010 (IBSP 176953); Cave N4E\_0015 (6°2'10"S; 50°9'35"W), 1<sup>Q</sup>, 20/IV-04/V/2010 (IBSP 176945); Cave N4E\_0022 (6°2'2"S; 50°10'4"W), 1<sup>Q</sup>, 20/IV-04/V/2010 (IBSP 176954); Cave N4E\_0033 (6°2'25"S; 50°9'36"W)  $1^{\circ}$ , 15–22/IX/2009 (IBSP 176927); Cave N4E 0043 (6°1'55"S; 50°9'50"W)  $1^{\circ}$ , 19/II-04/III/2010 (IBSP 176862); Cave N4E 0045 (6°2'25"S; 50°9'40"W), 1♀, 24–30/VII/2009 (IBSP 176928); Cave N4E\_0047 (6°2'15"S; 50°9'36"W), 13, 18/ VIII-03/IX/2009 (IBSP 176912); Cave N4E 0048 (6°2'15"S; 50°9'37"W), 1♀, 19/ II-04/III/2010 (IBSP 176931); Cave N4E\_0051 (6°2'22"S; 50°9'38"W), 1♀, 19/ II-04/III/2010 (IBSP 176932); Cave N4E\_0053 (6°2'3"S; 50°10'2"W), 1♀, 24–30/ VII/2009 (IBSP 176929); Cave N4E\_0055 (6°1'55"S; 50°9'59"W), 2♀, 19/II-04/ III/2010 (IBSP 176930); Cave N4E 0070 (6°1'56"S; 50°9'10"W), 1♀, 19/II-04/ III/2010 (IBSP 176870); Cave N4E 0070 (6°1'56"S; 50°9'10"W); 1∂1♀, 24–30/ VII/2009 (IBSP 176918, IBSP 176910); Cave N4E 0072 (6°1'56"S; 50°9'13"W), 1∂2♀, 24–30/VII/2009 (IBSP 176911, IBSP 176921); 1∂8♀, 19/II-04/III/2010 (IBSP 176871, IBSP 176873, IBSP 174074); Cave N4E 0074 (6°1'59"S; 50°9'21"W), 2<sup>Q</sup>, 19/II-04/III/2010 (IBSP 176933, IBSP 176934); Cave N4E 0079 (6°1'59"S; 50°9'5"W), 5<sup>Q</sup>, 19/II-04/III/2010 (IBSP 176936, IBSP 176935); Cave N4E\_0080 (6°1'58"S; 50°9'4"W), 3♀, 24–30/VII/2009 (IBSP 176920, IBSP 176919); 6♀, 19/ II-04/III/2010 (IBSP 174064); Cave N4E\_0085 (6°2'3"S; 50°9'26"W), 1♀, 19/II-04/III/2010 (IBSP 176937); Cave N4E 0092 (6°2'22"S; 50°9'31"W), 6Q, 24-30/ VII/2009 (IBSP 176924, IBSP 176926, IBSP 176923, IBSP 176925); 2♀, 19/II-04/ III/2010 (IBSP 176938, IBSP 176939); Cave N4E\_0093 (6°2'22"S; 50°9'31"W), 2♀, 24–30/VII/2009 (IBSP 176922); Cave N5S 04 (6°6'20"S; 50°8'2"W), 1♀, 14–23/ X/2009 (IBSP 177621); Cave N5S\_07 (6°6'20"S; 50°7'59"W), 7<sup>Q</sup>, 14–23/X/2009 (IBSP 177616, IBSP 177617, IBSP 177619, IBSP 177618, IBSP 177620); Cave N5S 12 (6°6'11"S; 50°7'31"W) 4♀, 14–23/X/2009 (IBSP 177622, IBSP 177623); Cave N5S 37 (6°6'22"S; 50°7'57"W), 1♀, 14/III-04/IV/2010 (IBSP 177624); Cave N5S 40 (6°6'19"S; 50°8'0"W), 2♀, 15–21/IX/2009 (IBSP 177615); Cave N5S 52/53 (6°6'28"S; 50°7'59"W), 2♀, 25/VIII-03/IX/2009 (IBSP 177613, IBSP 177614); Cave N5S\_55 (6°6′28″S; 50°7′57″W), 1♀, 25/VIII-03/IX/2009 (IBSP 177610); 1♀, 14/ III-04/IV/2010 (IBSP 177625); Cave N5S\_63/64/65 (6°6'12"S; 50°8'7"W), 1♀, 14/ III-04/IV/2010 (IBSP 177626); Cave N5S\_68 (6°6'3"S; 50°8'7"W), 12, 25/VIII-03/IX/2009 (IBSP 177608); Cave N5S 70 (6°6'5"S; 50°8'4"W), 32, 25/VIII-03/ IX/2009 (IBSP 177611, IBSP 177612, IBSP 177670) 2<sup>Q</sup>, 14/III-04/IV/2010 (IBSP 177627); Cave N5S\_74 (6°6'2"S; 50°8'5"W), 1<sup>o</sup>, 14/III-04/IV/2010 (IBSP 177628); Cave N5S-79 (6°6'8"S; 50°8'13"W), 2♀, 14/III-04/IV/2010 (IBSP 177629); Cave N5S\_85 (6°5'12"S; 50°7'35"W), 1♀, 25/VIII-03/IX/2009 (IBSP 177609); all collected by R. Andrade & I. Cizauskas et al.

**Etymology.** The specific name refers to Little Miss Spider, a very popular spider around the world and the main character of the children's books by David Kirk.

**Diagnosis.** *Ochyrocera misspider* is the smallest among the species from Floresta Nacional de Carajás and resembles *O. caeruleoamethystina* Lopez & Lopez and *O. thibaudi* Emerit & Lopez by the small projection in the cymbium (see Lopez and Lopez 1997, fig. 8; Emerit and Lopez 1985, fig. 1A). It can be distinguished by the male palp with an elongated tibia, twice as long as the cymbium, and by the bifid embolus (Figs 13C–D, 14A–B). Females are distinguished from other species of the genus by the genitalia with a very long and narrow medial columnar uterus externus, internally with approximately 12 chambers, and an elongated, erect and sinuous spermathecae (Fig. 13F–G).

**Description. Male** (holotype). Total length 1.8. Carapace length 0.6, ovoid; narrowing gradually anteriorly; with purplish pattern pars cephalica flat; fovea not visible (Fig. 13A). Clypeus length 0.7, curved forward. Eyes: PME elongated oval; ALE and PLE rounded. Chelicerae light yellow; promargin with seven teeth, attached to long lamina (Fig. 16A), retromargin without teeth. Sternum light yellow. Endites white yellow suffused. Legs: light purple, formula 1423, total length: I 7.0; II 5.9; III 4.1;



**Figure 16.** SEM images of *Ochyrocera misspider* sp. n. male IBSP 176911 (**A**, **C**), female, IBSP 178561 (**B**, **D–F**) **A** chelicerae, frontal view, **B** colulus, ventral view **C** epiandrous, abdomen, ventral view **D** chelicerae, frontal view **E** pedipalp, distal, prolateral view **F** same, tarsal organ. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 μm (**E**, **F**).

IV 6.5. Male palp: palpal femur length 0.4; palpal tibia twice as long as cymbium (Fig. 13C–D); cymbial apophysis long and slightly curved with elongated cuspule at tip, two retrolateral long hairs on projected base, one tarsal organ and three basal setae

(Figs 13C, 14B, E–F, 15A); cymbial prolateral extension rounded (Fig. 14A); bulb oval; embolus elongated, flattened at base and with distal area bifid, with short branch notched (Figs 14A–B, 15B–D). Abdomen length 1.3, oval; evenly purplish-green color (Fig. 13A). Six epiandrous spigots, with short base (Fig. 16C).

**Female** (paratype IBSP 176870). Total length 1.9; carapace length 0.74, as in male (Fig. 13B). Pedipalp without claw with conical tip, subdistal trichobothrium and tarsal organ (Figs 16E–F). Clypeus 0.68 diameter. Eyes, chelicerae, sternum, endites (Fig. 16D) and labium as in male. Legs as in male, formula 4123, total length I 6.3; II 4.7; III 3.6 IV4.3. Abdomen length 0.96, globular (Fig. 13B). Colulus triangular, with 9 bristles (Fig. 16B). Internal genitalia with elongated and sinuous spermathecae; long medial columnar uterus externus, longer than spermathecae, with visible chambers. Narrow neck in the columnar uterus externus. Small oval pore-plates on the spermathecae base, with approximately 8–10 glandular ducts (Fig. 13E–F).

**Distribution.** Recorded exclusively from caves in the Carajás region, state of Pará, northern Brazil (Fig. 19B).

#### Ochyrocera charlotte sp. n.

http://zoobank.org/7D7713DC-9B56-4B8F-994C-69299390BCA0 Figures 17, 20B

**Types.** Holotype male from Cave N8\_0038 (6°10'24"S, 50°8'49"W), 02–29/IV/2015 (IBSP 188897) and paratype female from Cave N1\_0247 (6°1'14"S, 50°16'22"W), 03–17/XII/2014 (IBSP 188896), both from Serra Norte, Floresta Nacional de Carajás Parauapebas, Pará, Brazil, Equipe Carste et al.

# Other material examined. None.

**Etymology.** The specific name refers to Charlotte, the spider from the classic "Charlotte's Web" by E.B. White and a great friend of the pig named Wilbur.

**Diagnosis.** Males and females of *Ochyrocera charlotte* sp. n. resemble those of *O. ungoliant* and *O. viridissima* Brignoli in having a subapical cuspule in the distal area of the cymbium (Fig. 17E–F; Brignoli 1974: fig. 6) but can be distinguished from these species by their yellowish cephalic area and cream body color pattern. Males can be distinguished by their pentagonal cymbium, with cylindrical tegulum (Fig. 17E–H). Females are diagnosed by the genitalia with a very narrow medial columnar uterus externus and an elongated and medially curved spermathecae (Fig. 17C–D).

**Description. Male** (holotype). Total length 2.3. Carapace length 0.7, ovoid; narrowing gradually anteriorly; with yellowish cephalic area, and cream pattern poteriorly, pars cephalica flat (Fig. 17A); fovea not visible. Clypeus length 0.7, curved forward. Eyes: PME elongated oval; ALE and PLE rounded. Chelicerae light yellow, promargin with eight teeth, attached to long lamina; retromargin without teeth. Sternum yellowish. Endites dark yellow. Legs: light yellow; formula 1423; total length: I 7.0; II 5.9; III 4.1; IV 6.5. Male palp: palpal femur length 0.4; palpal tibia narrow, same length as cymbium; cymbial apophysis not curved distally, retrolateral long hair on



**Figure 17.** *Ochyrocera charlotte* sp. n., male holotype (**A**, **E–H**), female paratype, IBSP 188896 (**B**, **C–F**) **A–B** habitus, dorsal view **C** genitalia, enzyme cleared, dorsal view **D** same, dorsal view **E** same, retrolateral view **F** same, prolateral view **G** left palp, prolateral view **H** same, retrolateral view. Abbreviations: NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 μm (**E**, **F**).

non-projected base, next to the tarsal organ; with a long hair; with three basal setae; cymbial prolateral extension squared; very coiled spermatic ducts, embolus elongated and flattened (Fig. 17E–H). Abdomen length 1.3, oval; uniformly cream with dorsal light brown band (Fig. 17A). Six epiandrous spigots, with short base.

**Female** (paratype IBSP 188896). Total length: 2.0; carapace length: 0.74; Carapace pattern as in male (Fig. 17B). Pedipalp without claw with conical tip and sub-

distal trichobothria. Clypeus: 0.68 diameter; Eyes, chelicerae, sternum, endites, and labium as in male. Legs as in male; leg formula 4123, total length: I 6.3; II 4.7; III 3.6 IV4.3. Abdomen length 0.96. Colulus triangular with 8–10 bristles. Internal genitalia with weakly sclerotized, long, curved spermathecae; long medial columnar uterus externus, shorter than spermathecae, with two distal chambers. Narrow neck in the columnar uterus externus. Small oval pore-plates far from the spermathecae base, with approximately 8–10 glandular ducts (Fig. 17C–D).

**Distribution.** Recorded exclusively from two caves in the Carajás region, state of Pará, northern Brazil (Fig. 20B).

#### Ochyrocera ungoliant sp. n.

http://zoobank.org/5FBC7B7B-B21D-4B3B-8921-C1A7659E4AA5 Figures 18, 20A

**Types.** Holotype male from Cave N4E\_0062 (6°2'1"S, 50°9'12"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 24–30/VII/2009, R. Andrade & I. Cizauskas et al. (IBSP 174063). Paratype: female from Cave N4E\_0046 (6°2'16"S, 50°9'36"W), Serra Norte, Floresta Nacional de Carajás, Parauapebas, Pará, Brazil, 18/VIII-03/IX/2009, R. Andrade & I. Cizauskas et al. (IBSP 174062).

**Other material examined.** BRAZIL. Pará: **HYPOGEAN SAMPLES**: Parauapebas, Floresta Nacional de Carajás, Serra Norte, Cave N4E\_0046 (6°2'16"S; 50°9'36"W), 1 $\bigcirc$ , 18/VIII-03/IX/2009 (IBSP 188868); Canaã dos Carajás, Floresta Nacional de Carajás, Serra Sul, Cave S11D-0064 (6°23'31"S, 50°18'48"W), 1 $\bigcirc$ , 13–30/I/2010, R. Andrade & I. Cizauskas et al. (IBSP 175488); Níquel Vermelho, Cave NV 07 (6°28'41"S, 49°54'20"W), 1 $\bigcirc$ , 22–28/II/2005, R. Andrade & I. Armoni (IBSP 55367).

**Etymology.** The specific name in apposition refers to Ungoliant, an evil spider spirit created by J. R. R. Tolkien in the book "The Silmarillion".

**Diagnosis.** Males and females of *Ochyrocera ungoliant* resemble those of *O. charlotte* and *O. viridissima* Brignoli in having a subapical cuspule in the distal area of the cymbium (Fig. 18G–H; Brignoli 1974: fig. 6), but can be distinguished from these species by their intense dark green color pattern and carapace with two longitudinal yellowish-green dorsal bands (Fig. 18A–B). Males can be diagnosed by their short cymbial apophysis with a very narrow tip and embolus with lamellar area in the distal third (Fig. 18E–H); and females by their genitalia with very short medial columnar uterus externus and spermathecae with broad and furrow apex (Fig. 18C–D).

**Description.** Male (holotype). Total length 2.3. Carapace length 0.7, ovoid; narrowing gradually anteriorly; pars cephalica flat; fovea not visible (Fig. 18A). Clypeus length 0.7, curved forward. Eyes: PME elongated oval; ALE and PLE rounded. Chelicerae green; promargin with eight teeth, attached to long lamina; retromargin without teeth. Sternum light green. Endites dark green suffused. Legs: light green; formula 1423; total length: I 7.0; II 5.9; III 4.1; IV 6.5. Male palp: palpal femur length 0.4; palpal tibia not enlarged basally; cymbial apophysis same length as tibiae, with subdistal cuspule at



**Figure 18.** *Ochyrocera ungoliant* sp. n., male holotype (**A**, **E**–**H**), female paratype, IBSP 174062 (**B**–**D**) **A–B** habitus, dorsal view **C** genitalia, enzyme cleared, dorsal view **D** same, dorsal view **E** same, prolateral view **F** same, retrolateral view **G** left palp, prolateral view **H** same, retrolateral view. Abbreviations: NUE = neck of uterus externus, PP = pore-plate, SP = spermathecae. Scale bars: 0.5 mm (**A**, **B**); 0.7 mm (**C**, **D**); 500 μm (**E**, **F**).

tip, retrolateral long hair on projected base, next to the short tarsal organ; with three basal setae, and cymbial prolateral extension squared; bulb oval; embolus elongated, wide at base and curved forward, with sinuous tip (Fig. 18E–H). Abdomen length 1.3, oval; uniformly purplish green color (Fig. 18A). Six epiandrous spigots, with short base.

**Female** (paratype IBSP 174062). Total length 2.0. Carapace length 0.74. Carapace as in male (Fig. 18B). Pedipalp without claw with conical tip and subdistal trichobothria. Clypeus 0.68 diameter. Eyes, chelicerae, sternum, endites, and labium as in male. Legs as in male, formula 4123, total length I 6.3; II 4.7; III 3.6 IV 4.3. Abdomen length 0.96, with pattern as in male (Fig. 18B). Colulus triangular, with 8–10 bristles.



Figure 19. A Distribution map of *Ochyrocera varys* sp. n., yellow star **B** *Ochyrocera misspider*, yellow diamond, and *O. aragogue* sp. n., white triangle.



Figure 20. A Distribution map of *Ochyrocera laracna* sp. n., yellow square and *O. ungoliant*, white star B *Ochyrocera atlachnacha*, white diamond and *O. charlotte* sp. n., yellow circle.

Internal genitalia with weakly sclerotized; short columnar uterus externus, with inconspicuous chambers. Uterus externus ending in a rounded neck in the columnar uterus externus. Small oval pore-plates far from the spermathecae base, with approximately 10–12 glandular ducts (Fig. 18C–D).

**Distribution.** Recorded exclusively from three caves in the Carajás region, state of Pará, northern Brazil (Fig. 20A).

## Discussion

*Relationships.* The inclusion and maintenance of these species in the genus *Ochyrocera* is mainly justified by the male palps following the standard of the type-species, *Ochyrocera arietina* (see Simon 1891: plate XLII, fig. 10): cymbium with a prolateral extension and a distinct distal apophysis, bearing an apical cuspule. Simon (1891, plate XLII, fig. 11) also describes *Ochyrocera quinquivittata* from the same region; however, males of this species have a conical palpal cymbium with a conspicuous retrolateral apophysis.

Since the proposition of the genus, as observed by Hormiga et al. (2007: 16), *Ochyrocera* can be separated into two different groups of species: those with an entire cymbium, with no retrolateral apophysis (proposed here as *arietina* group) and those with an apparently bifid retrolateral apophysis (*quinquivittata* group). Pérez-González et al. (2016) questioned the ambiguous relationship between *Ochyrocera* and *Fageicera* Dumitrescu & Georgescu, 1992, suggesting these genera could be synonymous. Species of *Fageicera* resemble those of the *quinquivittata* group due to the presence of a bifid cymbium, and this characteristic may be a putative synapomorphy for this group. In this case, all *Ochyrocera* species with this feature should be transferred to *Fageicera*. In addition to the three species described by Dumitrescu and Georgescu (1992), species such as *Ochyrocera cachote* Hormiga et al., 2007 and *O. otonga* Dupérré, 2015 could also be included in the genus *Fageicera*. Nevertheless, this can only be solved by an accurate examination of type-species of genera such as *Pandeus* Keyserling, 1891 and *Ceruleocera* Marples, 1955, currently synonyms of *Ochyrocera* (see comments in Pérez-González et al. 2016: 41), and by a cladistic analysis of all species under these generic names.

The aim of this work is not to propose a phylogeny of the genus, but to show that all species herein described have affinities with the *arietina* group. Among the newly described species, *Ochyrocera varys* sp. n., *O. atlachnacha* sp. n., and *O. misspider* seem to be related to the type species, since the male palpal cymbium have distinct distal apophysis, bearing a typical apical cuspule. This character state appears to be the most common among American species (see Dupérre 2015, Pérez-Gonzáles et al. 2016, Valdez-Mondragón 2017). In this scenario, *Ochyrocera misspider* sp. n. may be a sister group of *O. caeruleoamethystina* Lopez & Lopez, 1997, from French Guyana, and *O. thibaudi* Emerit & Lopez from Antilles, since they share the same type of retrolateral projection with long setae on the cymbium (see Lopez and Lopez 1997, fig. 8; Emerit and Lopez 1985, fig. 1A). *Ochyrocera charlotte* and *O. ungoliant* also have a distinct distal apophysis, such as *O. viridissima* Brignoli (see Brignoli 1974: fig.6); however, they have subapical cuspules, a unique characteristic of these three species among those described so far for the Neotropical region.

Ochyrocera laracna sp. n. and O. aragogue sp. n. form a distinct group of species among those with distal apophysis. In these species, the cymbium of the male palps bears two apical cuspules and this characteristic seems to be exclusive of these two species among the Neotropical Ochyrocera.

*Distribution and ecological notes.* In general, the Ochyroceratidae are poorly known in the Neotropical region. Data on the diversity and ecological features of the group are lacking, and most studies are carried out in restricted areas. The group's known diversity should be larger than it currently is. In this paper, we decribe seven new species of the genus *Ochyrocera* with different distributions and patterns collected in ferruginous caves in the region of Floresta Nacional de Carajás, in the state of Pará, Brazil.

The specimens were manually collected inside caves of Floresta Nacional de Carajás, with the aid of brushes and tweezers, and with pitfall and Vulcan traps placed inside and around cave entrances (Piló and Andrade 2007; Cizauskas and Giroti 2011; Bichuette et al. 2015). Collections have been carried out annually in the Carajás region since 2005, usually comprising thirty-day visits with random sampling, aiming at the production of faunistic and environmental reports for the Brazilian environmental protection agency (IBAMA 2017). In general, the cave specimens occupy ground areas near the base of the lateral walls of the caves, where they construct their small webs of refuge (Fig. 21A–F). Spiders collected outside the caves are usually in the shallow litter layer on the ground, building their webs among dead leaves as observed by Jocqué & Dippenaar-Schoeman (2006).

Floresta Nacional de Carajás is part of a conservation unit comprising a large forest island in the southwest of Pará. The area is currently surrounded by pastures, which replaced the original forest (Martins et al. 2012; Campos and Castilho 2012), and comprises 411,949 hectares, covering the municipalities of Parauapebas, Canaá dos Carajás and Água Azul do Norte. The climate type is Montano or Serrano Amazon, with average annual temperatures between 21–22°C. The predominant phytophysiognomy in Floresta Nacional de Carajás is the Equatorial Forest of Terra Firme, with natural clearings such as rupestrian fields or cangas (Ab'Saber 1986).

All species of *Ochyrocera* found in Floresta Nacional de Carajás can be classified as edaphic troglophiles, organisms that can complete their life cycle in the soil, shallow subterranean habitats or in caves (Fig. 21E–F; Sket 2008; Culver and Pipan 2009, Gavish-Regev et al. 2016). During the last five years, we have collected ca. 2000 adult specimens in caves (see examined material above). *Ochyrocera varys* sp. n. (35233, 875 99) and *O. atlachnacha* sp. n. (13133, 37199) were the most abundant in our collections. Only the species *O. varys* sp. n. and *O. laracna* sp. n. were observed both in caves and epigeal environments, with 933, 1699 and 4533, 2899 collected, respectively. Besides these species, only *O. misspider* sp. n. exceeded 180 specimens collected in caves (533, 18299). Other species were represented by ten specimen's maximum (see examined material).

Floresta Nacional de Carajás has two great ferruginous blooms, Serra Norte (Paruapebas) and Serra Sul (Canaã dos Carajás) (Beisiegel 2006). The species *Ochyrocera charlotte* sp. n. and *O. aragogue* sp. n. were found only in caves from Serra Norte, whereas



**Figure 21. A** *Ochyrocera varys* sp. n., predating a Diptera **B** *Ochyrocera atlachnacha* sp. n., on the web **C** *Ochyrocera misspider* sp. n., couple in the web **D** *Ochyrocera varys* sp. n., carrying the egg sac **E** Entrance of an iron cave **F** Canga vegetation on rocky outcrop.

*O. atlachnacha* sp. n., the second most abundant species, was exclusively found in Serra Sul caves. The other species were sampled in both cave areas (Figs 19–20). Another result of these collections was that five of these species were also found in caves located outside Floresta Nacional de Carajás, with the exception of *Ochyrocera charlotte* sp. n. and *O. atlachnacha* sp. n., which were restricted to the caves of the Floresta Nacional de Carajás (Fig. 20B). Among all species collected, we found no characteristic that infers isolation to the underground environment and these species can be classified as edaphic troglophiles, capable of completing its life cycle in soil, shallow subterranean habitats, or caves.

Most specimens of all the species herein described are located in caves in the Floresta Nacional de Carajás. This area has been an environmental impact target due to the mining process. The effect of this impact in local spider populations has not yet been fully evaluated, especially as large samplings are lacking in other areas, especially those outside caves.

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RESEARCH ARTICLE



# New eurymeline leafhoppers (Hemiptera, Cicadellidae, Eurymelinae) from Eocene Baltic amber with notes on other fossil Cicadellidae

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# Abstract

Two new extinct fossil cicadellid taxa from Eocene Baltic amber, representing the subfamily Eurymelinae (*sensu lato*), are described and illustrated, and their relationships to modern leafhoppers are discussed. *Eoidiocerus emarginatus* **gen.** and **sp. n.** is the oldest known representative of the tribe Idiocerini. The new genus resembles some modern Afrotropical and Indomalayan idiocerine genera but differs in having the gena relatively narrow. *Archipedionis obscurus* **gen.** and **sp. n.**, is the first well-preserved fossil representative of Macropsini to be described in detail. Previous reports of this tribe from Baltic amber, while credible, included too little morphological information to assess their relationships. Additional comparative notes are provided for previously described fossil taxa belonging to Idiocerini and Macropsini from the Oligocene of Germany. The new combinations *Oncopsis sepultus sepultus* (Statz, 1950), **comb. n.** are proposed for taxa previously included in *Bythoscopus* Germar. The previously unplaced cicadellid fossil taxon *Priscacutius denticulatus* Poinar & Brown, 2018 from mid-Cretaceous Myanmar amber is newly placed in subfamily Signoretiinae, tribe Phlogisini, and represents the oldest known member of this subfamily, the only one known from the fossil record and only the second modern cicadellid subfamily confirmed by direct fossil evidence to have been present during the Cretaceous period.

## Keywords

Auchenorrhyncha, Idiocerini, Macropsini, morphology, Phlogisini, Signoretiinae

## Introduction

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The fossil record of leafhoppers (Cicadellidae), a group of sap-sucking hemipteran insects comprising >20,000 described extant species worldwide, is poorly documented, with fewer than 100 confirmed fossil species having been formally described so far from the Cretaceous (Oman 1937, Shcherbakov 1986, Hamilton 1990, 1992, Poinar and Brown 2018), Eocene (reviewed by Szwedo 2002, 2005, Dietrich and Gonçalves 2014, Gröhn 2016) and Oligo-Miocene (Statz 1950, Dietrich and Vega 1995). Several additional fossils listed as belonging to "Cicadelloidea" by Metcalf and Wade (1966) have either been transferred to other families or require further confirmation.

Recent molecular phylogenetic studies of leafhoppers have attempted to estimate the ages of various cicadellid lineages (Krishnankutty 2012, Catanach 2013, Krishnankutty et al. 2016, Wang et al. 2016, Dietrich et al. 2017) but have been hindered by the paucity of well-preserved fossil leafhoppers available for calibrating the ages of nodes on phylogenies. Although molecular divergence time estimates consistently suggest that many modern cicadellid subfamilies originated during the Cretaceous, only one such subfamily, Ledrinae, has been reported previously from Cretaceous-age fossil material (Hamilton 1990, Shcherbakov 1992).

Six modern cicadellid subfamilies (Aphrodinae, Bathysmatophorinae, Eurymelinae, Megophthalminae, Mileewinae and Typhlocybinae) have their oldest representatives recorded from Eocene Baltic amber (Dietrich and Gonçalves 2014). A record from Baltic amber of subfamily Coelidiinae (based on "*Jassus*" *immersus* Germar and Berendt, 1856), considered to "very probably" represent this subfamily (Szwedo and Sontag 2009), requires further verification. Records of Deltocephalinae (as "Deltacephalinae cf. Paralinnini") and Neocoelidiinae (a nymph, as "Neocoelidinae") based on photographs by Gröhn (2016) also require further study and confirmation.

Judging from the numerous amber specimens offered for sale online over the past few years by dealers in Lithuania, Poland and elsewhere in Europe, most of the leafhoppers preserved in Baltic amber are nymphs, and many of these are difficult to place taxonomically and phylogenetically, given the still highly incomplete knowledge of the morphology of modern leafhopper nymphs (reviewed by Dmitriev 2002). One earlyinstar nymph was placed by Szwedo and Gebicki (2002) in its own subfamily, Nastlopiinae, but this placement cannot, at present, be thoroughly evaluated because the early (first and second) instar nymphs of most extant leafhopper subfamilies have never been described in the literature and very few specimens are preserved in collections.

The new fossil taxa described herein include the oldest known representative of the leafhopper tribe Idiocerini and the first report of the tribe from fossil amber. Idiocerini, at present, are distributed worldwide with 105 genera and ~700 known extant species. Previous records of fossil Idiocerini consist of three rock fossils from the Oligocene of Germany (Statz 1950, see comparative notes below). Also described below is a new fossil representative of Macropsini, a tribe previously recorded from Baltic amber (as "Macropsinae", Szwedo 2002) but based on specimens very incompletely described and illustrated by Germar and Berendt (1856).

# Material and methods

Fossil specimens were obtained from amber dealers in Palanga, Lithuania. Morphological characters were assessed by examination of the specimens using an Olympus SZX-12 dissecting microscope. Specimens were prepared by grinding flat facets in strategic locations to obtain a clear field of view for detailed photomicrographs according to Nascimbene and Silverstein (2000) and Bisulca et. al. (2012). Photomicrographs were taken using a Zeiss SteREO Discovery V20 zoom stereomicroscope with a Plan-Apochromat S 0.63x f/Reo WD=81 mm objective. Drawings were prepared either with a camera lucida or by tracing over photographs of the specimens. For bilaterally symmetrical parts of the head and thorax obscured by fractures and other flaws in the amber, drawings (Fig. 2A, B, F, G) were prepared by tracing one half (the fully visible side) with the camera lucida and reconstructing the other half using its mirror image. All specimens examined are deposited in the Paleontological Collection of the Illinois Natural History Survey, Champaign, Illinois, USA. Morphological terminology follows Dietrich (2005).

# Taxonomy

# Subfamily Eurymelinae Amyot & Serville, 1843

**Note.** The concept of Eurymelinae adopted here is narrower than that of Hamilton (1983) but broader than those of Oman et al. (1990) and Dietrich (2005). It includes Eurymelinae, Idiocerinae and Macropsinae, sensu Oman et al. (1990) and the latter two taxa are treated as tribes of Eurymelinae (following Hamilton 1983). A recent large-scale molecular phylogenetic analysis of Membracoidea (Dietrich et al. 2017) placed Idiocerini and Macropsini within a well-supported monophyletic group also including Eurymelinae, *sensu* Oman et al. (1990).

# Tribe Idiocerini Baker, 1915

*Eoidiocerus* gen. n. http://zoobank.org/690668B2-F892-4941-BD6E-BC715DE087CE

Type species. *Eoidiocerus emarginatus* sp. n.; by present designation and monotypy.

**Diagnosis.** This genus differs from other described genera of Idiocerini in having the following combination of traits: head with fine arcuate striations above ocelli, ocelli situated above mid-height of eye, gena strongly emarginate below eye; hind femur macrosetal formula 2+1; female abdominal sternite VII strongly emarginate, exposing base of ovipositor; length of ovipositor more than half that of entire abdomen.

**Description.** Head in dorsal view with crown slightly shorter medially than next to eyes; face slightly longer than width across eyes, texture shagreen, area of vertex above

ocelli with inconspicuous, fine arcuate parallel striations; ocelli approximately equidistant between eyes and midline, situated above mid-height of eyes; lateral frontal suture nearly straight, extended from antennal pit to ocellus; antennal ledge carinate but only weakly produced over antennal base; antenna shorter than head width, arista attenuate, with conspicuous preapical seta extended mesad; gena strongly concave and narrow below eye, partly exposing small proepisternum; lorum convex, extended nearly to lateral margin of face; anteclypeus broadened near apex; rostrum extended slightly past middle coxae, distal segment somewhat expanded toward apex. Pronotum shagreen, with indistinct transverse rugae. Forewing elongate, appendix broad, extended to wing apex, bordering first and second apical cells; vein R with three branches extended to wing margin; crossvein s absent; with two r-m and three m-cu crossveins (two closed subapical cells); vein CuA reaching submarginal vein near midlength of appendix; claval veins distinct. Front femur with AM1 strongly reduced; intercalary row with several long, fine setae; tibia cylindrical, with conspicuous setae only at apex. Middle femur and tibia without macrosetae. Hind femur macrosetal formula 2+1; tibia strongly flattened, distance between dorsal setal rows much less than distance between dorsal and ventral rows, row AD with fewer macrosetae than PD, row AV macrosetae distributed along distal 3/4 of tibia, row PV with alternating short and long tapered setae through most of length, tarsomere I with dorsoapical pair of macrosetae well developed, without plantar setae, pecten with 2 platellae. Female pygofer and ovipositor narrow and elongate, occupying 3/4 total length of abdomen; sternite VII with deep median parabolic emargination, exposing base of ovipositor.

**Etymology.** The genus name, a masculine noun, combines the Greek word *eos* (dawn) with *Idiocerus*, the name of the type genus of Idiocerini, referring to the status of the fossil as the oldest known representative of Idiocerini.

Notes. Placement of *Eoidiocerus* in Idiocerini is unequivocal and supported by the presence of several synapomorphic features diagnostic for that tribe, including: head broader than pronotum, crown short, ocelli on face distant from dorsal margin and well separated from eyes, lateral frontal sutures present and extended to ocelli; pronotum in dorsal view with anterior margin not extended anteriad of eyes; chaetotaxy of front and middle legs strongly reduced; forewing appendix broad and extended to wing apex. Eoid*iocerus* resembles several modern idiocerines in most external structural features. Its most distinctive diagnostic traits are the arcuate series of fine striations on the vertex above the ocelli, present in several modern genera (e.g., Idiocerus Lewis, 1834, Idioceroides Matsumura, 1912; see also Webb 1983b), the relatively long and narrow face (occurring also in some Paleotropical genera, e.g., Chunra Distant, 1908), the distinctly emarginate, relatively narrow gena (broad and not, or very weakly, emarginate in most modern idiocerines), and the greatly elongated female pygofer and ovipositor, which occurs also in some modern species of Idiocerus. The forewing venation of the only available specimen is poorly delimited but the visible parts suggest that the venational pattern in this genus is similar to that exhibited by most modern genera of the tribe, i.e., only two closed anteapical cells are present and the appendix borders only two apical cells rather than three as in some genera from South Asia and Madagascar (Viraktamath 2007, Krishnankutty and Dietrich 2011). In the structure and proportions of the head, pronotum and mesonotum, the new genus is perhaps most similar to *Cafixia* Webb, 1983b, a genus represented by a single species occurring in South Africa, but *Eoidiocerus* differs in having the gena distinctly emarginate below the eye and exposing the small, flaplike proepisternum. Modern idiocerine genera known to have the gena distinctly emarginate below the eyes are *Idioceroides* from East Asia, and *Tumocerus* Evans, 1941 and *Quilopsus* Webb, 1983a from western Australia. *Idioceroides* differs in having the ocelli relatively high and laterad on the face and the lateral frontal sutures reduced. *Tumocerus* and *Quilopsus* differ in numerous respects, including having the face much wider than long, the lateral frontal sutures nearly vertical in orientation, and the portion of the vertex dorsad of the ocelli relatively short and lacking arcuate striations (Webb 1983a: figs 449, 464). The emarginate gena of *Eoidiocerus* also approaches the condition found in Macropsini, but in that tribe the gena is even more strongly emarginate, the proepisternum is enlarged and the lateral frontal sutures are poorly delimited or absent and not extended to the ocelli.

Previously reported fossil Idiocerini include *Oligoidiocerus pronotumnalis* Statz, 1950, *Idiocerus goeckii* Statz, 1950 and an additional unnamed "*Idiocerus*?" species from the Oligocene of Germany (Statz 1950). *Oligoidiocerus* apparently lacks an appendix in the forewing (Statz 1950: fig. 17) and, therefore, probably does not belong to this tribe. Its forewing venation is consistent with that of tribe Macropsini but other characters that could confirm its placement in that tribe do not appear to be visible on the fossil (Statz 1950: fig. 58). *Idiocerus goeckii* has the forewing venation well preserved and resembling that of modern species of *Idiocerus* (Statz 1950: fig. 18), differing from *Eoidiocerus* in the apparent lack of vein R1 and crossvein m-cu2. According to the photograph provided by Statz (1950: fig. 59) the specimen he identified as "*Idiocerus* ? sp." is too poorly preserved to confirm its placement in Idiocerini.

#### Eoidiocerus emarginatus sp. n.

http://zoobank.org/FAFF0AC4-8C8F-441E-860E-9E8D5581ECE0 Figs 1A–B, 2A–E

**Description.** Measurements (mm): body length including wings 4.8; head width across eyes 1.4; height of face (crown apex to anteclypeus apex) 1.5; forewing length 3.8; forewing maximum width (across approximately midlength) 1.1 mm; front tibia length 0.7; hind tibia length 1.7; hind tarsus length 0.7; ovipositor length (portion exposed posterad of sternite VII) 1.3. Hind tibia rows PD, AD and AV with 10, 9 and 11 macrosetae, respectively. Other structural features as described for genus. Body apparently uniformly pale brown, without discernible markings or pattern.

Etymology. The species name refers to the emarginate gena.

**Material examined.** Holotype female, Eocene Baltic amber (37–44 Ma), purchased by the first author from an amber dealer in Palanga, Lithuania. Deposited in the Paleontological Collection of the Illinois Natural History Survey (INHSP 10320).

The exoskeleton of the holotype is well preserved and intact except the femorotibial joints and adjacent parts of the left legs have been sheared off, apparently during



**Figure 1. A–B**, *Eoidiocerus emarginatus* gen. and sp. n. holotype, dorsolateral and ventrolateral habitus **C–D** *Archipedionis obscurus* gen. and sp. n. holotype, dorsal and ventral habitus.

initial processing of the amber piece, and are missing; most of the tibia and the entire tarsus of the left middle leg are also missing. Variable preservation of different parts of the integument give the impression that the holotype specimen has a pattern of dark markings but these appear to be artifacts.

## Tribe Macropsini Evans, 1935

## Archipedionis gen. n.

http://zoobank.org/9B6D4F13-9484-4861-A8F1-CE80BED0516D

Type species. Archipedionis obscurus sp. n.; by present designation and monotypy.

**Diagnosis.** This genus differs from other Macropsini in having the following combination of traits: crown shorter medially than next to eye; face with epistomal suture visible; ocelli slightly mesad of antennal pits, coronal pits dorsolaterad of ocelli; lorum not fused to frontoclypeus or anteclypeus; rostrum extended beyond middle coxae. Pronotum angulately produced medially but extended only slightly anterad of eyes in dorsal view, irregularly rugose. Forewing outer anteapical cell open, veins without markings.

**Description.** Head in dorsal view with crown shorter medially than next to eyes; face relatively broad and short, texture minutely and more or less evenly punctate, ocel-



**Figure 2. A–E** *Eoidiocerus emarginatus*: **A** head, anteroventral view **B** head, pronotum and mesonotum, slight anterodorsal view **C** forewing **D** prothoracic femur and tibia, anterior view **E** hind femur, tibia and tarsus, anterior view **F–J** *Archipedionis obscurus* **F** head, pronotum and mesonotum, slight anterodorsal view **G** head, ventral view **H** forewing **I** visible part of hind wing **J** hind femur, tibia and tarsomere, anterior view.

li slightly closer to eyes than to midline; coronal pits present dorsolaterad of ocelli; epistomal suture visible; gena strongly concave and narrow below eye, exposing flaplike proepisternum; lorum convex, extended nearly to lateral margin of face, not fused to anteclypeus; anteclypeus parallel-sided with apex truncate; rostrum extended past middle coxae, slender. Pronotum shagreen, with irregular transverse rugae. Forewing elongate, appendix narrow, extended around wing apex; most of membrane opaquely sclerotized; veins somewhat obscure, without obvious markings; inner and middle anteapical cells closed, outer anteapical cell open (crossvein s absent); claval veins distinct. Visible portion of hind wing apex with two closed apical cells, anterior branch of R absent. Front femur and tibia without conspicuous setae. Middle femur and tibia without macrosetae. Hind femur macrosetal formula 2+1; tibia strongly flattened, distance between dorsal setal rows much less than distance between dorsal and ventral rows, row AD with 8 preapical macrosetae (PD not visible in fossil), row AV macrosetae extended most of length of tibia, row PV with numerous close-set slender setae subequal in length, tarsomere I with dorsoapical pair of macrosetae well developed, with two rows of plantar setae, pecten with 2 platellae. Female pygofer relatively short, occupying < half total length of abdomen; sternite VII angulately emarginate, covering base of ovipositor.

**Etymology.** The genus name, a masculine noun, combines the prefix *archi*- derived from the Greek *archaeos*, meaning old, with *Pedionis*, the name of a modern macropsine genus with similar forewing venation.

**Notes.** This genus has forewing venation resembling that of the modern genus *Pedionis* Hamilton, 1980, i.e., with the s crossvein delimiting an outer anteapical cell absent, but differs in having the structure of the head more plesiomorphic, resembling *Zelopsis* Evans, 1966. Specifically, the face has the epistomal suture visible and arcuate and the anteclypeus is well delimited laterally and basally by sutures. The pronotum is not strongly produced anteromedially, although it still extended slightly anterad of the eyes medially, and the transverse rugae are only slightly arched anterad medially. Unfortunately, because only one female specimen is known, it is not known whether the structure of the lower part of the face is sexually dimorphic in *Archipedionis*, as is usual among modern macropsines. The elongate rostrum of this genus is apparently unusual in the modern macropsine fauna and has been reported only in *Galboa* Distant, 1909 (Seychelles Islands) and *Paragalboa* Yang, Dietrich & Zhang, 2016 (Madagascar), but also occurs in some species of *Pedionis*.

Three previously described fossil species from Baltic amber have been included in Macropsini: *Bythoscopus homousius* Germar & Berendt, 1856, *B. punctatus* Bervoets, 1910, and *Pediopsis minuta* Bervoets, 1910 (Szwedo 2002). Unfortunately, the only known specimens of these species were apparently destroyed during World War II and the original descriptions and illustrations are not sufficiently detailed to facilitate placement or detailed comparison with the species described here. Nevertheless, information provided in the original descriptions appears to indicate that these previously described species are different from the one described here. According Germar and Berendt (1856), *B. homousius* has the outer anteapical cell of the forewing closed distally (open in *Archipedionis*). *Pediopsis minuta* is much smaller (3 mm vs. 4.5 mm) and has the anterior margins of the head and pronotum much more strongly angulate.

*B. punctatus* is similar in size and in the shape of the head and pronotum to *Archipedionis obscurus* but the ocelli are closer to the midline of the face and the frontal sutures are not delimited. Collectively, these three species and the new species described below are the oldest representatives of Macropsini known from the fossil record.

Statz (1950) reported another species of Macropsini, *Macropsis pectoralis* Statz, 1950, from the Oligocene of Germany. The photograph of the holotype provided by Statz (1950: fig. 57) indicates that this fossil is correctly placed in Macropsini based on overall size and the form of the head and pronotum (pronotum angulately extended anterad of eyes) but its forewing venation is only partly visible and other traits that would facilitate detailed comparison with modern taxa are not visible. *Bythoscopus sepultus* Statz, 1950 may also be confidently placed in Macropsini based on the hind wing venation (absence of vein R2+3, submarginal vein not extended along costal margin basad of R4+5). The shape of the head and pronotum (Statz 1950: fig. 7) are indistinguishable from those of the modern Holarctic macropsine genus *Oncopsis* Burmeister, 1838; therefore the new combinations *Oncopsis sepultus sepultus* (Statz), comb. n. and *Oncopsis sepultus austerus* (Statz), comb. n. are proposed here. Two additional fossils placed by Statz (1950) in *Bythoscopus* Germar, 1833 (an isogenotypic junior synonym of *Iassus* Fabricius, 1803), *B. lunatus* Statz, 1950 and *B. robustus* Statz, 1950, also appear to belong to Macropsini but their correct generic placements cannot be determined due to the poor condition of the fossils.

#### Archipedionis obscurus sp. n.

http://zoobank.org/EF78935C-60F2-4F05-83DC-615570BDA0E5 Figs 1C–D, 2F–J

**Description.** Length including forewing 4.6 mm. head width across eyes 1.6; pronotum width: 1.3; height of face (crown apex to anteclypeus apex, approximate) 1.0; forewing length 3.4; forewing maximum width (across approximately midlength) 1.2 mm; front tibia length 0.7; hind tibia length 1.7; hind tarsus length 0.7; ovipositor length (portion exposed posterad of sternite VII) 0.9. Hind tibia rows AD, AV and PV with 8, 8 and >17 macrosetae, respectively (PD not visible and PV only partly visible in holotype). Other structural features as described for genus. Dorsal coloration uniformly black except pale distal third of forewing (possibly an artifact of preservation), legs testaceous except for black macrosetal sockets on hind tibia. Female sternite VII only slightly longer than sternite VII, posterior margin shallowly obtusely emarginate.

Etymology. The species name, *obscurus*, refers to the dark overall coloration.

**Material examined.** Holotype female, Eocene Baltic amber (37–44 Ma), purchased by the first author from an amber dealer in Palanga, Lithuania. Deposited in the Paleontological Collection of the Illinois Natural History Survey (INHSP 10321).

The holotype is well preserved and intact with the right side of the body well visible in dorsal view but the left side largely obscured by a fracture in the amber extended along the midline. In ventral view, much of the head and parts of the legs are obscured by fractures and a milky veil also obscures parts of the legs and abdomen.

# Subfamily Signoretiinae Baker, 1915 Tribe Phlogisini Linnavuori, 1979

#### Priscacutius denticulatus Poinar & Brown, 2018, new placement

Notes. This recently described fossil taxon from mid-Cretaceous Myanmar (Burmese) amber (~99 Ma) was originally considered unplaced to subfamily (Poinar and Brown 2018). The holotype fossil was not re-examined but, based on the original photos and description, this species may be confidently placed in the modern subfamily Signoretiinae (new placement) based on the enlarged, punctate pronotum that extends to the scutellar suture (Poinar and Brown 2018: fig. 4). Poinar and Brown (2018) labeled the posterior part of the pronotum as the mesonotum but we interpret the entire sclerite (labeled "P" and "M" in their fig. 4) as the pronotum. Priscacutius Poinar & Brown, 2018 runs to tribe Phlogisini in the key of Takiya et al. (2013) based on the position of the ocelli on the crown, distant from the anterior margin and the lack of distinct carinae on the crown and face. On this basis it is here included in Phlogisini although it exhibits several unique features. The tuberculate sensillum adjacent to the antenna, the relatively broad forewing with truncate apex, and the enlarged preapical teeth and elongate apical spines of the first hind tarsomere distinguish *Priscacutius* from previously known genera of Signoretiinae (Takiya et al. 2013, Viraktamath and Dietrich 2017).

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RESEARCH ARTICLE



# The first fossil brown lacewing from the Miocene of the Tibetan Plateau (Neuroptera, Hemerobiidae)

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# Abstract

A new species of Hemerobiidae, *Wesmaelius makarkini* Yang, Pang & Ren, **sp. n.** is described from the Lower Miocene, Garang Formation of Zeku County, Qinghai Province (northeastern Tibetan Plateau), China. The species is assigned to the widely distributed extant genus *Wesmaelius* Krüger (Hemerobiinae). The species represents the first named fossil of this family from China, which sheds light on the historical distribution of *Wesmaelius* and early divergences within Hemerobiinae.

# Keywords

Cenozoic, China, Wesmaelius

# Introduction

Hemerobiidae, commonly known as brown lacewings, are the third largest family of Neuroptera, with about 520 species assigned to 27 genera (Oswald 2017). Hemerobiids are the most widely distributed lacewings, from subpolar tundra to tropical regions

(Oswald 1993, 2017; Monserrat 2000; Makarkin et al. 2016). The extant brown lacewings have been comprehensively studied by Oswald (1993) including a taxonomic revision, a genus-level phylogeny based on morphology, and the establishment of a subfamilial classification. Recently, Garzón-Orduña et al. (2016) provided a total evidence phylogeny of the family based on combined data of morphological characters and DNA. As a result, seven known subfamilies were recovered to be monophyletic, with the addition of a new subfamily and the revision of Notiobiellinae (Garzón-Orduña et al. 2016).

Compared with its putative sister group Chrysopidae, Hemerobiidae have a relatively sparse and recent fossil record (Haring and Aspöck 2004; Winterton et al. 2010; Wang et al. 2016), although in a recent study, Hemerobiidae are not sister to Chrysopidae, but to the clade including Mantispoidea, Chrysopoidea, and Myrmeleontoidea (Winterton et al. 2017). Only four species have been described from the Mesozoic, with the earliest from the Late Jurassic. All the other 19 species have been described from the Cenozoic, from the Eocene to the Miocene (Makarkin et al. 2003, 2016; Engel and Grimaldi 2007) (Table 1). The Mesozoic hemerobiids comprise four monotypic extinct genera, one from the Late Jurassic of Kazakhstan, two from the Early Cretaceous of Mongolia and England, and one from the Late Cretaceous of Canada (Panfilov 1980; Ponomarenko 1992; Jepson et al. 2012; Klimaszewski and Kevan 1986; Makarkin et al. 2003, 2016). In the Cenozoic, 10 genera have been described from Russia, Baltic amber, Denmark, England, Canada, USA, and Dominican amber (Pictet-Baraban and Hagen 1856; Scudder 1878, 1890; Henriksen 1922; Krüger 1923; Jarzembowski 1980; Makarkin 1991; Oswald 2000; Makarkin et al. 2003; Makarkin and Wedmann 2009; Jepson et al. 2010), as well as China in this paper.

Fossil hemerobiids have never been described from China. *Mesohemerobius jeholen*sis Ping, from the Lower Cretaceous, Yixian Formation of China, was previously placed in Hemerobiidae, but was later excluded from the family by Makarkin et al. (2003) and referred as Neuroptera *incertae sedis*. Wang et al. (2014) mentioned hemerobiids in Fushun amber, but with no descriptions or figures. Herein, we describe a new species of *Wesmaelius* Krüger (Hemerobiidae: Hemerobiinae) from the Lower Miocene of the northeastern Tibetan Plateau in China. The species is the first named fossil of the family Hemerobiidae in China.

#### Materials and methods

The specimen was collected from the Guide Group at Caergen Village, Duohemao Town, Zeku County, eastern Qinghai Province, China (34°56'N, 101°48'E, 3700 m a.s.l.) (fig. 1 in Li et al. 2017). The stratum is a papery oil shale deposit and constitutes a lacustrine–fluvial sedimentary succession (fig. 1 in Li et al. 2016), belonging to the Garang Formation (<16–19 Ma), the Lower Miocene. The deposit yielded abundant, exquisitely preserved fossil plants (Guo 1980; Fang et al. 2005, 2007), bird feathers (Yang 1975), and insects including representatives of Hemiptera (Li et al. 2017), Diptera, Hymenoptera, Neuroptera, Mecoptera, Odonata and Coleoptera (pers. obs.).

Taxon	Deposit	Reference
Drepanepteryx oedobia Makarkin	Miocene, Russia	Makarkin 1991
Drepanepteryx ramosa Makarkin	Miocene, Russia	Makarkin 1991
Hemerobius incertus Makarkin	Miocene, Russia	Makarkin 1991
Hemerobius prohumulinus Makarkin	Miocene, Russia	Makarkin 1991
Megalomus caucasicus Makarkin	Miocene, Russia	Makarkin 1991
Megalomus sikhotensis Makarkin	Miocene, Russia	Makarkin 1991
Notiobiella thaumasta Oswald	Miocene, Dominican amber	Oswald 1999
Wesmaelius makarkini sp. n.	Miocene, China	This paper
Bothromicromus lachlani Scudder	Oligocene, Canada	Scudder 1878
Drepanepteryx resinata (Krüger, 1923)	Eocene, Baltic amber	Krüger 1923; Makarkin et al. 2016
Megalomus tinctus (Jarzembowski)	Eocene, England	Jarzembowski 1980; Makarkin 1991
Prolachlanius resinatus (Hagen)	Eocene, Baltic amber	Pictet-Baraban and Hagen 1856; Krüger 1923; Makarkin et al. 2012
<i>Proneuronema gradatum</i> Makarkin, Wedmann et Weiterschan	Eocene, Baltic amber	Makarkin et al. 2016
Proneuronema minor Makarkin, Wedmann et Weiterschan	Eocene, Baltic amber	Makarkin et al. 2016
Proneuronema wehri (Makarkin, Archibald et Oswald)	Eocene, USA	Makarkin et al. 2003; Makarkin et al. 2016
Prospadobius moestus (Hagen)	Eocene, Baltic amber	Pictet-Baraban and Hagen 1856; Krüger 1923
<i>Sympherobius completus</i> Makarkin and Wedmann	Eocene, Baltic amber	Makarkin and Wedmann 2009
Sympherobius siriae Jepson, Penney et Green 2010	Eocene, Baltic amber	Jepson et al. 2010
<i>Wesmaelius mathewesi</i> Makarkin, Archibald et Oswald 2003	Eocene, Canada	Makarkin et al. 2003
Megalomus densistriatus Henriksen	Eocene, Denmark	Henriksen 1922
<i>Plesiorobius canadensis</i> (Klimaszewski et Kevan, 1986)	Late Cretaceous, Canadian amber	Klimaszewski and Kevan 1986; Makarkin et al. 2016
Cretomerobius disjunctus Ponomarenko	Early Cretaceous, Mongolia	Ponomarenko 1992
<i>Purbemerobius medialis</i> Jepson, Makarkin et Coram	Early Cretaceous, England	Jepson et al. 2012
Promenalomus anomalus Panfilov	Late Jurassic Kazakhstan	Panfilov 1980

Table 1. List of Named Fossil Hemerobiidae (updated from Engel and Grimaldi 2007).

The specimen is housed in the collection of the Key Laboratory of Insect Evolution & Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China (CNUB; Dong Ren, Curator).

The specimen was examined using a Zeiss Discovery V20 stereomicroscope and photographed with an AxioCam HRc digital camera attached to the Zeiss Discovery V20 stereomicroscope (both instruments Carl Zeiss Light Microscopy, Göttingen, Germany). Line drawings were prepared with the CorelDraw 12 graphics software and with the aid of Adobe Photoshop CS6. The vein terminology in general follows Yang et al. (2012, 2014). Terminology of wing spaces and details of venation (e.g., veinlets, traces, 'oblique radial branches' ("ORB") concept) follows Oswald (1993).

AA1-AA3	first to third anterior anal	MP	posterior branche of media;	
	vein;	ORB1,	ORB2, ORB3 first to third	
CuA	anterior cubitus;		oblique radial branches;	
CuP	posterior cubitus;	RA	anterior radius;	
hv	humeral veinlet;	RP	posterior sector;	
fl	flexion fold line;	ScA	subcosta anterior;	
MA	anterior branche of media;	ScP	subcosta posterior.	

Venation abbreviations used in the text and figures:

# Systematic palaeontology

Class Insecta Linnaeus, 1758 Order Neuroptera Linnaeus, 1758 Family Hemerobiidae Latreille, 1802 Subfamily Hemerobiinae Latreille, 1802 Genus *Wesmaelius* Krüger, 1922

*Wesmaelius makarkini* Yang, Pang & Ren, sp. n. http://zoobank.org/4B084F07-9F9E-4EDF-B900-31B2133F1F2F Fig. 1

Holotype. CNU-NEU-QZ2017001 (holotype), a complete forewing (Fig. 1).

**Diagnosis.** Forewing with transparent spots on veins, and dark spots on the graduate crossveins, darker pigmentation along wing margin, subcostal veinlets, and longitudinal veins with dark intervals or dots. MA and MP pectinately forked, 2m-cu located at the fork of MA and M, the crossveins of the third gradate series more oblique.

Description. Holotype CNU-NEU-QZ2017001. Only forewing preserved.

Forewing oval, 8.31 mm long, 3.17 mm wide. Trichosors prominent, along the entire wing margin. Setae distinct, scarce on the veins and dense on the margin. Costal space relatively broad, dilated basally. Humeral veinlet recurrent, with two pectinate branches. Presumable ScA present. Majority of subcostal veinlets branched once, several basal veinlets branched twice, with no crossveins between them. Subcostal space moderately broad, with two prestigmal sc-r crossveins: basal 1sc-r and distal 3sc-r. Posterior trace of RA forked apically, with two distal branches. One RA branch forked once, the other twice. RP with three branches (ORBs) originated from RA; ORB1with two pectinate branches between 3r-m and 4r-m, all with distal forks; ORB2 dichotomously forked between the third and fourth gradate series of crossveins, each branch dichotomously forked; ORB3 forked between the second and third gradate series, with two dichotomously forked branches. M appear to be fused basally with R. M forked at 2m-cu; MA, MP configuration similar, parallel for a long distance, then each with two pectinate branches between the third and fourth gradate series. The second branch of MP dichotomously forked. Forewing with three m-cu crossveins. Crossvein 2r-m present and positioned distally to crossvein 2m-cu;





**Figure 1.** *Wesmaelius makarkini* sp. n., holotype CNU-NEU-QZ2017001. **A** photograph of forewing under alcohol **B** Line drawing of forewing. Scale bars: 2 mm.

2m-cu at the fork of MA and MP. Cu divided into CuA and CuP proximal of the first gradate series, close to wing base; CuA with four pectinate branches distal to 2cua-cup, all branches with marginal forks; CuP simple, only with marginal fork. AA1 with three pectinate branches, all with marginal forks. AA2 with two simple branches, forked proximal to aa1-aa2. AA3 simple. Three flexion fold (line) distinct between RP and MA, MP and CuA, CuP and AA1. The third gradate series with nine crossveins and the fourth gradate

with seven crossveins. Forewing with transparent spots on veins, and dark spots at the graduate crossveins; margined with darker pigmentation, and no other distinct maculation; wing margin, subcostal veinlets and longitudinal veins with dark intervals or dots.

**Etymology.** The specific epithet is in honor of the entomologist Dr. Vladimir Nikolaevich Makarkin to acknowledge his great help to the first author in his study of Neuropteran.

**Type locality and horizon.** Caergen Village, Zeku County, Qinghai Province, China; Garang Formation; The early Miocene.

**Remarks.** The species can be easily attributed to the genus *Wesmaelius* due to the following characters: two prestigmal sc-r crossveins, three RP branches (ORBs), crossvein 2r-m present and positioned distally to crossvein 2m-cu; intersection of crossvein 2m-cu with M not more than the crossvein's length distal to fork MA/MP (sometimes anterior to this fork), resulting in cell c2m-cu broad distally; forewing with three m-cu crossveins (Oswald, 1993).

In the genus, *Wesmaelius makarkini* sp. n. is most similar to the extant species of *W. nervosus* (Fabricius, 1793), *W. subnebulosus* (Stephens, 1836) and *W. reisseri* U. Aspöck & H. Aspöck, 1982. The new species with two ORB3 branches, 2m-cu located at the division of MA and MP; while *W. nervosus* with three ORB3 branches, 2m-cu distal to the division of MA and MP; and *W. makarkini* with a distinct large darker pigmentation at the apex of forewing. The new species differs from *W. subnebulosus* and *W. reisseri* in the pectinately forked MA and RP1, 2m-cua located at the division of MA and MP in *W. subnebulosus* and *W. reisseri*. Moreover, the new species has seven crossveins in the fourth series, while *W. reisseri* has four crossveins.

# Discussion

Named fossil Hemerobiidae have been described from the Late Jurassic to the Miocene, including four extinct genera from the Mesozoic and 11 genera from the Cenozoic (Table 1). Among the Cenozoic genera, six of them are extant genera, belonging to five subfamilies (Drepanepteryginae, Sympherobiinae, Megalominae, Hemerobiinae, Notiobiellinae), which are distributed in the three main clades of Hemerobiidae according to Garzón-Orduña et al. (2016). The earliest fossil record of each of the five subfamilies are from the Eocene of Europe and North America, indicating these extant subfamilies have been well differentiated and widely distributed across the Northern hemisphere by the Eocene.

*Wesmaelius* is an extant genus with approximately 62 extant species and two fossil species from the Eocene of Canada (Makarkin et al. 2003) and the Miocene of China (as afore-described). The extant species are widely distributed in the Palearctic, Nearctic, Afrotropic, and Indomalaya, with the majority of species widely distributed across the northern hemisphere; only four species are found in the southern hemisphere (Makarkin et al. 2003; Oswald 2017). Nearly all of them are restricted from the tropical to the temperate zone, and most of them restricted to higher elevation montane region.

The genus apparently is distributed from the north to the south, but the center of origin of *Wesmaelius* is questionable, mainly because the generic assignment of the oldest species (i.e., *W. mathewesi*) from the Eocene of Canada is uncertain (Makarkin et al. 2003). Nevertheless, *W. mathewesi* shows high affinity to the genera of *Wesmaelius* and *Hemerobius*, both of which belong to the subfamily Hemerobiinae. Therefore, it represents one of the earliest fossil records of the subfamily to date. *Hemerobius* also has fossil records extending back to the Miocene (Makarkin 1991). The geological history of the Hemerobiinae is still uncertain, due to the undetermined subfamilial assignment of the extinct genera, which requires further study.

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