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(Zoraptera, Spiralizoridae)

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COUVERTURE / *COVER*:

Collecting of *Zoraptera* in Montagne des Singes (above Marek Jankásek, below Petr Kočárek).

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# A new cryptic species of *Brazilozoros* Kukalova-Peck & Peck, 1993 from French Guiana (Zoraptera, Spiralizoridae)

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## KEY WORDS

Polyneoptera,  
Zoraptera,  
Spiralizorinae,  
Neotropical region,  
molecular phylogeny,  
new species.

## ABSTRACT

The genus *Brazilozoros* Kukalova-Peck & Peck, 1993 (Zoraptera, Spiralizoridae) currently includes three species distributed in the Neotropical region. Here we describe a new species, *B. kukalovae* n. sp., from French Guiana. It is related to *B. weidneri* (New, 1978) but differs by the morphology of the male genitalia and the sensilla basiconica on 10th abdominal tergite. We also present a molecular phylogenetic reconstruction that confirms the position of the new species in *Brazilozoros* and the inclusion of *Brazilozoros* in the Spiralizoridae Kočárek, Horká & Kundera, 2020. The study provides further evidence for the high level of cryptic diversity in the Zoraptera.

## RÉSUMÉ

*Une nouvelle espèce cryptique de Brazilozoros Kukalova-Peck & Peck, 1993 de Guyane (Zoraptera, Spiralizoridae).*

Le genre *Brazilozoros* Kukalova-Peck & Peck, 1993 (Zoraptera, Spiralizoridae) comprend actuellement trois espèces originaires de la région néotropicale. Nous décrivons ici une nouvelle espèce : *B. kukalovae* n. sp. de Guyane. Elle est apparentée à *B. weidneri* (New, 1978), mais s'en différencie par la morphologie des genitalia mâles et des sensilla basiconica sur le 10<sup>e</sup> tergite abdominal. Nous présentons également une reconstruction phylogénétique moléculaire corroborant la position de la nouvelle espèce dans *Brazilozoros* et l'inclusion de *Brazilozoros* dans les Spiralizoridae Kočárek, Horká & Kundera, 2020. L'étude fournit une preuve supplémentaire du degré élevé de diversité cryptique chez les Zoraptera.

MOTS CLÉS  
Polyneoptera,  
Zoraptera,  
Spiralizorinae,  
région néotropicale,  
phylogénie moléculaire,  
espèce nouvelle.

## INTRODUCTION

Although several fundamental studies dealing with the evolution and phylogeny of Zoraptera Silvestri, 1913 have been recently published (e.g. Kočárek *et al.* 2020; Matsumura *et al.* 2020), our knowledge of this insect order is still poor, and we are only beginning to recognize its true diversity. All current studies suggest that the order Zoraptera is much more diverse than previously thought (Kočárek *et al.* 2020; Matsumura *et al.* 2020; Kočárek & Horká 2023), and detailed taxonomic studies will likely increase the number of described extant species (Kočárek *et al.* 2020; Kočárek & Horká 2022, 2023).

Zoraptera are relatively common in tropical and subtropical areas of all biogeographical regions, but due to their small size and cryptic lifestyle, they are often overlooked by researchers, and their diversity thus remains inadequately explored (Mashimo *et al.* 2013, 2014 and others). A high level of the zorapteran cryptic diversity is indicated by a recent study of *Latinozoros* Kukulova-Peck & Peck, 1993 (Zoraptera, Spiralizoridae Kočárek, Horká & Kunderata, 2020) (Kočárek & Horká 2023), where three morphospecies previously considered to belong to a single species were identified based on morphological and molecular data.

Zorapteran uniformity in general morphology has led to the persistence of a conservative classification of extant Zoraptera, with only a single nominotypical genus in a single family for >100 years (Mashimo *et al.* 2014). Kočárek *et al.* (2020) and Matsumura *et al.* (2020) conducted molecular phylogenetic studies using a combination of nuclear and mitochondrial markers. Both of these independent analyses revealed two major phylogenetic lineages, which Kočárek *et al.* (2020) classified as families (Zorotypidae Silvestri, 1913 and Spiralizoridae), with each family divided into two robustly supported subclades, i.e., subfamilies (Kočárek *et al.* 2020). The recognition of two families and four subfamilies was supported by synapomorphies in the structure and shape of the male genitalia and in other taxonomically valuable characters including the number of spurs on the metatibia and the relative lengths of the first three antennomeres.

The genus *Brazilozoros* Kukulova-Peck & Peck, 1993 was recently reinstated as a valid genus by Kočárek *et al.* (2020) and comprises three species: *B. huxleyi* (Bolívar y Pieltain & Coronado, 1963), *B. weidneri* (New, 1978), and *B. brasiliensis* (Silvestri, 1946), all of which are distributed in the Neotropical region. A synapomorphy documented in *B. huxleyi* and *B. weidneri* is the enlarged basal plate of the male genitalia, which lacks an elongated intromittent organ that is typical for other Spiralizoridae species (Kočárek *et al.* 2020). *Brazilozoros brasiliensis* was described based on the morphology of adult females and has been assigned to the genus based on a molecular comparison (Matsumura *et al.* 2020). The classification of this genus also supports the typical arrangement of sensilla basiconica of 10th tergite, which was partly documented based on examination of a gynandromorph (Rafael *et al.* 2017). It seems that this species is mainly parthenogenetic (Silvestri 1946; New 1978; Rafael *et al.* 2008), although the finding of a gynandromorph suggests that at

least some populations contain both females and males (Rafael *et al.* 2017).

Here, we describe a new species of *Brazilozoros* Kukulova-Peck & Peck, 1993 from French Guiana.

## MATERIAL AND METHODS

### SAMPLING AND MORPHOLOGICAL STUDY

An aspirator was used to collect zorapteran specimens from under the bark of different tree species; the specimens were stored in 96% ethanol. For observation of morphological and anatomical structures, specimens were placed in 10% KOH at room temperature for 0.5 h and then washed with distilled water and returned to 96% ethanol for storage. Type specimens were slide-mounted in Euparal (BioQuip Products, Rancho Dominguez, California) or stored in 96% ethanol. The zorapteran specimens were studied and photographed with a Leica Z16 APO macro-scope equipped with a Canon 6D Mark II camera; slide-mounted body parts and genitalia were observed and documented with an Olympus CX41 microscope equipped with a Canon D1000 camera. Micrographs of 20 to 30 focal layers of the same specimen were combined with Helicon Focus software and finally processed with Adobe Photoshop CS6 Extended v13.

We compared the material of the new species described below with male and female apterous paratypes of *B. weidneri* (New, 1978) deposited in BMNH and labelled 'Brazil, Manaus, Reserva Duche, INPA, 12.XII.1975, W. D. Hamilton'.

Classification and nomenclature follow Kočárek *et al.* (2020); the nomenclatural terminology of wing venation follows Kukulova-Peck & Peck (1993).

### DNA ANALYSIS

Genomic DNA was extracted from tissues using the Qiamp DNA Micro Kit (Qiagen, Inc.) and following the manufacturer's protocols. Partial sequences of 18S and 16S rRNA were amplified and sequenced. The primers and PCR conditions follow Kočárek & Horká (2023). The amplified DNA was purified using a Gel/PCR DNA Fragments Extraction Kit (GENAID, Taiwan). Sanger sequencing reactions were performed using an ABI3730XL DNA Sequencer by Macrogen (Amsterdam, The Netherlands).

The chromatograms were visually checked and manually edited where appropriate using ChromasPro v2.1.9 (Technelysium, Brisbane, Australia).

### PHYLOGENETIC ANALYSES

Sequences of 16S, 18S, and histone 3 (H3) were aligned in MEGAX (Kumar *et al.* 2018). The protein coding sequences (H3) were translated into amino acids to check for potential stop codons within the open reading frames. Substitution saturation was tested in DAMBE v6.4 (Xia 2017) using the index proposed by Xia *et al.* (2003). Gblocks v0.91b was used to detect and eliminate poorly aligned and highly divergent regions in 16S and 18S rRNA alignments (Talavera & Castresana 2007). Genetic divergences between sequences were detected using the Kimura 2-parameter model within MEGAX software.

The multigene dataset was concatenated by SequenceMatrix v1.8 (Vaidya *et al.* 2011). Maximum likelihood (ML) and Bayesian inference (BI) analyses were used to estimate phylogenetic relationships, and both analyses were conducted with the on-line CIPRES Science Gateway v3.3 (Miller *et al.* 2011). BI analysis was performed based on gene partitions using molecular evolution models (GTR+G+I for 16S, and GRT+G for 18S and H3) selected with PartitionFinder v2.1.1. (Lanfear *et al.* 2016).

The ML analysis using the GTR+G nucleotide model was conducted in RAxML-HPC BlackBox v8.2.12 (Stamatakis 2014). The obtained trees were rooted using outgroup taxa from other polyneopteran orders and were displayed using iTOL (interactive Tree Of Life) v6.5.6 (Letunic & Bork 2021).

Bayesian analysis was conducted with MrBayes v3.2.7a in XSEDE (Ronquist *et al.* 2012) using a Markov chain Monte Carlo (MCMC) method. Two independent MCMC runs of four chains were run for  $20 \times 10^6$  generations until the standard deviation of the split frequencies reached 0.001. Trees were sampled every 100 generations, and 25% of the trees were discarded as burn-in. The convergence of BI analysis was confirmed in Tracer v1.6 (Rambaut *et al.* 2014).

Sequences of specimens used in the phylogenetic analysis published in GenBank under the name *B. huxleyi* and *B. weidneri* without description or illustration of critical diagnostic characters (which would allow correct assignment of these species) are presented in quotation marks. Details of analysed taxa including isolation numbers and GenBank accession numbers are indicated in Table 1.

#### ABBREVIATIONS

##### *Type depositories*

BMNH	The Natural History Museum, London;
MNHN	Muséum national d'Histoire naturelle, Paris;
NMPC	National Museum, Prague, Czech Republic.

##### *Morphological terms used in the text and/or figures*

Cu	cubitus vein;
CuA	anterior cubitus vein;
M	media vein;
R	radius vein;
S	abdominal sternite;
T	abdominal tergite.

#### RESULTS

##### Order ZORAPTERA Silvestri, 1913

##### Family SPIRALIZORIDAE Kočárek, Horká & Kundera, 2020

##### Subfamily SPIRALIZORINAE Kočárek, Horká & Kundera, 2020

##### Genus *Brazilozoros* Kukalova-Peck & Peck, 1993

##### *Zorotypus* Silvestri, 1913: 196, partim.

*Brazilozoros* Kukalova-Peck & Peck, 1993: 338 (description, illustration). — Engel & Grimaldi 2000: 151 (synonymy with *Zorotypus* Silvestri, 1913). — Kočárek *et al.* 2020: 11 (reinstated as valid genus, diagnosis, illustration).

TYPE SPECIES. — *Brazilozoros brasiliensis* Silvestri, 1946; designated by Kukalova-Peck & Peck (1993): 342.

TAXA INCLUDED. — *Brazilozoros brasiliensis* (Silvestri, 1946), *B. weidneri* (New, 1978), *B. huxleyi* (Bolívar y Peltain & Coronado, 1963), and *B. kukalovae* n. sp.

DISTRIBUTION. — Brazil, Peru, French Guiana (Silvestri 1946; Bolívar y Peltain & Coronado 1963; New 1978; Hubbard 1990; Matsumura *et al.* 2020).

##### *Brazilozoros kukalovae* n. sp.

(Figs 1–4)

[urn:lsid:zoobank.org:act:F896840D-F6EE-4BA2-8293-80A47942990B](https://doi.org/10.21203/rs.3.rs-2900000/v1)

TYPE LOCALITY. — French Guiana: Montagne des Singes.

MATERIAL EXAMINED. — **Holotype.** French Guiana • ♂; French Guiana, Kourou env., Montagne des Singes; 5°41'17.11"N, 52°41'50.26"W; 40 m a.s.l.; 7.VI.2022; P. Kočárek, M. Jankásek, I. H. Tuf leg.; NMPC. **Paratypes.** French Guiana • 1 ♀; same data as for holotype; NMPC • 1 ♂; French Guiana, Montsinéry env., Sentier du Bagne des Annamites track; 4°49'37.23"N, 52°30'58.24"W; 25 m a.s.l.; 11.VI.2022; P. Kočárek, M. Jankásek, I. H. Tuf leg.; NMPC • 1 ♂, 1 ♀; French Guiana, Cacao env., Molokoï track; 4°33'39.70"N, 52°27'44.52"W; 40 m a.s.l.; 10.VI.2022; P. Kočárek, M. Jankásek, I. H. Tuf leg.; MNHN.

DIAGNOSIS. — *Brazilozoros kukalovae* n. sp. (Figs 1–4) is morphologically similar to *B. weidneri* (New, 1978), but males of the two species can be distinguished by the specific arrangement of sensilla basiconica on 10th tergite and by the shape of the male genitalia. The anterior group of sensilla basiconica on the 10th abdominal tergite are composed of four sensilla in *B. kukalovae* n. sp., and the anterior sensilla are apparently shorter than those of the posterior group (Fig. 3D). The anterior group of sensilla basiconica are composed of only two sensilla in *B. weidneri*, anterior sensilla are longer than those of the posterior group; lateral sensilla basiconica of anterior group are divided basally (Fig. 3E). Differences in the morphology of the male genitalia are illustrated in Fig. 3A–C. Females of these two species can be distinguished based on the shape of the emargination of the distal edge of the 8th abdominal sternite. The emargination is deep, i.e., two times deeper than its maximal width, in *B. kukalovae* n. sp. In *B. weidneri*, in contrast, the emargination is as wide as deep and has a semicircular shape. *Brazilozoros huxleyi* is a visually conspicuous species, easily recognized by the hind legs, which bear three conspicuous spurs on the ventral edge in the case of *B. huxleyi*. In clear contrast only 10 short bristles are present in *B. kukalovae* n. sp. This character applies to both sexes. *Brazilozoros brasiliensis* is known only from the female sex, with the exception of only one described gynandromorphic specimen (Rafael *et al.* 2017). Based on this specimen, we suspect that the anterior group of sensilla basiconica on the 10th tergite of males is composed of two sensilla (which are not divided basally) in the case of *B. brasiliensis* but of four sensilla in the case of *B. kukalovae* n. sp. Females of the two species differ in the emargination of the distal edge of the 8th abdominal sternite, which is shallow, broadly concave, and wider than deep in *B. brasiliensis* vs. two times deeper than wide in *B. kukalovae* n. sp. All described species of *Brazilozoros* can be identified with the aid of the key provided in this study.

ETYMOLOGY. — This species is named after Dr Jarmila Kukalova-Peck in honour of her contribution to Zoraptera research.

TABLE 1. — Details of the material used in the phylogenetic analyses. Newly obtained sequences are marked in **bold**. Asterisk (\*) indicates the specimens published in GenBank as *B. weidneri* (New, 1978) (see Matsumura et al. 2020). Abbreviation: **N/A**, not available.

Analysed taxa	Sampling locality	Isolate	H3	16S	18S
<b>Zorotypidae</b> Silvestri, 1913					
<b>Spermozorinae</b> Kočárek, Horká & Kunderata, 2020					
<i>Spermozoros asymmetricus</i> (Kočárek, 2017)	Brunei	4Z	ON811689	MN790592	ON807040
<b>Zorotypinae</b> Silvestri, 1913					
<i>Zorotypus delamarei</i> Paulian, 1949	Madagascar	71Z	ON811690	MN790583	ON807041
<i>Usazoros hubbardi</i> (Caudell, 1918)	USA: Florida	89Z	ON811691	ON722350	ON807042
<b>Spiralizeridae</b> Kočárek, Horká & Kunderata, 2020					
<b>Latinozorinae</b> Kočárek, Horká & Kunderata, 2020					
<i>Latinozoros cacaoensis</i> Kočárek & Horká, 2023	French Guiana	48Z	ON811693	ON722349	ON807044
<i>Latinozoros gimmeli</i> Kočárek & Horká, 2023	Dominican Republic	41Z	ON811692	ON722348	ON807043
<b>Spiralizerinae</b> Kočárek, Horká & Kunderata, 2020					
<i>Brazilozoros brasiliensis</i> (Silvestri, 1946)	Brazil: Paraná	YK29	LC477128	LC476748	LC477104
<i>Brazilozoros "huxleyi"</i> (Bolívar y Pieltain & Coronado, 1963)	Ecuador	YK11	LC477129	LC476749	LC477105
<i>Brazilozoros "huxleyi"</i>	Peru	YK20	LC477131	LC476751	LC477106
<i>Brazilozoros "huxleyi"</i>	Peru	YK21	LC477132	LC476752	LC477107
<i>Brazilozoros kukalovae</i> n. sp.	French Guiana	94Z	N/A	<b>OP562643</b>	<b>OP558030</b>
<i>Brazilozoros kukalovae</i> n. sp.	French Guiana	YKFG	LC477127*	LC476747*	LC477103*
<i>Brazilozoros kukalovae</i> n. sp.	Brazil: Ubajara	YK18	LC477126*	LC476746*	N/A
<i>Brazilozoros</i> sp.	French Guiana	93Z	N/A	<b>OP562642</b>	<b>OP558031</b>
<i>Brazilozoros "weidneri"</i> (New, 1978)	Brazil: Amazonas	YK7	LC477124	LC476744	LC477101
<i>Brazilozoros "weidneri"</i>	Brazil: Roraima	YK17	LC477125	LC476745	LC477102
<i>Spiralizeros cervicornis</i> (Mashimo, Yoshizawa & Engel, 2013)	Brunei	1Z	ON811694	MN790588	ON807046
<i>Spiralizeros magnicaudelli</i> (Mashimo, Engel, Dallai, Beutel & Machida, 2013)	Malaysia: Sabah	6Z	ON811695	MN790600	ON807047
<b>Outgroup (Polyneoptera)</b>					
Blattodea: Gromphadorhina portentosa	N/A	B01; N/A	AY125216	Z97626	Z97592
Dermoptera: Euborellia arcanum	USA: Florida	E8	ON856150	MN790595	MN790614
Grylloblattodea: Grylloblatta campodeiformis	USA: MT	BYU_gb31.1	DQ457398	DQ457262	DQ457299
Mantodea: Mantis religiosa	Ghana	MN247	FJ806794	FJ806243	FJ806432

DISTRIBUTION, HABITAT, AND BIOLOGY. — *Brazilozoros kukalovae* n. sp. was collected from under the bark of rotting logs in a lowland rainforest in French Guiana at altitudes from 25 to 40 m a.s.l. (Fig. 5). The species is currently known only from French Guiana, but we expect its occurrence in similar habitats in neighbouring countries in Amazonia.

DESCRIPTION

*Apterous male*

**Measurements.** Total body length: 2.58-2.71 mm, head width 0.52-0.54 mm, head length 0.40-0.41, antenna length 1.42-1.44 mm, pronotal width 0.44-0.45 mm, metafemur length 0.65-0.66 mm, metatibia length 0.60, abdomen maximal width 0.55-0.57 mm, cerci length 0.11 mm.

**Colour.** Body color pale brown; tibiae and tarsi of all legs, cerci, and membranous regions lighter; antennae pale brown with antennomeres VII-IX lighter (Fig. 5E).

**Head.** Head subtriangular, about as long as broad posteriorly, and triangular in dorsal view, slightly wider than pronotum (Fig. 1A-C); coronal and frontal sutures invisible; y-shaped ecdysial cleavage lighter (Fig. 1A-C); cephalic setae (Fig. 1B) short and sparse, not grouped; compound eyes and ocelli absent; fontanelle (cephalic gland) absent; antennae 9-segmented (Fig. 1A, D), antennomeres II-VI with several long setae (longer than diameter of antennomere), antennomere I slightly curved outward, antennomere II short,

about half the length of antennomere I, antennomere III slightly longer than antennomere II; antennomeres IV-VIII longer than wide, distally narrowed, with maximal width in distal third, antennomere IX cylindrical, distally narrowed. Mandibles asymmetrical, triangular with rounded lateral margins, each with five teeth on distal half and serrated molar region; left mandible with apically branched stick-like prosthema, inserted on ventral surface of second (subapical) tooth; maxillary palpus five-segmented; labial palpus three-segmented (Fig. 1C).

**Thorax.** Pronotum subrectangular, wider than long, slightly narrowed posteriorly and setose, chaetotaxy as depicted in Fig. 1B; median ecdysial suture lighter; prosternum with peg-like lateral protuberance proximally on each side (Fig. 1C); mesonotum and metanotum trapezoidal, distinctly wider than long, shorter than pronotum.

**Legs.** Covered by short setae (Fig. 1A, E); distal half of protibia with row of flat sensilla ventrally, as long as setae on tibia; metafemur broad with maximal width in middle part and gradually tapering towards apex (Fig. 1E); dorsal surface broadly setose, ventral surface with 10 bristles; length of bristles I, III, and V-X similar to length of dorsal setae, bristle I and IV distinctly shorter and thinner (Fig. 1E); metatibia with densely arranged setae and two strongly sclerotized spurs ventrally of similar length as setae in distal third of tibia; distal

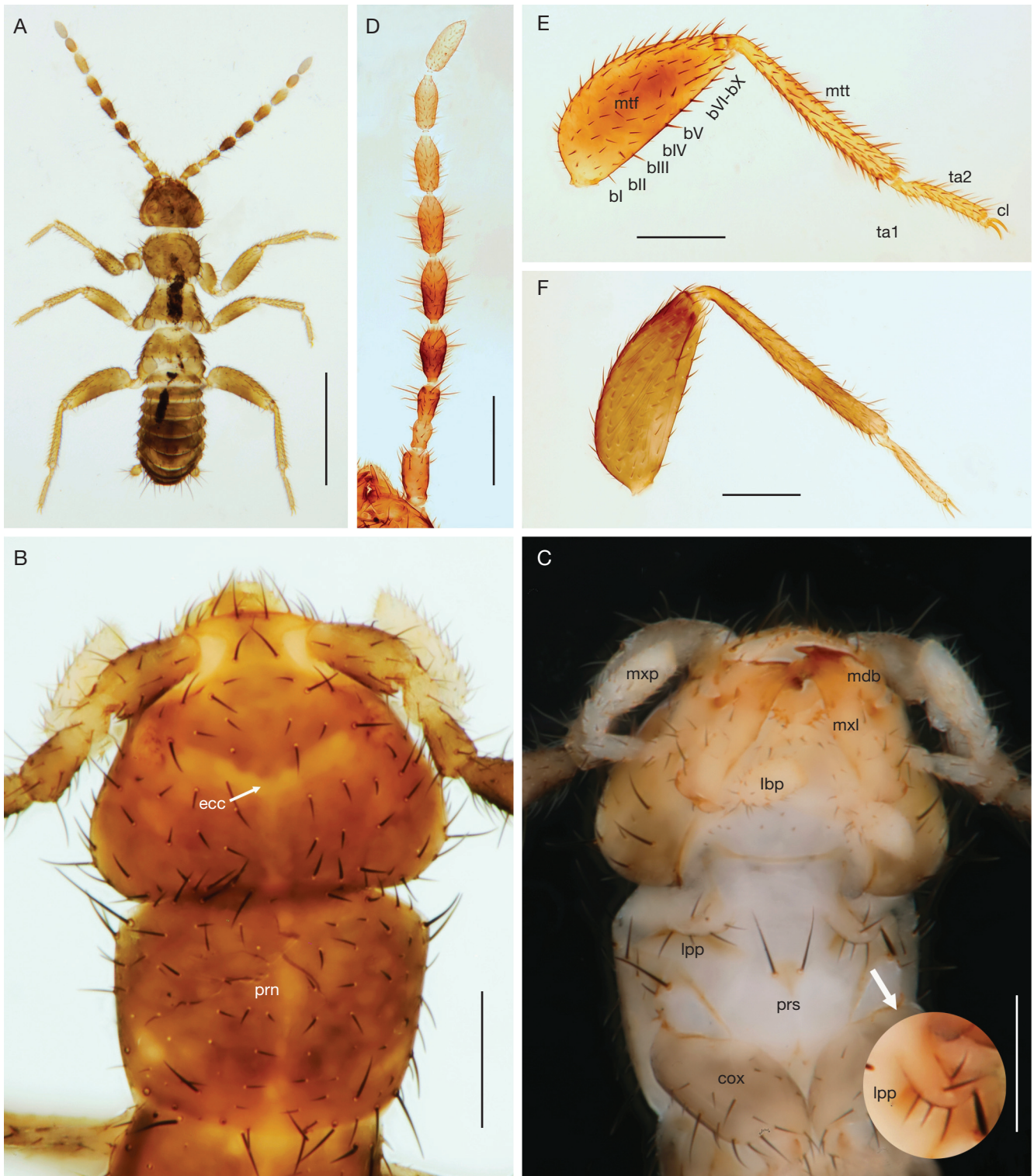


FIG. 1. — *Brazilozoros kukalovae* n. sp., apterous male and female paratypes: **A**, dorsal view of male; **B**, head and pronotum of male from dorsal view; **C**, head and pronotum of male from ventral view; **D**, right antenna of male; **E**, right hind leg of male, anterior view; **F**, right hind leg of female, anterior view. Abbreviations: **bl-bX**, bristles; **cl**, clavus; **cox**, coxa; **ecc**, ecdysial cleavage; **lbp**, labial palp; **lpp**, lateral protuberance of prosternum; **mdb**, mandibula; **mxl**, maxilla; **mxp**, maxillary palp; **mtf**, metafemur; **mtt**, metatibia; **prn**, pronotum; **prs**, prosternum; **ta**, tarsus. Scale bars: A, 1.0 mm; B, C, 0.2 mm.

end of tarsomere II with short unguitactor plate and hooked paired claws (Fig. 1E).

**Abdominal tergites.** I to VII (T1-7) with single transverse row of four to six setae of moderate length distally and few lateral, short setae on each side of midline (Fig. 2A,

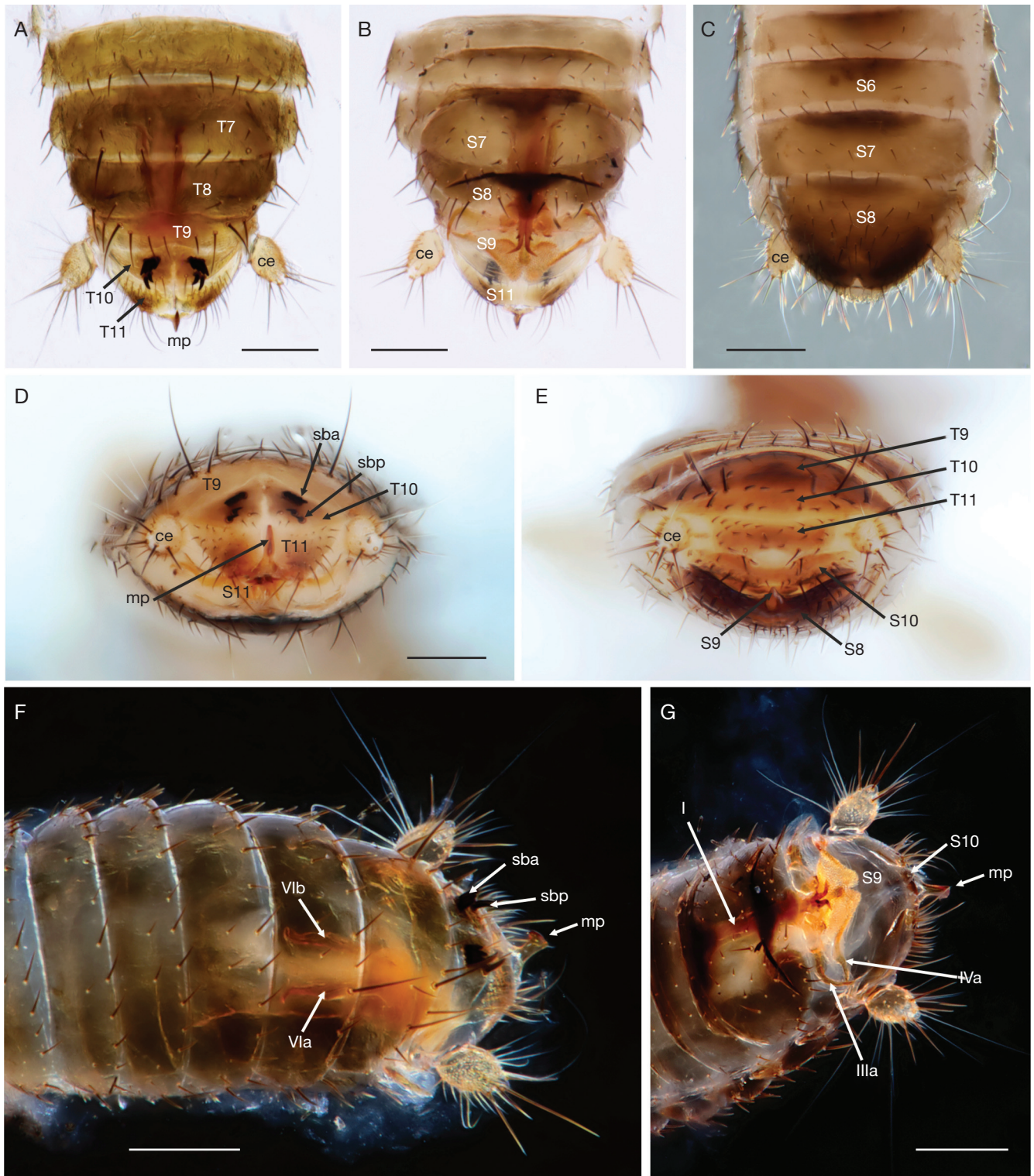


FIG. 2. — *Brazilozoros kukalovae* n. sp., apterous male and female paratypes: **A**, posterior segments of male abdomen, dorsal view; **B**, posterior segments of male abdomen, ventral view; **C**, posterior segments of female abdomen, ventral view; **D**, tip of male abdomen, posterior view; **E**, tip of female abdomen, posterior view; **F**, male abdomen in lateral view, dark field; **G**, latero-dorsal view of tip of male abdomen, dark field. Abbreviations: **ce**, cercus; **mp**, median up-curved projection; **S**, abdominal sternite; **sba**, anterior group of sensilla basiconica; **sbp**, posterior group of sensilla basiconica; **T**, abdominal tergite; **I-VI**, sclerites of male genital. Scale bars: 0.2 mm.

F); T8 with two setae posteriorly on each side of midline (Fig. 2A); T9 short, with anterior trapezoidal expansion extending beneath T8, with single transverse row of two

shorter and three longer setae posteriorly on each side of midline (Fig. 2A, F); T10 posteromedially incised, with three short setae on each side of midline and an anterior



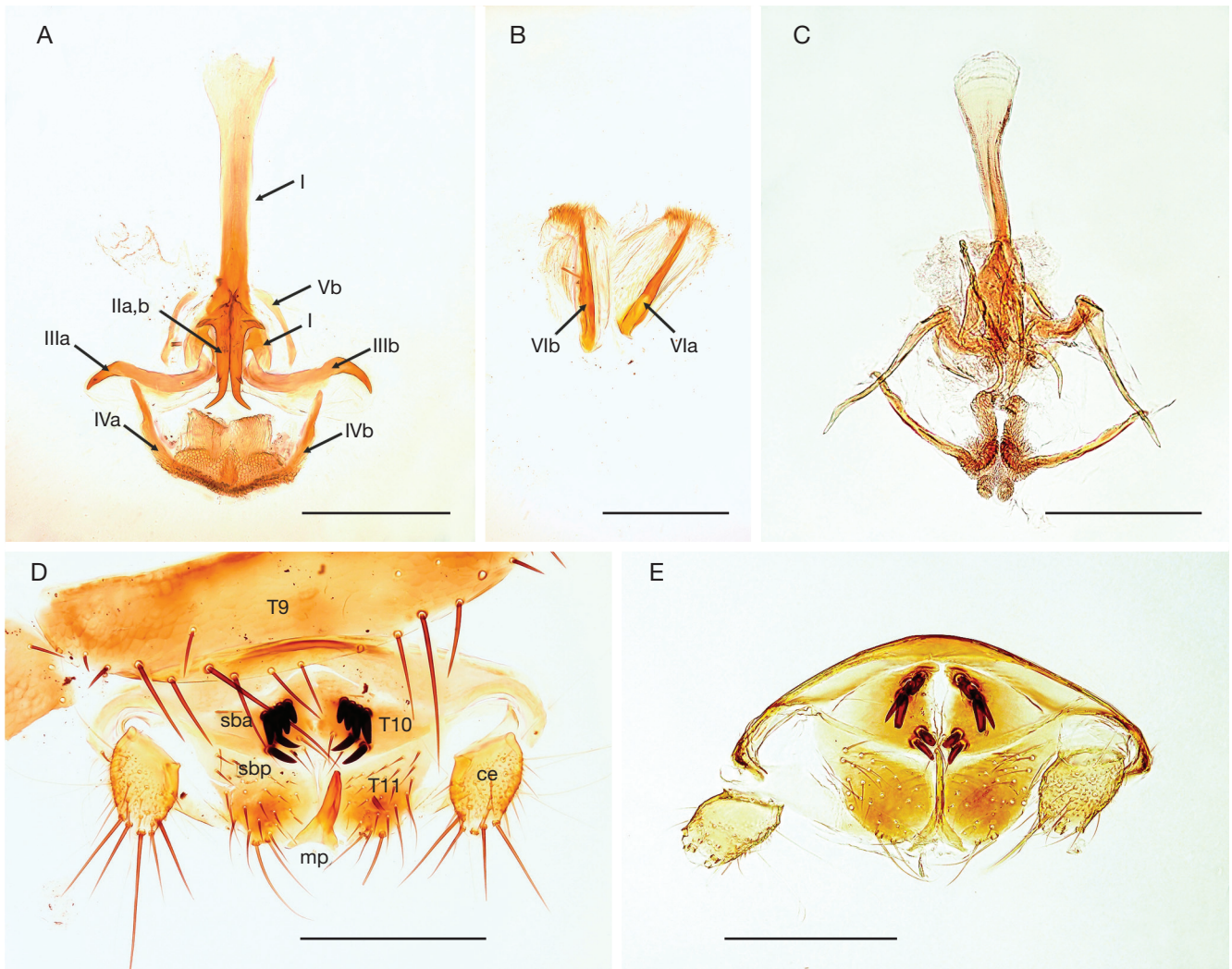


FIG. 3. — *Brazilozoros kukalovae* n. sp., apterous male paratype (A, B, D) and *B. weidneri* (New, 1978), apterous male paratype (C, E): A, ventral view of male genitalia of *B. kukalovae* n. sp.; B, anterior view of sclerites VIa,b of male genitalia in *B. kukalovae* n. sp.; C, ventral view of male genital of *B. weidneri*; D, tip of male abdomen in *B. kukalovae* n. sp., posterior view; E, tip of male abdomen in *B. weidneri*, posterior view. Abbreviations: ce, cercus; mp, median up-curved projection; sba, anterior group of sensilla basiconica; sbp, posterior group of sensilla basiconica; T, abdominal tergite; I–VI, sclerites of male genitalia. Scale bars: 0.2 mm.

and posterior group of thick, black sensilla basiconica; anterior group composed of four sensilla basiconica, posterior group of two sensilla basiconica (Fig. 2A, E–G); T11 with median upcurved projection (= male mating hook) and two lateral, subtriangular sclerites, covered by several short and moderate-length setae on each sclerite (Fig. 2D); epiproct and paraproct unsclerotized; cercus unsegmented, oval, with several moderate-length setae and several very long, fine setae (Fig. 2A, B, D–G); surface covered with numerous minute spicules except at base and apex (Fig. 2G); sternum I (S1) scarcely sclerotized; S2–S3 weakly sclerotized with single transverse row of several moderate-length setae distally; S4–6 with single transverse row of four to six moderate-length setae on each side of midline (Fig. 2A, F); S7 covered by moderate-length setae; posterior edge with 3–4 longer seta on each side of midline (Fig. 2B, D); S8 strongly transverse, with row of short setae anteriorly and row of several (3–4)

moderate-length setae posteriorly on each side of midline (Fig. 2A); S9 transverse, rounded, distally with small, fine setae (Fig. 2B); S10 invaginated beneath S9, weakly sclerotized, excised distally and not visible externally in living specimens – sclerite partly visible after maceration on Fig. 2G; S11 weakly sclerotized, composed of two lateral sclerites, each with small setae (Fig. 2B, D).

**Male genitalia.** Symmetrical (Fig. 3A, B). Composed of antero-posteriorly oriented basal plate (sclerite I) with long, anterior tongue-like process, and bifurcated posterior part; antero-posteriorly oriented pair of central ventral sclerites (sclerite IIa,b) attached to basal plate, with laterally oriented spines anteriorly, pointed and hooked (mutually divergent) distal ends, each with minute teeth on distal third, oriented laterally; pair of sinuous transverse sclerites attached to basal plate (sclerite IIIa,b), each with elongate outer projection; pair

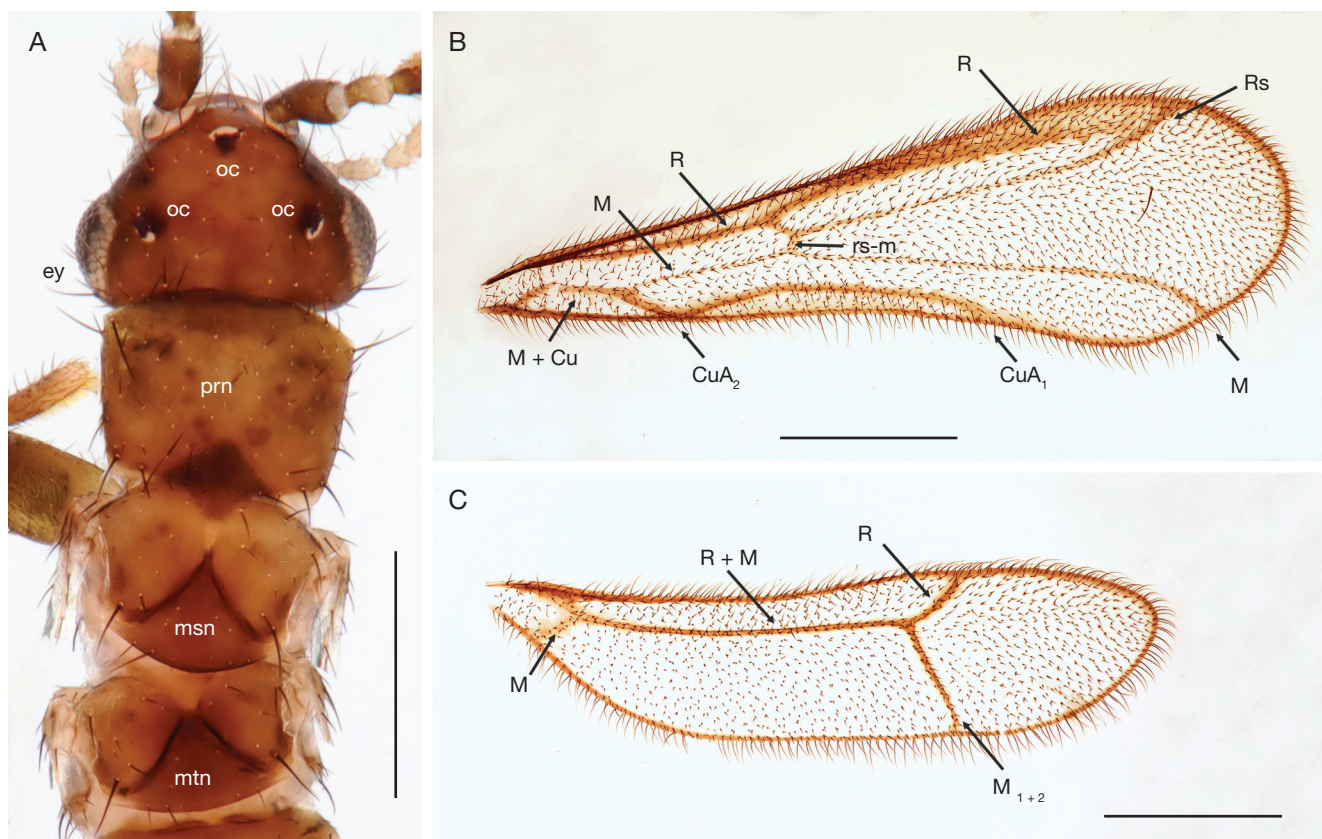


FIG. 4. — *Brazilozoros kukalovae* n. sp., dealate female paratype and wings of alate female: **A**, dorsal view of head and thorax; **B**, forewing, dorsal view; **C**, hind wing, dorsal view. Abbreviations: **Cu**, cubitus vein; **CuA**, anterior cubitus; **ey**, compound eye; **M**, media vein; **msn**, mesonotum; **mtn**, metanotum; **oc**, ocelli; **prn**, pronotum; **R (Rs)**, radius vein. Scale bars: 0.5 mm.

of transverse arms (sclerites IVa,b) gradually narrowing from base to apex and curved symmetrically towards sclerites III; medial part rugose, anteriorly two rugose lobes inserted on each arm, one smaller and rounded, second larger and truncated anteriorly; central part of genital apparatus bordered by narrow lateral sclerites (sclerites Va,b); dorsally placed pair of needle-like sclerites (sclerites VIa,b) not attached to rest of genital apparatus, sclerites oriented antero-posteriorly (Fig. 2A, E).

#### Apterous female

**Morphology and measurements.** Similar to male. Except for following features: metafemur slender, ventral surface with the same arrangement of spines as in males, but spines thinner (Fig. 1F); abdomen wider, maximum width 0.61–0.69 mm; T10 transverse, not incised posteromedially, with row of small scattered setae on proximal and distal edge; T11 trapezoidal, not divided into two halves (hemitergites), covered with small scattered setae and pair of paramedian setae of moderate length apically, distal edge weakly incised; S8 strongly trapezoidal, wider than long, with short setae evenly scattered and longer setae flanking the distal and lateral edges; distal end deeply triangularly excised (Fig. 2C); S9 transverse, mostly covered by S8, distal edge medially with triangular protuberance juxtaposed to excision of S8 basally (gonopore between excision

of S8 and protuberance of S9); S10 entire, not divided into hemitergites, transverse, weakly emarginate anteriorly, with row of small scattered setae on proximal and distal edge.

#### Description of alate/dealate specimens

**Morphology.** Generally similar to those of the apterous specimens except as follows: blackish brown coloration; compound eyes and three black ocelli present, ocelli arranged as triangle, two anterior to compound eyes, and one medially on anterior frons; distal edge of pronotum only weakly sclerotized; mesonotum and metanotum indistinctly divided into slightly pointed prescutum, large scutum, and smaller and posteriorly rounded scutellum (Fig. 4A).

**Wings.** Brown-hyaline with dense pubescence (Fig. 4B, C). Forewing 2.3 mm long, hind wing 1.8 mm. Forewing veins (Fig. 4B) M and Rs weak and indistinct; pterostigma present on anterior margin between R and C veins; Rs far from the wing tip, reaches C vein close to pterostigma; veins rs-m present, slightly shorter than abscissa of Rs; M thin, extended to posterior margin; M + Cu coarse and nebulous; CuA<sub>1</sub> extending from posterior wing margin; CuA<sub>2</sub> short, almost absent. Hind wing (Fig. 4C) with long R+M, R and M<sub>1+2</sub> veins well defined, M vein weaker; R and M<sub>1+2</sub> fused in distal third of wing; M short and indistinct, starting near the base of the wing; Cu absent.



FIG. 5. — Habitat and living specimens of *Brazilozoros kukalovae* n. sp.: **A**, rotting logs in type locality, Montagne des Singes (French Guiana: around Kourou); **B**, molokoi track (French Guiana: around Cacao); **C**, collecting of Zoraptera in Montagne des Singes (above Marek Jankásek, below Petr Kočárek); **D**, nymph of *B. kukalovae* n. sp. with a small unidentified isopod (above) from the family Philosciidae Kinahan, 1857, commonly occurring together with zorapterans in French Guiana; **E**, apterous female. Photographs: P. Kočárek (A, B, D, E) and I. H. Tuf (C).

#### MOLECULAR IDENTIFICATION

We obtained a partial 16S rRNA sequence (500 bp) of *Brazilozoros kukalovae* n. sp. as a DNA barcode for the purpose of

molecular identification of the species; the sequence has been deposited in GenBank under accession number OP562643 (isolate number: 94Z).

TABLE 2. — Genetic divergences of *Brazilozoros* Kukulova-Peck & Peck, 1993 species were analysed using the Kimura 2-parameter model. Molecular markers 16S (302 analysed positions) and 18S (459 analysed positions) are shown; values of 16S are below the diagonal. Symbol: \*, specimens published in GenBank as *B. weidneri* (New, 1978) (see Matsumura *et al.* 2020); **N/A**, not available.

		1	2	3	4	5	6	7	8	9	10
1	<i>B. kukalovae</i> n. sp. 94Z Fr. Guiana		0.00	N/A	0.00	0.00	0.02	0.02	0.03	0.02	0.03
2	<i>B. kukalovae</i> n. sp. YKFG Fr. Guiana *	0.00		N/A	0.00	0.00	0.02	0.02	0.03	0.02	0.03
3	<i>B. kukalovae</i> n. sp. YK18 Brazil *	0.04	0.04		N/A	N/A	N/A	N/A	N/A	N/A	N/A
4	<i>B. "weidneri"</i> YK7 Brazil	0.16	0.16	0.18		0.00	0.02	0.02	0.03	0.02	0.03
5	<i>B. "weidneri"</i> YK17 Brazil	0.17	0.17	0.20	0.03		0.02	0.02	0.03	0.02	0.03
6	<i>B. sp.</i> 93Z Fr. Guiana	0.23	0.23	0.25	0.24	0.25		0.00	0.01	0.00	0.01
7	<i>B. "huxleyi"</i> YK20 Peru	0.26	0.26	0.27	0.26	0.28	0.05		0.01	0.00	0.02
8	<i>B. "huxleyi"</i> YK11 Ecuador	0.23	0.23	0.26	0.26	0.27	0.07	0.07		0.01	0.02
9	<i>B. "huxleyi"</i> YK21 Peru	0.25	0.25	0.26	0.25	0.27	0.07	0.06	0.07		0.01
10	<i>B. brasiliensis</i> YK29 Brazil	0.19	0.19	0.21	0.22	0.22	0.27	0.27	0.25	0.26	

KEY TO SPECIES OF *BRAZILZOROS* KUKALOVA-PECK & PECK, 1993

1. Male ..... 2  
— Female ..... 5
2. 10th tergite with two sensilla basiconica (one anterior, one posterior) on each side of midline. Metafemur with three conspicuous spurs on ventral edge, much longer than diameter of tibia ..... *Brazilozoros huxleyi* (Bolivar y Pieltain & Coronado, 1963)  
— 10th tergite with two groups of sensilla basiconica on 10<sup>th</sup> tergite, each composed of at least two sensilla. Metafemur with number of bristles, length of bristles is less than diameter of tibia ..... 3
3. Anterior group of sensilla basiconica composed of 4 sensilla, anterior sensilla shorter than sensilla in posterior group ..... *Brazilozoros kukalovae* n. sp.  
— Anterior group of sensilla basiconica composed of 2(3) sensilla, anterior sensilla longer than sensilla in posterior group ..... 4
4. Lateral sensilla basiconica of anterior group divided basally (to two protrusions of similar length) .....  
..... *Brazilozoros weidneri* (New, 1978)  
— Anterior group of sensilla basiconica composed of two sensilla, not-divided basally (based on only one known gynandromorph male – see Rafael *et al.* 2017) ..... *Brazilozoros brasiliensis* (Silvestri, 1946)
5. Metafemur with number of bristles, length of bristles is less than diameter of tibia ..... 6  
— Metafemur with three conspicuous spurs on ventral edge, as long as or longer than diameter of tibia .....  
..... *B. huxleyi*
6. Distal emargination of 8<sup>th</sup> sternite deep, two times deeper than its maximal width ..... *B. kukalovae* n. sp.  
— Distal emargination of 8<sup>th</sup> sternite wider, shallow ..... 7
7. Distal emargination of 8<sup>th</sup> sternite semicircular, as deep as wide ..... *B. weidneri*  
— Sternite 8 with shallow, broadly concave emargination, significantly wider than deep ..... *B. brasiliensis*

PHYLOGENETIC POSITION OF *BRAZILZOROS KUKALOVAE* N. SP.

The final analysed dataset of the three markers (18S, 16S, and H3) consisted of 1,106 bp. The phylogenetic tree based on the ML method (Fig. 6) shows a strongly supported sister position of the new species *B. kukalovae* n. sp. with *B. "weidneri"*. The genetic divergences within *Brazilozoros* species based on 16S and 18S rRNA sequences using the Kimura 2-parameter model are presented in Table 2. The maximum divergence of *B. kukalovae* n. sp. (Fig. 6, clade 1) to *B. "weidneri"* (clade 2) is 20% for 16S and 0% for 18S. The maximum value between specimens of *B. kukalovae* n. sp. from French Guiana and specimens from Brazil is 4% for 16S; the 18S sequence from Brazil was not available.

DISCUSSION

Zoraptera is one of the less diverse insect orders, with the extant diversity (46 described species) much lower than that in almost all other groups of Hexapoda (Kočárek *et al.* 2020; Kočárek & Horká 2022, 2023). The low diversity of Zoraptera might result from poor collecting efforts, but also from the high level of morphological uniformity, and the real diversity of this lineage seems to be highly underestimated (e.g. Mashimo *et al.* 2013). Consistent with this possibility, the first molecular phylogenetic analyses (Kočárek *et al.* 2020; Matsumura *et al.* 2020) revealed a cryptic diversity in Zoraptera, especially in the genera *Centrozoros* Kukulova-Peck & Peck,

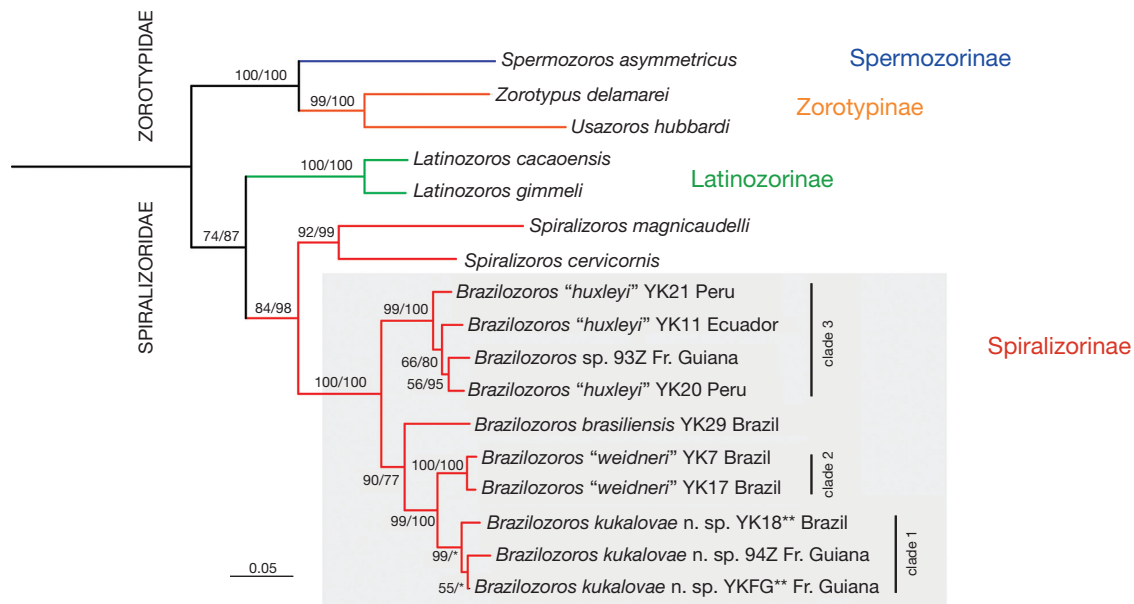


FIG. 6. — Phylogenetic tree of the genus *Brazilozoros* Kukulova-Peck & Peck, 1993 resolved by Maximum Likelihood based on the combined dataset for three molecular markers (16S, 18S, and H3). RAxML bootstrap supports and Bayesian posterior probabilities are expressed in percentages; a single asterisk (\*) indicates different topology of Bayesian tree, and two asterisks (\*\*) indicate the specimens published in GenBank as *B. weidneri* (New, 1978) (see Matsumura *et al.* 2020). For species in quotation marks, see materials and methods.

1993; *Brazilozoros* Kukulova-Peck & Peck, 1993; *Spiralizoros* Kočárek, Horká & Kundera, 2020; and *Latinozoros* Kukulova-Peck & Peck, 1993. A recent molecular phylogenetic reconstruction and detailed morphological comparison detected three genetically distinct lineages in *Latinozoros* (until then a monotypic genus) that were described as separate species (Kočárek & Horká 2023). Here, we described *Brazilozoros kukalovae* n. sp. (Spiralizoridae: Spiralizorinae), which is externally very similar to *B. weidneri* collected from Brazil and French Guiana (New 1978; Matsumura *et al.* 2020, 2022). The new species is well-defined mainly based on characters of the male genitalia, but also based on the morphology of the sesilla basiconia on the 10th abdominal tergite of males (Fig. 3D, E). The description of this new species is further evidence of the cryptic diversity of Zoraptera and of its little-explored taxonomy.

The results of our phylogenetic analysis confirmed the monophyly of the subfamily Spiralizorinae including *Brazilozoros* species, and its sister position to Latinozorinae (Fig. 6), which is in line with previous studies by Kočárek *et al.* (2020) and Matsumura *et al.* (2020). Together, both clades (subfamilies) represent the family Spiralizoridae, a family characterised by symmetrical male genitalia. Our phylogenetic tree also indicates the monophyly of *Brazilozoros*.

The genus *Brazilozoros* was described by Kukulova-Peck & Peck (1993) based on the wing venation of allate specimens. Engel & Grimaldi (2000) critically revised the supraspecific classification of Zoraptera and concluded that the proposed

generic characters are either continuous across taxa or variable within a given species; the authors synonymized the genus with nominotypic *Zorotypus* Silvestri, 1913. Kočárek *et al.* (2020) reinstated *Brazilozoros* as a valid genus based on results of molecular phylogeny reconstruction and a new diagnosis based on the morphology of male genitalia and the male abdomen. At present, the genus *Brazilozoros* comprises four neotropical species (including *B. kukalovae* n. sp. described here). *Brazilozoros kukalovae* n. sp. appears to be closely related to *B. weidneri*, with which it shares most morphological characteristics, and morphologically differs only in the details of the structure of the genitalia and sensilla on the abdomen (see Fig. 3).

We included all publicly available sequences of *Brazilozoros* species in our phylogenetic analysis (Fig. 6) to illustrate the diversity and phylogenetic relationships in the genus, but the taxonomic identity of *B. huxleyi* (clade 3) and *B. weidneri* (clade 2) specimens adopted from Matsumura *et al.* (2020) is unclear and requires rigorous comparison of DNA with type material, and ideally, with voucher sequences from the type locality. Because no morphological descriptions were provided for the specimens published in Matsumura *et al.* (2020) and because the specimens did not come from the type localities, we indicate these samples in the phylogenetic tree in quotation marks with the name of the country (region) of origin. The only species of the genus *Brazilozoros* for which we can confidently associate sequences with its name is *B. kukalovae* n. sp. described here, and a detailed

review of morphology is necessary to verify the identity of the remaining species.

The description of *B. kukalovae* n. sp. provided in the current study increases the number of described Zoraptera species to 61; this number includes 46 extant species and 15 fossil species known from Cretaceous (12) and Miocene (3) amber (Chen & Su 2019; Mashimo *et al.* 2019). The current study is further proof of the high degree of cryptic diversity in Zoraptera and points to the necessity of a critical assessment of newly found specimens and to the importance of detailed morphological comparison with the type material.

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### Author Contributions

PK collected the material, performed the morphological study, and prepared the figures. IH obtained the sequences and performed phylogenetic analyses. Both authors designed the research and participated in writing the final manuscript.

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