

Identity of *Zorotypus juninensis* Engel, 2000, syn. nov. revealed: it is conspecific with *Centrozoros hamiltoni* (New, 1978) (Zoraptera, Spiralizoridae)

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Abstract

Zorotypus juninensis Engel, 2000, was previously diagnosed based on the external morphology of female and male specimens without description of the male copulatory organ, which is an important character for classification in Zoraptera. Based on a detailed morphological study of the *Zorotypus juninensis* Engel, 2000 type collections deposited in the American Museum of Natural History in New York, and based on the comparison with the holotype male of *Centrozoros hamiltoni* (New, 1978), we have determined that these two species are conspecific. We therefore formally synonymize *Zorotypus juninensis* Engel, 2000, **syn. nov.** with *Centrozoros hamiltoni* (New, 1978). Morphological characters and phylogenetic relationships of *Centrozoros* Kukalova-Peck & Peck, 1993 are also discussed in this report.

Key Words

Neotropical region, Peru, Polyneoptera, synonymy, taxonomy

Introduction

Zoraptera is one of the smallest and least known of the insect orders (Mashimo et al. 2014b). The extant diversity of Zoraptera is much lower than that of almost all other groups of Hexapoda, with only 44 described species (Mashimo et al. 2014b; Choe 2018; Kočárek et al. 2020) that are distributed mainly in tropical regions (Hubbard 1990; Choe 2018). Zoraptera show extreme uniformity in general body morphology, and this 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. 2014b; Kočárek et al. 2020). All known extant species were described within a single genus, *Zorotypus* Silvestri, 1913 (Kočárek et al. 2020). Kukalova-Peck and Peck (1993) were the first to propose a classification of Zoraptera into seven genera based on wing venation, and Chao and Chen (2000) subsequently introduced a new genus, *Formosozoros*, based on a single

apomorphic species from Taiwan. Engel and Grimaldi (2000) critically revised the supraspecific classification of Zoraptera and concluded that the proposed generic characters concerning wing venation are either continuous across taxa or variable within a given species. In contrast to the external uniformity in Zoraptera, there are repeatedly documented conspicuous differences in the reproductive system (Dallai et al. 2012, 2014, 2015). These observations suggested the existence of deep evolutionary lineages within Zoraptera, although at the time, not enough information was available to reconstruct the phylogeny (Kočárek et al. 2020).

Matsumura et al. (2020) and Kočárek et al. (2020) conducted the first molecular phylogenetic studies using a combination of nuclear and mitochondrial markers. Both independent analyses revealed two major phylogenetic lineages with maximal statistical support. These two lineages were classified by Kočárek et al. (2020) as families (Zorotypidae Silvestri, 1913 and Spiralizoridae Kočárek, Horká

& Kundera, 2020), and each of them was divided into two robustly supported subclades, i.e. subfamilies (Kočárek et al. 2020). The recognition of two families and four subfamilies is supported by synapomorphies in the structure and shape of the male genitalia and other taxonomically valuable characters. Striking differences in the structure of male genitalia within the recovered monophyletic clades illustrate deep divergences of these old evolutionary lineages.

The classification proposed by Kočárek et al. (2020) comprises two families, four subfamilies, and nine genera, and is based mainly on a molecular phylogenetic analysis in combination with an analysis of the morphology of the male reproductive system. Unfortunately, researchers described some species based solely on immature or female specimens or provided insufficient information on male genitalia (Kočárek et al. 2020). Altogether, 9 species could not be properly assigned to a supraspecific rank and therefore remain *incertae sedis* until males are described or molecular phylogenetic studies are conducted. One of these species is *Zorotypus juninensis* Engel, 2000, for which the original diagnosis was based on female and male specimens without available information about the male reproductive system, i.e., without information required for generic classification.

In this contribution, we present the results of morphological analysis of the type series of *Zorotypus juninensis* Engel, 2000 which led to the clarification of the taxonomical status of this species.

Materials and methods

The type specimens of *Zorotypus juninensis* Engel, 2000, which were stored in 96% ethanol, were studied and photographed with a Leica Z16 APO macroscope equipped with a CANON 6D Mark II camera; a slide-mounted type specimen of *Centrozoros hamiltoni* (New, 1978) and genitalia of *Z. juninensis* 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 (version 13). Coiled flagella were measured with Corel Draw software. For observation of genital armature, the armature was placed in a 10% KOH solution at room temperature for 1 h before it was washed with distilled water and returned to 96% ethanol for observation and storage.

The classification and nomenclature are based on the study by Kočárek et al. (2020); abdominal morphology follows Mashimo et al. (2014a).

Total genomic DNA was isolated from the tissue of the paratype female of *Z. juninensis* (AMNH: IZS00343398) with courtesy of the museum. The isolation was performed with a QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. The mitochondrial markers, 16S rRNA and the cytochrome c oxidase subunit I (COI), and the nuclear marker, 18S rRNA, were amplified by PCR with minor modification of previously reported thermal cycling conditions (Kočárek et al.

2020). Partial segments of these markers were amplified using the primers listed in Kočárek et al. (2020).

Depositories for type specimens are abbreviated as follows: AMNH (American Museum of Natural History, New York, USA); BMNH (The Natural History Museum, London, United Kingdom); ZMH (Zoological Museum Hamburg, Germany). Classification and nomenclature follow Kočárek et al. (2020).

In addition to investigating the type material of *C. hamiltoni* (New, 1978) and *Z. juninensis* Engel, 2000 (see the next section), we compared the material with the following museum specimens of *Centrozoros neotropicus* (Silvestri, 1916): COSTA RICA · 1 ♂; San José; 14 Nov 1935; leg. F. Nevermann; coll. ZMH; COSTA RICA · 1 ♀; Farm Hamburg am Ravantazon; 4 Feb 1934; leg. F. Nevermann; coll. ZMH.

Taxonomy

Centrozoros hamiltoni New, 1978

Figs 1, 2

Zorotypus juninensis Engel, 2000 syn. nov.

Note. New 1978: 365–368 (description, illustration, keyed); Hubbard 1990: 52 (catalog of world species); Choe 1989: 150 (distribution map); Choe 1992: 250 (distribution map); Engel 2000: (description of *Z. juninensis*, syn. nov.); Choe 2018: 200 (distribution); Mashimo et al. 2019: 753 (distribution); Matsumura et al. 2020: 352–357 (distribution, phylogenetic relationships); Kočárek et al. 2020: 11–12, 14–15 (male genitalia, classification).

Studied type material. *Centrozoros hamiltoni* (New, 1978) – **Holotype:** COLOMBIA · 1 apterous ♂; nr. Purace, Marenberg, Huila; 30 Mar 1976; leg. W.D. Hamilton; coll. BMNH; *Zorotypus juninensis* Engel, 2000, syn. nov. – **Holotype:** PERU · 1 apterous ♀; Agueas Mellizas, Estancia Naranjal San Ramon, Dep. Junin; 1 500 m; July 1965; leg. P. & B. Wygodzinsky; coll. AMNH: IZS00343397; **Paratypes:** 1 apterous ♀; 1 apterous ♂: same locality data as in Holotype (AMNH: IZS00343398).

Diagnosis. Dark-brownish black Zoraptera, with anterior regions of abdominal tergites darker than posterior regions; antennal segment nine, and apex of segment eight pale. Body length ranges from 2.9 to 3.6 mm; antennal length ranges from 1.45 to 1.63 mm. Ventral side of metafemur with row of 8 to 10 thickened setae situated in the distal two-thirds of the femur; proximal third with several (5–8) slender setae (Fig. 1A, B). Metafemur of females with the same arrangement of setae with less pronounced thickening. Abdominal tergites T5–T8 (Fig. 2A–D) each with a single posterior row of 10 to 14 setae and 1 or 2 more anterior setae near each lateral border. Abdominal tergite T9 with group of 6 to 8 thickened setae on each side of the midline (Fig. 2A, D). Tergite T10+11 membranous medially (Fig. 2A, C, D), with about 20 short setae each side of the midline and a median insert bearing a short dorsally curved extension (mating hook).

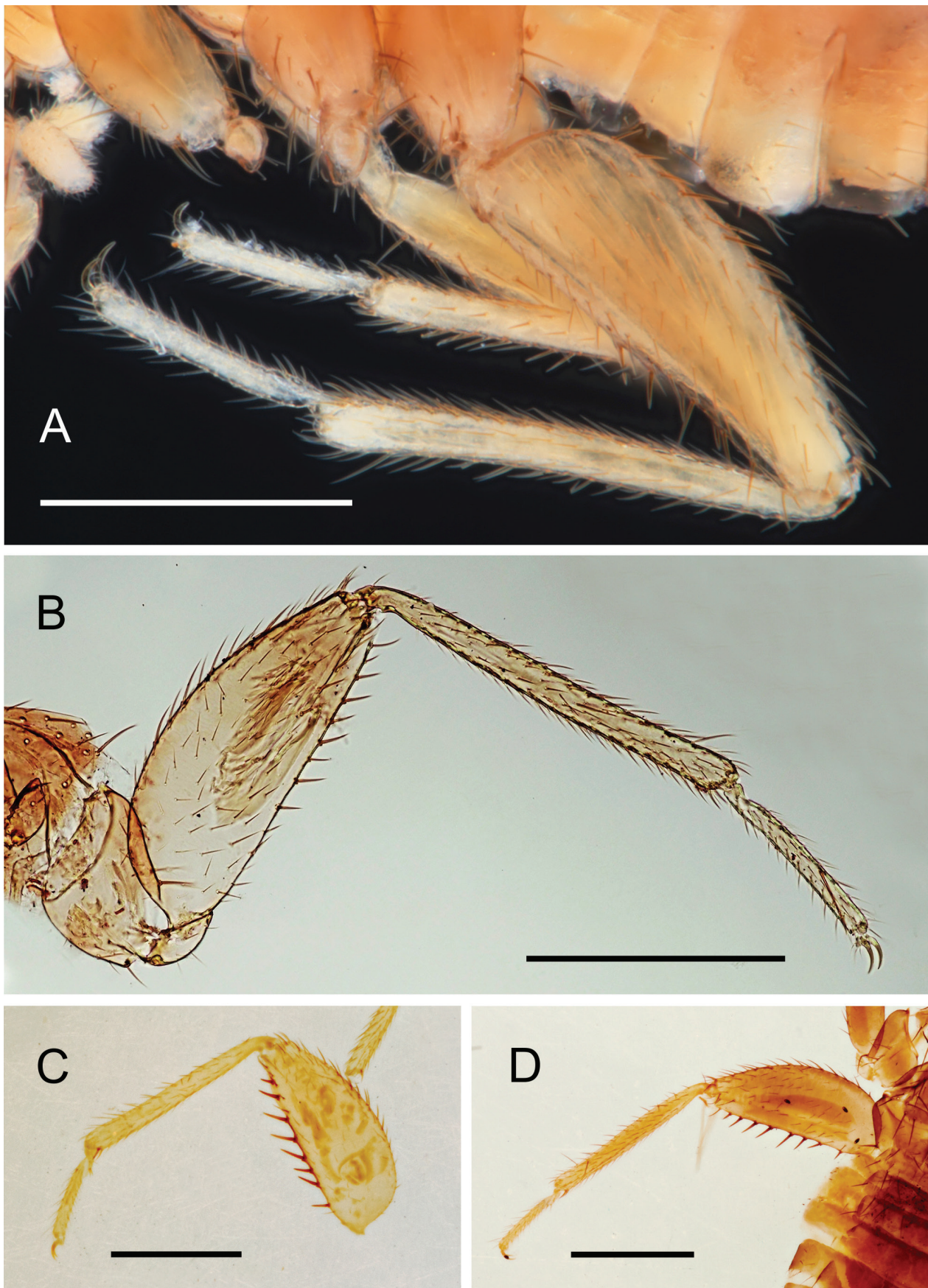


Figure 1. A. Hind legs of the paratype male of *Zorotypus juninensis* Engel, 2000 syn. nov.; B. Hind leg of the holotype male of *Centrozoros hamiltoni* (New, 1978); C. Hind leg of a male of *Centrozoros neotropicus* (Silvestri, 1916); D. Hind leg of a female of *C. neotropicus*. Scale bars: 0.5 mm.

Cerci (Fig. 2A–D) slightly longer than wide, tapered, with several long apical setae. Male genitalia symmetrical (Fig. 2E, F). Basal plate flat, 0.5 mm long, sclerotized, with an anterior conical process, and posteriorly bifurcated; flagellum sclerotized, coiled, and 1.4 mm long.

Taxonomic comments. The studied paratype male of *Z. juninensis* fully agrees with the holotype male of *C. hamiltoni* in external characters as well as in morphology of male genital armature. The spiral of the coiled flagellum is more open in paratype of *Z. juninensis* (Fig. 2E) than in *C. hamiltoni* (Fig. 2F); the less open flagellum of *C. hamiltoni* is probably an artefact of preparation. We measured the lengths of both flagella, and these were nearly identical (1.382 mm for *Z. juninensis* vs. 1.375 mm for *C. hamiltoni*).

Centrozoros hamiltoni (New, 1978) is morphologically similar to *C. manni* (Caudell, 1923), *C. neotropicus* (Silvestri, 1916), *C. cramptoni* (Gurney, 1938), and *C. gurneyi* (Choe, 1989). *C. manni* is known only from female specimens. Engel and Grimaldi (2000) reported that *C. hamiltoni* (*sensu* this study) differed from *C. manni* in lacking a medial cleft on the apex of S8 in females and in its broadly separated basal processes on S9 in females, its S9 setation pattern on females, its medial field of minute spicules on the cerci, and its long, sinuous setae on the cerci. *Centrozoros neotropicus* (Silvestri, 1916) is another species similar to *C. hamiltoni* in external morphology, including the setation and arrangement of abdominal tergites/sternites. This species is known only from Costa Rica (Silvestri 1916; Gurney 1938; Choe 1992; Kočárek et al. 2020), and it differs from *C. hamiltoni* in the arrangement of its metafemur setae (Fig. 1C, D), which are composed of 5–9 thick setae of similar length (as noted earlier, *C. hamiltoni* has 8–10 thickened setae in the distal two-thirds of its femurs and 5–8 slender and shorter setae in the basal one-third of its femurs). The morphology of only females has been described in literature (Silvestri 1916). We also studied the single male specimen of *C. neotropicus* deposited in the ZMH collections, but the permanent slide did not enable the detailed study of the copulatory organs necessary for clear species diagnosis. The validity of this species was verified molecularly (Kočárek et al. 2020), but the diagnosis should be augmented by the description of male genitalia after the next specimens of males are found. *Centrozoros cramptoni* and *C. gurneyi* seem to be most closely related to *C. hamiltoni* based on their similar morphology of male genital armature. The proximal part of the basal plate of both species has a conical shape as does the plate in *C. hamiltoni*, but the corners of the bifurcated basal part are continually divergent in *C. cramptoni* (Gurney, 1938) in contrast to the convergent tips of corners in *C. hamiltoni* (Fig. 2E, F); in the case of *C. gurneyi*, the bifurcated part regularly narrows towards the end, and the tips of the arms are narrower than the midregion of the basal plate (Choe 1989). These three species also differ in the arrangement of metafemur setae and in the setation of abdominal tergite T9. Like sternite 8 in *C. manni* females, sternite S8 in the females of *C. gurneyi* have an emarginated tip (in contrast to *C. hamiltoni* and *C. neotropicus* with not emarginated distal margin of S8). Relative to *C. hamiltoni*, *C. gurneyi* and *C. cramptoni*,

the other two *Centrozoros* species (*C. snyderi* (Caudell, 1920) and *C. mexicanus* (Bolivar y Pieltain, 1940)) differ substantially in the morphology of male genitals in that they have a broad rather than a conical basal plate (Gurney 1938; Bolivar y Pieltain 1940). *Centrozoros snyderi* and *C. mexicanus* appear to be closely related.

Molecular identification. For unequivocal species identification, we attempted to obtain a DNA barcode from the *Z. juninensis* paratype (AMNH: IZS00343398). For DNA isolation, we used the QIAamp DNA micro kit designed for a small amount of tissue. According to the voucher, the specimen was preserved in pure ethanol and was almost 60 years old when we examined it. Although we have attempted to amplify the DNA several times, our attempts to obtain partial sequences of 16S RNA, COI, and 18S RNA have failed.

Distribution. *Centrozoros hamiltoni* (New, 1978) was originally reported from Colombia (New 1978), and was additionally documented from Colombia by Villamizar and González-Montana (2018). The morphological characters of a single male from Barbados mentioned by New (1978) were similar to all of the characters described for *C. hamiltoni* except that basal plate was a little narrower and shorter on the Barbados male than on the *C. hamiltoni* males; Matsumura et al. (2020) described a specimen from Ecuador that they tentatively identified as *C. hamiltoni*. *Zorotypus juninensis* Engel, 2000 has been described from Junin Province in Peru (Engel and Grimaldi 2000), and was later reported from Peru by Matsumura et al. (2020). Published records indicate that the distribution of *C. hamiltoni* includes western Amazonia (Colombia, Peru, Ecuador), but the record from Barbados suggests a potentially wider distribution in the Neotropical region. Further studies are needed to clarify the distribution of *C. hamiltoni*.

Discussion

The genus *Centrozoros* (Kukalova-Peck & Peck, 1993) has been described based only on the wing venation of *C. gurneyi* (Choe, 1989). Engel and Grimaldi (2000) synonymized this genus with *Zorotypus* Silvestri, 1913 based on the variability of characters used for the generic diagnosis. Kočárek et al. (2020) reinstated the genus based on molecular phylogeny relationships and a critical evaluation of morphological characters. The genus *Centrozoros* (Kukalova-Peck & Peck, 1993) *sensu* Kočárek et al. (2020) includes species that are distributed in the Neotropical region and whose males have an enlarged basal plate with a coiled intromittent organ and an anterior tongue-like process that is not dilated anteriorly (Kočárek et al. 2020). *Centrozoros hamiltoni*, *C. cramptoni*, and *C. gurneyi* share a similar basic morphological plan of the male genitalia, and these three species probably compose a monophyletic group in *Centrozoros* along with *C. mexicanus* and *C. snyderi*. The remaining *Centrozoros* species are known only from females, and their taxonomic relationships are therefore unclear. *Centrozoros hamiltoni* fully fits the diagnostic characters of *Centrozoros*, and the

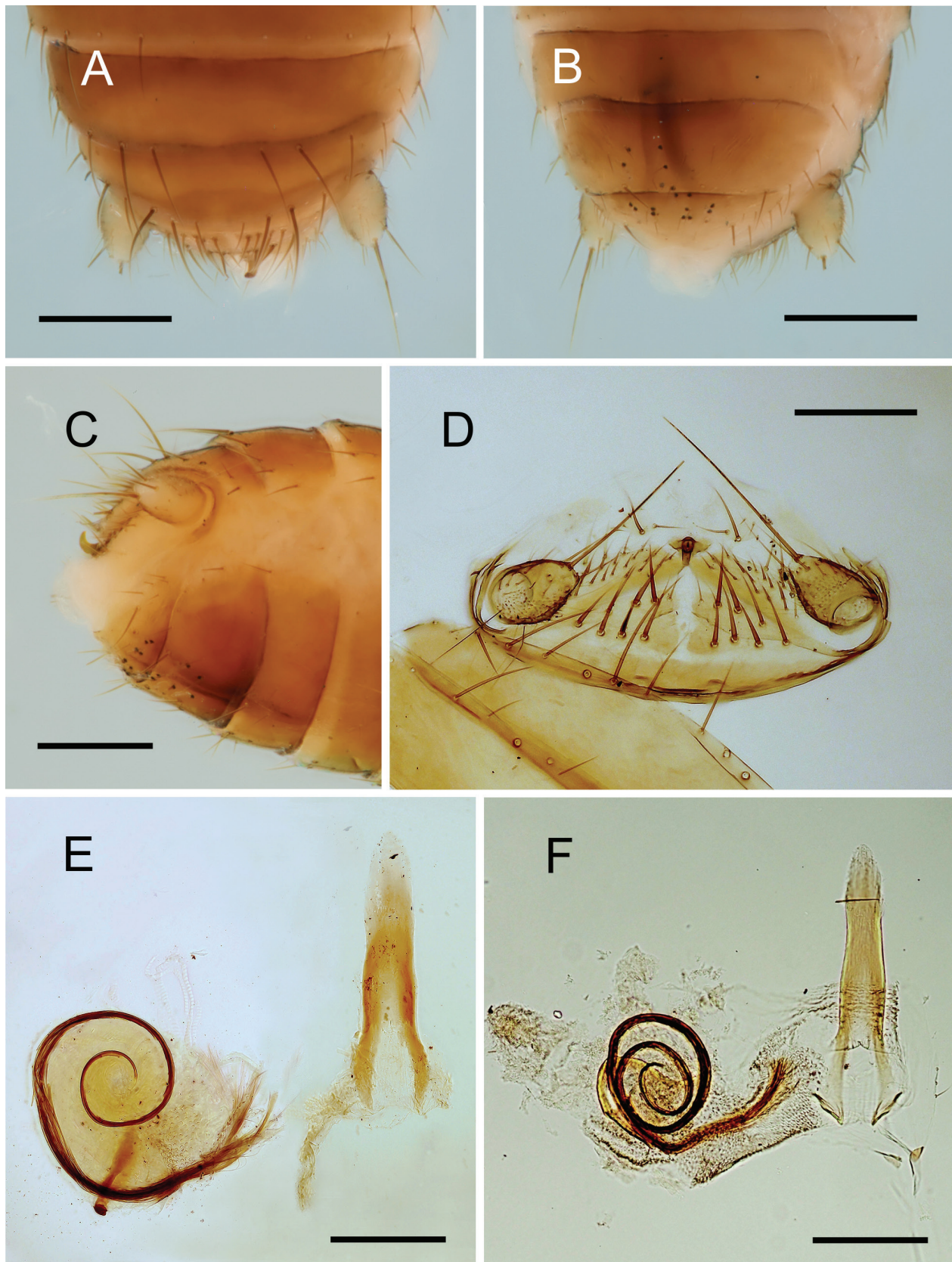


Figure 2. *Centrozoros hamiltoni* (New, 1978). **A, B, C, E.** The paratype male of *Zorotypus juninensis* Engel, 2000, syn. nov.; **D, F.** The holotype male of *C. hamiltoni*. **A.** Tip of the male abdomen of *C. hamiltoni*, dorsal view; **B.** Tip of the male abdomen of *C. hamiltoni*, ventral view; **C.** Tip of the male abdomen of *C. hamiltoni*, ventro-lateral view; **D.** Tip of the male abdomen of *C. hamiltoni*, dorsal view; **E.** Male genital armature of the *Z. juninensis* paratype; **F.** Male genital armature of the *C. hamiltoni* holotype. Scale bars: 0.2 mm.

placement to *Centrozoros* has been proved also by molecular phylogeny studies of Matsumura et al. (2020), with *C. manni*, *C. mexicanus* and *Z. juninensis* (= *C. hamiltoni*) included.

To date, molecular characterizations have been published for four of the seven described species of *Centrozoros*, i.e., for *C. manni*, *C. mexicanus*, *C. hamiltoni*, and *C. neotropicus* (Kočárek et al. 2020; Matsumura et al. 2020). However, both Matsumura et al. (2020) and Kočárek et al. (2020) reported several molecularly different, undescribed species of *Centrozoros*. It is therefore evident that the genus is more diverse than previously thought and requires extensive taxonomic revision. Because of the high level of external uniformity in Zoraptera (and supposed parthenogenetic reproduction in some species of *Centrozoros*), it is important to clarify the species identity by DNA barcodes, ideally extracted from the type specimens or at least from the type locality or from a locality that is geographically near the type locality.

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