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A new epigean species of the genus *Anelpistina* (Insecta: Zygentoma: Nicoletiidae) from Sierra de El Abra, Taninul, Mexico

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Abstract. *Anelpistina taninuli* sp. nov. (Insecta: Zygentoma: Nicoletiidae), a species from Taninul, Sierra de El Abra, San Luis Potosi, Mexico, is described. We also report the 16S rRNA sequence of this new species.

Keywords. Cubacubaninae, Thysanura, *Anelpistina quinterensis, Cubacubana, Neonicoletia*.

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Introduction

The caves in Sierra de El Abra and Sierra de Guatemala, in northern Mexico, are inhabited by the cave adapted nicoletiid *Anelpistina quinterensis* (= *Neonicoletia quinterensis* Paclt, 1979). A recent study (Espinasa *et al*. 2014) has shown that the species most likely migrated underground to reach both mountain ranges within the last 12,000 years. An alternative hypothesis is that the species can survive on the surface and have independently colonized both mountain ranges. The search for nicoletiids on the surface has successfully resulted in collecting another species of nicoletiid, but has never turned up a surface specimen of *A. quinterensis* (Espinasa *et al*. 2014). Further analysis showed that the surface specimens collected in that study belonged to a previously undescribed species. We describe the morphology of this new species and report on its 16S rRNA DNA sequence.

Material and methods

Dissections of the holotype and the largest female paratype were made with the aid of a Motic K series stereo microscope and were mounted as fixed preparations with Cytoseal™ 60 solution (Richard-Allan Scientific). The remaining samples were stored in a vial with ethanol. Type material will be deposited in the American Museum of Natural History (AMNH).

Genomic DNA samples were extracted using Qiagen's DNEasy® Tissue Kit by digesting a leg of the male holotype and of the largest female paratype in lysis buffer. Amplification and sequencing of the 16S rRNA fragment was done as in Espinasa *et al*. (2007), following standard protocols and using primers 16Sar and 16Sb for the 16S rRNA fragment (Edgecombe *et al*. 2002). Amplification was carried out in a 50 µL volume reaction, with 1.25 units of AmpliTaq® DNA Polymerase (Perkin Elmer, Foster City, California, USA), 200 µm of dNTPs and 1 µm of each primer. The PCR program consisted of an initial denaturing step at 94 ºC for 60 s, 35 amplification cycles (94 ºC for 15 s, 49 ºC for 15 s, 72 ºC for 15 s), and a final step at 72 ºC for 6 min in a GeneAmp® PCR System 9700 (Perkin Elmer). Success of amplifications was checked with agarose gel electrophoresis and PCR amplified samples were purified with the QIAGEN QIAquick PCR Purification Kit. Samples were then sent to SeqWright for direct sequencing. The sequence editing software Sequencher™ 3.0 was used to read the chromatograms obtained from the automated sequencer, make contigs and perform alignments of the sequences. External primers were excluded from the analyses. The Basic Local Alignment Search Tool (BLAST) from NCBI was used as described in Young (2008) to find and identify the most similar sequences in GenBank. Results provided identification of previously described species of nicoletiids whose 16S sequence may be closely related to the new species. The software SequencherTM 3.0 was also used to align these Genbank sequences with the new specimens and to obtain the number of base pair differences and its % of similarity (p value), estimated as bp differences / length of fragment.

Results

Molecular data

Molecular data was obtained from two individuals (GenBank# KR067394, KR067395). The 16S rRNA fragments with primers excluded were 499 bp long. The two specimens differed from each other by 1 bp (0.2 %). When the 16S rRNA fragments of the Taninul specimens were compared against the other Cubacubaninae whose 16S rRNA has been sequenced, BLAST analysis shows the new specimens most similar to *A. nandalumii* Espinasa *et al*., 2012 (GenBank# JQ340910) and *A. multispinata* Espinasa & Boyko, 2009 (GenBank# KR067393**)**, from which they differ by 102–104 bp (20.4% and 20.8%).

There are 52 species in six genera described within the subfamily Cubacubaninae and most have been sequenced for the 16S rRNA. Currently there are 120 sequences available in GenBank. Using the 16S rRNA fragment sequences of nicoletiid species across the subfamily Cubacubaninae, Espinasa *et al*. *(*2012) concluded that pairs of specimens within a population differ by an average of 1.7 nucleotides (0.3%; range 0–7 bp; n=29), by 3.4 nucleotides (0.7%; range 0–13 bp; n=22) in different populations of the same species, and by 31.2 nucleotides (6.2%; range 10–64 bp; n=14) between sister species. These parameters have subsequently been used several times (e.g., Espinsa & Mathes 2014; Espinasa & Socci 2014) and found to be reliable indicators to support species recognition. A difference of at least 102 bp (20.4%) between the Taninul specimens and any other described species, which had its 16S rRNA sequenced, supports the hypothesis that they belong to a different species, described below.

Taxonomic description

Class Hexapoda Blainville, 1816
Order Zygentoma Börner, 1904
Family Nicoletiidae Escherich, 1905
Genus *Anelpistina* Silvestri, 1905

Anelpistina taninuli sp. nov.
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Figs 1A–I, 2A–I

Etymology

The species epithet, *taninuli*, refers to the locality, Taninul, where the species is found.

Material examined

Holotype

MEXICO: □, 7.5 mm, Taninul Hotel, Highway Cd. Valles Tampico km 15, San Luis Potosi, Mexico, under rocks (21°56'15.20" N, 98°53'25.50' W, 75 masl), 16. Mar. 2013, L. Espinasa, A. Cahill and M. Yurgel colls (ESP2013Taninul1 to be deposited in AMNH).

Paratypes

MEXICO: 3 □□, 5, 4 and 3.3 mm long; 4 □□, 7, 5.5, 4.7 and 4 mm long; collecting data as for holotype. (ESP2013Taninul2–8 to be deposited in AMNH).

Description

Maximum body length 7.5 mm. Maximum conserved length of antenna and caudal appendages 4.5 mm. General color light yellow to white. Pedicellus about ⅔ as long as first article and with unicellular glands on ventral surface, clustered approximately in 4 groups and with a row of microchaetae bordering them in form of a U. On outside lateral border a cluster of about four groups forming a row of unicellular glands (Fig. 1A).

Head with macrochaetae and microchaetae as shown in Fig. 1B–C, with approximately 8 + 8 macrochaetae on border of insertion of antenna. Mouthparts short, labial palp as in Fig. 1D. Length of apical article equal to width and distinctly larger than penultimate article. Penultimate article with bulge containing two macrochaetae. Labium and first article of labial palp with macrochaetae. Maxilla as shown in Fig. 1E. Ultimate article of maxillary palp approximately equal in length to penultimate article. Apex of galea with two conules of different widths (Fig. 1F). Lacinia as in Fig. 1F. Mandible chaetotaxy as in Fig. 1G, with approximately four macrochaetae heavily sclerotized plus several macrochaetae less sclerotized.

Nota with about 6 macrochaetae on lateral borders apart from several setae of varied sizes (Fig. 1H–I). Legs as shown in Fig. 2A. Hind tibia approximately 3 times longer than wide and slightly shorter than tarsus. Claws with a hairy appearance (Fig. 2B), similar to other *Anelpistina* (Espinasa *et al*. 2007). Abdominal sterna II-VII subdivided into coxites and sternites and sterna VIII and IX of male entire (Fig. 2E–H), as in other members of subfamily. No apparent modifications in urosternum III of male. Urosternum IV of adult male with 1 + 1 articulated submedian appendages, their length being about 2× their width (Fig. 2C). Appendages acute, pointy and narrow, except at their base where they are broad, with a bulge on edge towards stylets. Appendages surpass stylets of this segment by half length of stylets (Fig. 2C). Apex of appendages slightly curved, but not quite hooked. Urosternum VIII of adult male deeply emarginated, its projections rounded (Fig. 2E). Urosternum IX of adult male as in Fig. 2D–E. Point of insertion of parameres in urosternum IX very deep. Coxal processes with a few slightly more

sclerotized setae (Fig. 2D–E). Stylets IX slightly larger than others, with two macrochaetae and extra subapical pair. Other stylets with one macrochaeta plus subapical pair (Fig. 2E, H). Terminal spine with small teeth. Stylets IX otherwise without modifications in males. Penis and parameres of adult males as in Fig. 2D–E. Parameres attaining about half length of stylets IX, bend distinctly outwards, tapering on

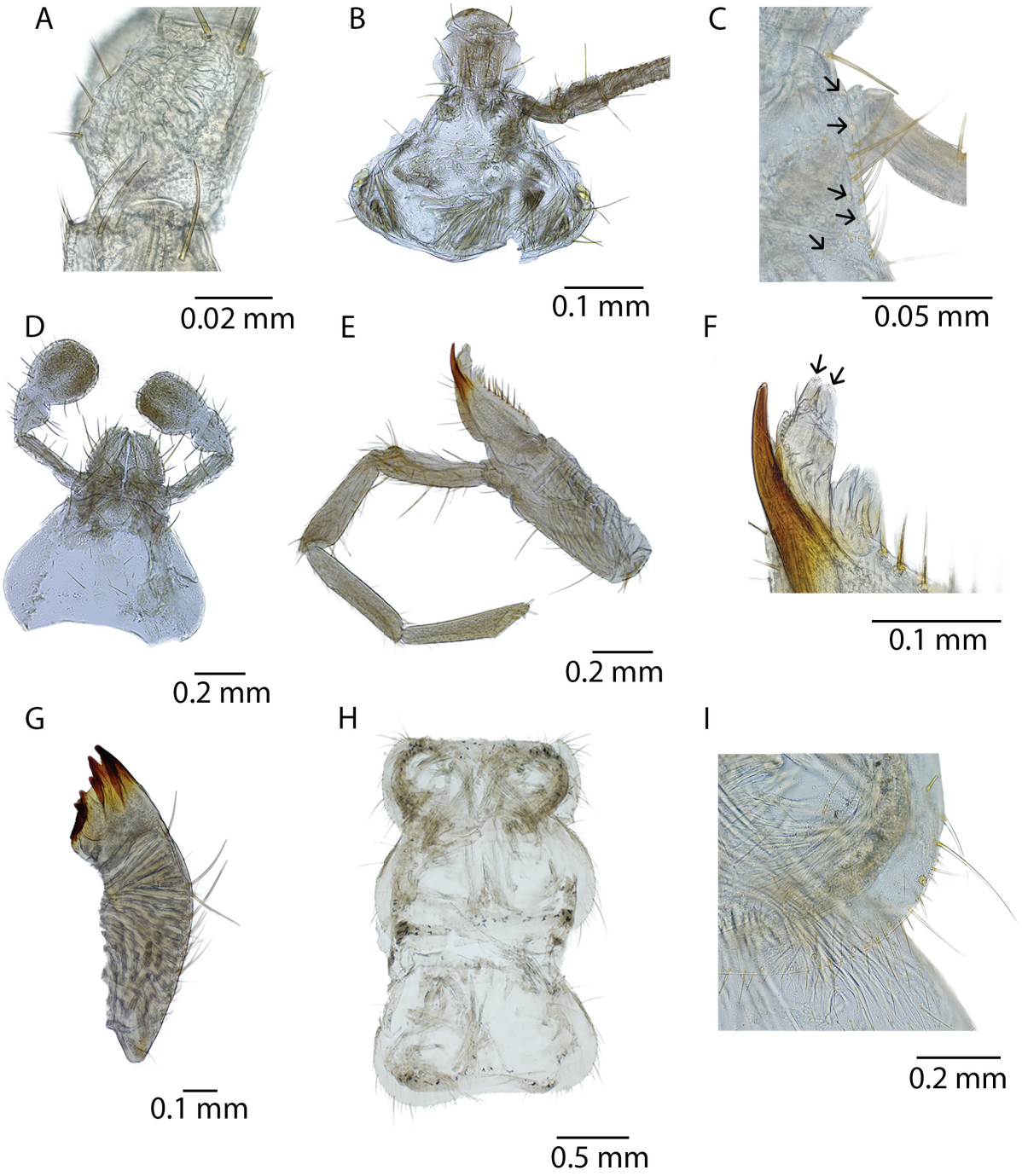

Fig. 1. *Anelpistina taninuli* sp. nov. Holotype, adult □. **A**. Pedicellus (outside lateral view). **B**. Head. **C**. Border of insertion of antenna (arrows point at alveoli where macrochaetae have fallen). **D**. Labium. **E**. Maxilla. **F**. Apex of lacinia and galea. Galea with two conules of different widths. **G**. Mandible. **H**. Nota. **I**. Border of nota.

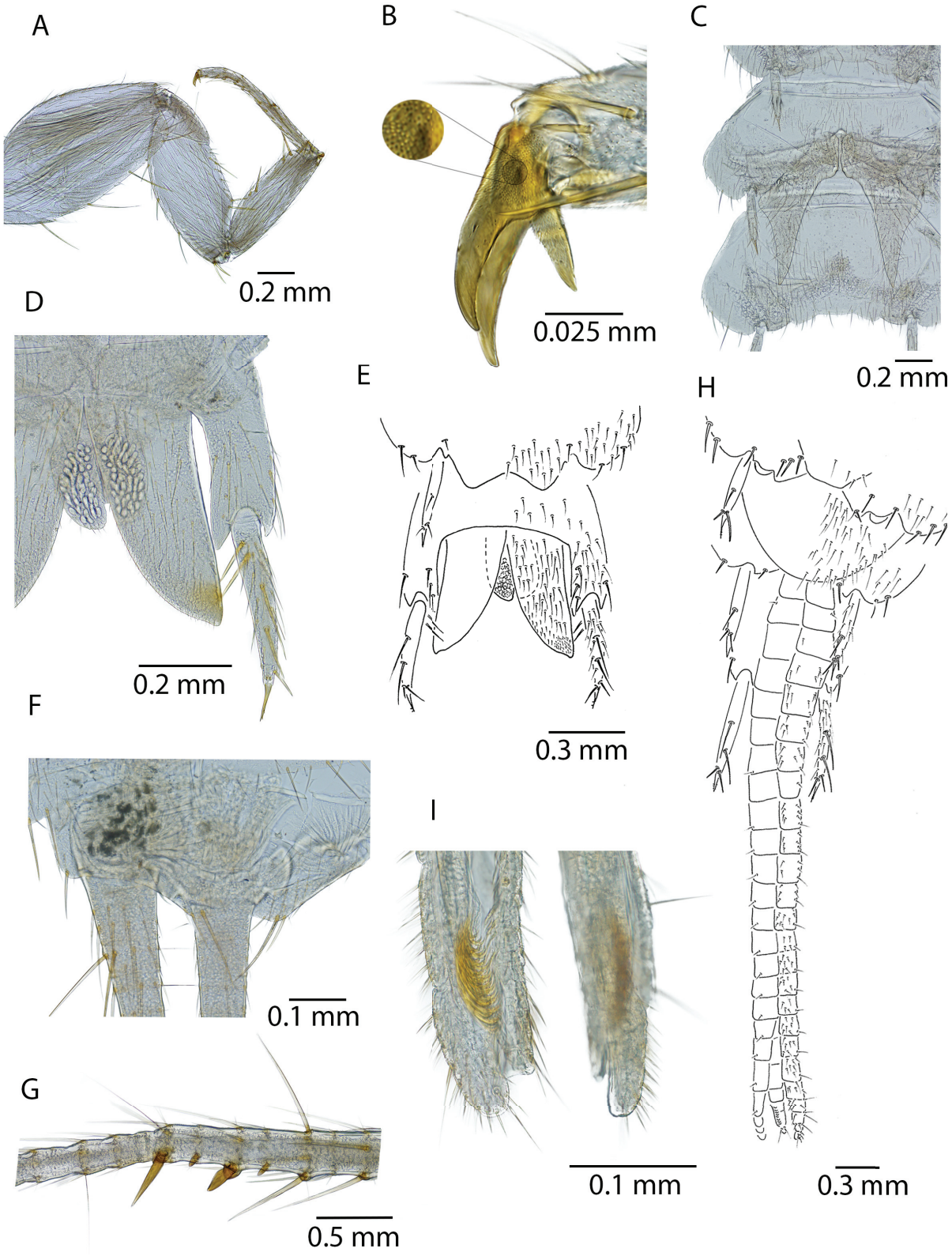

Fig. 2. *Anelpistina taninuli* sp. nov. Holotype, adult ♂ and paratype, ♀. **A**. Hind leg. **B**. Claws with enlarged section showing hairy appearance. **C**. Urosternum IV. **D**. Male genital area. **E**. Urosternum VIII-IX. **F**. Urotergite X. **G**. Cercus. **H**. Ovipositor and subgenital plate. **I**. Apex of ovipositor.

the inner side to create an acute and slightly hooked apex. Distal portion somewhat sclerotized and with small, specialized hairs (Fig. 2D).

Urotergite X shallowly emarginated, posterior angles with two macrochaetae, one distinctly longer, plus a few relatively strong setae (Fig. 2F). Length of inner macrochaetae slightly longer than distance between them. Cercus of adult male with longer than wide basal annulus, followed by three annuli of about equal length and width, and then a very long annulus with spines. Composition of spines includes 4 spines; a long, acute and slightly curved spine, a small one, a strong, subacute spine, and a small one. The two large spines inserted in tubercles (Fig. 2G).

Adult female genital area as in Fig. 2H. Subgenital plate rounded, half as long as wide. Ovipositor surpassing stylets IX by about 2.75 times the length of stylus and gonapophyses with about 25 pseudoarticles. Distal portion with modified setae (Fig. 2I).

Postembryonic development

The holotype male was 7.5 mm long and had glands in the pedicellus, an articulated appendage on urosternum IV, and spines on the cerci. All three other smaller males (5, 4 and 3.3 mm) lacked all these secondary sexual characters. In the largest female (7 mm), the ovipositor surpasses stylets IX by about 2.75 times their length and the gonapophyses have about 25 pseudoarticles. In the female which is 5.5 mm long, it surpasses them by once their length and has about 22 pseudoarticles. At a length of 4.7 mm they barely reach the tip of the stylets and no subdivisions are evident. At a length of 4 mm the ovipositor is just starting to form.

Distribution

Specimens of this species have been collected only from the type locality.

Discussion

This species is a member of the subfamily Cubacubaninae. It has stylets on urosternite II, but lacks scales, sensory pegs in the appendix dorsalis, and conspicuous lateral lobes bearing numerous glandular pores in the labium. As such, its generic allocation is within *Anelpistina* Silvestri, 1905 (= *Cubacubana* Wygodzinsky & Hollinger, 1977 = *Neonicoletia* Paclt, 1979) as defined by Espinasa *et al*. (2007).

Males of *Anelpistina taninuli* sp. nov. can be differentiated from all previously described *Anelpistina* by the unique articulated appendages of their urosternum IV, that are broad at the base, with a bulge on the edge towards the stylets, which then become acute, pointed and narrow. These appendages somewhat resemble those of *A. multispinata* and *A. boneti* Wygodzinsky, 1946 by being acute and narrow, but they differ in that they are less pointed and, most importantly, lack a bulge at their base. Another defining character of the new species shared with only two other species, *A. bolivari* Wygodzinsky, 1946 and *A. musticensis* Espinasa *et al*., 2009, is that their parameres, which are not extremely long, bend distinctly outwards, tapering on the inner side. The new species is easily differentiated from these two species because *A. bolivari* has broad articulated appendages on urosternum IV and *A. musticensis* does not have the articulated appendages, while the new species has pointed and narrow appendages. Furthermore, both species have a very different spine disposition on the cerci from that of the new species.

Finally, the new species can be differentiated from any other described species within the genus *Anelpistina* by the cercus of adult males. The typical cercus has a longer than wide basal annulus, followed by a very long annulus with spines. Sometimes, between these two, there is an annulus which is wider than long. In the new species, there are three annuli of about equal length and width between the two aforementioned annuli. Caution should be used with this character because the number of basal annuli can vary among individuals of the same population.

The assignment of the specimens to a previously undescribed species is supported by the BLAST analyses which show that their 16S rRNA sequence is very different from that of other *Anelpistina* species.

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