

Full Length Research Paper

A new species of genus *Squamigera* (Insecta: Zygentoma: Nicoletiidae) from the Mayan ruins of Palenque, Chiapas, Mexico

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Due to the difficulty in collecting specimens, the genus *Squamigera* (Insecta: Nicoletiidae: Cubacubaninae) remains one of the least studied groups of the American nicoletioid insects. Before this investigation, only six specimens were available for study. Furthermore, previous assessments of its phylogenetic relationship within the Cubacubaninae used incomplete 16S rRNA sequences that had ambiguous bases from only a single *Squamigera* specimen, thus reducing the resolution. We describe here a new species of *Squamigera* and provide for future studies, a more complete 16S rRNA sequence for the genus using this new species.

Key words: Silverfish, bristletails, thysanura, morphology, molecular data.

INTRODUCTION

When insects of the subfamily Cubacubaninae (Nicoletiidae) are encountered under rocks or in caves of Mexico and Central America, they are frequently abundant. There is one exception: the elusive members of the genus *Squamigera*. Field collections have yielded few samples. Before this study, only six specimens were available for study.

In 1988, the first single male *Squamigera* silverfish was collected in a Central Mexican cave. The specimen was distinctive in many ways. Measuring 22 mm in length, it was one of the largest specimens in the Nicoletioid family. Its body and head were also covered by smooth to serrated scales. Despite many subsequent visits to the same locality, no other specimens were found. Eleven years after the original discovery, the decision was made to describe a new genus with this single specimen (Espinasa, 1999). By 2004, five more specimens were available. Four were found in the collections of the American Museum of Natural History at New York, and one was finally collected from the original cave type

locality. To date, four species have been reported, all with no more than two specimens available for study:

- 1) *Squamigera latebricola* Espinasa, 1999 is a highly cave adapted species which, with a maximum body length of 29 mm, holds the distinction of being the largest nicoletioid in the world. Only two male specimens have ever been collected from a cave in Central Mexico.
- 2) *Squamigera jaureguii* Espinasa and Burnham, 2004 is a surface species from the Gulf of Mexico coastal plains from which only one female specimen is available.
- 3) *Squamigera cumcalcaris* Espinasa and Burnham, 2004 is a cavernicole species from Chiapas, Mexico, described with only one male and one female specimen.
- 4) *Squamigera* sp., Espinasa and Burnham, 2004 is a single juvenile of an undescribed species, reported from a second cave in Chiapas. Formal description of the species is awaiting collection of adult specimens.

In 2007, Espinasa et al. provided a phylogeny of the

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American silverfish Cubacubanae through a combined approach using morphology and five molecular loci. In this study, DNA was extracted from a single *Squamigera* individual; *S. latebricola*. In their results, *Squamigera* appears in between genus *Prosthecina* and *Anelpistina*. Regrettably, the reported sequence for the 16S rRNA in *S. latebricola* had incomplete and ambiguous bases, thus reducing its resolution.

For this study, two collecting field trips in a new locality took place in the ancient ruins of Palenque, Chiapas, Mexico. Four new specimens of a fifth unreported species of *Squamigera* were found. These specimens belong to a different species other than *S. cumcalcaris* and the undescribed juvenile from Chiapas mentioned before. In this paper, we describe a new species of *Squamigera* and we reported its 16S rRNA sequence.

MATERIALS AND METHODS

Morphology

The specimens were found under rocks in the rubble north of the Temple of the Cross in the Mayan ruins of Palenque, Chiapas, Mexico. Specimens were collected by hand and deposited in 100% ethanol. Dissections were made with a Motic K series stereo microscope and the different body parts were mounted in fixed preparations with Cytoseal™ 60 solution (Richard-Allan Scientific). Illustrations were made with the aid of a camera lucida attached to a compound microscope.

Molecular data

Genomic DNA was extracted using a Qiagen DNEasy® Tissue Kit by digesting a leg of the two male specimens in lysis buffer; one specimen is the paratype collected on 3/16/03 and the other is the holotype collected on 7/21/11. Amplification and sequencing of the 16S fragment was completed as described in Espinasa et al. (2007). Chromatograms obtained from the automated sequencer were read and contigs were made using the sequence editing software Sequencher™ 3.0. External primers were excluded from the analyses.

RESULTS AND DISCUSSION

Molecular data

16S rRNA molecular data were obtained for two specimens collected eight years apart and both had identical sequences. The 16S rRNA fragments were 509 bp long (with primers excluded). GenBank accession number is KC854406.

The previously reported partial 16S rRNA sequence (GenBank AY084073.1) for *S. latebricola* was 446 bp long and had multiple ambiguous base pairs. The new sequence reported here of a *Squamigera* member is longer and has better quality than what was previously reported. In their overlapping sequence, the new species and *S. latebricola* have 99 disagreements. While there is no “magical number” by which to determine how much

DNA sequence difference is needed to make two populations belong to the same or different species; comparison with other species can be informative. Using the 16S rRNA fragment sequences of nicoletioid species across the subfamily Cubacubanae (Espinasa and Giribet, 2009), it can be observed that pairs of specimens within a population differ by an average of 1.7 nucleotides (range 0 to 7; n = 29), by 3.4 nucleotides (range 0 to 13; n = 22) in different populations of the same species, and by 31.2 nucleotides (range 10 to 64; n = 14) among sister species. A 99 disagreements supports they are different species. Particularly because this is the disagreements in only their overlapping sequence. If a longer sequence was available for *S. latebricola*, a higher number may be expected. New species of *Squamigera pakali*, Espinasa, Botelho and Socci are shown in Figures 1, 2A-G, and 3A-F.

Type material

Under rocks, North of the Temple of the Cross, Palenque ruins, Chiapas, México, 17°28'59" N 92°02'42" W, 170 m above sea level, and in the nearby jungle by the side of one of the creeks. Male holotype (6.5 mm) and two female paratypes (11.3 and 8.5 mm) collected on 7/21/11 and one male paratype (6 mm) collected on 3/16/03. Espinasa L., Cahill A., Socci K, McCaffery S., Espinasa R., and Espinasa J. cols. types will be deposited in the collection of the American Museum of Natural History, New York.

Description

Maximum body length of adult female is 11.3 mm. Male holotype is 6.5 mm long and not fully mature. Maximum conserved length of antennae is 9 mm and of caudal appendages is 5 mm. General color is light yellow to white. Morphology of the body is as shown in Figure 1. There are smooth edged scales (Figure 2B) in the head, but become increasingly serrated (Figure 3B) in the rest of the body and proximal segments of the legs (Figure 2G).

Antennae of females and juvenile male are as shown in Figure 2A. Pedicellus is half as long as basal article. No adult males were available for study, but it is expected that pedicellus could be longer and with the unicellular glands typical of the genus (Espinasa and Burnham, 2004). Head is with approximately 8 + 8 macrochaetae on border of insertion of antennae (Figure 2A). Mouthpart appendages are relatively short. Labial palp is shown in Figure 2C. Apical article's width is equal to its length and longer than penultimate article. Penultimate article with bulge contain macrochaetae. Labium and first article of labial palp is with macrochaetae. Maxilla is shown in Figure 2D. Last article



Figure 1. *S. pakali* n. sp. Male holotype. Lines are 1 mm.

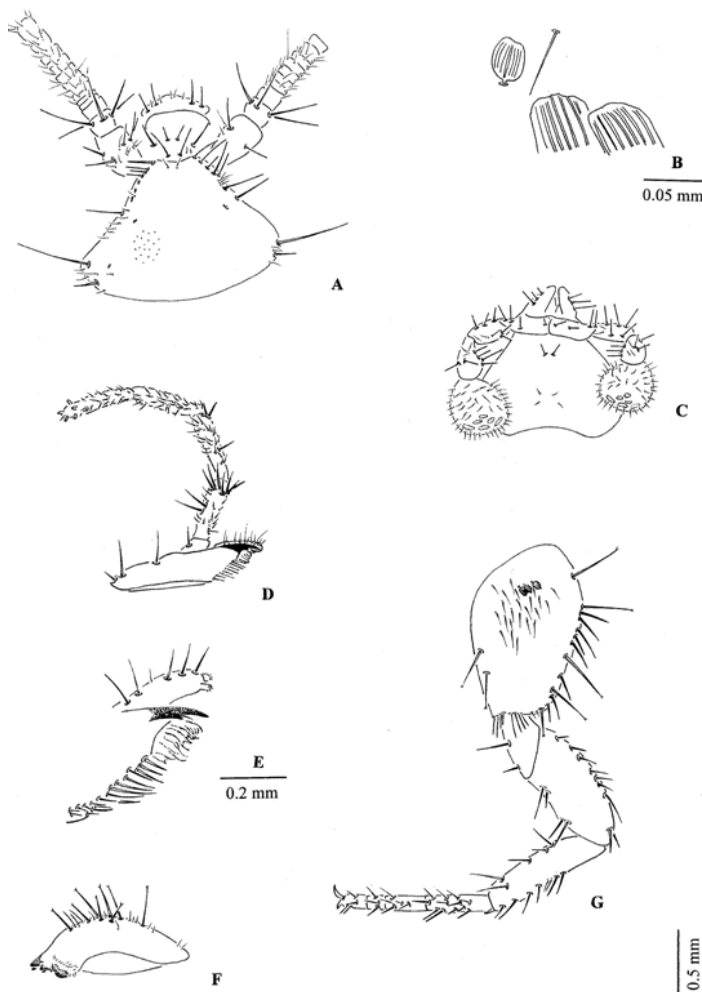


Figure 2. *S. pakali* n. sp.: Female paratype, scales and microchaetae are partially shown. A, Head; B, scales from base of head; C, labial palps and labium; D, maxilla; E, apex of maxilla; F, mandible; G, 3rd leg.

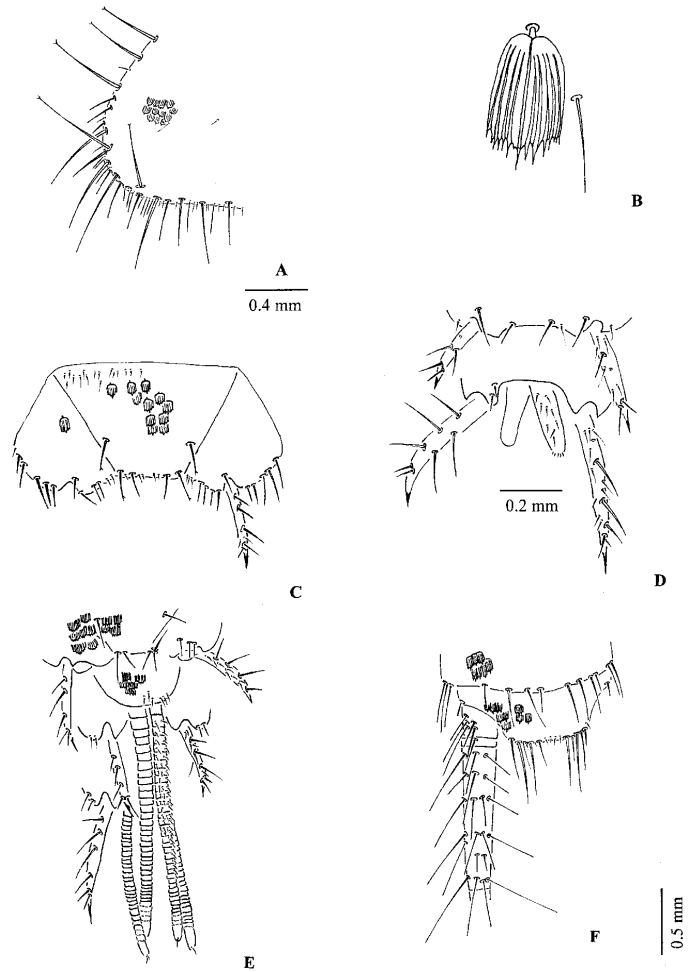


Figure 3. *S. pakali* n. sp.: D, Male holotype; A-C, E-F, female paratype. Scales and microchaetae are partially shown. A, Mesonotum; B, abdominal scale; C, urosternum IV; D, juvenile male genital area; E, female genital area; F, urotergite X and cercus.

of maxillary palp is equal in length to penultimate. Apex of galea is with two conules of similar width. No 3rd minute extra conule is apparent (Figure 2E). Two teeth on lacinia. Mandibles chaetotaxy as in Figure 2F, with about 8 macrochaetae.

Legs are relatively short and stout. Tibia on the 2nd leg with five macrochaetae, some of them stout, and approximately 3.3 times longer than wide and 1/5 shorter than tarsus. Tibia on 3rd leg with five macrochaetae, and approximately 3.75 times longer than wide and 1/4 shorter than tarsus (Figure 2G). Claws are smooth, without a hairy appearance and of normal length, as seen in Espinasa et al. (2007).

Thoracic nota with scales and many macrochaetae on lateral borders apart from several setae of varied sizes (Figure 3A), but no small sclerotized spines on posterior borders, as in *S. latebricola*.

Abdominal sterna I entire, sterna II-VII subdivided into coxites and sternites and sterna VIII and IX of male

entire, as in other members of subfamily (Mendes, 1988). Urosterna III and IV (Figure 3C) of examined juvenile male is without modifications. It is currently unknown if adult specimens will develop modifications, as in *S. latebricola*. Urosterna VIII posterior projections is acute to slightly rounded, subtriangular (Figure 3D).

Urosterna IX of male is shown in Figure 3D. Point of insertion of parameres deep and setae are slightly more sclerotized on internal face of coxal processes (Figure 3D). Stylets IX are bigger than the others, typically with four macrochaetae and an extra subapical pair. In both males and females there are no spines or other modifications. Other stylets have only three macrochaetae plus the subapical pair. Parameres are shown in Figure 3D. Parameres in the juvenile male attain less than $\frac{1}{3}$ of stylets IX length, although adult males may have larger paramera, as it is common for the subfamily. Parameres are straight and narrow with the normal distinct group of microchaetae on the tip. Subgenital plate of female is parabolic to slightly trapezoid (Figure 3E). Ovipositor surpassing apex of stylets IX by less than $\frac{1}{3}$ the length of stylets (Figure 3E). Gonapophyses are with approximately 38 articles, although the last segments are not yet fully defined, possibly due to a certain degree of immaturity.

Urotergite X posterior angles with several long macrochaetae and setae of different sizes (Figure 3F). Female and juvenile male caudal appendages are as shown in Figure 3F. It is likely that adult males will develop spines, as in other members of the genus (Espinasa and Burnham, 2004).

Postembryonic development and known range

Both available males (6.5 and 6 mm) are not yet fully mature. Pedicellus lack unicellular glands, paramera attain less than $\frac{1}{3}$ of stylets IX, and cercus without spines. In the largest female (11.3 mm), the ovipositor surpasses apex of stylets IX by less than $\frac{1}{3}$ the length of stylets and gonapophyses with approximately 38 articles. In the other female (8.5 mm), ovipositor barely surpasses the apex of the stylets and gonapophyses with more than 30 articles, but distal segments are not yet fully differentiated. Its range is known only from the type locality.

Etymology

The name *pakali* is derived from K'inich Janaab' Pakal (23 March 603 – 28 August 683), ruler of the Maya polity of Palenque in the Late Classic period. During a long reign of some 68 years, Pakal was responsible for the construction or extension of some of Palenque's most notable surviving inscriptions and monumental architecture.

DISCUSSION

S. pakali n. sp. can be differentiated from all described species of *Squamigera* by apparently lacking a third small conule in the maxillary palp. It can furthermore be differentiated from the other described species by the following characters:

S. latebricola is a troglobite, endemic to a cave in Central Mexico, whose adaptations include gigantism and highly elongated appendages (body up to 29 mm, tibia 5 times longer than wide, apical article of labium barely shorter than penultimate, and maxilla's last article shorter than penultimate), while *S. pakali* is a surface species with shorter and more stout body proportions (maximum body length available, 11.3 mm; tibia 3.75 times longer than wide; apical article of labium longer than penultimate; and maxilla's last article equal to penultimate).

S. jaureguii has a very long ovipositor, surpassing the apex of stylets IX by thrice the length of the stylets and its gonapophyses has approximately 53 articles in its longest female (9.5 mm). In the largest female available of the new species (11.3 mm), even though it is longer than the aforementioned species, it has a smaller ovipositor; the ovipositor surpasses the apex of stylets IX by less than $\frac{1}{3}$ the length of stylets and the gonapophyses has approximately 38 articles. Furthermore, the subgenital plate in *S. jaureguii* is also much flatter distally than in the new species.

S. cumcalcaris inhabits the same state of Chiapas in a cave about 80 km away from the new species. Its largest female (15 mm) also has a longer ovipositor than the new species, surpassing the apex of stylets IX by thrice the length of the stylets, but in both species, the gonapophyses is divided in approximately the same number of articles, 38. The two species can further be differentiated because in *S. cumcalcaris* the head has approximately 5 + 5 macrochaetae on border of insertion of antennae, and the labial palp's apical article is wider than it is long. In contrast, the new species has approximately 8 + 8 macrochaetae and the apical article's width is equal to its length. It remains to be seen if male adults of the new species develop the assortment of unique sexual secondary characters that *S. cumcalcaris* has. It is unlikely that *S. pakali* n. sp. will develop such globular parameres of *S. cumcalcaris* since the juvenile of the new species has slender parameres.

The new 16S rRNA sequences will also allow for better phylogenetic analyses to be performed in the future.

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