

When troglomorphism dupes taxonomists: morphology and molecules reveal the first pyramidopid harvestman (Arachnida, Opiliones, Pyramidopidae) from the New World

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Cavernicolous species that exhibit a high degree of troglomorphism often provide some of the most intriguing evolutionary riddles. For such taxa, the correct systematic arrangement is difficult to determine and becomes problematic when based solely on highly convergent external morphological characters, leading to exaggerated support of spurious relationships. For the arachnid order Opiliones, examination of male genitalia morphology often aids in determining the family to which a particular taxon belongs. However, many taxa described prior to the 1990s lack detailed descriptions or drawings of this important character and, for highly-derived species, it is may still be necessary to seek support from additional sources of characters (e.g. molecular data) to accurately assess systematic placement. The enigmatic species *Stygnomma pecki* Goodnight & Goodnight, 1977 from a cave in Belize proved to be especially difficult to place based on morphological characters alone. Thus, using a previously published dataset for laniatorean harvestmen, we carried out a robust phylogenetic analysis aiming to determine the evolutionary relationship of this Neotropical troglomorph species. Informed by the results of the molecular phylogenetic analysis of 88 terminals representing Laniatores, we describe ***Jarmilana* gen. nov.** and provide a redescription of the type species *Jarmilana pecki* (Goodnight & Goodnight, 1977) **comb. nov.** Morphological evidence, including male genitalia morphology, supports the inclusion of *J. pecki* in the family Pyramidopidae. This represents the first record for the family Pyramidopidae in the New World, raising the question of whether this represents transoceanic dispersal or a relict of an ancient widespread tropical Gondwanan distribution.

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INTRODUCTION

Systematics of laniatorean harvestmen (Opiliones: Laniatores) has received increasingly more attention in the last decade (Giribet & Sharma, 2015) and

recent major revisions have resulted in many new systematic arrangements and descriptions of supraspecific taxa (Kury & Pérez-González, 2002; Kury, 2003; Pinto-da-Rocha & Hara, 2009; Sharma & Giribet, 2011, 2014; Sharma, Prieto & Giribet, 2011; Kury, 2012, 2014; Pinto-da-Rocha *et al.*, 2014). This has been greatly facilitated by the inclusion of a detailed description of male genitalic morphology, a structure that has played an increasingly important

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role in defining natural groups in harvestmen (Macías-Ordóñez *et al.*, 2010; Pérez-González, 2011; Pinto-da-Rocha *et al.*, 2012). Additionally, molecular data are rapidly advancing our understanding of evolutionary relationships of harvestmen, particularly within and among diverse Laniatores groups, often providing important insights about the systematic position of odd or poorly known taxa (Giribet *et al.*, 2010; Sharma & Giribet, 2011; Sharma, Prieto & Giribet, 2011; Pinto-da-Rocha *et al.*, 2014; Bragagnolo, Hara & Pinto-da-Rocha, 2015). The datasets produced by these studies have become a powerful resource for testing the affinities of taxa that are difficult to place based only on morphological traits, especially when character homology is masked by strong convergent evolution; for example, as observed in some cavernicolous species that exhibit a high degree of troglomorphy.

Despite recent advances in our understanding of harvestmen systematics (Giribet & Sharma, 2015), within Laniatores, there are still many non-natural groups consisting of multiple diverse lineages that require major systematic revision. This is partly a result of the typological approach engendered by early classification systems (e.g. the Roewerian system) in which a restricted number of convergent morphologies (e.g. the presence of tarsal scopula, tarsal formula, armature of ocularium) were used in combination to create many non-natural groups or, in some cases, to create a 'wastebasket taxon' into which species were 'dumped' based on a lack of characters. Among laniatorean harvestmen, the family Stygnommatidae is the epitome of wastebasket taxa because it contains species without a common ocularium that also lack a tarsal scopula. The family currently comprises one genus and 33 species (Kury, 2013b), although several studies have indicated that this is a non-monophyletic assemblage (Pérez-González, 2006, 2007; Pérez-González & Kury, 2007; Sharma & Giribet, 2011).

Within the genus *Stygnomma* Roewer, 1912, one of the most remarkable and enigmatic species is *Stygnomma pecki* Goodnight & Goodnight, 1977, a troglobite from Belize. The species authors recognized the unusual morphology of this species and indicated their uncertainty regarding relationships with other species of the genus by stating that 'It is unusual in appearance ... It bears no obvious relationships to any forms we have previously observed.' (Goodnight & Goodnight, 1977: 148). In an unpublished thesis, Pérez-González (2006) recognized that the male genitalia of this species did not match that of the Stygnommatidae s.s. (as subsequently defined in Pérez-González, 2007), nor any of the families belonging to the clade Samooidea + Zalmoxoidea. Pérez-González (2006) considered that the male genitalic morphology related this species with a group formed by Stygnop-

sidae, Epedanidae, 'Pyramidopidae', and Assamiidae (but, for current superfamilial concepts, see also Giribet & Sharma, 2015) and suggested that it was most likely related to Stygnopsidae because this was the only one of the four known from the New World.

Recent field expeditions in Belize have provided fresh material that enabled us to carry out a detailed study of *S. pecki*. In the present study, we investigate the systematic position of *S. pecki* using a molecular phylogenetic approach. Based on results of our analysis, we propose new familial and generic assignments. We provide a diagnosis for ***Jarmilana* gen. nov.** and a redescription of the species ***Jarmilana pecki* comb. nov.** based on detailed observations of morphological characters that strongly support the new taxonomic changes.

MATERIAL AND METHODS

MATERIAL EXAMINED

The material examined has been deposited in the Colección Nacional de Arácnidos (CNAN) UNAM, Mexico; American Museum of Natural History (AMNH), New York, USA; Texas Memorial Museum (TMM), Texas, USA, and Museo Argentino de Ciencias Naturales (MACN), Buenos Aires, Argentina. We adopted the terms alpha and beta male commonly used for Opiliones (Kury, 2008, 2013a; Ferreira & Kury, 2010; Kury & Ferreira, 2012; Ázara, DaSilva & Ferreira, 2013) and equivalent to Major/Minor males (Zatz *et al.*, 2011; Buzatto & Machado, 2014) to describe the dimorphic male condition in which larger, more strongly armed males (= alpha) are distinct from smaller, weakly armed males (= beta). One beta male of ***J. pecki* comb. nov.** was dissected and prepared for scanning electron microscopy (SEM) as described by Acosta, Pérez-González & Tourinho (2007). For SEM preparation, the specimens were dehydrated in a series of increasing concentrations of ethanol (85, 90, 95 and 100%), and dried using hexamethyldisilazane (Brown, 1993). After drying, they were mounted on adhesive copper tape (EMS 77802; Electron Microscopy Sciences) affixed to a stub and sputter coated with Au-Pd. Specimens were examined at accelerating voltages of 10–20 kV under high vacuum with a FEI XL30 TMP (at the MACN) and a Hitachi SU1510 (at the Instituto de Biología, UNAM). Photographs of ethanol preserved specimens were obtained with a DFC 290 digital camera (Leica) attached to a M165C stereomicroscope (Leica), and the focal planes were combined using HELICON FOCUS PRO (www.heliconsoft.com). Male genitalia were temporarily mounted in glycerol and drawn using a camera lucida attached to a BH-2 compound microscope (Olympus). To

expand the glans, the male genitalia was immersed in a hot, saturated solution of KOH for 2 min, and afterwards transferred to distilled water. All images were edited using Photoshop CS5 software (Adobe Systems Inc.).

TAXON SAMPLING

We used a subset of data from Sharma & Giribet (2011) to include a broad sampling of 81 species of Grassatores plus five outgroup taxa represented by Triaenonychoidea and Travunioidea. Additionally, '*Stygnomma* sp.' (MCZ DNA 106176; Sharma & Giribet, 2012) was included as an additional representative of the South American fauna of Samooidea (although it is not *Stygnomma* or Stygnommatidae s.s. as defined in Pérez-González, 2007). We added sequence data for *J. pecki* comb. nov. (MACN-DNA-Op024) to these previously sequenced taxa for a total of 88 terminals in our molecular phylogenetic analysis.

MOLECULAR METHODS

Sequence data were retrieved from GenBank via Batch Entrez for 87 species of Laniatores. GenBank files were parsed using the perl script parseGB.pl (<https://sites.google.com/site/shannonhedtke/Scripts>). Reconstruction of the Laniatores phylogeny utilized eight of the ten markers employed by Sharma & Giribet (2011), including three mitochondrial genes (i.e. for 12S rRNA, 16S rRNA, and cytochrome *c* oxidase subunit I) and five nuclear genes (i.e. for 18S rDNA, 28S rDNA, histones *H3* and *H4*, and *U2* snRNA). We excluded cytochrome *b* and elongation factor-1 α from our analysis because a large number of taxa (> 70%) in the dataset lacked sequence data for the appropriate genes.

Total DNA was extracted from one adult female *J. pecki* comb. nov. (MACN-DNA-Op024) using DNEasy tissue kits (Qiagen) by soaking two legs in lysis buffer overnight (approximately 14 h) as described in Boyer, Karaman & Giribet (2005). Genomic DNA was used as a template for amplification by a polymerase chain reaction (PCR) of three gene fragments (*COI*, complete 18S, and partial 28S). *COI* was amplified using the primer pair LCO1490-HCOoutout (Folmer *et al.*, 1994; Prendini, Weygoldt & Wheeler, 2005). 18S was amplified using three primer pairs that produce overlapping regions, including 18S 1F-5R, 18S 3F-bi, and 18S a2.0-9R (Giribet *et al.*, 1996; Whiting *et al.*, 1997). For 28S, the region amplified was bounded by the primer pair 28S rd4.8a-rd7b1 (Schwendinger & Giribet, 2005). More information about primer sequences is provided in Sharma & Giribet (2011). Reactions were carried out in 15- μ L volumes con-

sisting of 0.5 μ M each primer, 200 μ M dNTPs, 1 \times PCR buffer, 1.5 mM MgCl₂, 1.5 U of Taq DNA Polymerase (Thermo Scientific), and 1–2 μ L of DNA template. For 28S and 18S, cycles were run using a step-down protocol (Evans & Paulay, 2012) that involved an initial denaturation step (95 °C for 5 min), 15 high-specificity cycles (95 °C for 30 s, 51 °C for 45 s, 72 °C for 45 s), and 20 standard-specificity cycles (95 °C for 30 s, 49 °C for 45 s, 72 °C for 45 s). For *COI*, annealing temperatures were modified to 45 °C, stepped down to 42–43 °C.

PCR products were visualized by agarose gel electrophoresis (1.2% agarose) and later purified by adding 0.5 μ L of Exonuclease I and 1.0 μ L of FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) and running reactions at 37 °C for 30 min followed by 85 °C for 15 min. Products were sequenced using the forward primers, carried out at the Instituto Nacional de Tecnología Agropecuaria (Buenos Aires, Argentina) or Macrogen (Seoul, South Korea).

Chromatograms were viewed and automated sequence reads were edited using SEQUENCHER (Gene Codes). Most sequences were aligned using MUSCLE (Edgar, 2004). Sequences for 28S and 12S were aligned using Q-INS-i and L-INS-i algorithms, respectively, implemented in MAFFT (Kato, 2013) through the online CBRC portal using the parameters: 200 PAM/*k* = 2 scoring matrix, gap opening penalty of 1.53, and an offset value of 0.1. Alignments were inspected and manually adjusted, and were treated with GBLOCKS, version 0.91b (Castresana, 2000) to identify and remove ambiguous sites with the allowed gap positions parameter set to 'with half', the minimum number of sequences for a flanking position set to 75% of the total number of sequences in a partition, and the remaining parameters at their default setting. Lengths of the original sequence alignment and final selected blocks are provided (Table 1). Sequences were concatenated, and file types were converted, using the perl script BeforePhylo.pl (<https://github.com/qiyunzhu/BeforePhylo>) customized to generate relaxed phylip format.

We assessed the substitution saturation in the third-position of codons for protein-coding genes (*COI*, *H3*, *H4*) using a test for substitution saturation (Xia *et al.*, 2003; Xia & Lemey, 2009) implemented in DAMBE5 (Xia, 2013). The third-position of *COI* was highly saturated by substitutions; thus, the phylogenetic signal was obscured, which is an expected outcome for a broad sampling of taxa across diverse lineages of Laniatores. Therefore, we partitioned *COI* by codon position, retained positions 1 and 2 as separate partitions, and excluded position 3 from our

Table 1. Selection of conserved blocks for each gene sequence after treatment with GBLOCKS

Gene	Number of sequences	Original sequence length	Length of selected blocks	Number of selected blocks
<i>12S</i>	42	387	143	8
<i>16S</i>	26	490	374	13
<i>18S</i>	88	1828	1728	10
<i>28S</i>	88	3761	2332	22
<i>COI</i>	56	814	642	1
<i>H3</i>	71	327	321	1
<i>H4</i>	74	159	159	1
<i>U2</i>	40	131	131	1

analysis. We retained all three codon positions for *H3* and *H4*.

Four errors were discovered among the sequence data available for Laniatores and we indicate those errors here so that future studies that utilize this dataset may take these changes into account. Sequence accession numbers are from GenBank, and we refer to accession numbers listed in Sharma & Giribet (2011). GenBank accession number FJ796492 refers to a *COI* sequence that belongs to *Caenocopus* sp. MCZ DNA102593, and thus *COI* sequence data for *Cynortula granulata* MCZ DNA100332 do not exist at this time. Additionally, we discovered that *16S* sequence data available for *Pellobunus insularis* MCZ DNA101421 (GenBank# JF786469) was identical to *Pyramidops* sp. MCZ DNA101432 (GenBank# JF786468), which is implausible. We determined that the sequence belongs to *Pyramidops* sp. and thus we omitted the *16S* sequence data for *P. insularis*. Similarly, we discovered that *H4* sequence data for *Glysterus* sp. MCZ DNA101422 (GenBank# FJ475957) were identical to *Icaleptes* sp. MCZ DNA101420 (GenBank# FR850199) and determined that the sequence belongs to the latter species. Finally, sequence data for *Neopygoplus siamensis* were previously only available in GenBank for three genes (*COI*, *18S*, and *28S*); however, sequences for three additional genes were presumably included in the analysis of Sharma & Giribet (2011) but never published. Sequence data have been made available for histone *H3* (KU049766), histone *H4* (KU049767), and *U2* snRNA (KU049768) from *N. siamensis* (MCZ DNA104858).

PHYLOGENETIC METHODS

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference. ML analysis was conducted using RAXML, version

8.1.11 on XSEDE (Stamatakis, 2014) through the online CIPRES portal. Data were partitioned by gene, with *COI* partitioned into first and second codon positions, resulting in nine data partitions for the ML analysis. We inferred trees using a GTR + GAMMA model of sequence evolution for each data partition and estimated support values with 1000 bootstrap replicates.

Bayesian inference analysis was conducted using MrBayes, version 3.2.3 (Ronquist *et al.*, 2012) on XSEDE through the online CIPRES portal. Data were partitioned by gene, and protein coding genes *H3* and *H4* were further partitioned by codon position, resulting in a 13 partition scheme. Best-fit models of molecular evolution were specified for each partition (Table 2) as selected under the Bayesian information criterion using PARTITIONFINDER, version 1.1.1 (Lanfear *et al.*, 2012). The analysis consisted of two simultaneous runs each with four chains for 20 000 000 generations sampling every 1000 trees. The initial 25% of sampled trees were discarded as burn-in. The average SD of split frequencies between runs was < 0.01. Stationarity of parameters was visually confirmed in TRACER, version 1.6 (Rambaut *et al.*, 2014) and stationarity of tree topologies was confirmed using compare plots generated by AWTY (Nylander *et al.*, 2008). To investigate whether missing data had an effect on the placement of the taxon of interest, we analyzed a dataset consisting of only the genes available for *J. pecki* **comb. nov.** in a four partition scheme (i.e. *18S*, *28S*, and *COI* codon positions 1 and 2). There was no change in the nodal support for taxa relevant to our study; thus, we proceeded to use the eight gene dataset that provided better resolution of deep

Table 2. Best-fit models for sequence evolution for each partition selected under Bayesian information criterion using PARTITIONFINDER

Partition	Model
<i>12S</i>	GTR + I + G
<i>16S</i>	GTR + I + G
<i>18S</i>	GTR + I + G
<i>28S</i>	GTR + I + G
<i>COI</i> , first codon position	GTR + I + G
<i>COI</i> , second codon position	GTR + I + G
<i>H3</i> , first codon position	GTR + I + G
<i>H3</i> , second codon position	JC
<i>H3</i> , third codon position	GTR + G
<i>H4</i> , first codon position	GTR + I + G
<i>H4</i> , second codon position	JC
<i>H4</i> , third codon position	GTR + G
<i>U2</i>	GTR + I + G

phylogenetic relationships and facilitated direct comparisons with previous studies.

RESULTS AND DISCUSSION

PHYLOGENETIC ANALYSIS

New sequences for *J. pecki* **comb. nov.** have been submitted to GenBank for three genes: *COI* (KU049765), complete *18S* (KU049764), and partial *28S* (KU049763). The tree topology obtained by Bayesian inference was generally in agreement with the previous analyses of Sharma & Giribet (2011) with respect to the monophyly of families and some major superfamilial clades (Fig. 1). However, in our analysis, utilizing a subset of genes from Sharma & Giribet (2011), making several corrections to sequence data, and employing a different partitioning scheme had some impact on family level relationships (Fig. 1). The implications for these findings are discussed below. The phylogenetic tree obtained by ML (see Supporting information, Fig. S1) was generally congruent with Bayesian analyses (Fig. 1).

FAMILIAL ASSIGNMENT IN PYRAMIDOPIDAE: MOLECULAR AND MORPHOLOGICAL EVIDENCE

The African family Pyramidopidae, represented in our analysis by *Conomma oedipus* Roewer, 1949 and *Pyramidops* sp. is recovered as monophyletic [bootstrap support (BS) = 100%, posterior probability (PP) = 1.00]. *Jarmilana pecki* **comb. nov.** was recovered as sister to African pyramidopids with strong support in both analyses (BS = 99%, PP = 1.00).

The external morphology of *J. pecki* **comb. nov.** is extremely misleading because it is highly convergent with other troglobitic species, as demonstrated by the strongly developed pedipalps, the loss of eyes, and the dubious limits of an ocularium (Fig. 3). These troglomorphic characteristics, combined with the hourglass-shaped *scutum magnum* and the disregard for examining penis morphology, erroneously led the species authors to conclude that it belonged to Stygnommatidae.

Detailed examination of the male genitalic morphology provides supporting evidence with respect to placement of *J. pecki* **comb. nov.** in Pyramidopidae. Pyramidopids typically exhibit complex and aberrant penis morphologies. The pars distalis is armed with characteristic large paired macrosetae (with exceptions) and the glans lacks prominent parastylar conductors (Sharma *et al.*, 2011). The penis of *J. pecki* **comb. nov.** does not have a strongly developed par distalis, as is found in many

pyramidopids (Sharma *et al.*, 2011), although it does exhibit the characteristically large macrosetae, a simple glans with a follis ending in two large dorsoapical lobes, and a simple stylus without parastylar conductors (Figs 10, 11). The penis morphology of *J. pecki* **comb. nov.** shares similarities with other pyramidopids that possess a shorter, less complex and slightly swollen pars distalis; compare Fig. 10A to fig. 5n in Sharma *et al.* (2011). The large dorsoapical lobes of the follis are strikingly similar to *Conomma* sp. from Cameroon (Sharma *et al.*, 2011: fig. 5a) and *Pyramidops pygmaeus* Loman, 1902 from Nigeria (for which the genitalia was illustrated and homology of setae was interpreted by Kury & Villarreal, 2015: fig. 22d–f). Also, the mode of hydraulic expansion shows the affinities between *J. pecki* **comb. nov.** and the pyramidopid *C. oedipus*. When expanded, the movement of the glans is very similar in both species. The base of the follis inflates and moves dorsally, whereas the dorsoapical lobes of the follis inflate, separate slightly, and curl apically. The stylus projects apically and is exposed between the tips of the two lobes (Fig. 11). Another striking similarity between *J. pecki* **comb. nov.** and *P. pygmaeus* is the dorsal pair of macrosetae adjacent to the glans (referred to as D1 in Kury & Villarreal, 2015). The placement of a large pair of setae next to a mobile and expandable glans may serve a proprioceptive function, and may therefore be conserved across closely-related taxa with similar penis morphologies. However, tracing the homology of the macrosetae, defining species groups and delimiting genera in Pyramidopidae will require a better understanding of the diverse penis morphologies. Studying the penis morphology in the expanded state will also be important with respect to future studies of this group.

An interesting morphological character that we observed is the presence of microtrichia on the distal half of the setae of the major setiferous tubercles on the pedipalps (Fig. 7D, F) herein termed ‘plumose setae’. These plumose setae also have a smooth base and small pores located near the socket on the tubercle (Fig. 7E). This type of major setiferous tubercle with plumose setae is also present in *C. oedipus*, widely exhibited throughout Samooidea, and absent in Gonyleptoidea (J. Cruz-López, pers. observ.). However, this character is poorly surveyed in the remaining laniatorean families and thus its potential phylogenetic utility remains unknown.

ELUSIVE INTERFAMILIAL RELATIONSHIPS FOR PYRAMIDOPIDAE

Previous molecular phylogenetic studies have indicated a close relationship between Pyramidopidae + Assamiidae, together forming the Assamioidea

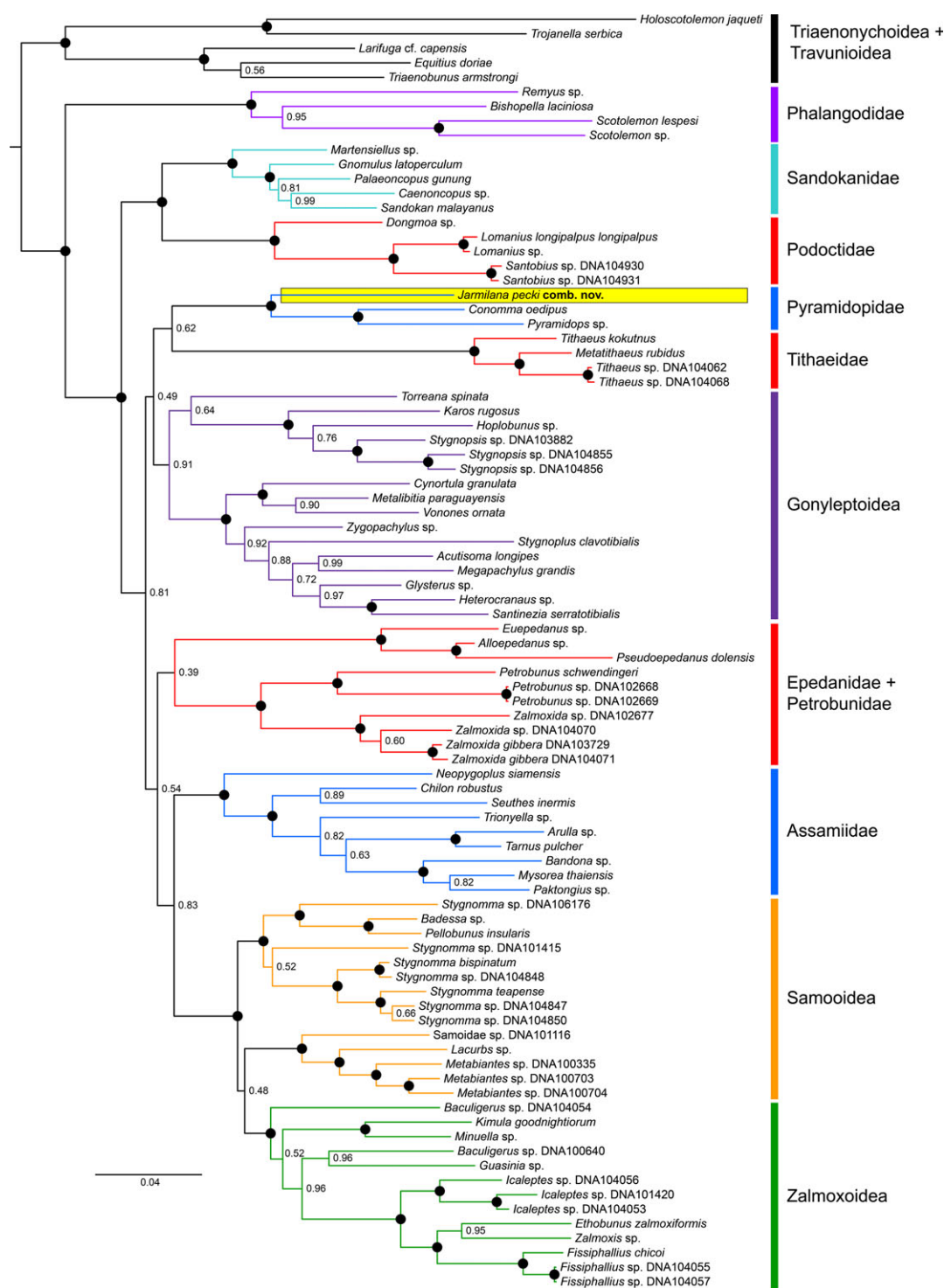


Figure 1. Phylogenetic relationships based on Bayesian inference analysis revealing the relationship of the Neotropical troglobite *Jarmilana pecki* comb. nov. (highlighted) with the African family Pyramidopidae. Numbers on nodes correspond to posterior probabilities; black circles at nodes indicate posterior probability of 1.00. Branch colours correspond to higher taxa (families or superfamilies) and reflect the same colour scheme used by Sharma & Giribet (2011) to facilitate direct comparison. The superfamilies Assamioidea (blue) and Epedanoidea (red) are recovered as polyphyletic.

(*sensu* Sharma & Giribet, 2011) and later Pyramidopidae was formalized and the family rank completed with a diagnosis for this diverse group (Sharma *et al.*, 2011). In the latter study, it was concluded that Pyramidopidae should be considered a separate family, sister to Assamiidae, rather than being included within Assamiidae. Inclusion in Assamiidae would have created a group without clear synapomorphies, thus making it difficult to diagnose (Sharma *et al.*, 2011). This was a sensible

decision, given that the clade Pyramidopidae + Assamiidae was partially justified based on the exclusion of Pyramidopidae from other superfamilies rather than clear synapomorphies that unite these families under Assamioidea.

In the Bayesian analysis, we recovered Assamiidae as sister to Zalmoxoidea + Samooidea, although with only moderate support (PP = 0.83), agreeing with the previous analysis of the full Laniatores dataset (i.e. Sharma & Giribet, 2011). Interestingly, however, we

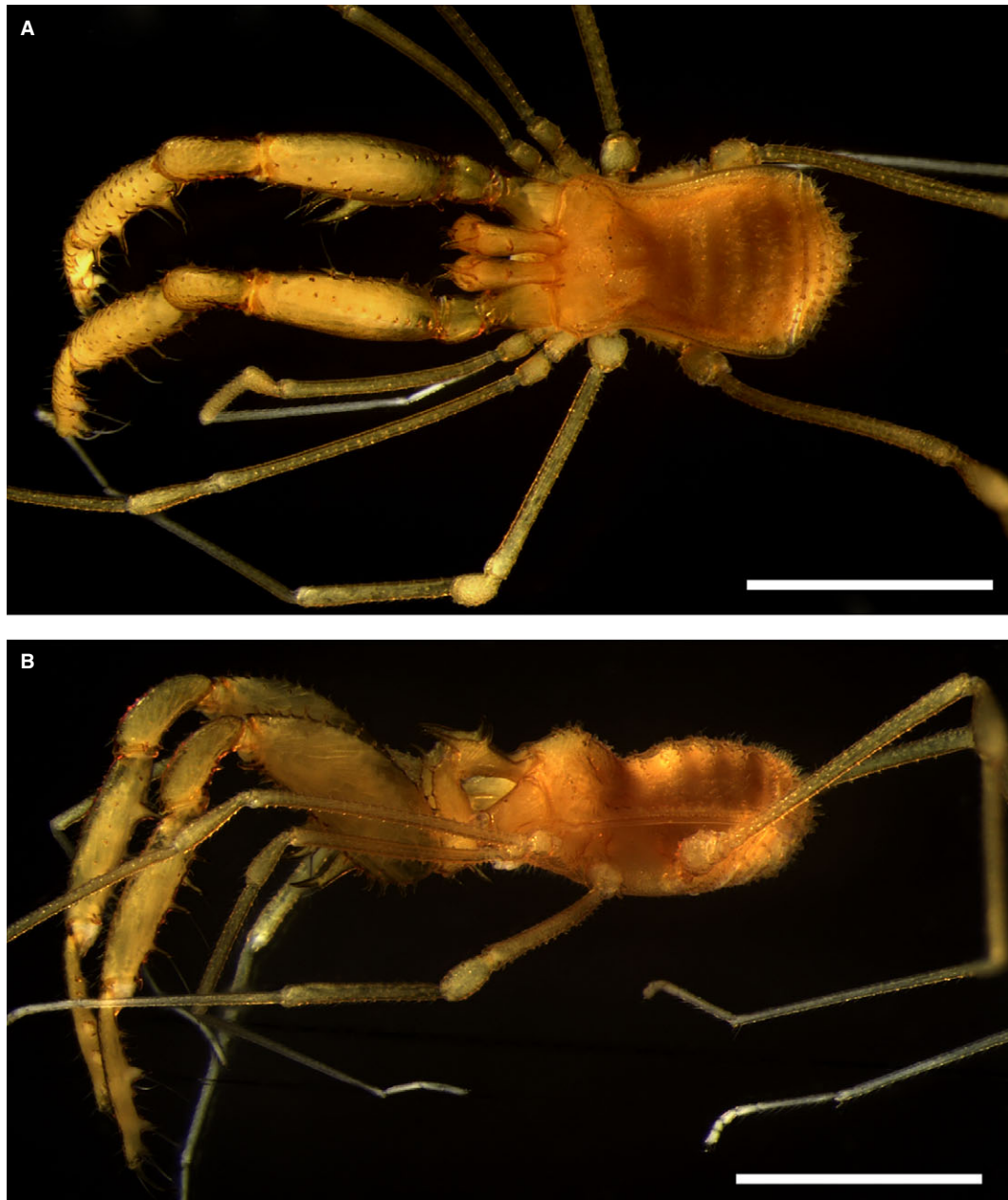


Figure 2. *Jarmilana pecki*, comb. nov. alpha male MACN_Ar 35555, habitus. A, dorsal view. B, dorsolateral view. Scale bars = 2 mm.

did not recover Pyramidopidae + Assamiidae as a clade. Instead, we recovered Pyramidopidae as sister to Tithaeidae (PP = 0.63), casting doubt on the sister-group relationship of Pyramidopidae + Assamiidae. Although this analysis does not offer well-supported alternatives to the sister-group of Pyrami-

dopidae, it clearly demonstrates that the evolutionary history of these lineages is much more complex than the current taxonomic sampling depicts, and the elusive relationships will only be resolved with broader taxonomic sampling for the African and South-east Asian harvestmen fauna.

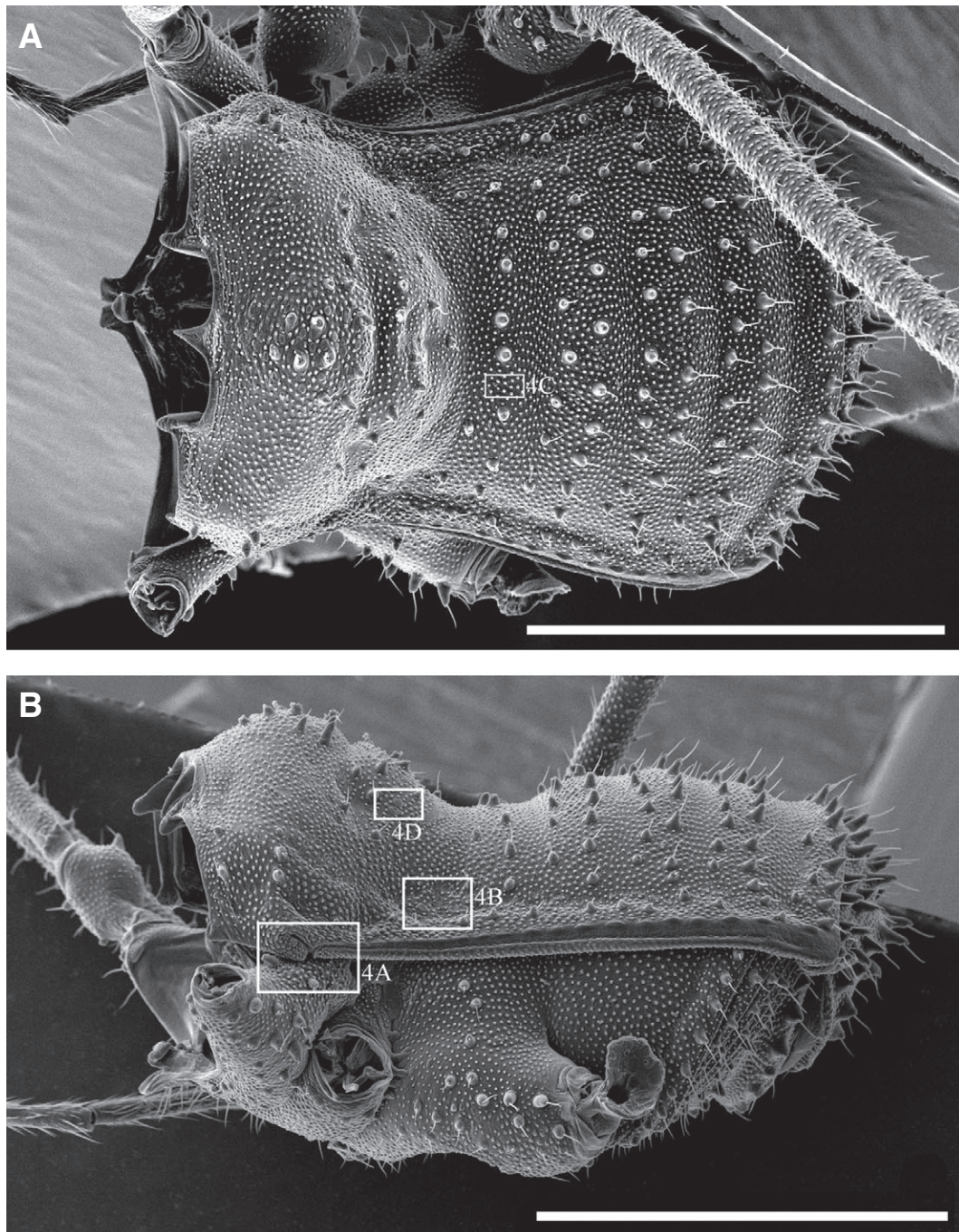


Figure 3. *Jarmilana pecki*, comb. nov. beta male MACN_Ar 35557, habitus. A, dorsal view. B, lateral view. Boxes indicate areas shown in detail in Fig. 4. Scale bars = 1 mm.

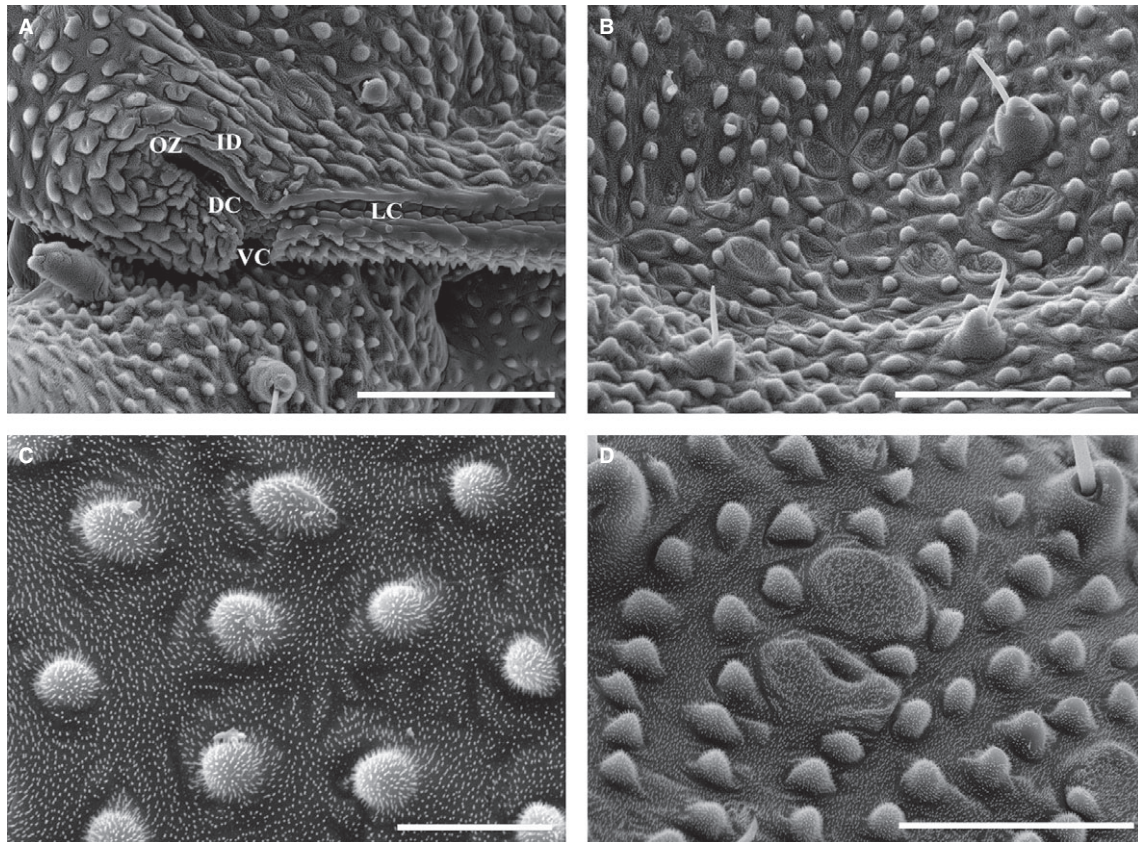


Figure 4. *Jarmilana pecki*, comb. nov. beta male MACN_Ar 35557, enlargements of Fig. 3 boxed areas. A, ozopore, lateral view. B, concentration of rough pit glands in depressed area adjacent to sulcus I, lateral view. C, microanatomy of the mesotergum showing verrucose cuticle with rounded granules covered in abundant hair-like microtrichia. D, rough pit glands concentrated in depressed area lateral to the position of sulcus I. Scale bars: (A, B) 100 µm; (C) 20 µm; (D) 50 µm. DC, descending channel; ID, integumentary dome; LC, lateral channel; OZ, ozopore; VC, vertical channel.

IMPLICATIONS FOR NEOTROPICAL PYRAMIDOPIDAE

Prior to this work, Pyramidopidae was formed by 45 species grouped in 12 genera with a geographical distribution restricted to central and western Africa (Sharma *et al.*, 2011). With the addition of *J. pecki* **comb. nov.** from the Cayo area in Belize, the family now consists of 46 species in 13 genera and shows a remarkable transoceanic distribution, spanning both Afrotropical and Neotropical regions. For sufficiently old lineages, this distribution is often explained by ancient vicariant events related to the break up of Gondwana, such as has been inferred for Ricinulei, Onychophora, Myriapoda, and other lineages of Opiliones (San Mauro *et al.*, 2004; Giribet & Edgecombe, 2006; Muriénne *et al.*, 2013, 2014; Fernández & Giribet, 2015). On the other hand, some disjunct biogeographical distributions for harvestmen families have been explained by transoceanic dispersal, as demonstrated by Zalmoxidae (Sharma & Giribet, 2012) and some Cyphophthalmi (Boyer *et al.*, 2007; Giribet *et al.*, 2012).

With respect to Pyramidopidae, either hypothesis may explain the transoceanic Gondwanan distribution. Previous estimates indicate that the Pyramidopidae lineage diverged from Assamiidae approximately 232 Mya and, within African pyramidopids (between *Conomma* and *Pyramidops*), the divergence dates to approximately 140 Mya (Sharma & Giribet, 2011); thus, the family appears to be sufficiently old. However, given that we have sequence data available for only three species of Pyramidopidae, we did not estimate divergence times using our dataset. Thus, it remains for future studies to determine whether *J. pecki* **comb. nov.** is an ancient relict from a widespread Gondwanan distribution or whether this is a case of transoceanic dispersal out of Africa.

REVISITING SANDOKANIDAE + PODOCTIDAE

The relationship of Sandokanidae with other families within Grassatores has long been a challenge. Sharma & Giribet (2009) provided strong support for

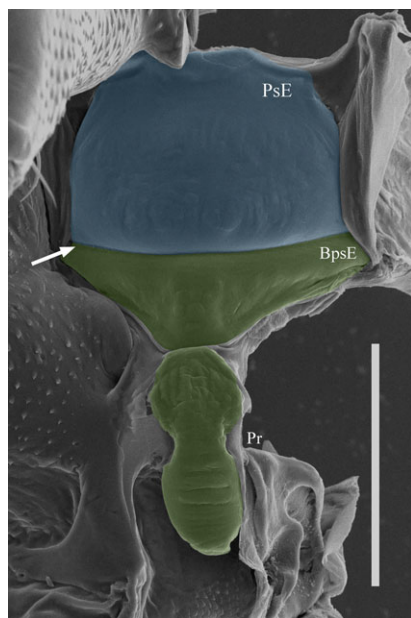


Figure 5. *Jarmilana pecki*, comb. nov. beta male MAC-N_Ar 35557, epistome, anterior view. Blue, post-sulcal epistome (PsE); green, pre-sulcal epistome consisting of two regions: a basal pre-sulcal epistome (BpsE) and pre-sulcal epistome process (Pr); arrow indicates the sulcus. Scale bar = 200 μ m.

the monophyly of Sandokanidae and the first molecular phylogenetic evidence to support a sister group relationship with Podoctidae. Subsequently, the first Laniatores phylogeny of Giribet *et al.* (2010), which represented many more families but utilized fewer markers, suggested that Phalangodidae, Sandokanidae, and Podoctidae form a grade near the base of the Laniatores tree and found only weak support for Sandokanidae + Podoctidae in the ML analysis. However, in both of those studies, Podoctidae was represented by only a single species. Adding a number of Laniatores taxa, including four more podoctids, and utilizing data from ten molecular markers, Sharma & Giribet (2011) clarified many important laniatorean relationships, although placement of Sandokanidae was still not well-resolved. They recovered Sandokanidae as either sister to all nonphalangodid Grassatores, or as sister to Podoctidae nested within other Epedanoidea. Given that we used a large subset of the data (including all Grassatores) from Sharma & Giribet (2011), it is interesting to note that we consistently recovered the clade Sandokanidae + Podoctidae with high nodal support in both analyses (BS = 88, PP = 1.00) and, together, they are recovered as sister to all nonphalangodid Grassatores (BS = 18; PP = 0.81). With respect to the basal placement of Sandokanidae, this outcome is consistent with the original analysis of this data-

set (i.e. Sharma & Giribet, 2011), although the new-found strong support for Sandokanidae + Podoctidae reiterates the idea that the relationship of this group with other Grassatores is sensitive to the analytical methods employed (Sharma & Giribet, 2009, 2011; Giribet & Sharma, 2015). As is the case for Pyramidopidae, resolving these relationships awaits a broader taxonomic sampling of African and South-east Asian lineages.

SYSTEMATICS

PYRAMIDOPIDAE SHARMA, PRIETO & GIRIBET, 2011

JARMILANA GEN. NOV.

Stygnomma (partim): Goodnight & Goodnight, 1977: 148; Reddell, 1981: 166; Rambla & Juberthie, 1994: 219; Kury, 2003: 235.

Type species: *Stygnomma pecki* Goodnight & Goodnight, 1977.

Etymology: The genus name is a tribute to Dr Jarmila Kukalová-Peck, recognized paleontologist and collector of the type series, who, in company with her husband, Dr Steward Peck, made important collections of cave dwelling animals across the Central American and Caribbean countries. Gender feminine.

Diagnosis: Eyeless harvestman (Fig. 2). Carapace highly elevated with a large anterior hump and smaller posterior hump (Fig. 3B). Male pedipalps very strong with palpal femur laterally compressed and high (Figs. 7A, C, 8). Male basichelicerite armed with two dorsal apophyses, cheliceral hand with one dorsal apophysis (Fig. 6A, B). Trochanter IV without sexually dimorphic spurs. Pars distalis of male genitalia slightly swollen and bulbous, without a marked ventral plate as in Gonyleptoidea, apical margin terminating in a small lip, curled ventrally. Pars distalis armed with six pairs of macrosetae, with one lateral pair in close proximity to the follis. Stylus subapical, long and curved, arising from the mid-follis, concealed below two huge dorsoapical lobes of the follis (Figs 10, 11). Several morphological features clearly separate this genus from other Pyramidopidae genera, such as the cheliceral and pedipalpal armature and the absence of an inflated dorsodistal bulla in the basichelicerite. The male genitalic morphology is also distinctive of this genus. The combination of a pars distalis swollen with one small and rounded lamina apicalis and a follis with dorsoapical portion modified into two large lobes that conceal (when retracted) a stylus ending with a dorsal barb appears to be unique to *Jarmilana* and not presented in any other known genera of Pyramidopidae.

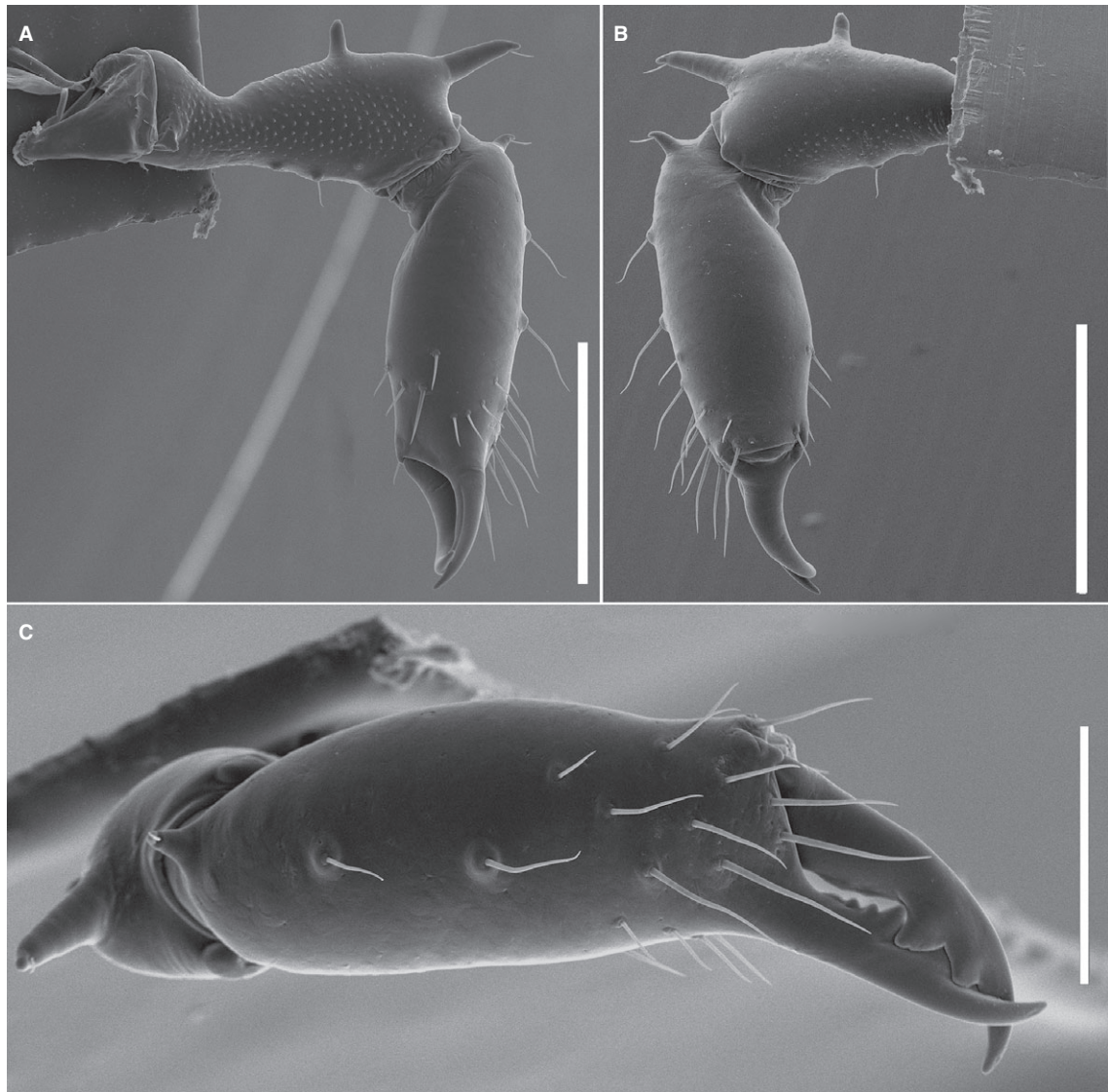


Figure 6. *Jarmilana pecki*, comb. nov. beta male MACN_Ar 35557, left chelicera. A, mesal view. B, ectal view. C, frontal view. Scale bars: (A, B) 500 μ m; (C) 250 μ m.

JARMILANA PECKI
(Goodnight & Goodnight, 1977) **COMB. NOV.**
(FIGS 2–11)

Stygnomma pecki Goodnight & Goodnight, 1977: 148; fig. 11; Reddell, 1981: 166; Rambla & Juberthie, 1994: 219; Kury, 2003: 235.

Type material:. One male holotype from Belize, Distrito de Cayo, Cave Branch, St Herman's Cave, 23 July to 21 August 1972, S. & J. Peck (AMNH, examined). One male and one female paratypes from Belize, Distrito de Cayo, Caves Branch, Mountain Cow Cave, 5 August 1972, S. & J. Peck (AMNH, examined).

Other material examined:. One male, one female, and one juvenile (CNAN-Op1702), one alpha male (MACN_Ar 35555), one beta male (MACN_Ar 35557, SEM voucher) and one female (MACN_Ar 35556, DNA voucher, extraction code Op024) from the same locality as the holotype, Belize, Distrito de Cayo, Caves Branch, St Herman's Cave, 6 November 2013, 17°08'48.76"N, 88°40'29.06"W, R. Monjaraz & C. Santibáñez. Two females from Belize, Distrito de Cayo, Caves Branch, St Herman's Cave, August 1972, S. & J. Peck (TMM-33.316, examined).

Diagnosis:. See diagnosis of the genus.

Redescription:. Based on one alpha male (MACN_Ar 35555). Body measurements: total body length 2.50,

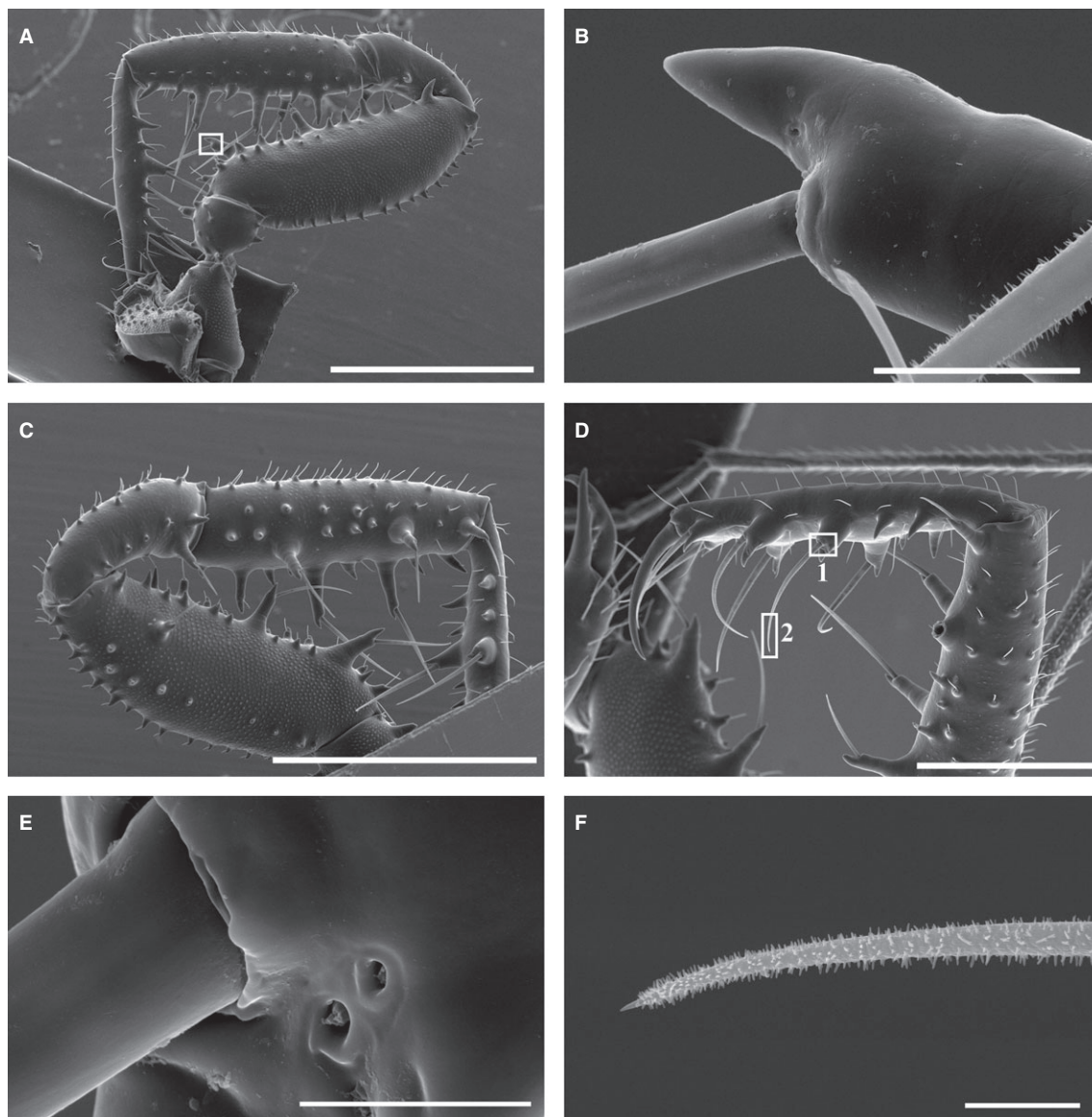


Figure 7. *Jarmilana pecki*, comb. nov. beta male MACN_Ar 35557, pedipalp. A, left pedipalp, ectal view. B, enlargement of box in (A), smooth base of plumose seta inserted on major setiferous tubercle. C, left pedipalp, mesal view. D, right pedipalpal tarsus, mesal view. E, enlargement of box 1 in (D), small pores at base of plumose seta inserting on major setiferous tubercle. F, enlargement of box 2 in (D), microtrichia on the distal part of a plumose seta associated with major setiferous tubercle. Scale bars: (A, C) 1 mm; (D) 500 μ m; (B) 50 μ m; (E, F) 20 μ m.

scutum magnum length 2.14, carapace length 1.00, carapace maximum width 1.37, mesotergal scute maximum width 1.60.

Dorsum:. Completely lacks eyes. *Scutum magnum* hourglass shaped with the constriction between prosoma and opisthosoma slightly pronounced (Figs 2, 3). Posterior margin of the *scutum* slightly convex, almost straight. Carapace with anterior margin straight, cheliceral sockets narrow but well marked (Fig. 3). Carapace and mesotergum in lateral

view convex, sulcus I not marked. Carapace highly elevated with a large anterior hump and smaller posterior hump, ornamented with small setiferous tubercles (Figs 2, 3). The absence of a cornea (or any evidence of eye position) makes it impossible to recognize whether one of these humps represents a vestigial ocularium. Mesotergal scutum with five recognizable, slightly convex areas. Sulci between mesotergal areas are not well marked. Areas I–V with transverse rows of setiferous tubercles: area I

with one row, II–IV with two rows, and V with one row. Lateral margins of mesotergum with a row of setiferous tubercles from sulcus I to sulcus V that increase slightly in size from anterior to posterior. Free tergites each with one transverse row of tubercles (Fig. 3). Ozopore region with well-marked descending, vertical and lateral channels, and a rounded integumentary dome covering the anteriodorsal area of ozopore (Fig. 4A). Lateral pegs (*sensu* Gnaspini & Rodrigues, 2011) present (Fig. 3B). Lateral to the sulcus I position, there is a depressed area that forms a wide trough (Figs 3, 4B). *Venter*. Coxa IV visible in dorsal view, terminating adjacent to sulcus II. Free sternites each with a transverse row of setiferous tubercles (Fig. 3). Spiracles crescent-shaped, with a mass of granules and tubercles protruding from the posterior border (Fig. 9D); not concealed by coxa IV. Anal operculum covered by many large, scattered setiferous tubercles. *Epistome*. Epistome with sulcus well marked. Post-sulcal epistome wider than tall, appearing subrectangular, arched dorsally. Basal pre-sulcal epistome wide and short, almost triangular. Pre-sulcal epistome process hourglass-shaped, with remarkable median constriction (Fig. 5). *Chelicera*. Chelicera normal, neither swollen, nor obviously hypertelic. Basicheicerite slightly elongated, without well-marked bulla, dorsally with two strong spiniform setiferous tubercles, the subdistal tubercle oriented dorsally and curved, and the distal tubercle larger, straight and pointed anteriorly (Fig. 6). Cheliceral hand with a proximodorsal setiferous tubercle oriented anteriodorsally. Mobile finger of cheliceral hand with two wide teeth, fixed finger with two small teeth proximally and one distally (Fig. 6). *Pedipalp*. Coxa almost as long as the basicheicerite, without remarkable armature. Trochanter globular, with a large ventral and small dorsal setiferous tubercle, among other scattered tubercles. Femur strong, distinctly widened dorsoventrally. Femur armed with three ventral and one subdistal mesal major setiferous tubercles, and with one dorsal, one mesal, and two ventral rows of setiferous tubercles; ventral ectal row terminates distally with one curved

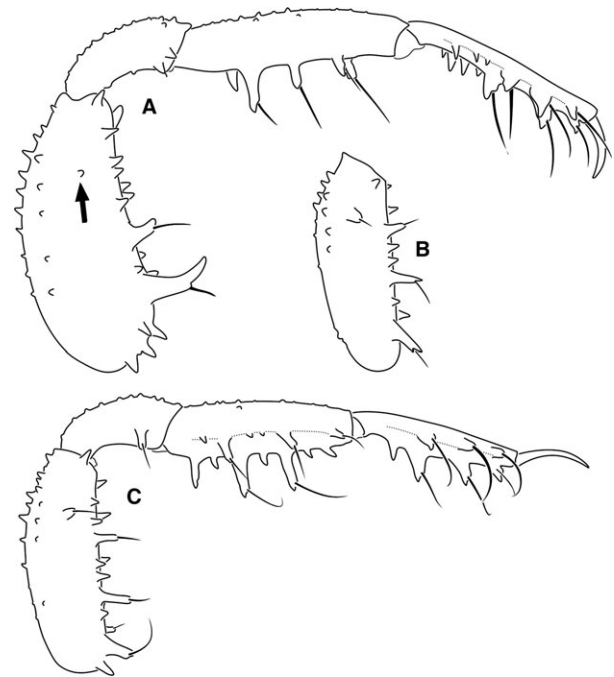


Figure 8. *Jarmilana pecki*, comb. nov. comparison of left pedipalps. A, entire palp, alpha male MACN_Ar 35555, mesal view. B, palpal femur of beta male MACN_Ar 35557, mesal view. C, entire palp, female MACN_Ar 35556, mesal view. All drawings are to same scale. Arrow indicates the mesal subdistal major setiferous tubercle.

spiniform apophysis (Figs 7, 8). Patella cylindrical, armed with one major setiferous tubercle ventrodistally; with several rows of small setiferous tubercles on dorsal and mesal surfaces. Tibia armed ventrally with two ectal and three mesal major setiferous tubercles, and multiple apophyses interspersed in ectal row; with several rows of small setiferous tubercles on mesal, dorsal, and ectal surfaces. Tarsus armed ventrally with three ectal and three mesal major setiferous tubercles; with multiple apophyses interspersed in mesal and ectal rows (Figs 7, 8). All major setiferous tubercles possess a 'plumose seta' that inserts subapically in a socket on the tubercle. Plumose setae are defined

Table 3. Length of appendages (in mm) for *Jarmilana pecki*, comb. nov. alpha male MACN_Ar 35555

	Coxa	Trochanter	Femur	Patela	Tibia	Metatarsus	Tarsus
Leg I	0.70	0.25	1.75	0.55	1.15	1.80	0.75
Leg II	0.90	0.30	2.72	0.75	2.20	2.30	3.20
Leg III	0.70	0.32	2.05	0.50	1.40	1.95	0.95
Leg IV	1.00	0.45	2.55	0.67	1.85	2.40	1.15

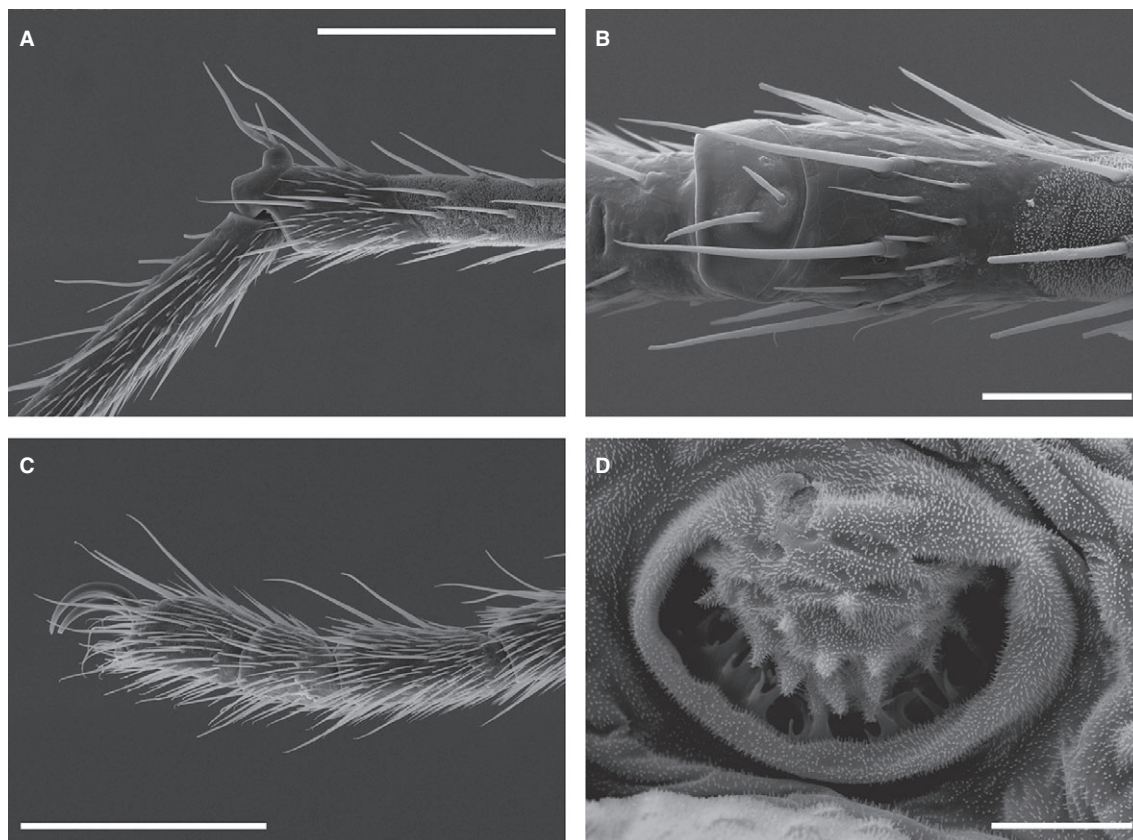
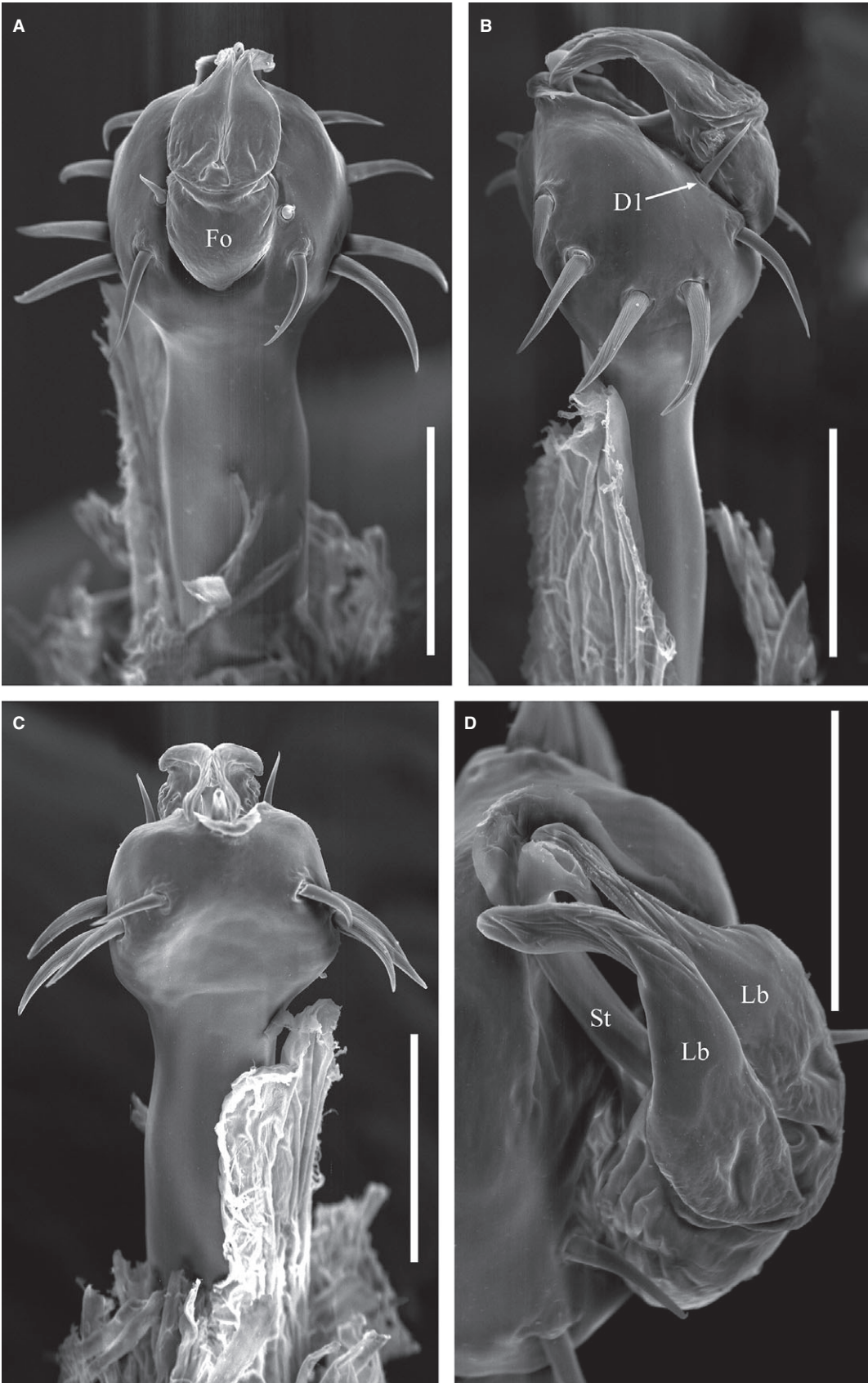


Figure 9. *Jarmilana pecki*, comb. nov. beta male MACN_Ar 35557, details of legs and spiracle. A, B, right metatarsus IV showing the division between calcaneus and astragalus, and the dorsodistal tubercle with two large setae, from (A) prolateral view and (B) dorsal view. C, distitarsus of leg IV, prolateral view. D, spiracle. Scale bars: (A, C) 200 µm; (B) 50 µm; (D) 20 µm.

here as setae with microtrichia covering the majority of the shaft (Fig. 7F) but with a smooth base (Fig. 7B, E); small pores are associated with plumose setae, found near the socket on the tubercle (Fig. 7B, E). *Legs.* Measurements in Table 3. Legs I–IV slender, cylindrical, equal in diameter, without remarkable armature. Calcaneus restricted to the distal portion of legs (Fig. 9A, B), not expanded throughout the ventral portion of the metatarsus in leg III (as in many samoid harvestmen; e.g. Pérez-González & Kury, 2007, fig. 4.37 d). Without scopula (Fig. 9C). Tarsal count: 3(2):9(4):4:5, the first tarsomere on legs III and IV is very long compared to the remaining tarsomeres, more than three times the length of the second tarsomere. Metatarsus IV has a large dorsodistal tubercle with two large setae inserted (Fig. 9A, B). *Microanatomy of cuticle.*

Cuticle of entire body and coxa to metatarsus (calcaneus only) of legs I–IV covered in small rounded granules (i.e. verrucose) that are < 10 µm in diameter and height (Figs 3, 4). Cuticle is further modified with dense microtrichia (Fig. 4C). Femur of pedipalps verrucose but with few microtrichia. Palpal patella, tibia, and tarsus relatively smooth, although some flattened granules are visible. On the pedipalps, plumose setae of the major setiferous tubercles possess microtrichia on the medial and distal portion (Fig. 7F), with smooth base (Fig. 7E). Rough pit glands (*sensu* Murphree, 1988; see also Willemart, Chelini & Gnaspini, 2007; Rodriguez *et al.*, 2014) are distributed on the entire carapace, along the lateral margins of the mesotergal scutum, and along sulcus V of the mesotergum; these structures are especially concentrated within a

Figure 10. *Jarmilana pecki*, comb. nov. genitalia of beta male MACN_Ar 35557, non-expanded. A, dorsal view. B, lateral view. C, ventral view. D, detail of stylus and apical lobes of follis. Scale bars: (A, B, C) 100 µm; (D) 50 µm. D1, macrosetae D1; Fo, follis; Lb, lobe of follis; St, stylus.



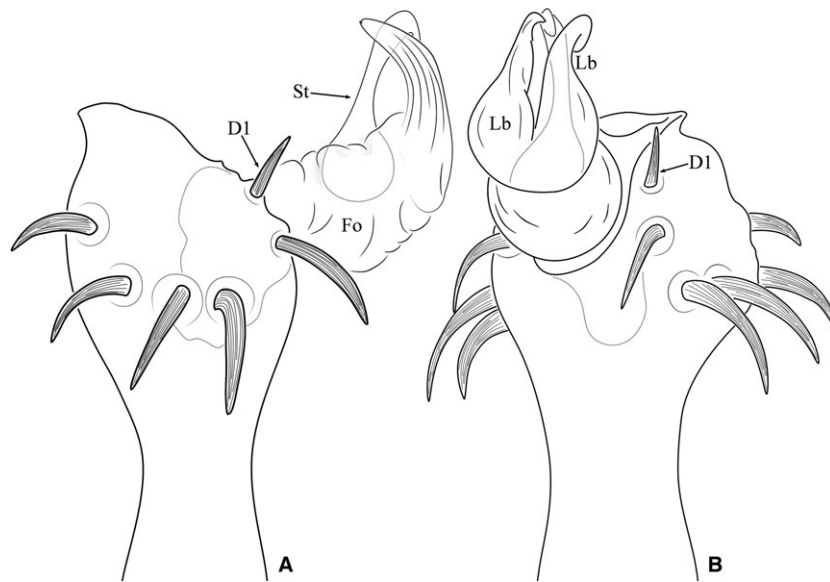


Figure 11. *Jarmilana pecki*, comb. nov. genitalia of alpha male MACN_Ar 35555, expanded. A, lateral view. B, dorso-lateral view. D1, macrosetae D1; Fo, follis; Lb, lobe of follis; St, stylus.

Table 4. Comparative size of the pedipalps between alpha male (MACN_Ar 35555), beta male (MACN_Ar 35557), and female (MACN_Ar 35556) for *Jarmilana pecki* comb. nov.

	Femur length	Femur width	Femur length/width	Patella length	Tibia length	Tarsus length
Alpha male	2.10	0.84	2.5	1.05	1.77	1.55
Beta male	1.42	0.47	3.02	0.72	1.23	1.10
Female	1.41	0.46	3.06	0.67	1.21	1.00

depressed area lateral to the position of sulcus I (Fig. 4B, D). *Male genitalia*. Pars distalis swollen, with slight constriction at base. Ventral plate not differentiated, as in Gonyleptoidea, although with one small and rounded lamina apicalis. Follis with dorsoapical portion modified into two lobes that conceal the stylus when retracted. Capsula interna without accessory sclerites (e.g. parastylar conductors). Stylus arising subapically from follis (i.e. at base of lobes), long and curved, tip of stylus ending with a dorsal barb. Pars distalis armed with two dorsal and four lateral pairs of macrosetae, with rounded tips and numerous longitudinal striae along the entire length. One pair of dorsal setae (D1 setae *sensu* Kury & Villarreal, 2015) insert directly adjacent to the follis, projecting dorsoapically; the second dorsal pair is inserted basolaterally to the glans and directed dorsobasally (Figs 10, 11). When the genitalia is expanded, the follis unfolds and inflates dorsally, whereas the lobes inflate, separate and curl apically; the stylus is directed apically between the tips of the two lobes (Fig. 11).

Beta male (MACN_Ar 35557). Body measurements: Total body length 1.70, *scutum magnum* length 1.61, carapace length 0.70, carapace maximum width 1.12, mesotergal scute maximum width 1.28. Similar to the alpha male but with the differences: smaller body size; pedipalps not strongly developed, and more similar in shape and size to the palps of the female (Table 4); palpal femur with mesal subdistal setiferous tubercle more strongly developed (Fig. 8B); cheliceral armature slightly reduced in size. *Female* (MACN-35556). Body measurements: Total body length 2.00, *scutum magnum* length 1.76, carapace length 0.70, carapace maximum width 1.15, mesotergal scute maximum width 1.36. Tarsal formula same as males. Similar in general appearance to males but with less developed sexual dimorphic characters, such as the size and armature of pedipalps (more similar to the beta male) (Fig. 8C, Table 4) and chelicerae.

Distribution: Known only from two caves: Mountain Cow Cave and St Herman's Cave – located in Caves Branch, Cayo District, Belize.

Natural history:. Individuals were observed in the twilight zone of the cave, actively walking and climbing on the floor and walls of the cave. When disturbed, they exhibited thanatosis.

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REFERENCES

- Acosta LE, Pérez-González A, Tourinho AL. 2007. Methods for taxonomic study. In: Pinto-da-Rocha R, Machado G, Giribet G, eds. *Harvestmen: the biology of Opiliones*. Cambridge and London: Harvard University Press, 494–505.
- Ázara LN, DaSilva MB, Ferreira RL. 2013. Description of *Mitogoniella mucuri* sp. nov. (Opiliones: Gonyleptidae) and considerations on polymorphic traits in the genus and Gonyleptidae. *Zootaxa* **3736**: 069–081.
- Boyer SL, Karaman I, Giribet G. 2005. The genus *Cyphophthalmus* (Arachnid, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. *Molecular Phylogenetics and Evolution* **36**: 554–567.
- Boyer SL, Clouse RM, Benavides LR, Sharma P, Schwendinger PJ, Karunarathna I, Giribet G. 2007. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. *Journal of Biogeography* **34**: 2070–2085.
- Bragagnolo C, Hara MR, Pinto-da-Rocha R. 2015. A new family of Gonyleptoidea from South America (Opiliones, Laniatores). *Zoological Journal of the Linnean Society* **173**: 296–319.
- Brown BV. 1993. A further chemical alternative to critical-point-drying for preparing small (or large) flies. *Fly Times* **11**: 10.
- Buzatto BA, Machado G. 2014. Male dimorphism and alternative reproductive tactics in harvestmen (Arachnida: Opiliones). *Behavioural Processes* **109**: 2–13.
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
- Evans N, Paulay G. 2012. DNA barcoding methods for invertebrates. *Methods in Molecular Biology* **858**: 47–77.
- Fernández R, Giribet G. 2015. Unnoticed in the tropics: phylogenomic resolution of the poorly known arachnid order Ricinulei (Arachnida). *Royal Society Open Science* **2**: 150065.
- Ferreira CP, Kury AB. 2010. A review of *Roquettea*, with description of three new Brazilian species and notes on *Gryne* (Opiliones, Cosmetidae, Discosomaticinae). *Zoological Science* **27**: 697–708.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek RC. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.
- Giribet G, Edgecombe GD. 2006. The importance of looking at small-scale patterns when inferring Gondwanan biogeography: a case study of the centipede *Paralamyctes* (Chilopoda, Lithobiomorpha, Henicopidae). *Biological Journal of the Linnean Society* **89**: 65–78.
- Giribet G, Sharma PP. 2015. Evolutionary biology of harvestmen (Arachnida, Opiliones). *Annual Review of Entomology* **60**: 157–175.
- Giribet G, Carranza S, Baguña J, Riutort M, Ribera C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Molecular Biology and Evolution* **13**: 76–84.
- Giribet G, Vogt L, Pérez-González A, Sharma PP, Kury AB. 2010. A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores. *Cladistics* **26**: 408–437.
- Giribet G, Sharma P, Benavides LR, Boyer SL, Clouse RM, de Bivort BL, Dimitrov D, Kawauchi GY, Murienne J, Schwendinger PJ. 2012. Evolutionary and biogeographical history of an ancient and global group of arachnids (Arachnida: Opiliones: Cyphophthalmi) with a new taxonomic arrangement. *Biological Journal of the Linnean Society* **105**: 92–130.
- Gnaspini P, Rodrigues GCS. 2011. Comparative study of the morphology of the gland opening area among

- Grassatores harvestmen (Arachnida, Opiliones, Laniatores). *Journal of Zoological Systematics and Evolutionary Research* **49**: 273–284.
- Goodnight CJ, Goodnight ML. 1977.** Laniatores (Opiliones) of the Yucatán Peninsula and Belize (British Honduras). *Bulletin of the Association for Mexican Cave Studies* **6**: 139–166.
- Katoh S. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Kury AB. 2003.** Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones). *Revista Ibérica de Aracnología*, Volumen especial monográfico **1**: 5–337.
- Kury AB. 2008.** A review of *Soaresia* H. Soares, 1945, with the description of a new species from Serra da Mantiqueira, Brazil (Opiliones, Gonyleptidae, Pachylinae). *Zootaxa* **1687**: 51–59.
- Kury AB. 2012.** First report of the male of *Zamora granulata* Roewer 1928, with implications on the higher taxonomy of the Zamorinae (Opiliones, Laniatores, Cranidae). *Zootaxa* **3546**: 29–42.
- Kury AB. 2013a.** The first species of *Roquettea* from Maranhão, Brazil (Opiliones: Cosmetidae: Discosomatinae). *Zoologia* **30**: 569–573.
- Kury AB. 2013b.** Order Opiliones Sundevall, 1833. In: Zhang Z-Q, eds. *Animal Biodiversity: An outline of higher-level classification and survey of taxonomic richness* (Addenda 2013). *Zootaxa* **3703**: 27–33.
- Kury AB. 2014.** Why does the Tricommatinae position bounce so much within Laniatores? A cladistic analysis, with the description of a new family of Gonyleptoidea (Opiliones, Laniatores). *Zoological Journal of the Linnean Society* **172**: 1–48.
- Kury AB, Ferreira CP. 2012.** Two new species of *Roquettea* Mello-Leitão, 1931 from northern Brazil (Opiliones: Laniatores: Cosmetidae). *Zootaxa* **3328**: 35–46.
- Kury AB, Pérez-González A. 2002.** A new family of Laniatores from Northwestern South America (Arachnida, Opiliones). *Revista Ibérica de Aracnología* **6**: 3–11.
- Kury AB, Villarreal O. 2015.** The prickly blade mapped: establishing homologies and a chaetotaxy for macrosetae of penis ventral plate in Gonyleptoidea (Arachnida, Opiliones, Laniatores). *Zoological Journal of the Linnean Society* **174**: 1–46.
- Lanfear R, Calcott B, Ho SYW, Guindon S. 2012.** PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695–1701.
- Macías-Ordóñez R, Machado G, Pérez González A, Shultz JW. 2010.** Genitalic evolution in Opiliones. In: Leonard J, Córdoba-Aguilar A, eds. *The evolution of primary sexual characters in animals*. Oxford, UK: Oxford University Press, 285–306.
- Murienne J, Benavides LR, Prendini L, Hormiga G, Giribet G. 2013.** Forest refugia in Western and Central Africa as ‘museums’ of Mesozoic biodiversity. *Biological Letters* **9**: 20120932.
- Murienne J, Daniels SR, Buckley TR, Mayer G, Giribet G. 2014.** A living fossil tale of Pangean biogeography. *Proceedings of the Royal Society B* **281**: 20132648.
- Murphree CS. 1988.** Morphology of the dorsal integument of ten opiloid species (Arachnida, Opiliones). *Journal of Arachnology* **16**: 237–252.
- Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL. 2008.** AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**: 581–583.
- Pérez-González A. 2006.** Revisão Sistemática e Análise Filogenética de Stygnommatidae (Arachnida: Opiliones: Laniatores). Unpublished D. Phil. Thesis, Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil, 308 pp.
- Pérez-González A. 2007.** Stygnommatidae Roewer, 1923. In: R Pinto-da-Rocha, G Machado, G Giribet, eds. *Harvestmen: the biology of Opiliones*. Cambridge and London: Harvard University Press, 229–232.
- Pérez-González A. 2011.** New familial assignment for two harvestmen species of the infraorder Grassatores. *Zootaxa* **2757**: 24–28.
- Pérez-González A, Kury AB. 2007.** Samoidae Sørensen, 1886. In: R Pinto-da-Rocha, G Machado, G Giribet, eds. *Harvestmen: the biology of Opiliones*. Cambridge and London: Harvard University Press, 224–226.
- Pinto-da-Rocha R, Hara MR. 2009.** New familial assignments for three species of Neotropical harvestmen based on cladistic analysis (Arachnida: Opiliones: Laniatores). *Zootaxa* **2241**: 33–46.
- Pinto-da-Rocha R, Benedetti AR, Gomes de Vasconcelos E, Hara MR. 2012.** New systematic assignments in Gonyleptoidea (Arachnida, Opiliones, Laniatores). *ZooKeys* **198**: 25–68.
- Pinto-da-Rocha R, Bragagnolo C, Marques FPL, Antunes Junior M. 2014.** Phylogeny of harvestmen family Gonyleptidae inferred from a multilocus approach (Arachnida: Opiliones). *Cladistics* **30**: 519–539.
- Prendini L, Weygoldt P, Wheeler WC. 2005.** Systematics of the *Damon variegatus* group of African whip spiders (Chelicerata: Amblypygi): evidence from behaviour, morphology and DNA. *Organisms, Diversity & Evolution* **5**: 203–236.
- Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014.** Tracer v1.6. Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Rambla M, Juberthie C. 1994.** Opiliones. In: Juberthie C, Decu V, eds. *Encyclopaedia Biospeologica, I*. Barcelona, Spain: University of Barcelona, 215–230.
- Reddell JR. 1981.** A review of the cavernicole fauna of Mexico, Guatemala and Belize. *Museum, Speleological Monographs* **27**: 69–257.
- Rodriguez AL, Townsend VR Jr, Johnson MB, White TB. 2014.** Interspecific variation in the microanatomy of cosmetid harvestmen (Arachnida, Opiliones, Laniatores). *Journal of Morphology* **275**: 1386–1405.
- Roewer CF. 1912.** Beitrag zur Kenntnis der Weberknechte Kolumbiens. In: Fuhrmann O, Mayor E (eds.). *Voyage d'exploration scientifique en Colombie. Mémoires de la Société neuchâteloise des Sciences naturelles* **5**: 139–159.

- Roewer CF. 1949.** Über Phalangodiden I: Subfam. Phalangodinae, Tricommatinae Samoinae; weitere Weberknechte XIII. *Senckenbergiana* **30**: 11–61.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- San Mauro D, Gower DJ, Oommen OV, Wilkinson M, Zardoya R. 2004.** Phylogeny of caecilian amphibians (Gymnophiona) based on complete mitochondrial genomes and nuclear RAG1. *Molecular Phylogenetics and Evolution* **33**: 413–327.
- Schwendinger PJ, Giribet G. 2005.** The systematics of the south-east Asian genus *Fangensis* Rambla (Opiliones: Cyphophthalmi: Stylocellidae). *Invertebrate Systematics* **19**: 297–323.
- Sharma PP, Giribet G. 2009.** Sandokanid phylogeny based on eight molecular markers – the evolution of a southeast Asian endemic family of Laniatores (Arachnida, Opiliones). *Molecular Phylogenetics and Evolution* **52**: 432–447.
- Sharma PP, Giribet G. 2011.** The evolutionary and biogeographic history of the armoured harvestmen – Laniatores phylogeny based on ten molecular markers, with the description of two new families of Opiliones (Arachnida). *Invertebrate Systematics* **25**: 106–142.
- Sharma PP, Giribet G. 2012.** Out of the neotropics: late Cretaceous colonization of Australasia by American arthropods. *Proceedings of the Royal Society B* **279**: 3501–3509.
- Sharma PP, Giribet G. 2014.** A revised dated phylogeny of the arachnid order Opiliones. *Frontiers in Genetics* **5**: 1–13.
- Sharma PP, Prieto CE, Giribet G. 2011.** A new family of Laniatores (Arachnida: Opiliones) from the Afrotropics. *Invertebrate Systematics* **25**: 143–154.
- Stamatakis A. 2014.** RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Whiting MF, Carpenter JM, Wheeler QD, Wheeler WC. 1997.** The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology* **46**: 1–68.
- Willemart RH, Chelini MC, Gnaspini P. 2007.** An ethological approach to a SEM survey on sensory structures and tegumental gland openings of two Neotropical harvestmen (Arachnida, Opiliones, Gonyleptidae). *Italian Journal of Zoology* **74**: 39–54.
- Xia X. 2013.** DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* **30**: 1720–1728.
- Xia X, Lemey P. 2009.** Assessing substitution saturation with DAMBE. In: P Lemey, M Salemi, A-M Vandamme, eds. *The phylogenetic handbook: a practical approach to DNA and protein phylogeny*, 2nd edn. Cambridge University Press, New York, USA 615–630.
- Xia X, Xie Z, Salemi M, Chen L, Wang Y. 2003.** An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* **26**: 1–7.
- Zatz C, Werneck RM, Macías-Ordóñez R, Machado G. 2011.** Alternative mating tactics in dimorphic males of the harvestman *Longiperna concolor* (Arachnida: Opiliones). *Behavioral Ecology and Sociobiology* **65**: 995–1005.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Phylogenetic relationships based on Maximum Likelihood revealing the relationship of the Neotropical troglobite *Jarmilana pecki* comb. nov. (highlighted) with the African family Pyramidopidae. Numbers on nodes correspond to bootstrap support; black circles at nodes indicate bootstrap values of 100. Branch colors correspond to higher taxa (families or superfamilies) and reflect the same color scheme used in Figure 1, and by Sharma & Giribet (2011). The superfamilies Assamioidea (blue) and Epedanoidea (red) are recovered as polyphyletic.