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Phylogeny of the family Withiidae (Arachnida: Pseudoscorpiones) and evolution of the male reproductive system

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A mi madre Nancy, mi hermana Luisa y mis abuelos Ricardo y Alba.

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Ingrid Catalina Romero Ortiz

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Resumen

Título en español: Filogenia de la familia Withiidae (Arachnida: Pseudoscorpiones) y evolución del sistema reproductor masculino

La evolución de los genitales animales ha sido un tema de investigación interesante desde que Darwin propuso que la selección sexual actúa sobre los rasgos morfológicos. En este contexto, tomando los pseudoescorpiones de la familia Withiidae como modelo para estudiar la influencia de las presiones selectivas en la variación de los genitales masculinos, 1) caracterizamos la morfología de los genitales masculinos proponiendo declaraciones de homología entre géneros después de un examen detallado de especímenes, 2) exploramos las relaciones filogenéticas internas de la familia Withiidae utilizando datos morfológicos y moleculares, 3) datamos la divergencia de los clados utilizando tres fósiles como puntos de calibración, y 4) exploramos las tasas de cambio de los caracteres de los genitales masculinos. Encontramos un fuerte apoyo para la monofilia de Withidae así como para dos clados internos, el grupo neotropical y el no neotropical; en cuanto al grupo hermano, no hay evidencia conclusiva pues los datos moleculares sugieren a Atemnidae, apoyando estudios moleculares previos, pero los datos combinados, moleculares más morfológicos, apuntan a Cheliferidae. Los resultados sugieren que Withiidae surgió en el Cretácico. También encontramos que los rasgos sensoriales i.e. posición de las tricobotrias *isb* e *ist*, y no los rasgos genitales, son los que cambian más rápido, desafiando la predicción de mayor velocidad de cambio en ellos como resultado de la selección sexual como la principal fuerza moldeadora. Finalmente,

encontramos varias novedades taxonómicas: primero, redescribimos *Metawithius nepalensis* dentro de la revisión del género; segundo, transferimos una especie originalmente descrita como un withido al género *Verrucachernes*; tercero, elevamos a género el subgénero *Oligowithius* y cuarto, describimos cinco nuevas especies. Creemos que, con el desarrollo de la genómica, nuevos resultados podrían darnos más pistas acerca de la influencia de diferentes presiones selectivas sobre cada grupo de caracteres morfológicos en esta familia de pseudoescorpiones.

Palabras clave: Filogenia, selección sexual, genitales masculinos, presiones selectivas, homología, nuevos taxones.

Abstract

Título en inglés: Phylogeny of the family Withiidae (Arachnida: Pseudoscorpiones) and evolution of the male reproductive system

The evolution of animal genitalia has been an intriguing topic of research since Darwin proposed that sexual selection is acting upon morphological traits. In this context, taking Pseudoscorpions of the family Withiidae as a model for studying the influence of selective pressures in male genitalia variation we, 1) characterized the morphology of male genitalia proposing homology statements between genera after detailed examination and dissection of male specimens; 2) we explored the internal phylogenetic relationships for the family using morphological and molecular data; 3) we dated the divergence times of clades of the family using three fossils as calibration points; and 4) we explored the rates of change of male genitalia characters. We found a strong support for the monophyly of Withidae as well as for the two internal clades, the neotropical and the non-neotropical clades; as for the sister group of Withiidae, there is no conclusive evidence since molecular data suggest Atemnidae, supporting previous molecular studies, but the combined morphological and molecular data pointed to Cheliferidae. Results suggest that Withiidae arose in the Cretaceous. We also found that sensorial traits i. e. position of the trichobothria *isb* and *ist*, and not the genital ones, changed faster, challenging the prediction of higher change rates as consequence of the role of sexual selection as the main shaping force. Finally, we found several taxonomic novelties: first, we redescribed *Metawithius nepalensis* as part of the revision of the genus; second, we transferred a species originally described as a withiid to the genus *Verrucachernes*; third, we raised to genus the subgenus *Oligowithius*, and fourth, we described five new species. We think that with the development of genomics in phylogenetics, new results could give us more hints about the influence of different selective pressures over each set of morphological characters in this family of pseudoscorpions.

Keywords: Phylogeny, sexual selection, male genitalia, selective pressures, homology, new taxa.

Content

Introduction

In Animalia, the male genitalia is the most variable and divergent structure of the body mainly because of sexual selection (Eberhard 1985, Hosken & Stockley 2004); consequently, the morphology of these structures is the focus of analyses for several studies in evolutionary biology (e.g. Klaczko et al. 2015, Langerhans et al. 2016, Simmons & Garcia-Gonzalez 2011, Soto et al. 2007). To explore the influence of sexual selection in shaping genital variation, several topics should be properly addressed: First, a clear relationship between transformation series of genital structures should be established to propose homology statements. Second, a phylogenetic framework for the study group should be provided, and third, characters should be traced along the tree to test homology statements and to infer the rates of change for character evolution dependency.

Homology is defined as similarity due to common ancestry (Göpel & Richter 2023, Nelson 1994, Nixon & Carpenter 2012, Patterson 1982, Richter 2017, De Pinna 1991), and homology statements are crucial in evolutionary studies since its definition is an essential step in the process of inferring phylogenies. Ochoterena et al. (2019) proposed a conceptual framework in which a character can be considered homologous once it has been tested in at least two of three hierarchical levels: organisms, populations, and species. According to these authors, the test at species level (e.g. phylogenetic analysis) is the only one that fulfills the concept thoroughly. For structures this means that we can propose homologies based on similarity, but their status can be proved as such only under a phylogenetic analysis.

Phylogenies are built using different sources of information as well as different methods. In recent times, most of the information used is genomic (several loci, UCE's, transcriptomics, mitogenomes), mainly due to the ease of gathering massive amounts of data to work with, slowly setting morphological data aside, however, morphology can have a strong influence in the results (Cabra-García & Hormiga 2020, Donoghue et al. 1989, Neumann et al. 2021, Pyron 2015, Wipfler et al. 2016, Wright & Hillis 2014), and allows the use of fossil taxa in

the analyses (Wiens et al. 2010, Wright & Hillis 2014). Fossils provide the crucial advantage of facilitating the establishment of dates for the tree and provide a reference for the estimation of evolutionary rates for different sets of characters.

Studying the role of sexual selection in phenotypic variation of male genitalia, must compare the rates of change between genital and non-genital structures in different species, since a higher rate in those characters could suggest the influence of a sexual selective pressure acting upon them (De-Lima et al. 2019, Klaczko et al. 2015, Reuland et al. 2021, Schultz et al. 2016); if this prediction is not fulfilled, it may be suggestive of more complex evolutionary processes.. For this purpose, we should use a reference of time that could be fossil records or geological events, and a model that estimates the rate at which the characters have evolved; this model could be fixed, relaxed or mixed clock (Wheeler 2012). All these models are based on the premise that DNA or proteins have evolved at a certain rate in different groups. Examples of these studies go from tracing the evolution of key phenotypic traits (e.g., Genevcius et al. 2021), integration of phenotypic traits for microhabitat specialization (e.g., Yuan et al. 2019), to testing parallel evolution of ecotypes (e.g., de Aranzamendi et al. 2022), among others.

The evolution of animal genitalia has been an intriguing topic of research since Darwin's first proposal of sexual selection acting upon morphological traits (Darwin 1871), and many other authors have largely contributed by defining different models of sexual selection that can explain the striking diversity of animal genitalia shapes, sizes, and complexity (Eberhard 1985, 1996, 2010, 2011; Arnqvist 1998; Hosken & Stockley 2004; Simmons 2014). Among the more studied mechanisms of genitalia evolution are the lock-and-key hypothesis (Dufour 1844), the pleiotropy hypothesis (Mayr 1963), the sexual selection hypothesis (Eberhard 1985, Lloyd 1979), the female choice, and the male-male genital competition hypotheses (Eberhard 1985). Although Eberhard (1985, 2010) rejected the first three, showing various examples across Arthropoda, the latest research on this topic has shown that it is difficult to completely refute any of those mechanisms (Simmons 2014).

Pseudoscorpions are an interesting taxon for studies on selective pressures, including sexual selection and the evolution of animal genitalia. They have indirect sperm transfer through a spermatophore and exhibit several courtship behaviors that range from spermatophore deposition with no contact, to complex mating dances (Weygoldt 1969). The spermatophore, which is the structure that will have direct contact with the female, is

formed in the male genitalia which acts as a mold for it (Heurtault 1994). These animals have rudimentary eyes, but a very sensitive system of trichobothria in their chela that helps them to sense their world (Weygoldt 1969).

Those small arachnids inhabit under rocks, in fallen logs, bark, and leaf litter (Weygoldt 1969); in this sense, they have a very developed sensorial system composed of a network of mechanical and chemichal receptors. Their phylogenetic relationships at the level of family are stable, but few studies have been published beyond that level. One of the families, Withiidae (Chamberlin 1931) has about 170 species arranged in two subfamilies. Thirteen of the thirty-seven genera are monotypic, and nine genera include only two species, while *Withius* Kew, 1911, is exceptionally large with 50 species. Withiidae is cosmopolitan, but its diversity is higher in tropical and subtropical environments (Harvey 2015). Despite the size of the family, it has been modestly represented in the most recent phylogenetic analyses (Benavides et al. 2019, Harvey et al. 2016, Murienne et al. 2008); thus, the monophyly of the family or its genera has not been rigorously tested. Harvey (2015) guessed that only 13 genera of the Withiidae could be monophyletic; the other proposed taxa at several ranks, such as genera, subfamilies, and tribes, are neither supported by morphology nor by molecules.

Within the superfamily Cheliferoidea, which includes Withiidae, complex reproductive behaviors and courtship dance repertoires that include many sensorial steps can be observed (Weygoldt 1969), and even though members of Withiidae exhibit a great diversity of sexual behaviors (Weygoldt 1969), few descriptions of their reproductive structures have been published. Such a study would be interesting, as the morphology of the male genitalia has a direct effect on courtship and thus may be crucial in sexual selection, as we have explained. For example, in two spider species of the family Salticidae, Lai et al. (2021) found that genital traits have high intra-specific variation, suggesting strong and perhaps divergent selective pressures.

In this context and taking Pseudoscorpions of the family Withiidae as a model for studying the influence of sexual selection in male genitalia and other selective pressures, we wanted to characterize the morphology of Withiidae through detailed examination and dissection of male specimens; then, to explore the relationships between the genera of the Withiidae, testing the monophyly of the family and study the intrafamily relationships, as well as dating the divergence of the clades; and finally, to explore the evolution of the male genitalia trough

comparative methods that allow us to infer a possible influence of sexual selection or another selective pressure.

Then, this study focuses on proposing a phylogenetic framework to test whether sexual selection is acting upon male internal genitalia, having in mind that pseudoscorpion genital atrium acts as a mold for spermatophore shaping (Heurtault 1994). In this sense, finding a difference in evolutionary rates over characters associated with male genital atrium, could give us a hint of sexual selective pressures acting upon these species, or in another case, a hint upon a different selective pressure. In addition, expanding the number of taxa where these studies are conducted significantly improves the generality and reliability of the models proposed.

During the research, we looked for specimens in collections from Australia, Austria, Colombia, Germany, Switzerland, and the United States, to sample representatives of a large geographical coverage and as many genera as possible. We built a character matrix with morphological characters and another with data of three molecular markers, two nuclear and one mitochondrial, and did phylogenetic analyses with several optimization approaches. Then we dated the phylogeny obtained and explored the rates of change of genital, versus non-genital characters, as well as assessing evolutionary dependency between groups of traits. Additionally, as we examined more than 150 specimens we found several taxonomic novelties that are part of this work: first, we redescribed *Metawithius nepalensis*, a species which description was outdated; second, we transferred two species to the genus *Verrucachernes* (Chernetidae), one of them described originally for the family Withiidae under the genus *Metawithius*; and third, we propose one genus and five new species of Withiidae that we describe as endemic for Colombia. Although we extensively sampled withiids of collections around the world, specimens of the African subfamily Paragoniochernetinae are missing from the molecular matrix. Yet, this is an important contribution to the knowledge on the relationships and evolution of the group.

We hypothesize that genital evolution correlates with phylogenetic relatedness in this family and that genitalia characters are changing faster than other characters, giving us a hint on sexual selection acting upon them. Thus, in the next three chapters we present the results of the work in 1) a morphological description of the Cacodemoniini (Withiidae) male genitalia, with propositions of homology (Romero-Ortiz & Sarmiento 2021), 2) a phylogenetic inference of the family Withiidae exploring both, morphological and molecular

characters and an exploration of selective pressures acting upon them; and 3) a taxonomic description of a new genus and five new species of the family, as well as other taxonomic novelties (Romero-Ortiz & Harvey 2019, Johnson et al. 2019).

1. Chapter 1: Comparative study of the male genitalia of the Cacodemoniini (Pseudoscorpiones: Withiidae) and proposals of homology*

1.1 Abstract

Propositions of homology are fundamental in systematics, since they provide the basis for supporting clades. Consequently, such phylogenetic propositions rely on correct character and character state definitions. Although male genital morphology is a key source of information for understanding the phylogeny and classification of the Withiidae (Pseudoscorpiones), they have only been subjected to examination in six of the 170 species of the family. The suprageneric classification of the Withiidae is unstable, as subfamilies and tribes are not well supported by morphological characters, and only the unranked group of genera Cacodemoniini is currently accepted. The aim of the present work is to characterize the male genital armature of the Cacodemoniini and propose homology statements for these structures based upon their morphological correspondence. Through direct examination and literature review of 12 of the 13 genera of the Cacodemoniini, we provide the first structural correspondence statements and descriptions of variation for the dorsal apodemes, the ejaculatory canal, the lateral apodemes, and the lateral rods; we also conclude that unlike other pseudoscorpions, the Cacodemoniini have paired, independent lateral rods and a long ejaculatory canal formed not by the dorsal apodemes exclusively, but by a fusion of the dorsal and the lateral apodemes. The proposed interpretations lay the groundwork for phylogenetic testing of homologies and may allow a better understanding of its formation of the spermatophore, given that it is molded by the genital armature.

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Keywords: Chelonethi, male genital armature, homology.

1.2 Introduction

In the phylogenomic era, large datasets of molecular information are being used to solve systematic problems. However, phylogenies need morphological data since they serve as independent sources of information for testing hypotheses (Pyron 2015; Reeder et al. 2015; Wipfler et al. 2016) and improving estimations of topology and branch lengths (Donoghue et al. 1989; Wright & Hillis 2014; Pyron 2015), as well as allowing the inclusion of fossil taxa (Wiens et al. 2010; Wright & Hillis 2014). Additionally, when the phylogeny is used in historical reconstructions of the phenotype, ecology, physiology and behavior of the taxa under study, morphology becomes crucial (Giribet 2015).

Since homology propositions are fundamental to phylogenetic systematics and provide the basis for supporting clades in an evolutionary framework (Patterson 1982; De Pinna 1991; Nixon & Carpenter 2012), authors have focused on the importance of correct character and character state definitions (Sereno 2007; Vogt et al. 2010) standardizing terminology to facilitate understandability, character statement construction and information exchange (Vogt et al. 2013).

Pseudoscorpiones is a meso-diverse arachnid group with about 3600 valid species (Harvey 2002, 2013). They are small animals (less than 1.2 cm long) with cryptic habits and are present in all terrestrial biogeographical regions except for the polar regions (Weygoldt 1969; Harvey 2013). The reproductive behavior of pseudoscorpions show a wide array of patterns from simple deposition of the spermatophore without direct interaction between the sexes, to complex courtship dances where the male takes the female by the chelae and moves her to the place where he has deposited the spermatophore (Weygoldt 1969). These complex patterns are especially seen in the superfamily Cheliferoidea (e.g., Weyoldt 1969; Harvey 1992; Andrade & Gnaspini 2003), which in turn is the sister-group to (Sternophoroidea + Cheiridioidea) (Harvey 1992; Benavides et al. 2019).

Withiidae is one of the four cheliferoid families, that has about 171 species, which are arranged into two subfamilies: Paragoniochernetinae, with five genera and 11 species, restricted to southern Africa, and Withiinae, with four tribes (Cacodemoniini, Juxtacheliferini, Protowithiini and Withiini), 32 genera and 159 species distributed worldwide (Harvey 2015). Thirteen of the 37 genera of the family are monotypic and nine

include only two species. *Withius* Kew, 1911 is an exceptionally large genus with 50 species. The Withiidae are cosmopolitan but their highest diversity occurs in tropical and subtropical regions (Harvey 2015).

Although the Withiidae appears to be a monophyletic group (Harvey 1992; Murienne et al. 2008; Harvey et al. 2016; Benavides et al. 2019) and is morphologically characterized by the presence of patches of glandular setae in the abdomen (absent in *Juxtachelifer* Hoff, 1956, *Termitowithius* Muchmore, 1990, and *Protowithius* Beier, 1955) (Figure 1) and by the perpendicular division of the femur and patella of leg I (Weygoldt 1970; Harvey 2015), the classification of its subfamilies and tribes was questioned by Harvey (2015), who stated that there are no synapomorphies for most of these taxa.

The Cacodemoniini is the only group of Withiidae that can be morphologically characterized: the long lateral apodemes in their male genital armature are developed into an extended triangle. Although this feature is not found in other pseudoscorpions, its monophyletic status has not been tested phylogenetically (Harvey 2015). The name was proposed by Chamberlin (1931a) based on the Neotropical genus *Cacodemonius* Chamberlin, 1931, and defined by several somatic character states. However, Harvey (2015) broadened the definition to include those withiids with the long, triangular lateral apodemes, and included 12 additional genera from the Americas, Australasia and Africa: *Balanowithius* Beier, 1959, *Cystowithius* Harvey, 2004, *Dolichowithius* Chamberlin, 1931, *Metawithius* Chamberlin, 1931, *Microwithius* Redikorzev, 1938, *Parawithius* Chamberlin 1931, *Pycnowithius* Beier, 1979, *Rexwithius* Heurtault, 1994, *Rugowithius* Harvey, 2015, *Thaumatowithius* Beier, 1940, *Trichotowithius* Beier, 1944, and *Victorwithius* Feio, 1944.

The morphology of the male reproductive system has been described for a variety of pseudoscorpions (e.g., Vachon 1938; Legg 1971, 1974a, b, c, 1975a, b, c), and the most detailed comparative study was presented by Klausen (2005) for the family Atemnidae. For the Withiidae, Mahnert (1975) and Dashdamirov (1992) suggested that a thorough examination of the male and female genitalia will be important for understanding the classification within the family. Nevertheless, only 13 of the 170 species belonging to six genera, have been subjected to detailed studies (Harvey 1988, 2004, 2015; Heurtault 1971; Mahnert 1988; Dashdamirov 1992), perhaps because it is difficult to examine the reproductive system in situ (Klausen 2005) and its extraction entails destruction of other traditionally used characters.

Legg (1974c) provided a generalized account of the male genitalia and associated glands for pseudoscorpions and established the correspondence between the terminologies employed by different authors such as Schtschelkanovzeff (1910), Kästner (1927), Chamberlin (1931b), Vachon (1938), and Weygoldt (1966, 1969). Legg (1974c) noted that the genitalia consist of the genital atrium, the genital armature, the accessory glands, and the genital sacs. The genital atrium is constituted of a series of apodemes which serve as muscle attachments and support the diverticula and the ejaculatory canal; the atrium also receives the products of the testis and accessory glands. Although the genitalia of Withiidae fits this description in general terms, their complexity and poor sclerotization make a proper understanding of its organization difficult.

In view of the above, the aims of this study are to 1) characterize the morphology of the male genital armature in several genera of the Cacodemoniini, and 2) propose homology statements for these structures based on morphological correspondence.

1.3 Methods

We studied 19 cacodemoniine specimens, representing all six Neotropical genera and thus about half of the global genera of Cacodemoniini (Table 1). This included: two specimens of *Balanowithius egregius* Beier, 1959; for *Cacodemonius* Chamberlin, 1931 and *Cystowithius* Harvey, 2004 two clearly differentiated specimens from different localities of each genus were included. For *Dolichowithius* Chamberlin, 1931, four specimens belonging to three clearly differentiated morphospecies (msp) were examined. For *Parawithius* Chamberlin, 1931, a single specimen was studied. For *Victorwithius* Feio, 1944 seven specimens belonging to five clearly differentiated morphospecies (msp) were examined. However, two of these morphospecies (msp. 4 and 5) exhibit significant differences in somatic and genital morphology and thus are treated separately from that genus and named as nr. *Victorwithius* (see below).

The specimens were identified based on published descriptions (e.g. Chamberlin 1931a; Feio 1944; Beier 1959; Harvey 2004), comparison with specimens lodged in the Western Australian Museum, and the advice of Dr. Mark Harvey (Western Australian Museum). We also reviewed the available descriptions and illustrations of six of the seven genera of non-Neotropical Cacodemoniini (e.g. Harvey 1988, 2015; Mahnert 1988; Dashdamirov 1992; Heurtault 1994; Johnson et al. 2019). We had no access to specimens of *Thaumatowithius*

Beier, 1940 and no descriptions are available for the male genitalia for either of the two described species. Thus, we had data for 12 of the 13 cacodemoniine genera.

The Neotropical specimens came from the arachnological collection of the Instituto de Ciencias Naturales-Universidad Nacional de Colombia (ICN-APs) and the Instituto Alexander von Humboldt invertebrate collection (IAvH) and were preserved in 75% or 100% ethanol.

We cleared and dissected males using the following protocol: 1) immerse the specimen in KOH 10% at room temperature for 4 hours or until the soft tissues disappeared; two lateral incisions in the abdomen of the individual were cut beforehand to facilitate the penetration of the KOH; 2) rinse in distilled water for 3 minutes; 3) transfer to 5% acetic acid for three minutes to neutralize the KOH; 4) rinse in distilled water for 1 minute; 5) the genitalia was extracted as follows: with the specimen upside down, forceps were used to create a gentle push over the proximal and distal regions of the genital opercula and simultaneously, a minuten pin was used to pull the opercula anteriorly such that the genital armature popped out; once out the minuten pin was used to sever the connections between the opercula and the armature; 6) after examination in glycerin, the genitalia is stored in a PCR tube in glycerin that was placed inside the vial with the body in ethanol. We took multifocal photographs of the genitalia with a Leica MC-170 HD digital camera attached to a Leica M205A stereomicroscope, which were processed with Leica Application Suite version 4.6.0.

Morphological nomenclature mainly follows Legg (1974c). We followed Klausen (2005) in using the terms “anterior” for the region of the genital armature that faces the prosoma, and “posterior” for the region that faces the opisthosoma. However, we use “ventral” to refer to the region attached to the genital opercula, and “dorsal” for the opposite region (Figure 2). As suggested by Mahnert (1975), we concentrated on the chitinized parts of the genitalia, because the shape and location of soft tissue such as the diverticula could vary according to the method of preparation. Structural equivalence between genitalia components of different genera was based on their relative position, without further considerations on the history of the character which would require a thorough phylogenetic study.

Abbreviations used: *da*—dorsal apodemes, *ejc*—ejaculatory canal, *ejca*—ejaculatory canal atrium, *la*—lateral apodemes, *lr*—lateral rods.

1.4 Results and discussion

As mentioned above, we identified two morphospecies that are morphologically similar to *Victorwithius*. They share important characters such as the presence of a patch of glandular setae only on sternite VIII of the male, and the presence of an inconspicuous tactile setae on leg IV. However, they differ in other characteristics including the shape of the patch of glandular setae, which circular in *Victorwithius* but oblong to elongated in the other taxa, and in the location of the patch which lies in the pleural membrane in *Victorwithius* but lies within the sternite in the others. To signify these differences, which are likely to be of generic significance, we refer these two species as nr. *Victorwithius*. Further phylogenetic analysis is needed to test their status and relationships.

Structural correspondence and variation.—Overall, the male genital armature of Cacodemoniini comprises the dorsal apodemes (*da*) that extend posteriorly to form the ejaculatory canal (*ejc*), which is elongated, giving a triangular shape to the genitalia, and the lateral apodemes (*la*) which enclose the ejaculatory canal atrium (*ejca*), which is supported in its anterior region by the lateral rods (*lr*) (Figure 3).

Figures are presented in alphabetical order according to the genera, which means figure 4 belongs to *Balanowithius egregius* Beier, 1959, figure 5 to *Cacodemonius* msps., figure 6 to *Cystowithius* msps., figure 7 to *Dolichowithius* msps., in ventral, lateral and dorsal views, and figure 8 to the anterior view; figure 9 to *Parawithius* msp., figure 10 to *Victorwithius* msps. in ventral, lateral and dorsal views, and figure 11 to the anterior view; figure 12 to nr. *Victorwithius* msps.

Dorsal apodemes (da): For Atemnidae, the closest family to the Withiidae (Benavides et al. 2019), Klausen (2005) defines the dorsal apodemes as an elongated fusion of the lateral rods.In contrast, cacodemoniines, they are not fused and appear as structures separate to the lateral rods (Fig. 3C–D). The genital atrium and the ejaculatory canal are formed by the dorsal and the lateral apodemes. In the genital atrium, the dorsal and lateral apodemes are separated surrounding it, while in the ejaculatory canal, the apodemes are fused (Figure 3).

The degree and extent of sclerotization and the shape of the dorsal apodemes vary between genera. Sclerotization is very marked in *Cacodemonius* (Fig. 5A, D) and nr. *Victorwithius* (Fig. 12A, D), while it is moderate in the genera *Balanowithius* (Fig. 4A),

Cystowithius (Fig. 6A, D), *Dolichowithius* (Fig. 7A, D), and *Victorwithius* (Fig. 10A, D, G), and weak in *Parawithius* (Fig. 9A). The sclerotization covers the entire genital atrium in *Cacodemonius* (Fig. 5B, E), *Dolichowithius* (Fig. 7C, F), *Victorwithius* (Fig. 10C, F), and nr. *Victorwithius* (Fig. 12B, C, E, F), while in the genera *Balanowithius*, *Cystowithius* and *Parawithius* it covers less than half of the genital atrium (Figs. 4A, 6A, 9A). The first half of the *da* are sinuous in *Dolichowithius* (Fig. 7D) and straight in the other taxa. The *da* are wider in the proximal third in *Cystowithius* (Fig. 6A), while they are uniform in the other genera.

The dorsal apodemes in *Metawithius* and *Rugowithius* were labeled by Harvey (2015) and Johnson et al. (2019) as median diverticula; however, these structures appear in the corresponding position of the dorsal apodemes of the Neotropical Cacodemoniini. In addition, their illustrations suggest that these are sclerotized structures, whereas the diverticula are not sclerotized. The dorsal apodemes of *Metawithius* are short and straight while these are long and straight in *Rugowithius* (Harvey 2015) and *Trichotowithius* (Dashdamirov 1992). The illustration of the latter did not name this structure. In *Microwithius* the dorsal apodemes cannot be seen due to a wide lateral apodeme covering the structures (Harvey 1988). Finally, in *Pycnowithius* and *Rexwithius* these structures are not visible in the published illustrations [Mahnert (1988) and Heurtault (1994) respectively].

Ejaculatory canal (ejc): Unlike that of other pseudoscorpions (e.g. Vachon 1938; Legg 1974c; Klausen 2005), the ejaculatory canal of cacodemoniines extends posteriorly as an inverted triangle seen in either dorsal or ventral view. The ejaculatory canal is formed by the elongation of the dorsal and lateral apodemes. This is the diagnostic character for the Cacodemoniini (Harvey 2015), and Klausen (2005) proposed that this is a functional extension of the ejaculatory canal atrium. This ejaculatory canal atrium (*ejca*) is a capsule surrounded by both the lateral apodemes and the dorsal apodemes (Figure 3); as stated by Legg (1974c), it receives products of the testis and the accessory glands.

We found differences for this structure in its length, shape, sclerotization of the posterior tip, and its direction in lateral view. The *ejc* is shorter than the width of the ejaculatory canal atrium in *Cystowithius* (Fig. 6) and *Parawithius* (Fig. 9), while it is longer in the other genera. The posterior end of the *ejc* is acute in nr. *Victorwithius* (Fig. 12A, D), wide or even lanceolate in *Dolichowithius* (Fig. 7D) and rounded in the other Neotropical taxa. The posterior tip of the *ejc* is sclerotized in *Cystowithius* (Fig. 6A) and nr. *Victorwithius* (Fig. 12A)

while it is not in the other genera (e.g. Fig. 7A, D, G, 10A). The *ejc* is straight in *Cacodemonius* (Fig. 5C, F), *Victorwithius* (Fig. 10E, H), and some species of *Dolichowithius* (Fig. 7E), while it is curved ventrally in *Balanowithius* (Fig. 4B), *Cystowithius* (Fig. 6B, E), and *Parawithius* (Fig. 9B), and curved dorsally in nr. *Victorwithius* (Fig. 12B, E) and some species of *Dolichowithius* (Fig. 7B, H).

The *ejc* is longer than the ejaculatory canal atrium in *Trichotowithius* and *Metawithius* (Dashdamirov 1992; Harvey 2015) and is either as long as or shorter than the ejaculatory canal atrium in *Pycnowithius* and *Rugowithius* respectively (Mahnert 1988; Harvey 2015). It is straight in ventral view in all non-Neotropical genera, but the illustrations and descriptions do not indicate whether it is directed dorsally or ventrally in lateral view. The posterior end is clearly obtuse in *Trichotowithius* (Dashdamirov 1992), but no information is available for the other genera. For *Microwithius* and *Rexwithius* the *ejc* is not visible in the illustrations (Harvey 1988; Heurtault 1994). In *Rugowithius* the *ejc* is labeled as *ejca* (Harvey 2015) but the structure does not correspond to our definition.

Lateral apodemes (la): According to Klausen (2005) the lateral apodemes are formed by the anterolateral sides of the two dorsal and medial diverticula. Legg (1974b, c) described that the lateral apodemes support the dorsal and medial diverticula and serve as attachment sites for the sterno-coxal and coxal muscles, specifically the segmental dorso-ventral muscles, the longitudinal-ventral muscles, the appendicular muscles, and the transverse muscle. We could not observe whether Neotropical specimens follow Klausen's and Legg's interpretation as we did not study the diverticula due to the clearing process.

We observed that in the Neotropical Cacodemoniini the lateral apodemes (*la*) are paired and together with the dorsal apodeme form a bowl that protects the ejaculatory canal atrium. In lateral view, the apodemes exhibit different levels of curvature and in ventral view, they seem to merge with the bifid dorsal apodeme. Together with the dorsal apodeme they extend posteriorly to form the ejaculatory canal. This general description of the lateral apodemes applies to the non-Neotropical Cacodemoniini.

The *la* vary between genera in their extension, shape of the basal region, and orientation of the concavity in lateral view. The *la* cover almost the whole genital atrium in *Balanowithius* (Fig. 4B), *Cacodemonius* (Fig. 5C, F), *Victorwithius*, and nr. *Victorwithius* (Fig. 10B, E, H, 12B, E), but they do not go beyond half of the genital atrium in *Cystowithius* (Fig. 6B, E), *Dolichowithius* (Fig. 7B, E, H), and *Parawithius* (Fig. 9A, B). The shape of the

basal region is clearly angled in *Cacodemonius* (Fig. 5D, E), *Dolichowithius* (Fig. 7A, D, G), and nr. *Victorwithius* (Fig. 12A, D), but it is rounded in the other genera. The curvature is more pronounced anteriorly in *Balanowithius* (Fig. 4B) and *Cacodemonius* (Fig. 5C, F), but it is more pronounced dorsally in the other genera.

Heurtault (1971) named the lateral apodemes "*arc chitinisé*" for *Withius hispanus* (L. Koch, 1873), *W. faunus* (Simon, 1879) and *W. neglectus* (Simon, 1878). Using the English version, "chitinized arch", Harvey (2015) and Johnson et al. (2019) follow this nomenclature for *Metawithius*. Since the chitinized arch corresponds to Legg's (1974c) lateral apodemes, we follow this original nomenclature. The la are wide in *Microwithius, Pycnowithius* and *Rugowithius* (Harvey 1988, 2015; Mahnert 1988), while they are thin in *Metawithius* and *Trichotowithius* (Dashdamirov 1992; Harvey 2015; Johnson et al. 2019). The only figure available for *Rexwithius* (Heurtault 1994) does not allow us to locate the *la*.

Lateral rods (lr): The lateral rods are only visible in anterior view. In the Cacodemoniini, they are placed between the anterior ends of the dorsal and lateral apodemes, and vary in shape, alignment and length. They are sinuous only in *Balanowithius* (Fig. 4C). They diverge dorsally in *Cystowithius* (Fig. 6G, H) and *Parawithius* (Fig. 9D), while they are parallel in the other genera. The lateral rods do not extend beyond the lateral apodemes in *Balanowithius* (Fig. 4C), whereas they are longer in the other genera. In many of the studied specimens they were not visible, due to the high amount of tissue around this area.

Concerning the non-Neotropical genera, the lateral rods are only visible in the illustrations of *Metawithius* and *Microwithius* provided by Johnson et al. (2019) and Harvey (1988), respectively. In *Metawithius* they are sinuous and parallel, while in *Microwithius* they are divergent dorsally. In *Trichotowithius*, Dashdamirov (1992) labels as lateral rods two longitudinal projections located together with the ejaculatory canal; however, this location does not fit our observations in other Cacodemoniini and we were unable to establish their correspondence to known structures. Since lateral rods are present in all the other cheliferoid families (e.g. Legg 1974c; Klausen 2005), it is likely that these structures are present in all Cacodemoniini.

Taxonomic characterization of the male genital armature for each genus.—A description of the genital armature of each of the Neotropical genera of Cacodemoniini is provided below.

Balanowithius (Figure 4)

Dorsal apodemes with sclerotization moderate to almost absent, stronger in the first half of its length; straight and of uniform width throughout its length; (Fig. 4A). Lateral apodemes covering most of the ejaculatory canal atrium; rounded basally; projecting anteriorly in lateral view (Fig. 4B). Ejaculatory canal long, two thirds length of genital armature; tip rounded, not sclerotized posteriorly; curved ventrally in lateral view (Fig. 4A). Lateral rods sinuous; parallel; not extending beyond lateral apodemes in dorsal view (Fig. 4B-C). This characterization is based on two specimens of *Balanowithius egregius*.

Cacodemonius (Figure 5)

Dorsal apodemes intensely and entirely sclerotized; straight and of uniform width throughout its length; (Fig. 5A, D). Lateral apodemes extending over most of ejaculatory canal atrium; angular basally; projecting anteriorly in lateral view (Fig. 5A, C, D). Ejaculatory canal long, two thirds length of genital armature; tip rounded, not sclerotized posteriorly; straight in lateral view (Fig. 5A, C, D, F). Lateral rods extending beyond lateral apodemes in dorsal view (Fig. 5G, H). This characterization is based on two specimens of two morphospecies of the genus *Cacodemonius*.

Cystowithius (Figure 6)

Dorsal apodemes with sclerotization moderate to almost absent, reaching half or less of its length; straight throughout its length; the sclerotization of its border is wider at the ejaculatory canal atrium (Fig. 6A, D). Lateral apodemes at most half length of the ejaculatory canal atrium; rounded basally; projecting dorsally (Fig. 6B, C, E, F). Ejaculatory canal short, one third of genital armature length; tip rounded, sclerotized posteriorly; curved dorsally in lateral view (Fig. 6A, B, D, E). Lateral rods straight; divergent dorsally; extending beyond lateral apodemes in dorsal view (Fig. 6G, F). This characterization is based on two specimens of two morphospecies of the genus *Cystowithius*.

Dolichowithius (Figures 7-8)

Dorsal apodemes with sclerotization moderate to almost absent, extending over half or less of its length; sinuous in proximal third and straight distally; of uniform width throughout (Fig. 7A, D, G). Lateral apodemes extending over at most half of ejaculatory canal atrium; angular basally; projecting dorsally (Fig. 7). Ejaculatory canal long, two thirds of genital

armature length; tip acute; not sclerotized posteriorly; straight in lateral view, sometimes curved ventrally (Fig. 7A, B, D, E, G, H). Lateral rods straight; parallel; extending beyond lateral apodemes in dorsal view (Fig. 8). This characterization is based on four specimens belonging to three morphospecies of the genus Dolichowithius.

Parawithius (Figure 9)

Dorsal apodemes with sclerotization moderate to almost absent, extending over half or less of its length; straight throughout its length; of uniform width throughout (Fig. 9C). Lateral apodemes extending over at most half of ejaculatory canal atrium; rounded basally, projecting dorsally (Fig. 9A-C). Ejaculatory canal short, one third of genital armature length; tip rounded, not sclerotized posteriorly; curved dorsally in lateral view (Fig. 9B, C). Lateral rods straight; divergent dorsally; extending beyond lateral apodemes in dorsal view (Fig. 9D). This characterization is based on one specimen of the genus *Parawithius*.

Victorwithius (Figures 10-11)

Dorsal apodemes entirely sclerotized but the sclerotization is moderate to weak; straight and of uniform width throughout its length (Fig. 10A, D, G). Lateral apodemes extending over most of ejaculatory canal atrium; rounded basally; projecting dorsally (Fig. 10). Ejaculatory canal long, two thirds of genital armature length; tip rounded, not sclerotized posteriorly; straight in lateral view (Fig. 10A, B, D, E, G, H). Lateral rods straight, parallel; extending beyond lateral apodemes in dorsal view (Fig. 11). This characterization is based on five specimens belonging to three morphospecies of the genus *Victorwithius*.

nr. *Victorwithius* (Figure 12)

Dorsal apodemes intensely sclerotized; extending over its entire length; straight and of uniform width throughout its length (Fig. 12A, D). Lateral apodemes extending over most of ejaculatory canal atrium; angular basally; projecting dorsally (Fig. 12A-F). Ejaculatory canal long, two thirds of the genital armature length; tip acute, sclerotized posteriorly; curved dorsally in lateral view (Fig. 12A, B, D, E). Lateral rods straight, parallel; extending beyond lateral apodemes in dorsal view (Fig. 12G, H). This characterization is based on two specimens belonging to two morphospecies of this undescribed taxon.

Final remarks.—Our analysis explored for the first time the male genitalia of the Cacodemoniini using a comparative approach and provides hypotheses of structural correspondences, covering six of the 13 cacodemoniine genera by direct examination of specimens, and six genera using published descriptions and illustrations (Harvey 1988, 2015; Mahnert 1988; Dashdamirov 1992; Heurtault 1994; Johnson et al. 2019).

Structural correspondence conclusions: In agreement with Harvey (2015), our analyses allow us to characterize the Cacodemoniini genitalia as follows: The dorsal apodemes and the lateral apodemes are separated in the most anterior region and surround the ejaculatory canal atrium. Posteriorly, they are fused and extend to form the ejaculatory canal. The lateral rods are separated from the apodemes. Variation on the genitalia is found in the shape of the lateral apodemes, the length of the ejaculatory canal and its projection in lateral view.

Regarding the triangular projection of the ejaculatory canal, we disagree with Harvey (2015) who attributes this projection to the lateral apodemes, whenever we could inspect the genitalia in sufficient detail, through direct examination or drawings, we observed that such elongation includes the fusion of the dorsal apodemes with the lateral apodemes.

Taxonomic implications: Although we have not observed every species of Neotropical Cacodemoniini, our data suggest that the differences in the male genital armature of each genus follow the current classification of the group, agreeing with Mahnert's (1975) proposition that this is a helpful structure for taxonomy at this taxonomic rank. This result contrast with Klausen's (2005) findings for the genera of Atemnidae where there is little taxonomic correspondence.

Phylogenetic connotations: The taxonomic distribution of the characters of the male genital armature across the Cacodemoniini genera makes it difficult to even speculate on their relationships using this character system. For example, the dorsal apodeme sclerotization is moderate and straight throughout its length in *Balanowithius* and *Victorwithius*, while the lateral apodemes are projected anteriorly in *Balanowithius* as opposed to dorsally in *Cystowithius* and *Victorwithius*. Another example is the presence of a short ejaculatory canal in *Cystowithius* and *Parawithius*, while *Cystowithius* and nr. *Victorwithius* share a sclerotized tip of the ejaculatory canal which is absent in *Parawithius*.

Examining some species of *Withius*, Klausen (2005) found that, like in Atemnidae, the lateral rods are neither merged proximally nor directed anteriorly and concluded that both families could be closely related. Our more extensive sampling of the Withiidae concur with his observation on *Withius* and with the phylogenetic hypothesis of Benavides et al. (2019), who found strong molecular evidence to support a sister-group relationship between Atemnidae and Withiidae.

Despite the impressive advances using molecular data for phylogenetic and biodiversity studies, morphological analyses as the one provided here are still required to understand the history of the phenotypes as the substrate of evolution. A thorough understanding of the genitalia in pseudoscorpions may shed further light into the diversity and history of their courtship strategies.

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Table 1 . Material examined for the study of the male genitalia in Cacodemoniini. All specimens were collected in Colombia (South America). Morphospecies codes are shown as 'msp'.

Species	Department	Municipality	Locality	Collection number	No. of specimens
Balanowithius egregius	Nariño	Barbacoas	Vereda Altaquer, Reserva Natural Río Ñambí	ICN-APs-473	2
Cacodemonius msp1	Cundinamarca	Cachipay	--	ICN-APs-091	1
Cacodemonius msp2	Atlántico	Usiacurí	Reserva La Montaña	ICN-APs-614	1
Cystowithius msp1	Tolima	Juntas	Reserva Natural Ibanasca	ICN-APs-077	1
Cystowithius msp2	Cundinamarca	San Antonio del Tequendama	Parque Nacional Natural Chicaque	ICN-APs-298	1
Dolichowithius msp1	Cundinamarca	Cachipay	--	ICN-APs-027	2
Dolichowithius msp2	Sucre	San Marcos	La Florida	ICN-APs-145	1
Dolichowithius msp3	Santander	Suaita	San Jose de Suaita. Fundación San Cipriano	ICN-APs-413	1
Parawithius msp1	Cundinamarca	Cogua	Embalse del Neusa Teusa. Llano Grande	ICN-APs-082	1
Victorwithius msp1	Arauca	Arauca	Sede Universidad Nacional	ICN-APs-384	1
Victorwithius msp2	Meta	Vista Hermosa	Vereda La Reforma. Finca. Los Moriches	ICN-APs-405	2
Victorwithius msp3	Caquetá	Florencia	CIMAZ Macagual. Río Sarabando	ICN-APs-573	1
nr. Victorwithius msp1	Meta	San Martín	Vereda San Francisco. Hacienda La María	ICN-APs-076	1
nr. Victorwithius msp2	Cesar	Valledupar	Ecoparque Los Besotes. Campamento base	ICN-APs-597	1

FIGURE LEGENDS

Figure 1.–Habitus of a male pseudoscorpion of the family Withiidae (nr. *Victorwithius*): A. Dorsal. B. Ventral. Inset shows the patches of glandular setae, one of the diagnostic characters of the family. Scale lines = 500 µm.

Figure 2.–Schematic representation in lateral view showing the orientation of the male genitalia of Cacodemoniini. Abbreviations: A= Anterior. P= Posterior. D= Dorsal. V= Ventral face.

Figure 3.–Male genitalia of Cacodemoniini with indication of the main structures. A. Ventral. B. Lateral. C. Dorsal. D. Anterior views. Abbreviations: *da*: dorsal apodemes, *la*: lateral apodemes, *ejc*: ejaculatory canal, *lr*: lateral rods. Scale lines = 100 µm.

Figure 4.–Male genitalia of *Balanowithius egregius* Beier, 1959. A. Ventral. B. Lateral. C. Anterior views. Scale lines = 100 µm.

Figure 5.–Male genitalia of *Cacodemonius* msp. 1: A, B, C, G; and *Cacodemonius* msp. 2: D, E, F, H. A, D. Ventral. B, E. Dorsal. C, F. Lateral. G, H. Anterior views. Scale lines = 100 µm

Figure 6.–Male genitalia of *Cystowithius* msp. 1: A, B, C, G; and *Cystowithius* msp. 2: D, E, F, H. A, D. Ventral. B, E. Lateral, C, F. Dorsal. G, H. Anterior views. Scale lines = 100 µm.

Figure 7.–Male genitalia of *Dolichowithius* msp. 1: A, B, C; *Dolichowithius* msp. 2: D, E, F; and *Dolichowithius* msp. 3: G, H, I. A, D, G. Ventral. B, E, H. Lateral. C, F, I. Dorsal views. Scale lines = 100 µm.

Figure 8.–Male genitalia of *Dolichowithius* spp., anterior view: A. *Dolichowithius* msp. 1; B. *Dolichowithius* msp. 3; C. *Dolichowithius* msp. 2. Scale lines = 100 µm.

Figure 9.–Male genitalia of *Parawithius* msp1: A. Ventral. B. Lateral. C. Dorsal. D. Anterior views. Scale lines = 100 µm.

Figure 10.–Male genitalia of *Victorwithius* spp.: *Victorwithius* msp. 1: A, B, C. *Victorwithius* msp. 2: D, E, F. *Victorwithius* msp. 3: G, H, I. A, D, G. Ventral. B, E, H. Lateral. C, F, I. Dorsal views. Scale lines = 100 µm.

Figure 11.–Male genitalia of *Victorwithius* spp., anterior view: A. *Victorwithius* msp. 2, B. *Victorwithius* msp. 3. C. *Victorwithius* msp. 1. Scale lines = 100 µm.

Figure 12.–Male genitalia of nr. *Victorwithius* msp. 1: A, B, C, G. nr. *Victorwithius* msp. 2: D, E, F, H. A, D. Ventral. B, E. Lateral. C, F. Dorsal. G, H. Anterior views. Scale lines = 100 µm

FIGURE 1

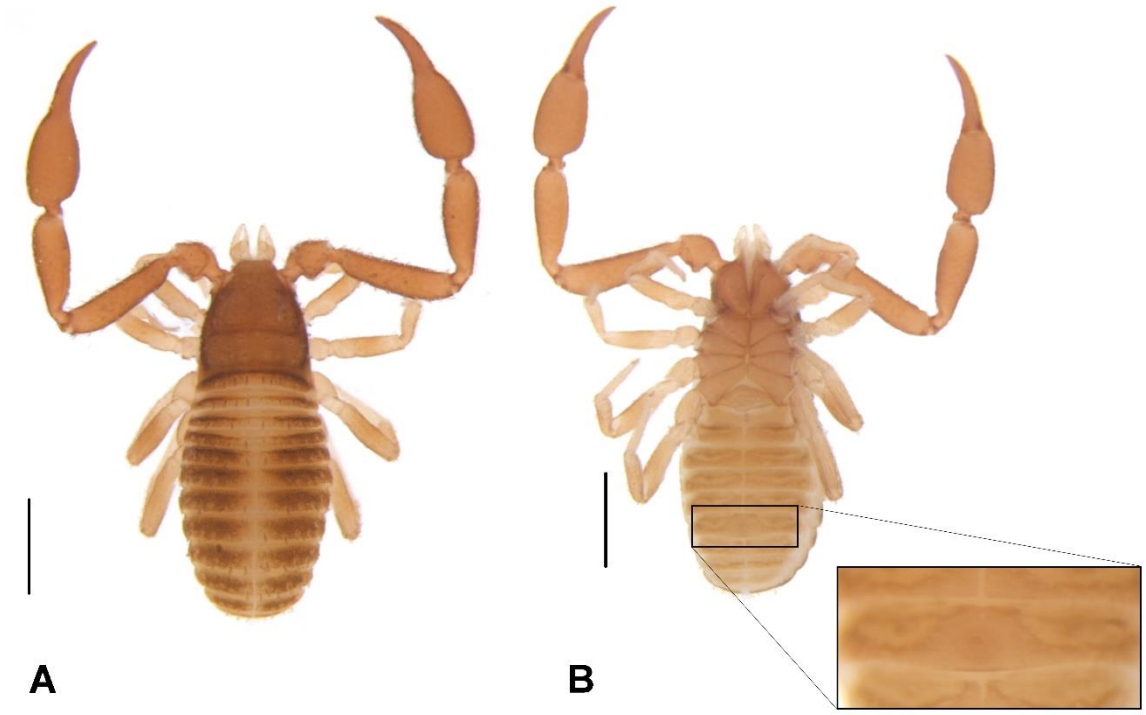


FIGURE 2

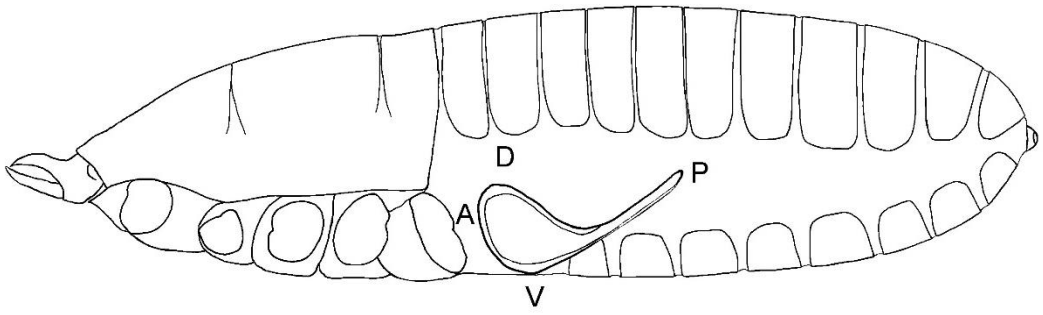


FIGURE 3

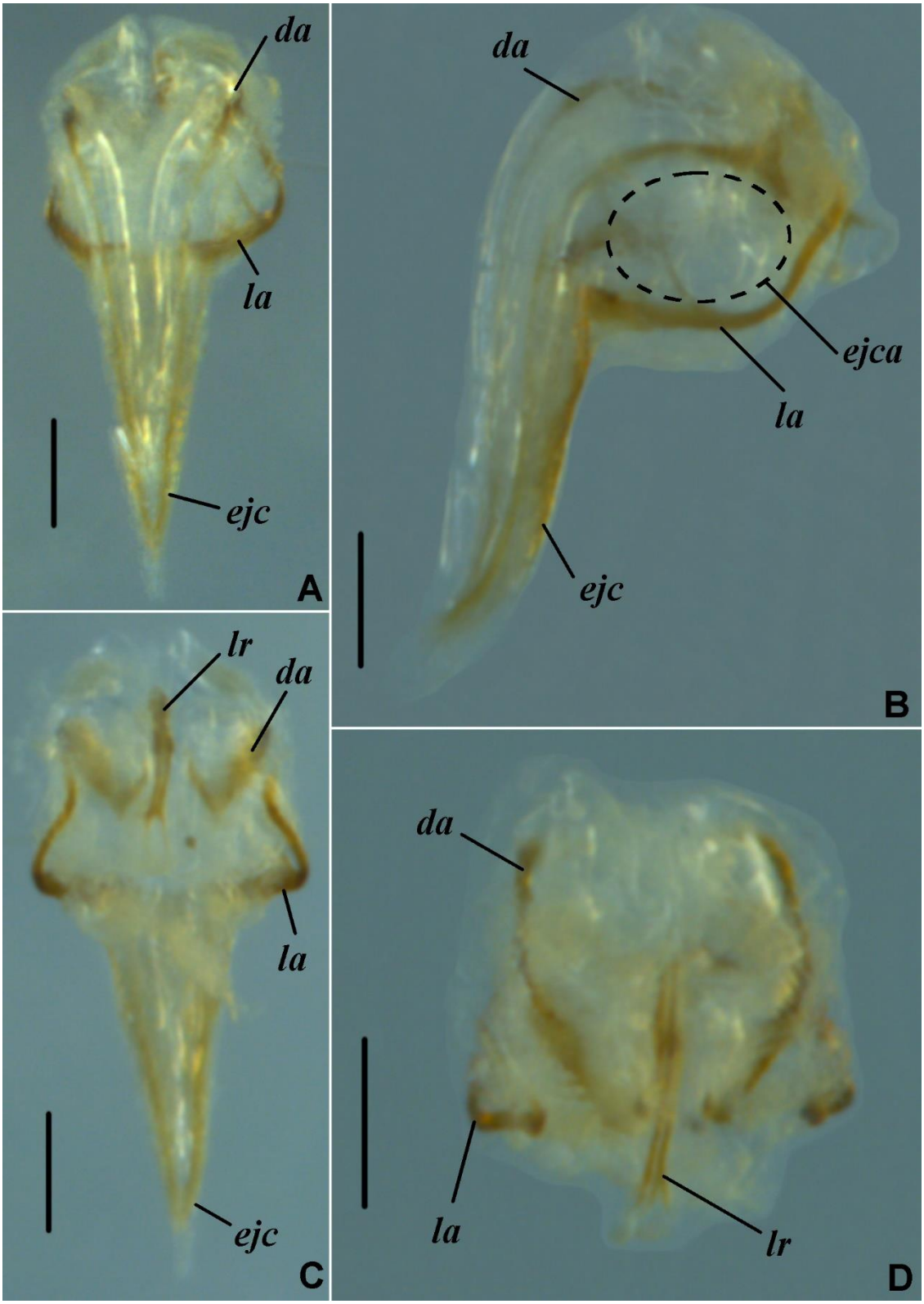


FIGURE 4

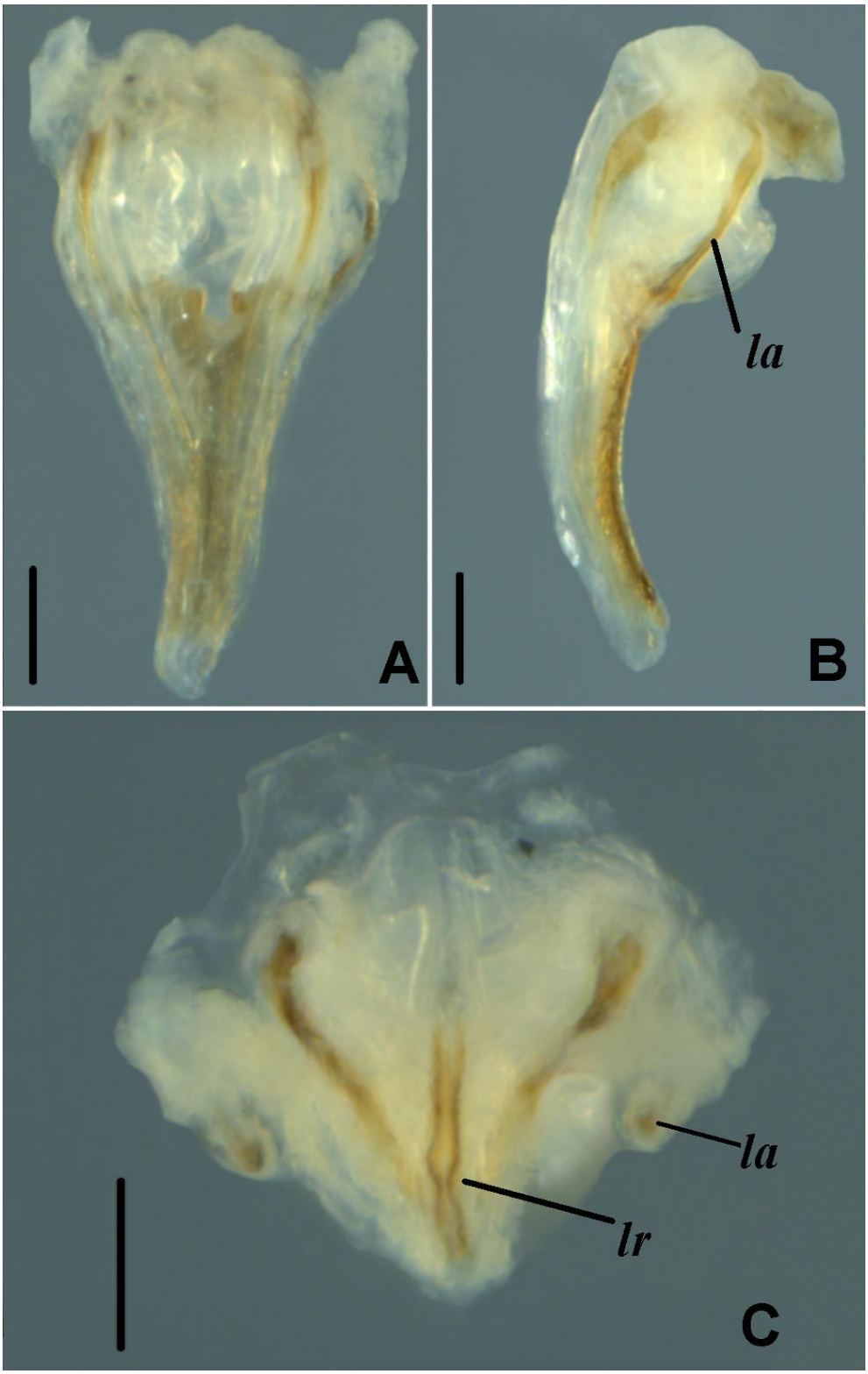


FIGURE 5

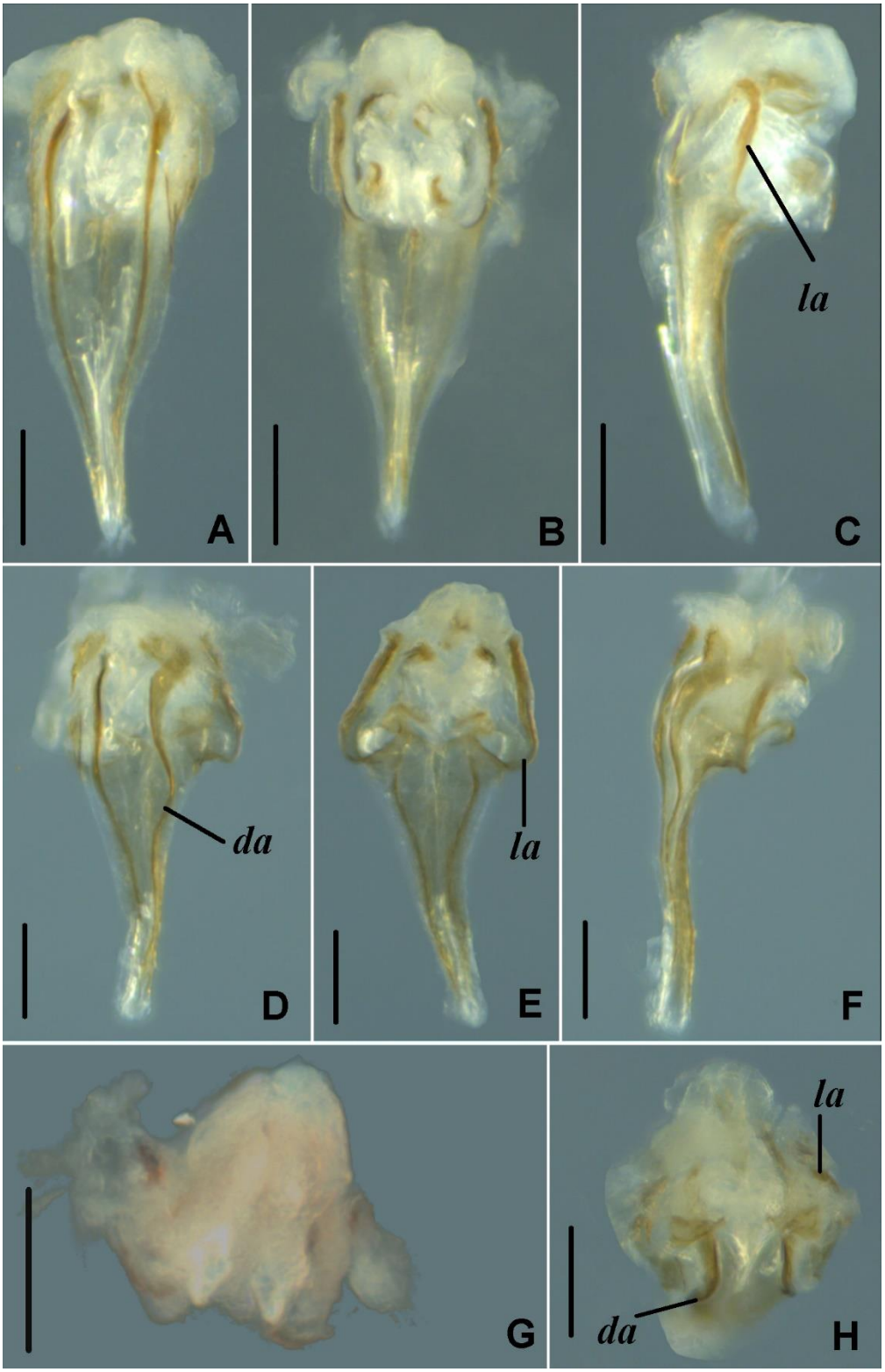


FIGURE 6

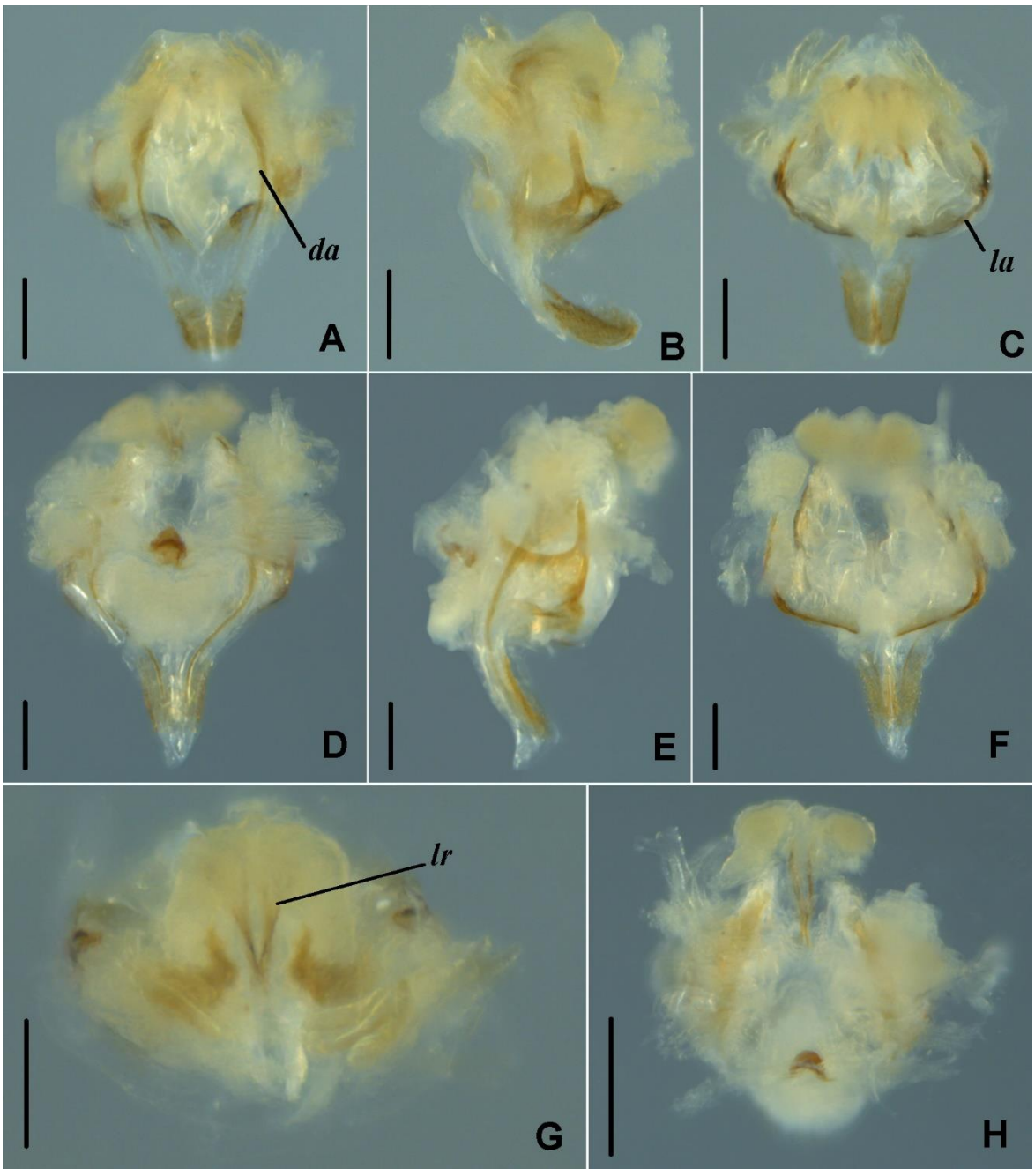


FIGURE 7

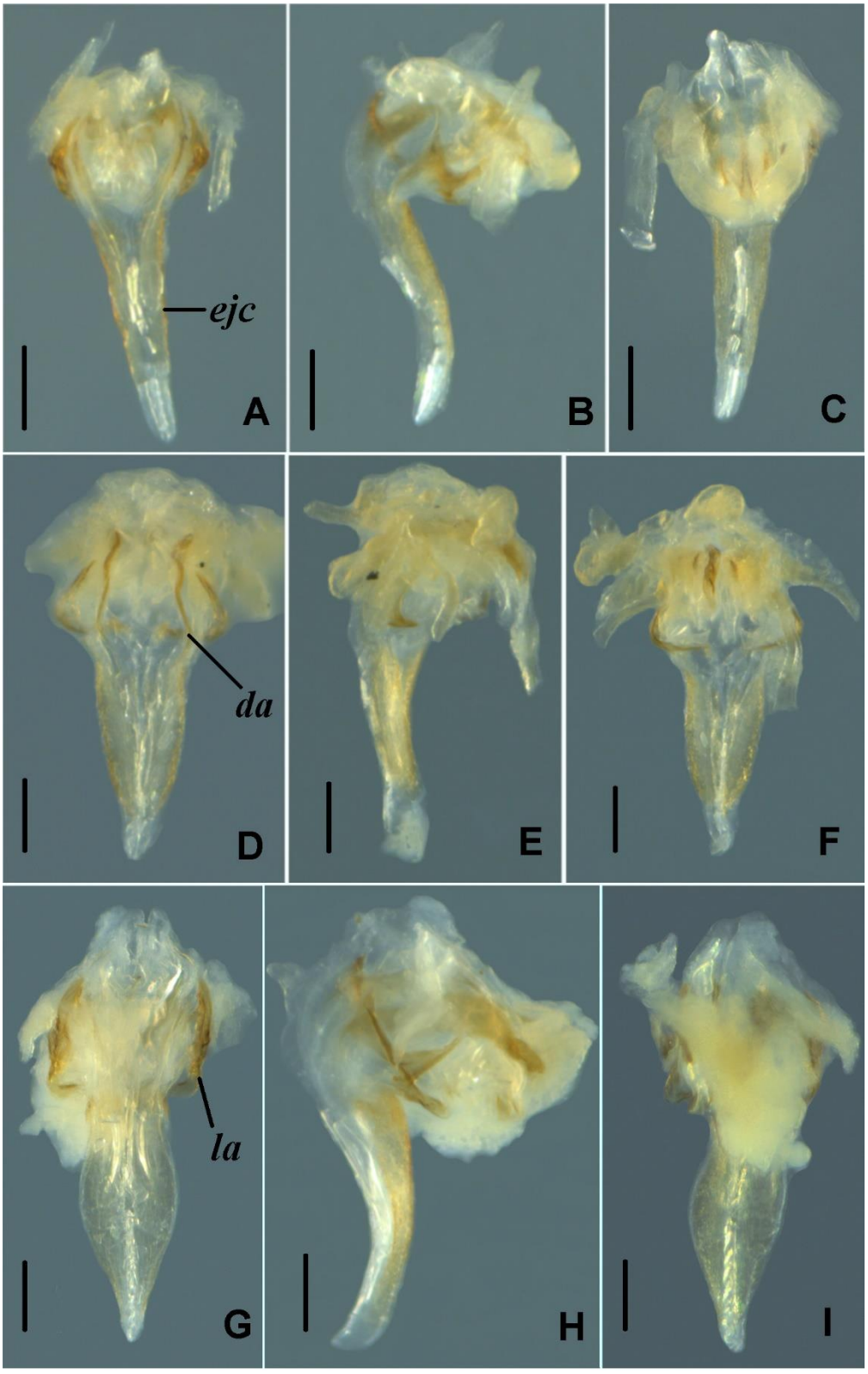
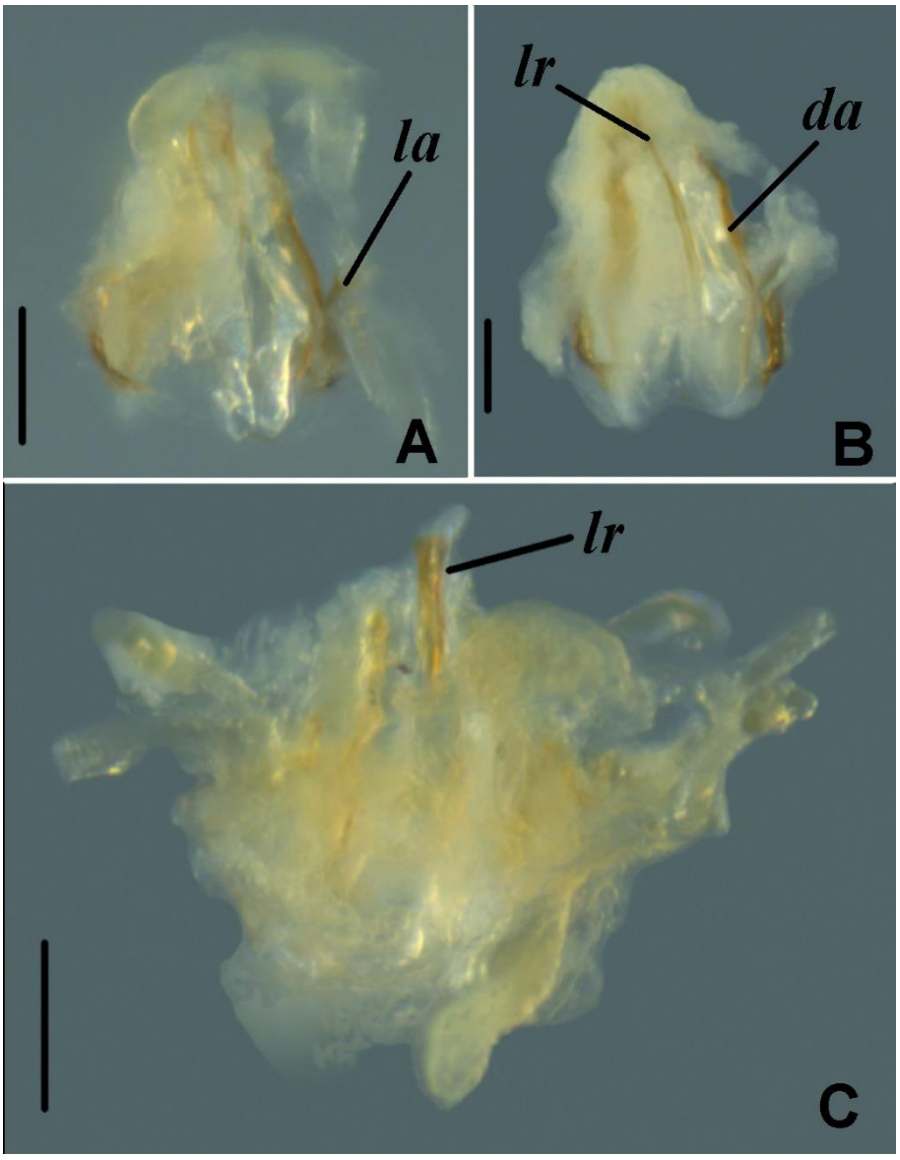


FIGURE 8



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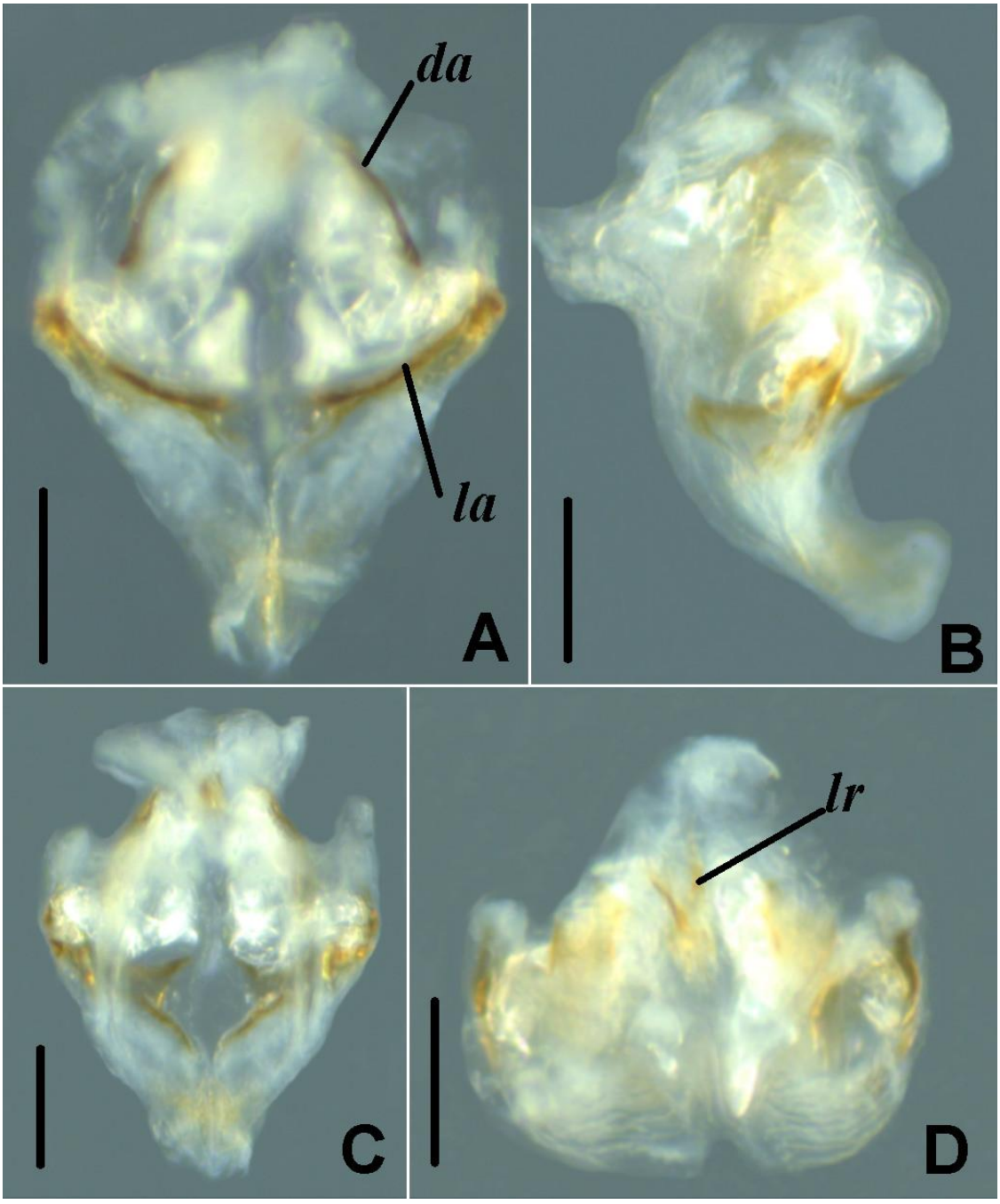

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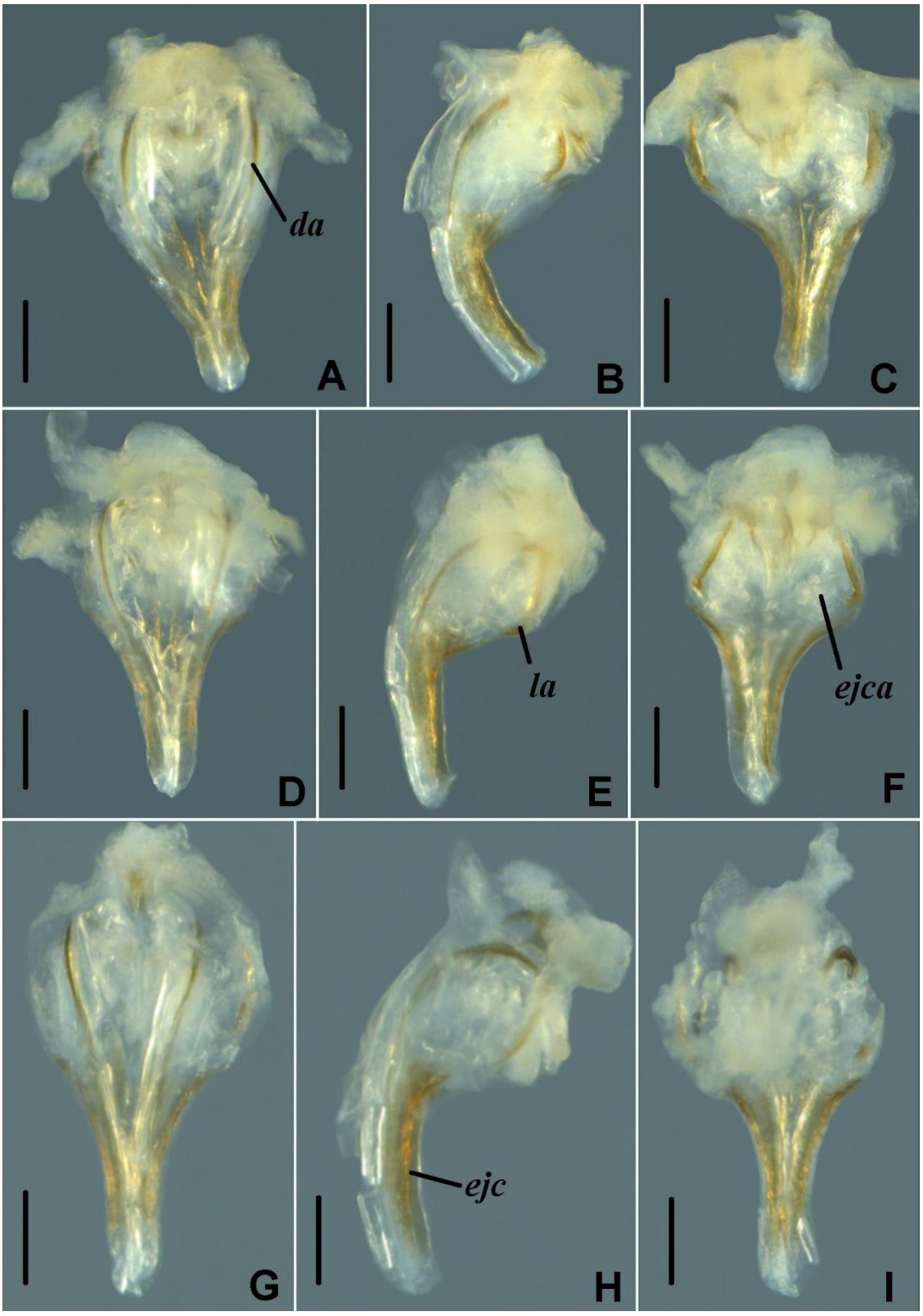


FIGURE 11

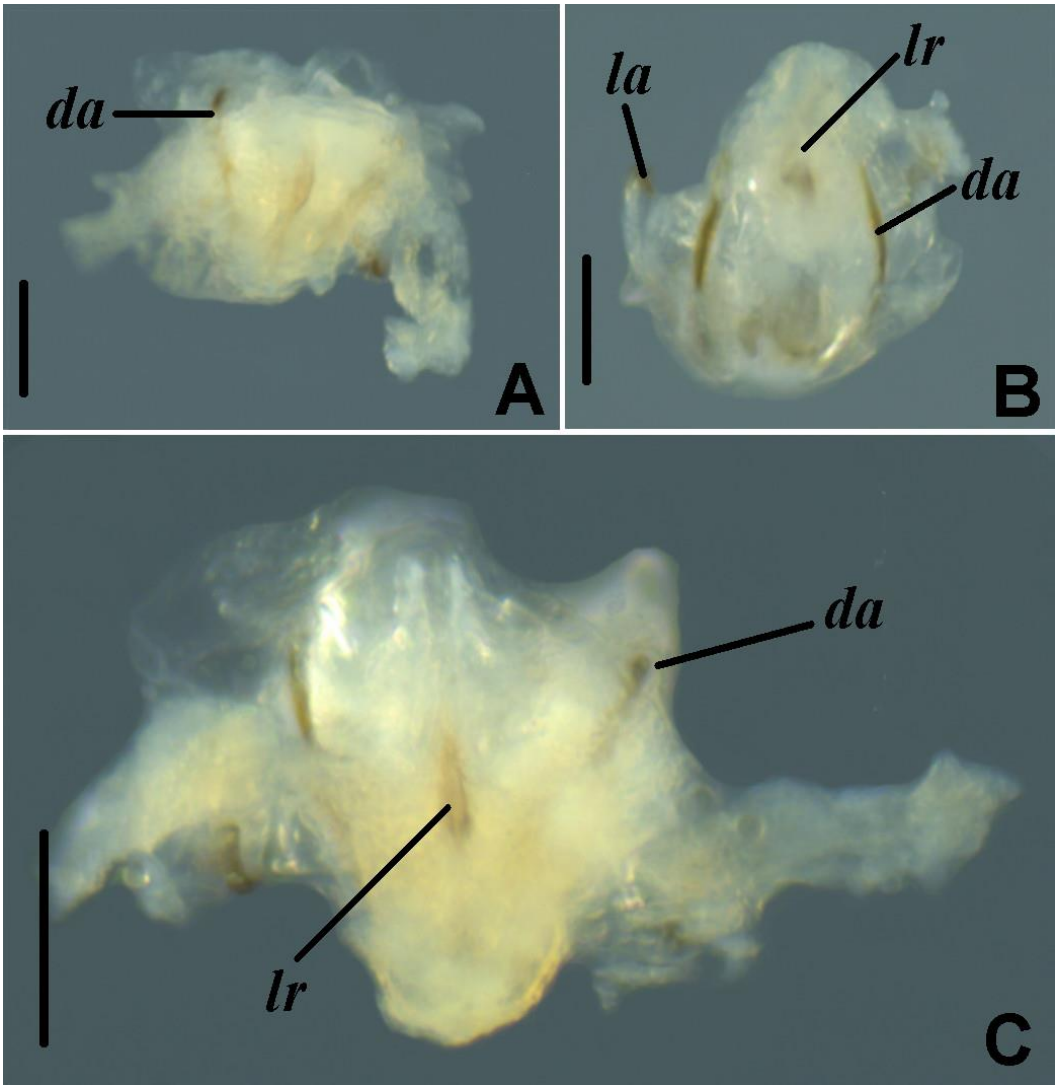
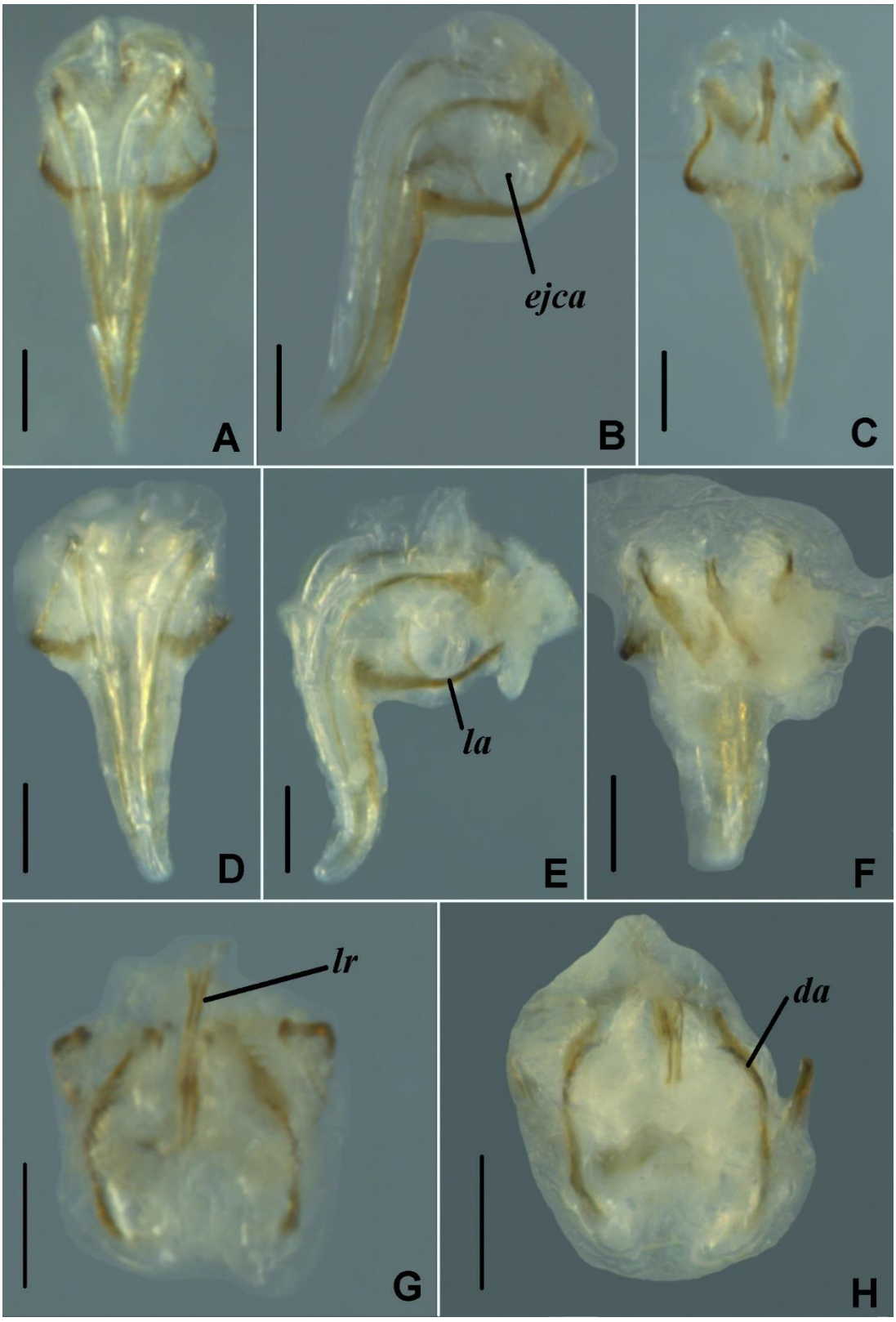


FIGURE 12



2. Chapter 2: Phylogeny of the Withiidae (Arachnida: Pseudoscorpiones) and evolution of its male genitalia

2.1 Introduction

Male genitalia are the most diverse and divergent structures in the animal kingdom (Simmons 2014). Eberhard (1985) proposed that this extraordinary phenotypic diversity is generated by sexual selection pressures. If sexual selection is acting over those characters, these will show a faster evolutionary rate than other structures (Arnqvist 1997, Eberhard 2010), otherwise, there could be a different selective pressure acting upon. To test this prediction, a comparative analysis dating the respective events is the proper analytical approach. This type of analyses has been used in vertebrates (Klaczko et al. 2015, Schutlz et al. 2016) but very few analyses are available in other animal groups, like arachnids, where most of the research is centered in assessing sexual dimorphism (Buzatto and Machado 2014, McLean et al. 2019, Soltan-Alinejad et al. 2021) or in the characterization of sexual behaviors (Ahtiainen et al. 2005, Nazareth and Machado 2010, Peretti et al. 2021).

An interesting model group to study this phenomenon are Pseudoscorpions. They are an ubiquitous mesodiverse arachnid group composed of 3700 species distributed in 26 families and more than 1700 genera. They are small sized, ranging between 0.2 to 12 mm with indirect sperm transfer, and a variety of sexual behaviors ranging from species with no physical interaction, groups where silk threads are set around the spermatophore to guide a future visiting female, up to cases of complex and extended courtship dances involving chelicerae and pedipalp contact, and male guidance towards the spermatophore (e.g. Weygoldt 1969, Muchmore 1990, Harvey et al. 2016). The shape of the spermatophore is

formed in the male genitalia which acts as a mold due to a complex interaction of muscles, glands, diverticula, and apodemes (Weygoldt 1969, Legg 1974, Heurtault 1971, 1994).

Withiidae (Chamberlin 1931) is one of the pseudoscorpion families where sexual behaviors are more complex. It is a cosmopolitan family, but its diversity is higher in tropical and subtropical environments (Harvey 2015). It is composed by about 170 species arranged in two subfamilies: Paragoniochernetinae with five genera and eleven species, restricted to the south of the African continent; and Withiinae with four tribes (Cacodemoniini, Juxtacheliferini, Protowithiini and Withiini), 37 genera and 170 species (Table 1) (World Pseudoscorpiones Catalog 2022). Thirteen of the thirty-seven genera are monotypic, nine genera have two species and *Withius* Kew, 1911, is exceptionally large with 50 species.

Withiidae was first proposed as a subfamily within the family Cheliferidae Risso, 1827. Later, Weygoldt (1970) raised it to the family rank based on the differences between their reproductive systems. According to Weygoldt (1970) the taxonomic characters that define the family are: 1) absence of Ram's horn organs, structures that the Cheliferidae have in the abdomen and use when they mate, 2) presence of patches of glandular setae in the posterior sternites, and 3) a complex spermatophore that exhibits several associated structures.

Withiidae appeared monophyletic in previous studies (Murienne et al. 2008, Harvey et al. 2016), however, the sampling of the family in those papers has been at minimum (a single taxa, described as *Withius* sp. and Withiidae sp., were included in each paper). In addition, Harvey (2015) called into question the proposed classifications within the family and claimed the need of a phylogenetic analysis to clarify the status of the subfamilies and tribes. He proposed two groups of genera within Withiidae based on the differences of the genital organs. The Cacodemoniini with long lateral apodemes and all the other withiids with short apodemes, but he found that species of the larger genus *Withius*, may have both, short or long apodemes. Recently, Romero-Ortiz and Sarmiento (2021) studied the male genitalia of the Cacodemoniini and found that there are genera-specific differences associated with the genital structures.

The fossil record of the Pseudoscorpions, Withiidae included, is relatively scarce, the oldest can be traced back to the Devonian (ca. 390 MY; Schawaller et al. 1991), and then we found most of the fossils from the late Cretaceous and the Cenozoic, ranging

between 100 to 16 MY with an important gap in between (Harms and Dunlop 2017). Regarding the Withiidae there are only two fossils, one belongs to the extinct genus *Beierowithius* dated 23 to 48 MY from the Eocene-Oligocene, the other is a *Parawithius* sp. dated 16 MY from the Neogene (Harms and Dunlop 2017). According to Benavides et al. (2019), most of the pseudoscorpion families originated in the Mesozoic with exception of the early divergence of those belonging to the Chthonioidea and Feaelloidea, whose origin could be Paleozoic. Divergence between Withiidae and its sister group Atemnidae, is thought to be around the Cretaceous (Benavides et al. 2019) however, dates for the clades below the family level have not been proposed yet.

To summarize, the Withiidae is an ideal model group to study the influence of sexual selection in the morphology of the male genitalia since it exhibits a variety of sexual behaviors and a diverse genital morphology. If this influence is true for the Withiidae, we could expect a faster rate of evolution in genital characters compared to somatic characters; however, if we find that the rate of change of genital characters is slower than other ones, we could assume that other selective pressures may be acting upon. Thus, our aims were: 1) to provide a sound dated phylogenetic hypothesis for the group, 2) to test the monophyly of the family, 3) to propose a hypothesis for the intergeneric relationships of the family, and 4) to test whether male genitalia evolved to a faster rate than other body regions, suggesting an ongoing sexual selection process. If this prediction is not fulfilled, it may be suggestive of more complex evolutionary processes.

Genus	**Author**	**# Accepted Species**
Aisthetowithius	Beier, 1967	1
Balanowithius	Beier, 1959	2
Cacodemonius	Chamberlin, 1931	6
Cryptowithius	Beier, 1967	1
Cyrtowithius	Beier, 1955	2
Cystowithius	Harvey, 2004	5
Dolichowithius	Chamberlin, 1931	16
Ectromachernes	Beier, 1944	4
Girardwithius	Heurtault, 1994	1
Juxtachelifer	Hoff, 1956	1
Metawithius	Chamberlin, 1931	10
Microwithius	Redikorzev, 1938	4
Nannowithius	Beier, 1932	7
Neowithius	Beier, 1932	5
Nesowithius	Beier, 1940	3
Paragoniochernes	Beier, 1932	2
Parallowithius	Beier, 1955	2
Parawithius	Chamberlin, 1931	3
Plesiowithius	Vachon, 1954	n/a

Pogonowithius	Beier, 1979	1
Protowithius	Beier, 1955	2
Pseudatemnus	Beier, 1947	1
Pseudochernes	Beier, 1954	2
Pycnowithius	Beier, 1979	3
Rexwithius	Heurtault, 1994	1
Rugowithius	Harvey, 2015	2
Scotowithius	Beier, 1977	1
Sphaerowithius	Mahnert, 1988	6
Sphallowithius	Beier, 1977	2
Stenowithius	Beier, 1932	10
Termitowithius	Muchmore, 1990	1
Thaumatowithius	Beier, 1940	2
Trichotowithius	Beier, 1944	1
Tropidowithius	Beier, 1955	1
Victorwithius	Feio, 1944	12
Withius	Kew, 1911	46
† Beierowithius	Mahnert, 1979	1

Table 1. List of the genera and number of species per genera of the Withiidae. Taken from the World Pseudoscorpiones Catalog 2022.

2.2 Materials and methods

2.2.1 Taxon sampling

A total of 107 specimens of Withiidae were studied; from them two data matrices were generated, a morphological matrix (87 specimens), and a molecular matrix (20 specimens). The morphological matrix includes 72 species of Withiidae covering 32 of the 37 genera currently recognized in Whithiidae, and 15 species belonging to the families Larcidae Harvey, 1992, Sternophoridae Chamberlin, 1923, Garypidae Simon, 1879, Atemnidae Kishida, 1929, Chernetidae Menge, 1855 and Cheliferidae Risso, 1827 as the outgroup taxa. A total of 87 specimens were examined.

For the molecular matrix, the ingroup sampling includes 20 withiids, all of them newly sequenced. Most of the newly sequenced withiids were from the Arachnological collection of the Instituto de Ciencias Naturales-Universidad Nacional de Colombia, Bogotá (ICN) and the Western Australian Museum, Perth (WAM). We did not use GenBank sequences for the ingroup since they were determined as Withiidae or *Withius* sp., a large genus that is difficult to define and that includes species that are most likely misplaced (Harvey 2015), leaving doubts about its status. The outgroup includes 18 species, four of them newly sequenced and 14 from the GenBank database. The selection of the outgroup was based on the phylogenies of Murienne et al. (2008), Harvey et al. (2016), and

Benavides et al. (2019) and included representatives of Cheliferoidea, Sternophoroidea, and Garypoidea as they are the closest relatives. The list of specimens, GenBank accession numbers, and collection details is given in Table 2.

2.2.2 Methods for morphological character coding

The total of the 87 specimens studied for morphology come from the following collections: American Museum of Natural History, New York (AMNH); Natural History Museum, London (BMNH); California Academy of Sciences, San Francisco (CAS); Field Museum, Chicago (FMNH); Florida State Collection of Arthropods, Gainesville (FSCA); Muséum d'histoire naturelle de la Ville de Genève, Geneva (MHNG); Muséum national d'Histoire naturelle, Paris (MNHN); Royal Museum for Central Africa, Tervuren (MRAC); Lund Museum of Zoology, Lund (MZLU); Naturhistorisches Museum, Vienna (NHMW); Museum Victoria, Melbourne (NMV); Queensland Museum, Brisbane (QM); Western Australian Museum (WAM); and Zoologisches Museum, Museum fiir Naturkunde, Berlin (ZMB).

The specimens were studied by preparing temporary slide mounts by immersion in 75 % lactic acid at room temperature for one to four days and mounting them on microscope slides with 10 mm coverslips supported by small sections of 0.25 mm or 0.50 mm diameter nylon fishing line (i.e., Harvey 2015, Romero and Harvey 2019). Chelicerae, legs I and IV, pedipalp and chelae were dissected for detailed observation. After study the specimens were rinsed in water and returned to 75% ethanol, with the body and dissected portions placed in 12 × 3 mm glass genitalia microvials (BioQuip Products, Inc.).

For the definition of morphological characters, we followed the criteria of Sereno (2007) and Vogt et al. (2010) (Table S1); that is, these complied with the properties of independence and exclusivity and their definition follows a logical structure composed by a locator, a variable and a qualifier of the variable (Sereno 2007, Vogt et al. 2010). We explored three subsets of characters because they could be subject to different selective pressures. The first one comprised the pedipalp structures, as these are their main sensorial organ, being extremely important for pseudoscorpion hunting and mate recognition; this subset was named Sensorial and included nine characters. The second subset included characters of the cephalothorax, legs, and chelicerae, these are related with locomotion and feeding; this subset was named Somatic and included 14 characters.

The third subset comprised the genitalia and abdominal glandular setae, traits related with sexual recognition and reproduction; this was named Sexual and included nine characters. Matrix was created using Mesquite version 3.3 (Madison and Madison 2021).

2.2.3 Methods for molecular data

Whole genomic DNA was extracted from specimens stored in ethanol 96%; left legs I, IV, and chelae, or the whole specimen were used. The DNeasy blood and tissue kit was employed to extract DNA (Quiagen, Lake Constance, Germany) following the tissue protocol with overnight incubation and two final dilutions of 60 µL. Two nuclear genes, 18S and 28S, and one mitochondrial gene, CO1, were amplified. For the complete 18S (~1.8 kb), three contigs were amplified with the primers 18S 1F/5R, 3F/bi and a2.0/9R (Giribet et al. 1996, Whiting et al. 1997); for the 28S, a fragment of ~300 pb was amplified with the primers 28Sa (Nunn et al. 1996)/28SpsR1 (Murienne et al. 2008). CO1 primers LCO1490/HCO2198 (Folmer et al. 1994) were used to amplify a fragment of ~650 pb. These set of molecular markers and its primers were selected according to Murienne et al. (2008). PCR reaction was done in 25 µL volume, with 2.5 µL of 10X buffer, 1.5 µL of Mg^{++}, 0.5 µL of dNTPs, 2.5 µL of each primer, 11.375 µL of ddH_2O, 0.125 of Taq DNA Polymerase Recombinant (Invitrogen, California, USA), and 4 µL of DNA template. PCR reaction followed a process of denaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 95°C for 30 s, annealing at 49 °C for the 18S and 28S, or 50°C for CO1 for 30 s, elongation at 72°C for 60 s and a final extension at 72 °C for 10 m. PCR products were checked on an agarose gel stained with ethidium bromide, then cleaned with ammonium acetate and sent to Macrogen Inc. (South Korea) for sequencing. Sequences were blasted for confirmation of correspondence in the GenBank. The fragments were cleaned, edited and contigs assembled in Sequencher (Gene Codes Corporation, MI, USA).

2.2.4 Phylogenetic analyses

We built seven matrices for the analyses of Whithiidae evolutionary relationships. One matrix with morphological characters only (MOR, 33 characters, 87 taxa; Table S1), five with molecular characters (COI, 649 pb; 18S, 1791 pb; 28S, 328 pb; 18S-28S, 2119 pb; and these concatenated (MOL), 2770 pb; 32 taxa in all cases), and one with both types of characters combined (COM; 20 taxa, 2803 chars.). We used three optimization

approaches, maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) as described below.

For the analyses of morphological matrix (MOR) we conducted the parsimony analyses with implied weighting as implemented in TNT v1.5 (Goloboff et al. 2008, Goloboff and Catalano 2016), using the following parameters: Ratchet settings: 50 iterations, 10 substitutions (no more than 10 tree-rearrangements accepted in the perturbation phase), equally weighted cycle: yes, Probability of up-weighting: 4, Probability of down-weighting: 4, Autoconstrained cycles: 0, Stopping when 99% of perturbation phase completed. In all analyses we used the strict consensus tree for comparison with symmetric resampling or bootstrap as support; likewise (Goloboff et al. 2008).

We generated trees from the five molecular data matrices: for each gene, for the two nuclear genes combined, and for the three genes concatenated (MOL). SequenceMatrix was used for gene concatenation (Vaidya et al. 2011). Alignment of each gene was done using MAFFT v. 7.450 (Katoh et al. 2002, Katoh and Standley 2013) implemented in Geneious® Prime. Settings for the alignment were as follows: Algorithm: Auto; gap open penalty: 2; offset value: 0,5 and scoring matrix as 1 PAM/k=2 as recommended by Cosgrove et al. (2016). In addition, we performed variations in the algorithm parameters gap open and offset value, to test the strength of the alignment; the first described settings produced the more stable alignment (Table S2). Then, we used PartitionFinder v. 2.1.1 (Lanfear et al. 2016) to search for partitions in our matrices. Finally, we used the algorithms implemented in each of the programs to select the best model for ML analysis, that is, ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQTREE (Trifinopoulos et al. 2016) and Smart Model Selection: SMS (Lefort et al. 2017) implemented in PhyML 3.0 (Guindon et al. 2010); for BI we used the greedy algorithm (Lanfear et al. 2012) in PartitionFinder v. 2.1.1 with and AICc as the selection criteria. Support values for these trees are given in SH-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010) and Ultrafast Bootstrap values (UFBoot; Hoang et al. 2018).

We used TNT v1.5 for parsimony searches of the molecular matrices with the following script modified from Cabra and Hormiga (2020): xmult= replications 10 rss css xss ratchet 50 drift 20 fuse 20 consense 10 / xmu: rep 5 ratchet 50 fuse 20 drift 20; resample jak rep 1000 freq from 0 [xmult] with Bremer supports. Gaps were treated as a fifth state. For the molecular matrices we used bayesian inference with MrBayes v 3.2.1 (Ronquist et

al. 2012) with posterior probabilities as supports; we ran the analysis with four chains for 30 million generations, a sample frequency every 1000 trees and a 10% burn-in. The files obtained were analyzed in TRACER v 1.7.1 (Rambaut et al. 2018) to check convergence of the chains. For ML analyses of the molecular datasets, we have in consideration that there are different rearrangement algorithms, we used IQTREE and PhyML 3.0 for tree searching in that case; we ran the analyses remotely for ML (IQTREE and PhyML in their web servers) and for BI (MrBayes in the CIPRES Science Gateway (Miller et al. 2010)).

For the COM matrix we used two optimization methods. Parsimony in TNT with Implied weights, and BI in MrBayes with *mk* (Lewis 2001) as the model for morphological data plus the models defined by PartitionFinder for each gene. For the search in MrBayes we ran four chains for 10 million generations, a sample frequency every 1000 trees and a 10% burn-in. We are aware of the difficulties of biological interpretation in assuming the *mk* model for discrete character evolution, but since Bayesian analysis using this model outperforms parsimony in terms of topological accuracy (Wright and Hillis 2014, O'Reilly et al. 2016), we prefer to use it and having a baseline level of comparison.

2.2.5 Dating

For the estimation of divergence times, we used ML and BI approaches. First, the Least Square Dating algorithm (LSD2) (To et al. 2016) in IQTREE2 v. 2.0.6 (Minh et al. 2020) command line version, using the option - - date ci to calculate the 95 % confidence intervals of the obtained dates. Second, we performed the analysis using a log-normal relaxed clock in BEAST v 2.6.2 (Bouckaert et al. 2019) inside the CIPRES cluster with bModelTest v 1.2.1 (Bouckaert and Drummond 2017) to find the substitution model. The parameters for the run were: 100 million generations, sampling every 1000 generations, and a Birth-Death speciation process. We used BEAUti v 2.6.2 (Bouckaert et al. 2019) to generate the xml file for the analysis and checked the convergence of chains in TRACER v 1.7.1 (Rambaut et al. 2018).

To test the sensitivity of the resulting dates, we used either two fossils in the outgroup and repeated the analysis including a third fossil placed within the ingroup for a total of three calibration points. The fossils are: 1) a Chernetidae of 99 MY located at the node of the Chernetidae (Benavides et al. 2019); 2) a Cheliferidae species, *Heurtaultia rossiorum* (Judson 2009) of 100 MY located in the Cheliferidae clade (*Chelifer cancroides*

and *Lissochelifer* sp.); and 3) a *Parawithius* specimen from 16 MY Dominican amber (Schlee 1980, Schawaller 1981, Poinar 1992, Poinar et al. 1998) placed in the split of *Parawithius* + other cacodemoniines. The fossils of *Beierowithius sieboldtii* (Menge, 1854) from Baltic amber (49 MY) and the Withiidae in Bitterfield amber (Ahrens et al. 2019) were not used because we could not find any information in the descriptions that allowed us to contrast or compare them with any other genera placed in our phylogeny (Menge 1855, Beier 1955).

It is important to mention that there is a discussion on considering the calculations on speciation and extinction rates due to the possibility of obtaining the same likelihood in trees under different rate values (Louca and Penell 2020) and the scope and implications of these conclusions (Morlon et al. 2020). However, for the purposes of our work and for further comparison with other studies, we used the function implemented in BEAST for the birth-death model.

2.2.6 Evolution of sexually related characters: Ancestral states reconstruction and character coevolution tests

The phylogenetic tree based on the MOL matrix was pruned to eliminate outgroups and to match male genitalia data. We compiled information regarding the length of the ejaculatory canal from Klausen (2005) for the Atemnidae, from Harvey (2014) for *Chelifer cancroides*, and from Romero-Ortiz and Sarmiento (2021) for the Cacodemoniini; and the information was defined as a two-states character: long and short. This character definition follows the diagnostic character characterization for the Cacodemomiinini. To compare evolutionary rates, we also coded two non-sexually related traits: the position of trichobothria *ib* and *isb*, and the type of eyes (Table S1; characters 10 and 17 respectively). With each trait coded as a binary we compared two transition models, a first one that considers equal rates for all trait substitutions (ER), and a second one that considers all rates different (ARD). Estimations were performed using the function *fitDiscrete* from geiger (Harmon et al. 2008), and we estimated weighted Akaike information criterion (wAIC) values to pick the best model for subsequent ancestral states estimation. Best-fitted models were then used to estimate ancestral states with the function *make.simmap* from phytools (Revell 2012), which performs a Bayesian reconstruction with 50000 simulations to estimate posterior probability distribution for each trait along the phylogeny. For an easier

observation of posterior probability of each state on the edges and node, we plotted the ancestral states with the *densityMap* function from phytools (Revell 2012).

We fitted the Pagel's (1994) model of character evolution to test whether the length of the ejaculatory canal (the sexual trait) evolved dependently or independently of the non-sexual traits. We used the *fitPagel* function from phytools (Revell 2012). To test whether these traits evolved in a coordinated fashion, we fitted four models: one in which the sexual trait depends on the non-sexual trait and vice versa (≠ EJC), one in which our sexual trait depends on the non-sexual trait but not the converse (>EJC), one in which our non-sexual trait depends on the sexual trait but not the converse (<EJC), and a model in which both traits are interdependent (↔ EJC). Finally, we compared these different scenarios by their AIC values in R 4.1.2.

Species	Locality	Depository and registration no.	GenBank accession number		
			18S rRNA	28S rRNA	COI
Chernetidae					
Balgachernes occultus Harvey, 2018	AUSTRALIA:Western Australia, Ashendon Rd. 5.7 km E of Pickering Brook		------	MT994378	MW439256
Chelanops sp.	CHILE:Juan Fernandez Island	MCZ	MT994377	------	MW431017
Garypidae					
Synsphyronus apimelus Harvey, 1987	AUSTRALIA:Western Australia, Stirling Range National Park, Donelly Peak		MT994368	MT994379	MW428208
Olpiidae					
Pachyolpium sp.	COLOMBIA:Cesar. Valledupar. Ecoparque Los Besotes. Monte Puma	ICN-APs-574	MT994373	MT994387	------
Withiidae					
Cystowithius sp.	COLOMBIA:Caldas, Manizales, Jardín Botánico U. de Caldas	ICN-Aps-682	MT994374	MT994389	MT085822
Stenowithius sp.	TANZANIA:North Pare Mts., Kindoroko For.	Vasily GREBENNIKOV coll.	------	MT994380	------
Stenowithius sp.	TANZANIA:Kaguru Mts. At Masenge vil.	Vasily GREBENNIKOV coll.	------	MT994381	------
Withius piger (Simon, 1878)	AUSTRALIA:Western Australia, 14 km NE. of Narrogin	WAM ex T145081	MT994369	MT994382	MW451764
Withius sp.	ZAMBIA:Wildlives Game Farm	WAM ex T67087	MT994370	MT994383	------

Withius sp.	SOUTH AFRICA: KwaZulu-Natal. Ndumo Game Reserve, Banzi Pan, SW shore	WAM ex T143320	MT994371	MT994384	------
Dolichowithius sp.	COLOMBIA:Meta. Villavicencio. Vda. Barcelona. Campus Unillanos	ICN-APs-460	------	------	MW451765
Victorwithius sp.	COLOMBIA:Caquetá. Florencia. CIMAZ Macagual. Río Sarabando	ICN-APs-573	MT994372	MT994385	------
Cacodemonius sp.	COLOMBIA:Cesar. Valledupar. Ecoparque Los Besotes. Campamento base	ICN-APs-597	------	MT994386	MW451766
Cacodemonius sp.	COLOMBIA:Guaviare. San José del Guaviare. Vereda Playa Guio	ICN-APs-512	------	MT994388	------
Cacodemonius sp.	COLOMBIA:Bolívar.Turbaco.Jardín Botanico Guillermo Piñeres	ICN-APs-684	MT994375	MT994390	------
Withius sp.	SOUTH AFRICA:Eastern Cape Prov. Mazeppa Bay	WAM ex T86744	------	------	MW451767
Withius sp.	ZAMBIA:Wildlives Game Farm	WAM ex T143319	------	MT994391	MW451768
Victorwithius sp.	PERU:Loreto, Amazon river, Natamú Lodge	ICN-APs-741	MT994376	MW430767	------
Dolichowithius sp.	COLOMBIA:Amazonas, San Martin de Amacayacu	ICN-APs-695	------	MW430768	------
Parawithius sp.	COLOMBIA:Cundinamarca, Sesquilé, Cerro de Las Tres Viejas	ICN-APs-7??	------	MW430769	------

Table 2. Newly sequenced specimens used in this study.

2.3 Results

2.3.1 Phylogenetic analyses of morphological data

Non-weighted and implied weighting analyses with all the morphologic characters recovered a poorly resolved phylogeny (Figs. S1-S4); 1 tree; 153 steps; CI 0.248; RI 0.519), with *Cacodemonius* (bs 54), *Cystowithius* (bs 57), *Dolichowithius* (bs 37), *Rugowithius* (bs 94), and *Metawithius* (bs 48) monophyletic but Withiidae and *Balanowithius*, *Victorwithius,* and *Withius* non-monophyletic (Fig. S1). This lack of resolution was expected given the low number of characters (36) compared to the number of taxa (72) and the high level of homoplasy. The analysis with the sensorial characters (5 trees; 37 steps; CI 0.270; RI 0.400) only recovered *Rugowithius* as monophyletic (bs 61) (Fig. S2); the analysis with the sexual characters (5 trees; 61 steps; CI 0.180; RI 0.383) recovered the genera *Cacodemonius* (bs 90), *Cystowithius* (bs 86), *Dolichowithius* (bs 76), and *Rugowithius* (bs 78) as monophyletic each with high support (Fig. S3), while the analysis with the somatic

characters dataset (3 trees; 79 steps; CI 0.215; RI 0.451) showed only the genera *Rugowithius* (bs 32) and *Metawithius* (bs 74) as monophyletic each with uneven and low support values in both cases (bs 32 and bs 74) (Fig. S4).

2.3.2 Phylogenetic analyses of molecular data

For the sister group of Withidae, each optimization criteria recovered slightly different groups. Parsimony recovered *Chelifer cancroides* (Br3) as the closest taxon, while ML placed *Miratemnus* (SH-aLRT: 87.7%, UFBoot: 60%), and bayesian inference revovered Cheliferidae (*Lissochelifer* sp. + *Chelifer cancroides*; pp 0.63) (Fig. 1) as its sister. It is important to note that for ML, Atemnidae was non-monophyletic because its genera *Miratemnus* and *Oratemnus* are resolved in different clades.

Parsimony, ML, and BI recovered Withiidae as monophyletic with strong support in most of the analyses using the concatenated matrix (MOL) (Br 11, SH-aLRT and UFBoot 100%, pp1), and individual genes, except for the 28S alone with all the optimization criteria (Fig. 1). The family splits into two clades, the neotropical withiids (Br 1, SH-aLRT and UFBoot 100%, pp1) and the non-neotropical whithiids (Br 1, SH-aLRT 99.9%, UFBoot 99%, pp1) (Fig. 1). This division was recovered in all the analyses with the concatenated matrix (MOL) and with ML for the 18S and the nuclear genes. The gene 28S did not recover the splitting in any of the analyses (Fig. 1).

For the neotropical clade, which is composed by *Cystowithius*, *Dolichowithius*, *Parawithius*, and *Victorwithius,* we highlight the genus *Cacodemonius* as this was the only recovered with high support in 18 of the 20 analyses (Fig. 1; Br 4, SH-aLRT and UFBoot 100%, pp1). For the non-neotropical clade, composed by *Rugowithius*, *Stenowithius*, and *Withius*, the support and congruence of the analyses was high for each of the genus, but heterogeneous inside *Withius* (Clades 1 to 7 in Fig. 1).

In summary, parsimony recovered Withiidae as a clade, and some relationships were weakly supported. For ML, both PhyML and IQTREE offered the same tree topology, that was consistent with the multiple gene combinations, and more resolved than parsimony. Likewise, Bayesian inference results were pretty much consistent with ML, showing Withiidae as monophyletic, the internal clades, and similar supports with those obtained by ML.

2.3.3 Phylogeny with the combined dataset

Parsimony and Bayesian analyses produced similar topologies with the combined matrix, recovering Withiidae as monophyletic with strong support (pp 1, bs 99) and dividing the family into two clades, the neotropical (pp 1, bs 35) and the non-neotropical clade (pp 1, bs 51) (Fig. 2). Cheliferidae (*Lissochelifer* sp. + *Chelifer cancroides*) was recovered as the sister group of Withiidae (pp 0.96, bs 26) (Fig. 2). Surprisingly, Atemnidae appeared as sister of Chernetidae in the BI analysis (pp 0.79), or sister to (Cheliferidae (Withiidae + Chernetidae)) in the parsimony tree (bs 97) but not sister to Withiidae (Fig. 2). Regarding the neotropical Withiidae, both analyses show a polytomy for *Cystowithius*, *Dolichowithius* and *Parawithius*; parsimony analysis established the clade (*Victorwithius* (*Cacodemonius* (Cy+Do+Pa))) (bs 35) while bayesian inference established the clade ((*Cacodemonius* + *Victorwithius*) (Cy+Do+Pa)) (pp 1) (Fig. 2). For the non-neotropical withiids, parsimony recovered a polytomy for *Rugowithius bulbosus*, *Stenowithius* spp, *Withius piger* and *Withius* SA1, while the Bayesian analysis recovered *R. bulbosus* as sister of all the others and the two *Stenowithius* as sister of *Withius piger* + *Withius* SA1 (Fig. 2).

In general, the analyses of the combined dataset showed consistency with the trees obtained with the molecular dataset (Figs. 1 and 2). It is important to mention that the resolution level and support within each of the two main clades of Withiidae is better in the molecular data analyses than for the combined data sources (Figs. 1 and 2). For this reason, we chose to select the tree generated by IQTREE with the molecular dataset to work in the dating estimation (Fig. 1). It is worth mention that junction femur-patella of leg I and the presence of glandular patches of glandular seta in the sternites appear as synapomorphies of the Withiidae (Fig. 2).

2.3.4 Dating

As described above, we used the tree generated by IQTree with the MOL matrix (2770 pb; 32 taxa) for the dating analyses given its resolution and stronger support (Fig. 1). Divergence dates are summarized in Table 3. For the split between the Withiidae and its sister Atemnidae, dates generated by IQTREE2 were similar with both, the two and the three calibration points. The splitting of Whitiidae is placed in the Lower Cretaceous between the Valanginian and the Hauterivian (132.9 to 129.4 MY). This was not the case for dates generated by BEAST 2.6 where the split between Atemnidae and Withiidae is

placed in the late Jurassic (163.99 MY) with the three or even in the late Triassic (211.42 MY) with the two calibration points. The splitting of the neotropical and non-neotropical clades is also different if we compared them between two or three calibration points (Table 3); when two calibration points are used, the dates range between 104.22 and 114.96 MY, corresponding to the Albian, and when three calibration points are used, the dates range between 78.43 and 88.24 corresponding to the Campanian, both in the Cretaceous. The other divergence dates were more similar between approaches. The divergence between *Rugowithius* and the other non-neotropical withiids falls in the Paleocene (69.84 – 54.53 MY), and the split between *Cacodemonius* and other neotropical withiids falls in the Eocene (55.76 – 32.24 MY).

Node	IQTREE2 (2 calibration points)	IQTREE2 (3 calibration points)	BEAST 2.6 (2 calibration points)	BEAST 2.6 (3 calibration points)
Miratemnus; Withiidae	135.28 (147-123)	131.45 (147-121)	211.42	163.99
Neotropical; non-neotropical	104.22 (122-90)	78.43 (96-62)	114.96	88.24
Cacodemonius; other neotropical genera	55.76 (66-46)	32.24 (42-27)	51.49	38.07
Rugowithius; African Withiidae	65-35 (79-52)	54.53 (67-43)	69.84	55.30

Table 3. Summary of the dating results. The number in the first row is the Age Mean in MY for the corresponding node and the numbers in parenthesis are the 95% Confidence intervals. These nodes are highlighted with stars in Figure 1.

2.3.5 Evolution of sexually related traits

The model that considers all different rates fitted slightly better the transitions to our phylogeny for the ancestral state reconstruction analysis (ARD, Table 4). In the case of the non-sexual traits, an equal-rates model for all trait substitutions best fitted the transitions between states compared with the sexual trait length of the ejaculatory canal (Table 5). This transition model was then used to run the ancestral states reconstruction under a Bayesian approximation. Ancestral state reconstructions suggest that the common ancestor of Whitiidae had a long ejaculatory canal (PP=0.723076) and rounded, corneate eyes (PP=0.7483699); The widely separated position of the trichobothria *ib* and *isb*, had a slightly higher probability of being the ancestral state (PP=0.542480). The estimated

average of changes between states for the length of the ejaculatory canal was 3.17144, with 1.79706 changes from long to short, and 1.37483 changes from short to long. The position of trichobothria *ib* and *isb* was more plastic, with an average of 9.74072 changes between states, 4.83494 changes from widely separated to closer, and 4.90578 changes between closer to widely separated. In the case of the type of eyes, we found an average of 2.00612 changes between states, 1.69188 changes from rounded, corneate eyes to flat eye spots, and 0.31424 changes from flat eye spots to rounded, corneate eyes (Table S1, char 17).

The test for correlated evolution indicated that the model with the eyes (non-sexual trait) as a dependent variable provided the best value to changes in the phylogeny, suggesting that changes in our sexual trait preceded changes in the eye morphology (Table 4). Comparing the speed of change of the characters, we found that the fastest change occurs in the position of the trichobothria *ib* and *isb*, then is the change about the length of the ejaculatory canal, and last, the change about the eye morphology.

Character	ER	ARD
Length of Ejaculatory canal (EJC)	0.4915718	0.5080282
Position of trichobothria ISB-IST	0.7376952	0.2623048
Eye morphology	0.6552369	0.3447631

Table 4. Weighted Akaike information Criterion (wAIC) scores for three binary characters (see Methods) for two transition models: equal rates for all substitutions (ER), and all different rates (ARD).

Non-sexual trait	Model	Lt	p-value	AIC	wAIC
ISB-IST	> EJC	0.040228	0.980087	40.589775	0.15887673
	< EJC	0.716273	0.698978	39.9137	0.222777256
	↔ EJC	1.23134	0.872912	43.39863	0.03900484
	≠ EJC	1.30879	0.859883	38.00221	0.57934587
EYES	> EJC	2.47713	0.289799	28.30476	0.1289494
	< EJC	6.01273	0.0494712	24.76916	0.75537773
	↔ EJC	6.24227	0.181771	28.53962	0.11466213
	≠ EJC	8.28042	0.0818296	29.46599	0.0636166

Table 5. Comparison of different models of coevolution between a sexual (EJC: Length of Ejaculatory canal) and non-sexual (ISB-IST: Position of trichobothria ISB-IST and EYES:

Eye morphology) traits, using Pagel's method (1994). LR= likelihood-ratio, AIC= Akaike information criteria, wAIC= weighted Akaike information criteria.

2.4 Discussion

Since previous phylogenetic analyses of Pseudoscorpions included only one or two species of the family Withiidae (Murienne et al. 2008; Harvey et al. 2016), its monophyly required a more thorough test. In this study, including 18 species of Withiidae, the monophyly of the family was recovered in 17 of the 22 analyses; these analyses were as follows: the genes 18S and COI separately, the combination of the two nuclear genes 18S and 28S, the analysis with the concatenated matrix (MOL-all genes) (Fig. 1), and the combined matrix (Fig. 2). It is important to point out that the first four data sets were analyzed under the four optimization criteria while the combine matrix (genetic plus morphologic characters) was analyzed with parsimony and BI approaches. The five analyses where Withiidae was not recovered as monophyletic were those that include the gene 28S; this gene had good resolution for depth relationships only (Fig. 1) (e.g., CRO09_*Stenowithius*_spTa+ CRO10_Withiidae_spTa.). Taxonomically, Withiidae was recognized as a family in 1970 by Weygoldt, by separating the then subfamily Withiinae from Cheliferidae. His taxonomic decision was based on the presence of patches of glandular setae in the sternites and the parallel junction of the femur/patella of leg I (Weygoldt 1970), characters that were also recovered as synapomorphies by our combined analyses.

Our sampling covered a wide geographic area of the distribution of the family focusing on Withiinae. The other subfamily, Paragoniochernetinae (Beier, 1944), is composed by five genera, and is only 7 % of the species of the family; this enigmatic subfamily has very few records besides the species originally described and unfortunately, many of the types are missing and the descriptions are poorly informative, making it difficult to clarify its status.

Regarding the sister group of the Withiidae, we found three results, all of them affected by the data source. In ten of the 20 analyses conducted with the molecular data (MOL), Atemnidae was recovered as sister group to Withiidae (Fig. 1), agreeing with Murienne et al. (2008), Harvey et al. (2016), and Benavides et al. (2019). However, in the four analyses with COI, the sister group was the clade Cheliferoidea composed by

Atemnidae, Chernetidae and Cheliferidae. The analyses that used the gene 28S with all four reconstruction methods did not recover Withiidae as even monophyletic. In the analyses with the genes 28S and 18S combined (nuclear genes), and in the combined matrix, where we included all the genes plus morphology (COM), the sister taxon was Cheliferidae instead of Atemnidae (Figs. 1 and 2). The differences between the topologies derived from COI and those from the concatenated molecular matrix (i.e., out group definition) could be explained as due to a low resolution of COI at this level or as a result of taxonomic sampling incompleteness because the fragment could not be obtained from most taxa (Liu et al. 2015, Wiens 2006). In the combined matrix (MOL+MOR) analyses, this change in outgroup could be explained as the result of a swamping event of morphological characters over molecular ones, a phenomenon that occurs even in phylogenomic data (Fernández et al. 2016, Pyron 2017, Noguerales et al. 2018, Neumann et al. 2021). However, further studies are required to understand this case.

The morphologic dataset showed a low resolution mainly due to the low number of characters compared with the number of taxa. Moreover, in the analyses with molecular data, the recovered tree was completely solved with high support values (>70) for 14 of the 19 clades, including the monophyly of Withiidae. At the genus level, only *Cacodemonius* was properly tested with the inclusion of several species, and it was consistently recovered as monophyletic. The relationships between representatives of the genera *Victorwithius, Parawithius, Dolichowithius,* and *Cystowihitus* had a low support (<50). Given the number of species per genus, we could not test the monophyly of these genera.

2.4.1 Internal relationships of the non-neotropical clade

Rugowithius was recovered as a sister group to the other non-neotropical withids with high support in analyses with molecular data, and with the combined dataset (bs 100; pp 1 respectively). It is important to note that *Rugowithius* is restricted to the tropical forests of northern Australia, in contrast to the other taxa of the non-neotropical clade, *Withius* which has a widespread distribution, and *Stenowithius* which is restricted to sub-equatorial Africa (Harvey 2013). The closeness of these two genera had already been commented by Beier (1932), who described *Stenowithius* based on specimens of *Withius buttneri* (Ellingsen), highlighting their similarity.

2.4.1 Internal relationships of the neotropical clade

Cacodemonius is recovered as the single strongly supported neotropical clade in 18 of the 20 analyzes with molecular data (bs 100). The relationships between the other taxa are not stable, always recovering very short internal branches. These results can have two explanations. The first is that the genes do not have enough resolution for this tree (Fig. 1; 28S analyses for shallow relationships, nodes 1 to 9), therefore, the number of genes should be increased to explore more evidence. The second is that very short branches can be indicators of very rapid evolutionary changes in the group (Parins-Fukuchi et al. 2021). Consequently, it is important to increase the number of taxa to establish more stable relationships (Poe 1998), although there is a debate concerning the addition of taxa to increase phylogenetic accuracy (Rokas and Carroll 2005, Shen et al. 2017).

2.4.2 Dating and biogeography

We assessed estimated divergences for four nodes and found less variation in two of them: 1. the separation between *Cacodemonius* and other neotropical genera, and 2. the separation between *Rugowithius* and the African genera (Table 3). For the other two nodes, the variation on the dates generated between IQTREE2 and BEAST 2.6 was higher: first the splitting of *Miratemnus* and Withiidae, and second, the separation of the neotropical and non neotropical clade, which was around 10 to 80 my (Table 3).

As for the first splitting, *Miratemnus* + Withiidae, these discrepancies could be explained because of the difference in the treatment of the uncertainty of the estimation methods, as well as the high rate of variation between lineages (Duchene et al. 2016), this considering that this relationship is the oldest of those evaluated and the one with the highest variation rate.

It is important to highlight that the clades inside Withiidae, show a specific geographic distribution, one clade in the Neotropics and the other in Africa and Australia (Fig. 1); this split match the separation of Gondwana in the two supercontinents and is consistent with Benavides et al. (2019), who places the origin of the two clades in the Cretaceous.

As stated in the results (Table 3), when two or three calibration points are used the dates differ, even using the same method (IQTREE2 or BEAST 2.6). For example, the division of the neotropical an the non neotropical clades assessed with IQTREE2 gave an estimated date in the Albian age with two calibration points (104.22 my) and in the

Campanian age with three (78.43 my), both in the Cretaceous. As the higher the number of calibration points, the better the date estimates (Xiang et al. 2011), we prefer the dates using the three fossils.

2.4.3 Evolution of sexually related traits

The length of the ejaculatory canal has been proposed as a synapomorphy for the Cacodemoniini (Harvey 2015). For the first time, this character has been analyzed into a phylogenetic framework and found that in general, the length of the ejaculatory canal (*ejc*) changed once from long to short in our selected reconstruction (Fig. 3); nevertheless, *Withius piger* calls for a more detailed sampling as the state long *ejc* appears as a reversion in this species.

We suggest that a shortening in the *ejc* structure could result from resources invested in building a spermatophore. Since the genital atrium works as a mold for the spermatophore (Heurtault 1994), a short *ejc* could mean less investment for producing a short spermatophore, for plain surfaces like tree bark, compared with leaf litter or soil.

The pattern of change in the eye morphology follows a similar trend observed in the ejaculatory canal, where the eyes are changing dependent of the change in the length of the *ejc* (Table 5). This dependence of a somatic characteristic on a sexual trait could suggest the action of a different selective pressure.

Regarding the speed of change in the tested characteristics, the position of the trichobothria *isb* and *ib* had the fastest rate of change. The position of the trichobothria is important for pseudoscorpions since they are the main sensorial organ (Weygoldt 1969), and their arrangement is of high importance in taxonomy. Of the three tested traits, and independent of the direction of change, this character tripled the speed of change compared to the other two (Fig. 3). This result does not support the prediction that sexual selection is involved in genital evolution explaining structural complexity due to faster changes; second, this could mean that a different selective pressure is acting beyond sexual selection. Since these cryptic arachnids do not have very developed eyes, they rely entirely in the chemical and mechanical signals generated by the trichobothria in their dark environment (Judson 2007).

An additional possibility to explain these results, that requires further research, is that trichobothria are involved in sexual behaviors and thus will experience faster changes as those predicted for genitalia. However, this interpretation does not account for the difference in evolutionary speed between trichobothria and the other two-character sets, neither the similarity in change speeds between eye morphology nor genital traits.

FIGURES

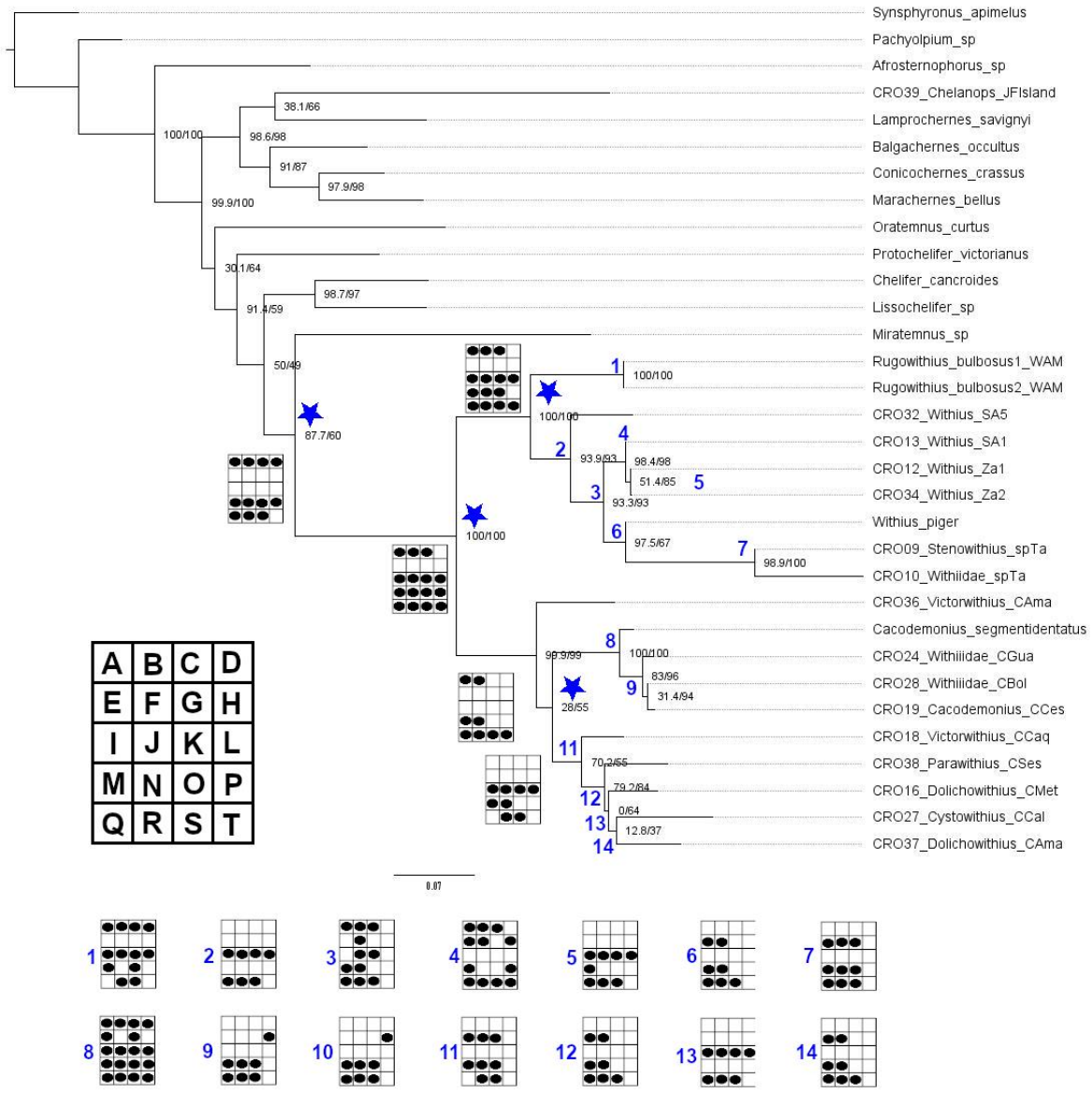


Figure 1. Phylogeny of the Withiidae inferred from the molecular datasets on IQTree. Support values are SH-aLRT/UFBoot. Dots in the rugs indicate that the clade was recovered in a specific analysis. Specific analyses: A,E,I,M,Q-PhyML. B,F,J,N,R-IQTree. C,G,K,O,S-Mr.Bayes. D,H,L,P,T-TNT. Matrices: A,B,C,D-18S. E,F,G,H-28S. I,J,K,L-COI. M,N,O,P-Nuclear (18S-28S). Q, R, S, T-MOL matrix. Stars indicate the nodes assessed for dates with IQTREE2 and BEAST 2.6.

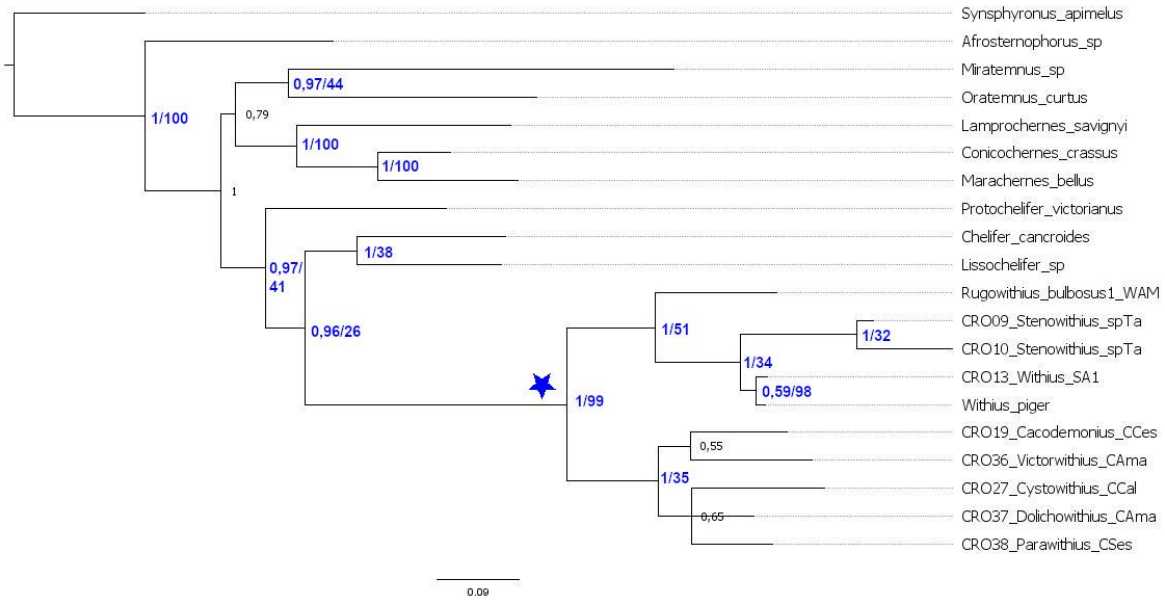


Figure 2. Phylogeny of the Withiidae inferred with the COM dataset (Molecular and morphologic characters) on MrBayes and TNT. Blue numbers show posterior probabilities/symmetric resampling. The clades that have only black numbers are the ones that were only recovered by Bayesian inference but not by Parsimony. The star indicates the node where state characters “junction femur/patella I: parallel” and “patches of glandular setae: present” are found as synapomorphies.

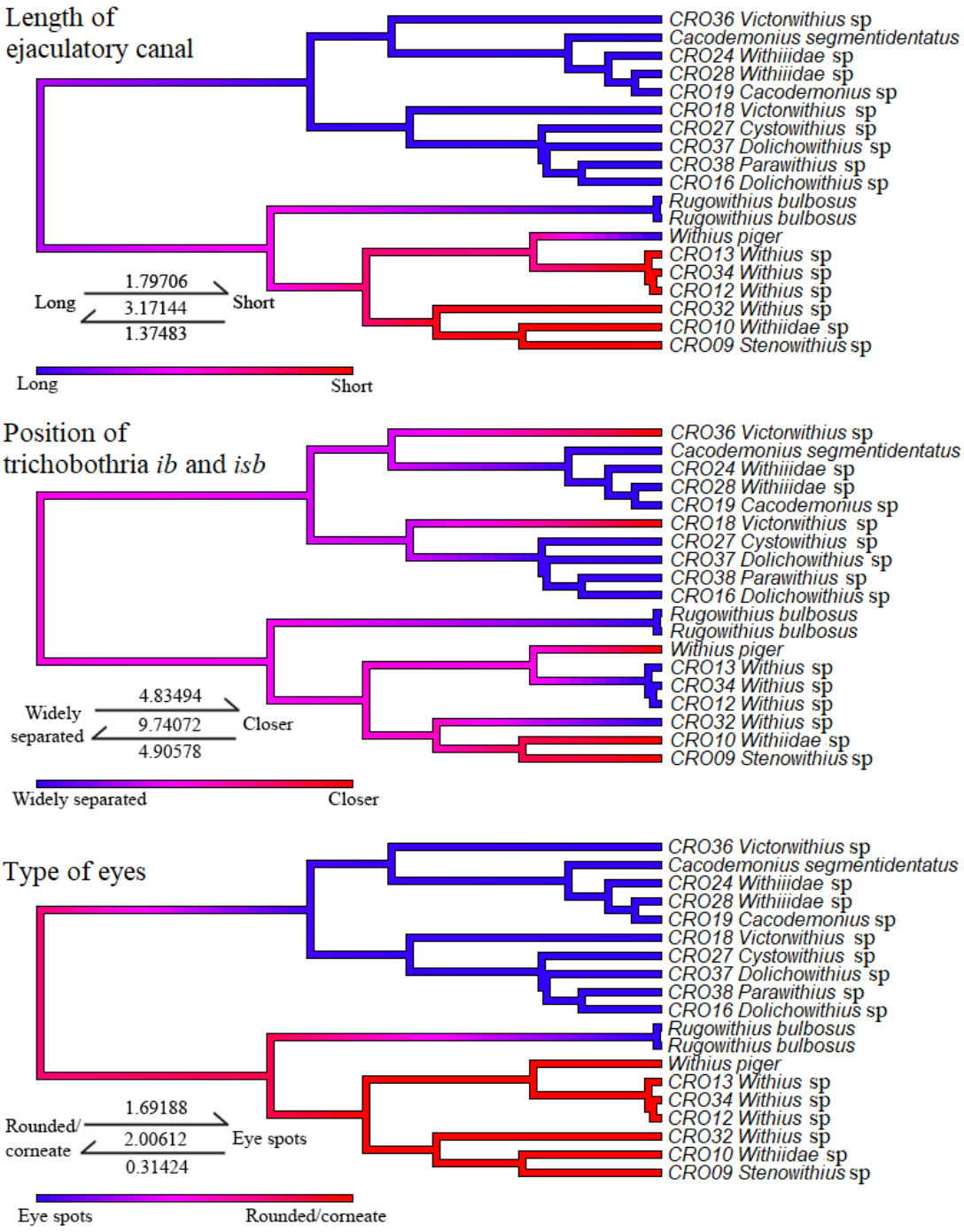


Figure 3. Ancestral state reconstruction of sexual (Length of ejaculatory canal) and non-sexual traits (position of trichobothria *ib* and *isb*, type of eyes), mapped on the MOL matrix tree to visualize the posterior probability of each state on the edges and nodes (plotted with the *densityMap* function from *phytools*; Revell 2012). Probability was obtained by the stochastic character mapping method with the model showed in table 4 as the best transition model. Values between the arrows represent the average of changes between states, and values over/under the arrows represent the estimated state transition in the direction given by the arrow.

2.5 Supplementary material

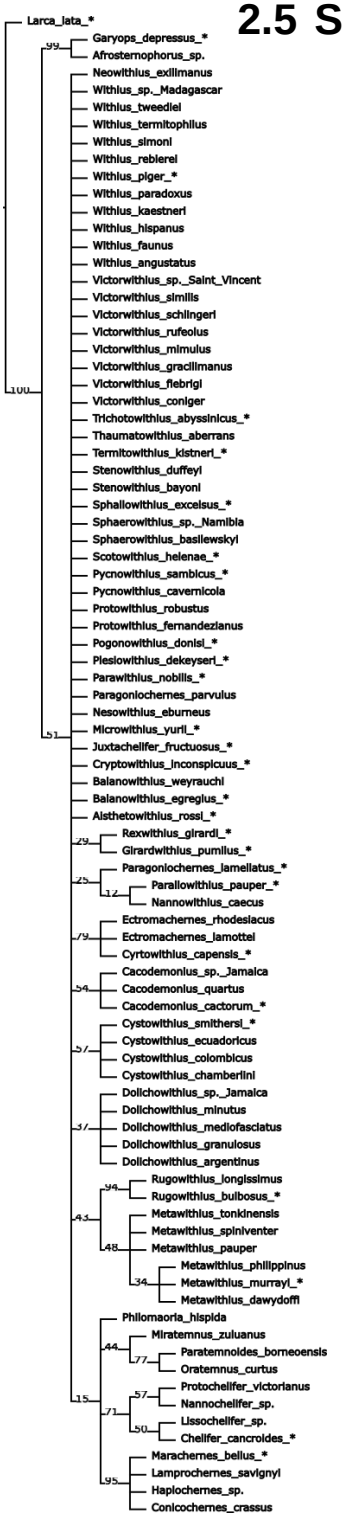


Figure S1. Parsimony tree with morphological characters under implied weighting

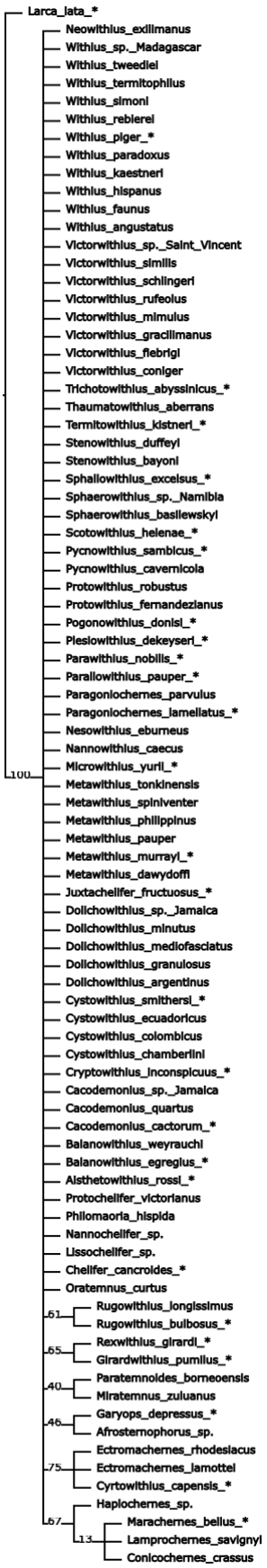


Figure S2. Parsimony tree with sensorial characters.

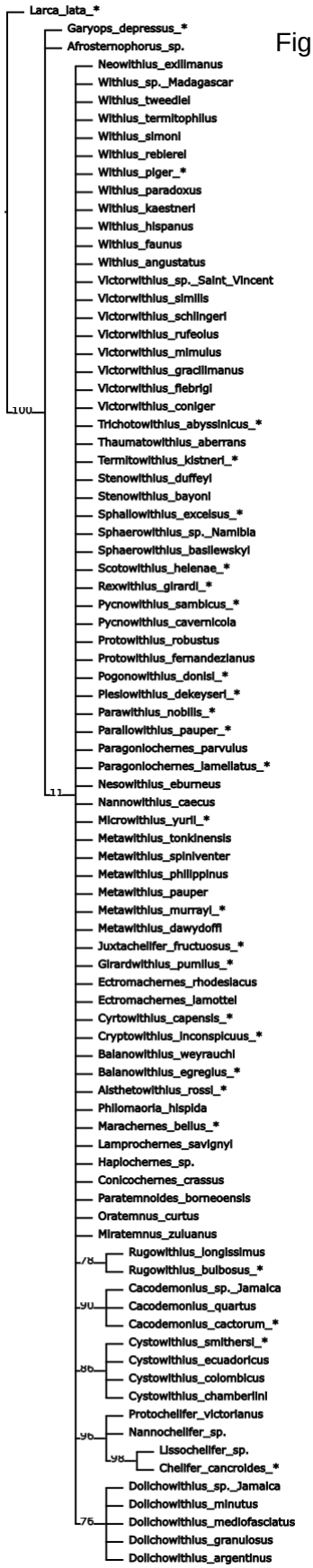


Figure S3. Parsimony tree with sexual characters.

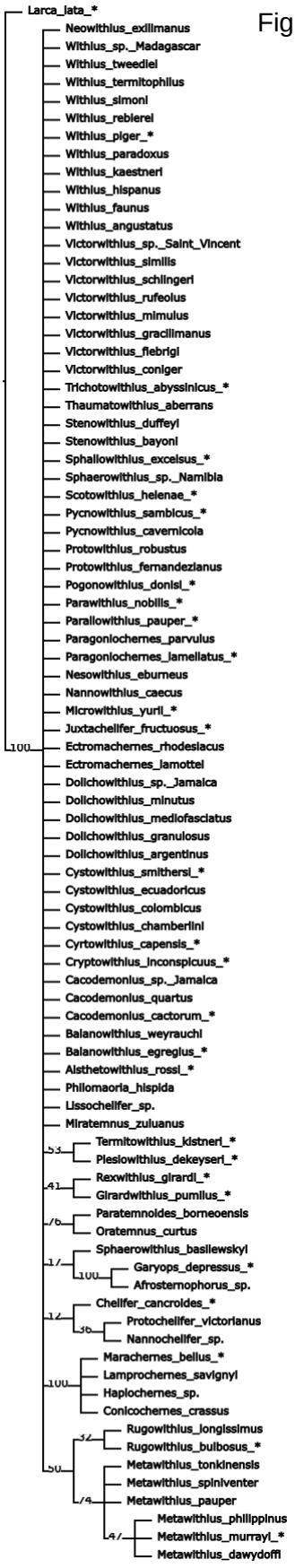


Figure S4. Parsimony tree with somatic characters.

Character	Character states
1 Chelicera: seta *sbs*	present absent
2 Chelicera: setae *bs*, morphology	smooth_and_acicular dentate_or_denticulate
3 Chelicera: seta *sbs*, morphology	smooth_and_acicular dentate_or_denticulate
4 Pedipalp: femur, males, morphology	not_hypertrophied hypertrophied
5 Pedipalps: mound on femur, male	absent present
6 Pedipalp: patella and chela, setation	setae_on_retrolateral_surface_of_patella_and_chela_of_males_not_modified setae_on_retrolateral_surface_of_patella_and_chela_of_males_long_and_slender
7 Pedipalp: chelal fingers, accessory teeth	absent present
8 Pedipalps: chelal fingers, teeth rows	teeth_in_straight_rows teeth_in_curved_rows
9 Pedipalps: movable chelal finger, number of trichobothria	4_trichobothria 3_trichobothria 2_trichobothria
10 Pedipalp: trichobothria *isb* (formerly *ist*) and *it*	situated_adjacent_to_each_other widely_separated
11 Pedipalp: venom apparatus, fixed chelal finger	present absent_or_very_reduced
12 Pedipalp: venom apparatus, movable chelal finger	present absent
13 Cephalothorax: carapace, posterior margin	straight_or_nearly_so angulate
14 Cephalothorax: maxillae, patch of rugose cuticle, males	absent present
15 Cephalothorax: maxillae, patch of rugose cuticle, position	on_internal_margin on_external_margin
16 Cephalothorax: eyes	absent present
17 Cephalothorax: eyes, morphology	rounded, corneate eyes  True eyes, dome shaped flat eye-spots  Not true eyes, white spots in their position
18 Cephalothorax: maxilla, sub-oral seta, male	sub-oral seta not on ''hooked'' mound sub-oral seta on ''hooked'' mound

19 Cephalothorax: "pseudosternum"'	absent present
20 Cephalothorax: coxal sac, male	absent present
21 Abdomen: lateral tergal margins, male	without_any_modification most_tergites_with_small_lateral_keels most_tergites_with_strong_lateral_keels
22 Abdomen: glandular setae, medial sternites, male	absent present
23 Abdomen: glandular setae, sternites, position, male	situated_on_sternites situated_in_membrane_between_sternites
24 Abdomen: glandular setae, morphology	glandular_setae_conical glandular_setae_distally_spatulate glandular setae distally rounded and medially slightly constricted
25 Abdomen: sternites, antero-lateral pockets, male	absent present
26 Legs: junction between femur and patella of legs I and II	perpendicular slightly_oblique strongly_oblique
27 Legs: tarsi III and IV, tactile seta, presence	absent present
28 Legs: tarsi III and IV, tactile seta, position	medial or sub-distal basal, very close to proximal end of tarsus
29 Legs: tarsi III and IV, slit sensillum	not_situated_on_mound situated_on_mound
30 Male genitalia: lateral apodemes, morphology	lateral_apodemes_not_elongated_or_triangular lateral_apodemes_elongated_and_triangular
31 Male genitalia: rams horn organs	absent present
32 Male genitalia: posterior diverticula	not short and finger-like short and finger-like
33 Female genitalia: spermathecae	absent present

Supplementary table 1. List of characters and character states used for the MOR analyses. The characters 10 and 17 were the ones used for the analysis of evolution of sexually related characters.

CLADE	**A**	**B**	**C**	**D**	**E**
Withiidae	X	X	X	X	X
Neotropical clade		X	X	X	X
Non-neotropical clade		X	X	X	X
Cacodemonius		X	X	X	X

Supplementary table 2. Clades recovered with five sets of alignment parameters as follows: A: Algorithm: Auto; Scoring matrix: 200; opening: 3; offset: 0,5/ B: Algorithm: Auto; Scoring matrix: 1; opening: 3; offset: 0,5 / C: Algorithm: Auto; Scoring matrix: 200; opening: 2; offset: 0,5 /D: Algorithm: Auto; Scoring matrix: 1; opening: 2; offset: 0,5 / E: Algorithm: E-INS-i; Scoring matrix: 1; opening: 2.

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3. Chapter 3: Taxonomic novelties about the Withiidae (Arachnida: Pseudoscorpiones)*

As a consequence of the morphologic study of samples around the world and the more complete phylogenetic analysis of the Withiidae, several novelties were found and updates were developed to improve the taxonomy of the group. Here we reported these results, some of these published.

3.1 A new genus and five new species of false scorpions (Arachnida: Pseudoscorpiones: Withiidae) from Colombia

3.1.1 Abstract

The pseudoscorpion family Withiidae is widely distributed around the world, with most of its diversity in tropical areas. Five new species and a new genus from Colombia are described: *Cystowithius florezi* sp. nov, *Parawithius bromelicola* sp. nov, *Oligowithius achagua* sp. nov., and the genus *Paciwithius* gen. nov. with two species *Paciwithius valduparensis* sp. nov. and *Paciwithius chimbilacus* sp. nov. A reassessment of the subgenus *Dolichowithius* (*Oligowithius*) Beier, 1936 allows us to elevate it to the generic level, and to transfer the only known species to *Oligowithius*, forming the new combination *Oligowithius abnormis* (Beier, 1936), comb. nov.

3.1.2 Introduction

With a surface area of over 1.1 million square kilometers, Colombia is considered a hot-spot of biodiversity with nearly 2,000 species of birds (ACO, 2020), 1,610 fish (DoNascimiento et al., 2019), 543 mammals (SCmas, 2021) and 27,713 plants and lichens (Bernal et al., 2020). However, the Colombian pseudoscorpion fauna is rather poorly

known even though its richness has been steadily rising over the past decade starting from 23 recorded species in 2007 (Ceballos & Florez 2007) to 65 species in 2019 (Romero-Ortiz et al., 2019). However, when compared with countries like Brazil with 174 species, Peru with 44 species, Ecuador with 61 species, Venezuela with 63 species, it is clear that there are considerable gaps to fill.

The pseudoscorpion family Withiidae comprises more than 170 species arranged in 37 genera. The family is divided into two subfamilies, Paragoniochernetinae and Withiinae, with the latter divided into four tribes, Cacodemoniini, Juxtacheliferini, Protocheliferini, and Withiini. The status of these groups is highly uncertain, with only the Cacodemoniini seemingly supported by a strong synapomorphy in the male genitalia (Harvey 2015). Withiidae has a cosmopolitan distribution with its highest diversity in the Tropics. For the Neotropical region, there are 10 genera recorded, six of them in the Cacodemoniini. Most of the taxonomic work on Neotropical withiids was undertaken in the 20th century and accompanied by few illustrations. One of the most recent studies concerning New World withiids examined the status of the genera *Parawithius* Chamberlin, 1931 and *Victorwithius* Feio, 1944, and described a new genus *Cystowithius* Harvey, 2004 (Harvey 2004). Most recently, a new species of the genus *Cystowithius* was described (García & Romero-Ortiz, 2021).

In this paper, we describe a new genus, *Paciwithius* gen.nov. with two new species, one new species of the genus *Cystowithius*, register a new record of the species *Cystowithius ankeri* Garcia & Romero 2021, propose an identification key for the species of this genus; describe a new species of the genus *Parawithius*, and provide a reassessment of the subgenus *Dolichowithius* (*Oligowithius*) Beier, 1936.

3.1.3 Materials and methods

The specimens examined for this study are lodged in the Arachnological collection at the Instituto de Ciencias Naturales – Universidad Nacional de Colombia (ICN-Aps). Specimens were studied by preparing temporary slide mounts by immersion in 75% lactic acid at room temperature for one to several days and mounting them on microscope slides with 10 mm coverslips supported by small sections of 0.25 mm or 0.50 mm diameter nylon fishing line. Specimens were examined with an Olympus BH–2 compound microscope and illustrated with the aid of a drawing tube. Photographs of the body were taken with a Leica MC-170

HD digital camera attached to a Leica M205A stereomicroscope, and then stacked by the Leica Application Suite version 4.6.0, with the specimen submerged in KY® gel. For the SEM pictures, specimens were critically point dried and coated with gold as standard protocols and mounted in copper tape (Alvarez-Padilla & Hormiga 2007). Pictures were taken with a Hitachi S-4700 in the American Museum of Natural History (AMNH) and a Hitachi T3030 Plus in the Western Australian Museum (WAM).

Measurements were taken at the highest magnification possible using an ocular graticule, taken to the nearest 0.005 mm. After study, the specimens were rinsed in water and returned to vials with the other body parts submerged in 75% ethanol. Dissected portions were placed in small capillary tubes sealed with cotton on both ends.

Terminology and measurements largely follow Chamberlin (1931b), except for the nomenclature of the pedipalps, legs, and, with some minor modifications, the terminology of the trichobothria where we follow Harvey (1992), chelicera (Harvey & Edward 2007; Judson 2007) and faces of the appendages (Harvey et al. 2012). Male genitalia terminology follows Romero-Ortiz & Sarmiento (2021).

The results of this work appear in the next section and the taxa are presented in the following order: First, the description of *Paciwithius* gen.nov. with two new species; next, the description of one new species of the genus *Cystowithius*, the new record of the species *Cystowithius ankeri* Garcia & Romero 2021, the identification key for the species of this genus; then the description of a new species of the genus *Parawithius*; and finally, the reassessment of the subgenus *Dolichowithius* (*Oligowithius*) Beier, 1936. Each taxa contains subsections that mention the type species, diagnosis, remarks, and etymology in the case of genera. For the new species, a detailed description is presented as well.

3.1.4 Systematics

FAMILY WITHIIDAE CHAMBERLIN, 1931

Genus *Paciwithius* gen. nov.

ZooBank Registration: http://zoobank.org/NomenclaturalActs/####

Type species.–*Paciwithius chimbilacus* sp. nov.

Diagnosis.–*Paciwithius* can be distinguished from all other withiids by the absence of tergal keels, absence of eyespots, absence of a tactile seta on tarsus IV, the trichobothrium *it* is midway between *isb* and *et*, the presence of a patch of glandular setae on sternite VIII with an elongated shape, and the first three to six tergites are entire. The male genitalia has dorsal apodemes extending over its entire length, lateral apodemes extending over most of the ejaculatory canal atrium, ejaculatory canal curved dorsally in lateral view and lateral rods straight, extending beyond the lateral apodemes in dorsal view. This genus was mentioned as *nr. Victorwithius* in Romero-Ortiz & Sarmiento (2021).

Remarks.–This new genus is placed within the Cacodemoniini as it has the triangular elongation made by the fusion of the dorsal and the lateral apodemes in the male genitalia that are characteristic of that tribe (Harvey 2015; Romero-Ortiz and Sarmiento 2021). The trichobothrial pattern has *it* and *isb* separated, and the only other Neotropical cacodemoniine genera with the same pattern are *Tropidowithius* Beier, 1955, *Balanowithius* Beier, 1959 and *Victorwithius* Feio, 1944. *Paciwithius* gen. nov. differs from *Tropidowithius*, *Balanowithius* and *Victorwithius* by the lack of eyespots and the lack of a tarsal tactile seta, which are present in these three latter genera; the only other withiid genera that lack a tactile seta on tarsus IV are *Nannowithius* Beier, 1932 and *Termitowithius* Muchmore, 1990 (see Harvey 2015). *Paciwithius* differs from *Victorwithius* by the patches of glandular setae on several sternites (restricted to sternite VIII in *Victorwithius*). For this unique combination of characters, we propose that the specimens belong to a new genus.

Etymology.–The genus name refers to the latin word for peace, *pax*. Colombia has had a long history of violence and since the peace agreement in 2016, lots of effort has been put into making this country a peaceful land, including the Truth commission (Comisión de la Verdad). We honor all this effort and believe that a change is possible. The name is masculine.

***Paciwithius chimbilacus* sp nov.**

Figures 1A, 2A–I, 8A

Material examined.–*Holotype* ♂. COLOMBIA: Meta: San Martín, Vda. San Francisco, Hacienda La María [3°39'55.5"N 73°39'29.7"W], 400 m, en guano de murciélagos (bat guano). [Neither date nor collector data] (ICN-APs-076). *Paratype*: 1 ♂, same data as the holotype (ICN-APs-076).

Diagnosis.–Males of *Paciwithius chimbilacus* sp. nov. can be distinguished from *P. valduparensis* sp. nov. by a slender pedipalpal femur and patella (femur: 4.10–4.35 x longer than broad in *P. chimbilacus* and 4.00 in *P. valduparensis*; patella: 3.33–3.43 x longer than broad in *P. chimbilacus* and 2.27 in *P. valduparensis*), and a stouter chela (3.16–3.33 x longer than broad in *P. chimbilacus* and 3.52 in *P. valduparensis*).

Description.–*Adults*: Color: Carapace brownish, darker than the tergites. Tergites yellow-brown, heavily granulated. Legs yellowish, proximal segments darker than the distal ones. Pedipalps reddish-brown, heavily granulated; chela and fingers reddish.

Chelicera (Fig. 2B): with 5 setae on hand, *bs* denticulate, all others including *sbs* acuminate; movable finger with 1 subdistal seta; galea simple; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth (Fig. 2C); lamina exterior present; 2 dorsal lyrifissures.

Pedipalp (Fig. 2D): trochanter, femur, patella and chelal hand granulate, fingers smooth; dorsal setae clavate; trochanter 1.72–1.88, femur 4.35–4.10, patella 3.43–3.33, chela (with pedicel) 3.56–3.38, chela (without pedicel) 3.33–3.16, hand 1.97–1.69 x longer than broad, movable finger 0.85-0.92 x longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 2D, 2H): *eb* and *esb* situated basally, as well as *ib* and *ist*; *isb* parallel to *est* both situated submedially, *it* midway between them and *et*; *b* and *sb* situated near one another, *st* closer to *sb* than to *t*, *t* situated submedially. Venom apparatus present in both chelal fingers, venom ducts not visible; nodus ramosus distal to *t* on movable finger, and not visible on fixed finger. Retrolateral margin of fixed finger with 2 sense-spots, one situated close to *esb* and *eb*, and the other distal to *est*. Chelal teeth squared; fixed finger with 42 teeth; movable finger with 41 teeth; accessory teeth absent.

Carapace (Fig. 2A): 1.28 x longer than broad; lateral margins not posteriorly widened; without eyes or eyespots; with ca. 62 setae, distributed: 32 anterior (2 near anterior margin), 20 in the mesozone, and 8 near posterior margin, all clavate; with 2 distinct furrows; posterior furrow slightly closer to posterior carapaceal margin than to median furrow (Fig. 2A).

Coxal region: coxal chaetotaxy: 20: 23: 24: 35; maxilla with 38 setae including 2 apical setae and 1 very small internal, sub-oral seta; median maxillary lyrifissure medial in position, posterior lyrifissure not visible.

Legs (Fig. 2F, G): junction between femora and patellae I and II parallel, junction in legs III and IV oblique; femur + patella of leg IV 3.45 x longer than broad; tarsal tactile seta of leg IV absent; arolium slightly shorter than claws; claws simple; legs with scale-like appearance, many clavate setae on leg IV.

Abdomen (Fig. 2I): tergites I–IV entire, the others with a faint medial suture, not keeled; sternites III–VII divided, sternites VIII–XI entire. Tergal chaetotaxy: 10: 10: 10: 11: 11: 12: 10: 12: 11: 8: 6: 4; mostly uniseriate but some tergites with a few setae placed anteriorly; all setae clavate. Sternal chaetotaxy: 10: (2) 13 (2): (2) 11 (2): 18: 19 + 1 gls: 20 + 24 gls: 13 + 84 gls: 9 + 4 gls+ 4 clavate: 10 + 7: 6 + 4 clavate setae: 2; sternites with many lyrifissures; sternite VI with 1 glandular seta, sternites VII–VIII of male with patches of glandular setae (Fig. 8A); glandular setae in extended patches (Fig. 8A); setae mostly uniseriate and acuminate but some clavate; male without paired invaginations on anterior margins of sternites.

Genitalia: see Romero-Ortiz & Sarmiento (2021) as “nr. *Victorwithius* 1”; male with elongated lateral apodemes although other structures not visible; female genitalia not visible.

Dimensions (mm) (L/W): male: holotype: body length 1.93. Pedipalps: trochanter 0.344/0.200, femur 0.696/0.160, patella 0.632/0.184, chela (with pedicel) 0.940/0.264, chela (without pedicel) 0.880, hand (without pedicel) length 0.520, movable finger length 0.440. Chelicera 0.192, movable finger length 0.136. Carapace 0.664/0.520 (width at medial area). Leg I: femur 0.128/0.128, patella 0.272/0.112, tibia 0.288/0.080, tarsus 0.280/0.064. Leg IV: femur + patella 0.552/0.160, tibia 0.488/0.096, tarsus 0.368/0.064.

Male: paratype: body length 1.78. Pedipalps: trochanter 0.360/0.192, femur 0.688/0.168, patella 0.640/0.192, chela (with pedicel) 0.920/0.272, chela (without pedicel) 0.860, hand (without pedicel) length 0.460, movable finger length 0.424.

Etymology.–This species epithet is derived from the common name of bats in the Meta region “chimbilaco”, due to the bat guano where the specimens were found.

***Paciwithius valduparensis* sp. nov.**

ZooBank Registration: http://zoobank.org/NomenclaturalActs/####

Figures 3A–I, 8C

Material examined.–*Holotype* ♂. COLOMBIA: Cesar: Valledupar, Ecoparque Los Besotes, campamento base [10°34'30.0"N 73°16'19.8"W], 600 m, 17 July 2015, captura manual, CarbiotTeam leg. (ICN-APs-597).

Diagnosis.–Males of *Paciwithius valduparensis* sp. nov. can be separated from *P. chimbilacus* sp. nov. by their stouter pedipalpal femur and patella (femur: 4.10–4.35 x longer than broad in *P. chimbilacus* and 4.00 in *P. valduparensis*; patella: 3.33–3.43 x longer than broad in *P. chimbilacus* and 2.27 in *P. valduparensis*), and a slender chela (3.16–3.33 x longer than broad in *P. chimbilacus* and 3.52 in *P. valduparensis*).

Description.–*Adults*: Colour: brown-yellowish, darker in carapace and tergites; carapaceal metazone without paired pale spots; pedipalps brownish, somewhat lighter than body, fingers reddish; legs yellow-brown, uniform color; all specimen heavily granulated.

Chelicera (Fig. 3B): with 5 setae on hand, *sbs* and *bs* denticulate, all others acuminate; movable finger with 1 subdistal seta; galea simple; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth (Fig. 3C); lamina exterior present; 2 lyrifissures on dorsal and 1 on ventral side.

Pedipalp (Fig. 3I): trochanter, femur, patella and chelal hand granulate, fingers smooth; dorsal setae clavate; trochanter 1.59, femur 4.00, patella 2.27, chela (with pedicel) 3.67, chela (without pedicel) 3.52, hand 1.72 x longer than broad, movable finger 1.07 x longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria, (Fig. 3E, H): *eb* and *esb* situated basally, as well as *ib* and *ist*; *isb* parallel to *est* both situated submedially, *it* midway between them and *et*; *b* and *sb* situated near one another, *st* closer to *sb* than to *t*, *t* situated subdistally. Venom apparatus present in both chelal fingers, venom ducts not visible; nodus ramosus not visible. Retrolateral margin of fixed finger with 4 sense-spots, three situated linearly between *esb* and *est*, and the other between *est* and *et*. Chelal teeth squared; fixed finger with 42 teeth; movable finger with 42 teeth; accessory teeth absent.

Carapace (Fig. 3A): 1.26 x longer than broad; heavily granulated lateral margins convex, not posteriorly widened; without eyes; with ca. 62 setae, distributed: 36 anterior (4 near anterior margin), 18 in the mesozone, and 8 near posterior margin, all clavate; with 2 distinct furrows; posterior furrow slightly closer to posterior carapaceal margin than to median furrow (Fig. 3A).

Coxal region: coxal chaetotaxy: 10: 14: 15: 19; maxilla with 32 setae including 2 apical setae and 1 very small internal, sub-oral seta; median maxillary lyrifissure medial in position, posterior lyrifissure not visible.

Legs (Fig. 3F, G): junction between femora and patellae I and II parallel, as well as legs III and IV; femur + patella of leg IV 3.44 x longer than broad; tarsal tactile seta of leg IV absent (Fig. 3G); arolium slightly shorter than claws; claws simple.

Abdomen (Fig. 3D): tergites I–III and X–XI entire, the others with faint medial suture, not keeled; sternites 3 to 6 divided, entire from 7 to 11. Tergal chaetotaxy: 10: 9: 9: 9: 12: 12: 12: 11: 12: 9: 4: 2; mostly uniseriate but some tergites with a few setae placed anteriorly; all setae clavate. Sternal chaetotaxy: 10: (2) 12 (2): (1) 6 (1): 19: 16 + 6 gls: 11 + 36 gls: 9 + 97 gls: 10 + 22 gls: 9 : 6: 2; sternites with many lyrifissures, X–XII with lines of peak-like waves; sternites VI–IX with patches of glandular setae (Fig. 8C); glandular setae in extended patches (Fig. 8C); setae uniseriate and mostly acuminate, but some clavate in the lateral region of sternites VIII to XII; without paired invaginations on anterior margins of sternites.

Genitalia: see Romero-Ortiz & Sarmiento (2021) as “nr. *Victorwithius* msp. 2”.

Dimensions (mm): male: holotype: body length 1.84. Pedipalps: trochanter 0.280/0.176, femur 0.608/0.152, patella 0.600/0.264, chela (with pedicel) 0.940/0.256, chela (without pedicel) 0.900, hand (without pedicel) length 0.440, movable finger length 0.472. Chelicera 0.206, movable finger length 0.150. Carapace 0.656/0.520 (width at medial area). Leg I: femur 0.144/0.112, patella 0.240/0.104, tibia 0.272/0.072, tarsus 0.288/0.048. Leg IV: femur + patella 0.496/0.144, tibia 0.400/0.088, tarsus 0.352/0.048.

Etymology.–This species is named after the city in which it was found. The Valledupar demonym is “valduparensis”. This is considered the place where the vallenato music was born. The specific epithet is an adjective.

Genus *Cystowithius* Harvey, 2004

Cystowithius Harvey, 2004: 440; García & Romero-Ortiz, 2021: 2.

Type species.–*Cystowithius smithersi* Harvey, 2004, by original designation).

Diagnosis.–See Harvey (2004).

Remarks.–The genus *Cystowithius* is endemic to Central and South America (Harvey 2004; García & Romero-Ortiz 2021), and currently contains five species: *C. colombicus* Harvey, 2004 and *C. ankeri* García & Romero-Ortiz, 2021 from Colombia, *C. ecuadoricus* (Beier, 1959) and *C. smithersi* Harvey, 2004 from Ecuador and Peru, and *C. chamberlini* Harvey, 2004 from Mexico and Guatemala. Males are easily recognized by the presence of sternal invaginations (Figs. 4D, 5A)

Cystowithius florezi sp. nov.

ZooBank Registration: http://zoobank.org/NomenclaturalActs/####

Figures 1B, 4A–I, 8D

Material examined.–Holotype ♂. COLOMBIA: Tolima: Juntas, Reserva Natural Ibanasca, [4°33'22.0"N 75°19'17.2"W], 1700 m, 12 February 2007, Plantación de pino [*Pinus* plantation], vegetación baja, captura manual, C. Cortes leg. (ICN-APs-077).

Diagnosis.–*Cystowithius florezi* can be separated from the other *Cystowithius* species as follows. *Cystowithius florezi* sp. nov. is very similar to *C. columbicus* Harvey, 2004 and *C. ecuadoricus* Harvey, 2004, so we show a detailed comparison for each one in Table 1. In general, *C. columbicus* has bigger pedipalp segments and the patches of glandular setae located in sternites VII–IX; in *C. florezi* the patches are just in sternite VIII. Also, the sternal pockets are in sternites VI and VII in *C. columbicus* and in V to VIII in *C. florezi*. It differs from *C. chamberlini* by the chelal hand being smooth rather than strongly granulated in *C. florezi*, and the position of the tactile setae on tarsus IV located close to midway of the tarsus, rather than distal in *C. florezi*; from *C. ankeri* by the length of the movable finger of the pedipalp which is longer in ankeri than in *C. florezi* (0.700 mm vs. 0.520 mm); and from *C. smithersi* by the length of the chela with pedicel which is longer in *C. smithersi* than in *C. florezi* (1.35 mm vs. 1.02 mm)

Description.–Colour: with sclerotized portions generally yellow-brown; carapace and pedipalps darker; carapaceal metazone with paired pale spots; legs darker at the edges.

Chelicera (Fig. 4B): with 5 setae on hand, *sbs* and *bs* denticulate, all others acuminate; movable finger with 1 subdistal seta; galea with 1 sub-terminal and 3 terminal rami; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth (Fig. 4I); lamina exterior present; 2 dorsal lyrifissures.

Pedipalp (Fig. 4G): trochanter, femur, patella and chelal hand granulate, chelal fingers smooth; setae clavate and denticulate; trochanter 2.04, femur 3.81, patella 3.30, chela (with pedicel) 3.64, chela (without pedicel) 3.42, hand 1.74 x longer than broad, movable finger 1.07 x longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 4E, H): *eb* and *esb* situated basally, as well as *ib* and *ist*; *isb*, *it* and *est* grouped together submedially; *et* near the distal end of the finger; *b* and *sb* situated near one another; *st* slightly closer to *sb* than to *t*; *t* parallel to *isb*. Venom apparatus present in both chelal fingers, venom ducts not visible in fixed finger; nodus ramosus distal to *t* on movable finger, not visible on fixed finger. Retrolateral margin of fixed finger with 3 sense-spots situated linearly between *esb* and *est*; movable finger with small structure between *t* and *st* that contains two small nubbins. Chelal teeth rounded with an apical spot; fixed and movable finger with 32 teeth; accessory teeth absent.

Carapace (Fig. 4A): 1.31 x longer than broad; lateral margins convex, not posteriorly widened; with 2 non-corneate eyes; with ca. 56 setae, including 4 near anterior margin, 7 near posterior margin, 13 in the medial zone and 32 in the anterior region; with 2 distinct furrows; posterior furrow slightly closer to posterior carapaceal margin than to median furrow (Fig. 4A); deeply granulated.

Coxal region: coxal chaetotaxy: 8: 8: 8: 12, with multiple small lyrifissures; maxilla with 2 apical setae, 1 very small internal, sub-oral seta and 12 setae; median maxillary lyrifissure medial in position, posterior lyrifissure present.

Legs (Fig. 4C, F): junction between femora and patellae I and II parallel, junction in legs III and IV oblique; femur + patella of leg IV 3.09 x longer than broad; tarsal tactile seta of leg IV situated distally, 0.74 of tarsus length (Fig. 4F); subterminal tarsal seta acute, distal to tactile seta; arolium same level as claws.

Abdomen (Fig. 4D): all tergites divided but the first two only with a faint medial suture; all sternites entire. Tergal chaetotaxy: 8: 7: 8: 14: 15: 13: 15: 15: 15: 13: 8 (including 2 tactile setae): 2; mostly uniseriate but some tergites with a few setae placed anteriorly; all setae foliate, except in the last tergite; tergites densely granulated. Sternal chaetotaxy: 10: (2) 9 (2): (2) 9 (2): 12: 12: 12: 2 + 40 gls: 9: 8: 9 (including 2 tactile and 3 clavate setae): 2; only sternite VIII with a small circular patch of glandular setae (Fig. 8D); with paired invaginations on anterior margins of sternites VI to VIII (Fig .4D).

Genitalia: see Romero-Ortiz & Sarmiento 2021 as "*Cystowithius* msp1".

Dimensions (mm): male: holotype: body length 1.94. Pedipalps: trochanter 0.360/0.176, femur 0.604/0.168, patella 0.608/0.184, chela (with pedicel) 1.020/0.280, chela (without pedicel) 0.960, hand (without pedicel) length 0.488, movable finger length 0.520. Chelicera 0.192, movable finger length 0.144. Carapace 0.672/0.512 (width at medial area); eye diameter 0.056. Leg I: femur 0.144/0.136, patella 0.288/0.136, tibia 0.204/0.096, tarsus 0.248/0.056. Leg IV: femur + patella 0.568/0.184, tibia 0.432/0.184, tarsus 0.344/0.072, TS 0.744.

Etymology.–This species is dedicated to Professor Eduardo Florez, considered the father of Arachnology in Colombia. He has also been the curator of the Arachnological collection of the Instituto de Ciencias Naturales, where all of the material used in this study is lodged.

	C. columbicus Harvey, 2004	*C. ecuadoricus* Harvey, 2004	*C. florezi* n. sp.
Body length	2.00–2.11	2.16–2.29	1.94
Carapace length/width	0.736/0.515 **1.43**	0.749–0.800/0.624 **1.20-1.28**	0.672/0.512 **1.31**
Pedip. femur	0.870–0.965/0.166–0.202 **4.78–5.25 x**	0.797–0.816/ 0.168–0.179 **4.47–4.74**	0,604/0,168 **3.595**
Pedip. patella	0.768–0.883/0.186–0.206	0.674–0.688/0.195–0.212	0.608/0.184 **3.304**

	3.88–4.46	**3.18–3.47**	
Chela with pedicel length/width	1.120–1.260/0.277–0.332 **3.80–4.04**	1.085–1.144/0.276–0.297 **3.65–4.01**	1,02/0,280 **3.64**
Mov. finger	0.493–0.606 **0.90–1.24**	0.497– 0.558 **0.86–1.11**	0,520
Hand	0.498–0.600	0.502–0.575	0,488
Femur/patella IV	0.674/0.166	0.600/0.205 **2.93**	0,568/0,184
Glandular setae	sternites VII–IX with patches of glandular setae [gls], arranged ca. 20: 27: 13 respectively	sternites VI–IX with patches of glandular setae [gls], arranged 6: 42: 10: 8 (lectotype) respectively	sternite VIII with patches of glandular setae [gls], arranged 40 in a small circle
Sternal pockets	on anterior margins of sternites VI-VII	on anterior margins of sternites V-VIII	on anterior margins of sternites VI-VIII
Sense spots in chelal finger	without sense spots on movable finger	without sense spots on movable finger but with small sense spot slightly distal to *st* that contains three small nubbins	without sense spots on movable finger but with small spot slightly distal to *st* that contains two small nubbins

Table 1. Comparative character states between three species of *Cystowithius*. Bold font denotes ratios.

New Record: *Cystowithius ankeri* Garcia & Romero-Ortiz, 2021

Figures 5A-C

Cystowithius ankeri Garcia & Romero-Ortiz, 2021: 2–5, figs 1–12, 15.

Material examined.–COLOMBIA: *Cundinamarca*: 3 ♂, 3 ♀, San Antonio del Tequendama, Parque Natural Chicaque, 04°36'51.3"N, 74°18'55.2"W, 2600 m, 1 June 2009, en tronco descompuesto de dosel a 25 m de altura, captura manual, F. Helbig leg. (ICN-APs-298).

Remarks.– Although these specimens were collected 320 km from the type locality in Caldas Department, we refer them to the species *C. ankeri*. They have similar pedipalp proportions, however their patches of glandular setae are different. The holotype of *C. ankeri* has two small patches in sternite VIII, and those examined here have one long patch of setae (Figs. 5A, C). We ascribe this characteristic to intraspecific variation.

Key to the species of the genus *Cystowithius*

1. Chelal hand smooth; setae on chelal hand only barely denticulate; tactile setae of tarsus IV situated closer to its mid-length (TS = 0.59–0.61).....*C. chamberlini*

1’. Chelal hand evenly granulate; setae on chelal hand distinctly denticulate; tactile seta of tarsus IV situated subdistally (TS = 0.68–0.79).....2

2. Movable finger of pedipalp more than 0.70 mm; males with sternal invaginations on sternites VI–VIII.....*C. ankeri*

2’. Movable finger of pedipalp less than 0.60 mm; males with sternal invaginations on sternites V–VIII or VI–VII.....3

3. Pedipalps longer and more slender, i.e. chela (with pedicel) longer than 1.35 mm.....*C. smithersi*

3’. Pedipalps shorter and more robust, i.e. chela (with pedicel) shorter than 1.20 mm.....4

4. Femur of the pedipalp stout (3.96 times longer than broad), males with sternal invaginations on anterior margins of sternites VI-VIII, patches of glandular setae on sternite VIII in a small circle……..… *C. florezi* sp. nov.

4’. Femur of the pedipalp slender (more than 4.47 times longer than broad), males with sternal invaginations on anterior margins of sternites VI-VIII, patches of glandular setae on sternite VIII in a small circle.....5

5. Setae on tergite XI short and strongly clavate; chelal hand without long, strongly denticulate setae.....*C. colombicus*

5’. Setae on tergite XI long and only slightly clavate; chelal hand with long, strongly denticulate setae.....*C. ecuadoricus*

Genus *Parawithius* Chamberlin 1931

Parawithius Chamberlin 1931: 292; Beier 1932: 212; Beier 1959: 216; Harvey 2004: 437.

The genus *Parawithius* was delimited by Harvey (2004). This genus includes three South American species: *P. nobilis* (With, 1908) from Colombia, *P. pseudorufus* Beier, 1932 and *P. iunctus* Beier, 1932 from Paraguay. We here add an additional species from Colombia

Type species.–*Chelifer nobilis* With 1908, by original designation.

Diagnosis.–See Harvey (2004).

Parawithius bromelicola sp nov.

ZooBank Registration: http://zoobank.org/NomenclaturalActs/####

Figures 1C, 6A-H, 8B

Material examined.–*Holotype* ♂. COLOMBIA: *Cundinamarca*: Cogua, Embalse del Neusa Tausa, Llano Grande, 2900 m, 7 March 2004, bajo corteza de tronco (under tree bark), A.L. Leon leg. (ICN-APs-082). *Paratypes:* COLOMBIA: *Cundinamarca*: 1 ♂, same data as the holotype (ICN-APs-082); 1 ♀, 1 ♂, Sesquilé, Camino al Cerro de Las Tres Viejas, 5°02'17.0"N 73°47'13.0"W, 2740 m, 8 September 2019, en bromelia (on bromeliad), C. Romero-Ortiz, F. Garcia, J.J. Lagos, A. Carvajal, D. Mayorga-Ch leg. (ICN-APs-836).

Other material. COLOMBIA: *Santander*: 1 ♀, Málaga, Vda. Buenavista, km 7 vía Bucaramanga, 6°42'23.7"N 72°44'52.6"W, 2620 m, 1 January 2020, en bromelia sobre

árbol “Loqueto” *Escallonia pendula* (Ruiz & Pav.) Pers. (on bromeliad), C. Romero-Ortiz, J.J. Lagos leg. (ICN-APs-847).

Diagnosis.–*Parawithius bromelicola* sp. nov. can be separated from *P. nobilis* (With, 1908) by the stouter pedipalpal segments (i.e. patella 3.36–3.44 x longer than broad compared with 3.24–3.30 x longer than broad, and the chela without pedicel 3.39–3.47 x longer than broad, compared to 3.58–3.85 x longer than broad); the extension of the strongly clavate setae on the dorsal surface of the fixed finger (i.e. distal to *it* and *est* compared to proximal to *it* and *est*); from *P. iunctus* Beier, 1932 and *P. pseudorufus* Beier, 1932 by the presence of pale spots on the carapaceal metazone, in addition to the size of the fingers compared to the hand (i.e. fingers shorter to the hand in *P. bromelicola* sp. nov., *P. nobilis* and *P. iunctus* and longer in *P. pseudorufus*).

Description.–*Adults*: Colour: brown yellowish, carapace darker than body, carapaceal metazone with paired pale spots; pedipalps brown reddish uniform in color, very granulated; tergites yellow-brown; big leg segments darker at posterior margin.

Chelicera (Fig. 6B): with 5 setae on hand, *sbs* and *bs* denticulate, all others acuminate; movable finger with 1 subdistal seta; galea of male with 3-4 very small terminal rami; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth (Fig. 6C); lamina exterior present; 2 dorsal lyrifissures.

Pedipalp (Fig. 6G): trochanter, femur, patella and chelal hand granulate, chelal fingers smooth; dorsal setae clavate and denticulate; trochanter 1.81 (1.59-1.74) (♂), 1.76 (1.73) (♀), femur 4.33 (4.38-4.17) (♂), 3.77 (4.14) (♀), patella 3.44 (3.36-3.42) (♂), 3.17 (3.5) (♀), chela (with pedicel) 3.99 (3.97-3.78) (♂), 3.52 (3.85) (♀), chela (without pedicel) 3.85 (3.78-3.58) (♂), 3.29 (3.65) (♀), hand 2.0 (1.82-1.78) (♂), 1.68 (1.92) (♀) x longer than broad, movable finger 0.95 (1.06-1.00) (♂), 1.01 (0.86) (♀) x longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 6D, H): *eb* and *esb* situated basally, *est* midway between *eb* and the fingertip, *et* situated distally; *ib* and *ist* situated basally, *it* directly behind *est* and close distal to *isb*; *b* and *sb* situated near one another; *st* closer to *sb* than to *t; t* parallel to *est*. Venom apparatus present in both chelal fingers, venom ducts not visible in ♂ and ♀. Retrolateral margin of fixed finger with 3 sense-spots situated distal to *esb* and linear in disposition. Chelal teeth very small and almost not developed; accessory teeth absent.

Carapace (Fig. 6A): 1.42 (1.18–1.11) (♂), 1.29 (1.16) (♀) x longer than broad; lateral margins convex, not posteriorly widened; with 2 non-corneate eyes; with ca. 57 (♂) setae, including 4 (♂) near anterior margin, 7 (♂) near posterior margin, 16 in the medial zone and 30 in the anterior region; with 2 distinct furrows; posterior fu-rrow slightly closer to posterior carapaceal margin than to median furrow (Fig. 6A).

Coxal region: coxal chaetotaxy: ♂, 10: 10: 5: 7; maxilla with 23 setae including 3 apical setae (1 tactile setae) and 1 very small internal, sub-oral seta; median maxillary lyrifissure medial in position, posterior lyrifissure present.

Legs (Fig. 6E, F): junction between femora and patellae I and II parallel as well as junction in legs III and IV; femur + patella of leg IV 3.46 (♂) x longer than broad; tarsal tactile seta of leg IV situated distally, 0.73 (♂) of tarsus length (Fig. 6F); 2 subterminal tarsal setae distal form TS arcuate and acute; arolium shorter than claws.

Abdomen : all tergites divided with a medial suture, with a row of spots in the middle of each hemItergite; sternites entire. Tergal chaetotaxy: ♂, 8: 9: 9: 10: 13: 13: 16: 16: 18: 12: 10 (including 2 tactile setae): 2; all setae foliate. Sternal chaetotaxy: ♂, 15: (2) 11 (2): (2) 13 (2): 16: 14: 16 + 21 gls: 13 + 86 gls: 11 + 2 gls: 10 (including 2 tactile setae): 8 (including 2 tactile setae and some dentate): 2; sternites VII and VIII of ♂ with patches of glandular setae (Fig. 8B); setae uniseriate and acuminate; ♂ without paired invaginations on anterior margins of sternites.

Genitalia: see Romero-Ortiz & Sarmiento 2021 as "*Parawithius* msp1".

Dimensions (mm): male: holotype (followed by male paratypes): body length 2.46 (2.58, 2.46). Pedipalps: trochanter 0.376/0.208 (0.368/0.232, 0.376/0.216), femur 0.832/0.192 (0.840/0.192, 0.768/0.184), patella 0.744/0.216 (0.752/0.224, 0.712/0.208), chela (with pedicel) 1.180/0.296 (1.240/0.312, 1.120/0.296), chela (without pedicel) 1.140 (1.180, 1.060), hand (without pedicel) length 0.592 (0.568, 0.528), movable finger length 0.560 (0.600, 0.528). Chelicera 0.212, movable finger length 0.170. Carapace 0.816/0.576 (0.848/0.720, 0.820/0.740); eyespot diameter 0.072. Leg I: femur 0.160/0.160, patella 0.328/0.144, tibia 0.352/0.096, tarsus 0.320/0.056. Leg IV: femur + patella 0.664/0.192, tibia 0.512/0.120, tarsus 0.368/0.072, TS 0.728.

Female: allotype (followed by female other material): body length 2.68 (2.70). Pedipalps: trochanter 0.408/0.232 (0.360/0.208), femur 0.784/0.208 (0.728/0.176), patella 0.736/0.232 (0.672/0.192), chela (with pedicel) 1.240/0.352 (1.140/0.296), chela (without pedicel) 1.160 (1.080), hand (without pedicel) length 0.592 (0.568), movable finger length 0.600 (0.488). Carapace 0.880/0.680 (0.740/0.640) (width at medial area).

Etymology.–This species is named after bromeliad plants, due to its association with them. Most of the specimens were collected by sifting bromeliads on white sheets.

Genus *Oligowithius* Beier, 1936, stat. nov.

Dolichowithius (*Oligowithius*) Beier, 1936: 447; Harvey, 1991a: 645.

Type species.–*Dolichowithius* (*Oligowithius*) *abnormis* Beier, 1936, by monotypy.

Diagnosis.–The genus *Oligowithius* can be distinguished from other Neotropical withiid genera by the presence of a patch of glandular setae on sternites VII and VIII either in one or two small circular patches in the middle of the sternites, the distal position of the tactile setae on tarsus IV, the presence of two non-corneate eyes, and the trichobothria *it* and *isb* located far apart. Moreover, the male genitalia is characterized by a pair of lateral apodemes that are not merged with the dorsal apodemes (Fig. 10).

Remarks.–Beier (1936) described four new species of pseudoscorpions from Venezuela and the Caribbean islands of Bonaire, Curaçao, and Aruba. Among them was *Dolichowithius* (*Oligowithius*) *abnormis* Beier, 1936 which represented the type species of the then newly described subgenus *Oligowithius* Beier, 1936. Unfortunately, the description was very short and the justification for the subgenus was limited. The holotype and only known specimen of *D. (O.) abnormis* cannot be traced in Naturalis Center, Amsterdam (Dr Bram van der BIjl, in litt. 10 July 2019) or the Naturhistorisches Museum, Vienna (Dr Christoph Hörweg, in litt. 9 July 2019), which renders the status of the species and subgenus difficult to assess.

We have studied three specimens of an unusual, small withiid from Colombia that closely resembles *D. (O.) abnormis* in the morphology of the male glandular setae and in the positions of the trichobothria. We found sufficient differences between these specimens and the description of *D. abnormis* to consider it as a new species. We also found sufficient

differences between the new species from Colombia and species of *Dolichowithius* to warrant the elevation of *Oligowithius* to full genus level, although we are aware that a test using a phylogeny will add support to our proposal. The differences between *Dolichowithius* and *Oligowithius* are as follows: patch of glandular setae on sternites VII and VIII in *Oligowithius* and on sternites VII–IX in *Dolichowithius*, trichobothria *isb* and *it* far apart in *Oligowithius* and close together in *Dolichowithius*, and the male genitalia with a pair of lateral apodemes that are not merged with the dorsal apodemes in *Oligowithius*, while they are merged in *Dolichowithius*.

***Oligowithius abnormis* (Beier, 1936), comb. nov.**

Dolichowithius (Oligowithius) abnormis Beier, 1936: 446–447, fig. 4.

Remarks.–With the recognition of *Oligowithius* as a distinct genus, *D. (O.) abnormis* is here transferred to the genus *Oligowithius*.

***Oligowithius achagua* sp. nov.**

ZooBank Registration: http://zoobank.org/NomenclaturalActs/####

Figures 1D, 7A-H, 8E-F, 9A-D, 10

Material examined.–*Holotype* ♂. COLOMBIA: *Meta*: Puerto Gaitán, Carimagua, 160 m, 22 April 2012, estero, D. Martinez leg. (ICN-APs-388). *Paratypes* ♂ and ♀ same data as the holotype.

Diagnosis.–*Oligowithius achagua* sp. nov. differs from *O. abnormis* by its smaller size (1.7 mm vs. 2.18 mm in *O. achagua*), a more slender patella in *O. achagua* (3.05 x longer than broad) than in *O. abnormis* (2.74 x), and a stouter chela in *O. abnormis* (3.7 x longer than broad) than in *O. achagua* (4.05 x).

Description.–*Adults*: Colour: yellowish-brown; carapace and pedipalp reddish-brown; legs yellowish, lighter than the abdomen, darker in the edges; carapaceal metazone without paired pale spots.

Chelicera (Fig. 7B): with 5 setae on hand, *sbs* missing, *bs* slightly denticulate, all others acuminate; movable finger with 1 subdistal seta; galea of male broken in holotype, galea with multiple rami in paratype (Fig. 9A); rallum of 4 blades, the most distal blade with

several serrations on leading edge, other blades smooth (Fig. 7E); lamina exterior present; 2 lyrifissures on dorsal side.

Pedipalp (Fig. 7C): trochanter, femur, patella and dorsal chelal hand granulate, ventral chelal hand and fingers smooth; dorsal setae clavate and denticulate; trochanter 2.05 (1.79) (♂), 1.75 (♀), femur 3.79 (3.58) (♂), 3.53 (♀), patella 3.05 (3.10) (♂), 2.95 (♀), chela (with pedicel) 4.05 (4.07) (♂), 3.79 (♀), chela (without pedicel) 3.79 (3.89) (♂), 3.55 (♀), hand 2.07 (2.0) (♂), 1.94 (♀) x longer than broad, movable finger 0.93 (1.00) (♂), 0.92 (♀) x longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 7H): *eb* and *esb* situated basally, as well as *ib* and *ist*; *est* situated midway between *it* and *isb* in the middle of the finger, *et* close to fingertip; *b* and *sb* situated near one another, *st* located midway between *sb* and *t, t* parallel to *it*. Venom apparatus present in both chelal fingers, venom ducts not visible in ♂; nodus ramosus not visible. Retrolateral margin of fixed finger with 2 double sense-spots situated midway between *esb* and *est,* movable finger with one double sense-spot close to *sb*. Chelal teeth rounded; fixed finger with 40 (♂) teeth; movable finger with 41 (♂) teeth; accessory teeth absent.

Carapace (Fig. 7A): 1.28 (1.20) (♂), 1.07 (♀) x longer than broad; posterior lateral margins slightly widened; with 2 non-corneate eyes; with ca. 57 (♂) setae, including 2 (♂) near anterior margin, 11 (♂) near posterior margin, 22 in the medial zone and 24 in the anterior region; with 2 distinct furrows; posterior furrow slightly closer to posterior carapaceal margin than to median furrow (Fig. 7A).

Coxal region: coxal chaetotaxy: ♂, 6: 7: 9: 18; maxilla with 19 setae including 2 apical setae and 1 very small internal, sub-oral seta; median maxillary lyrifissure medial-anterior in position, posterior lyrifissure present.

Legs (Fig. 7F, G): junction between femora and patellae I and II parallel, junction in legs III and IV oblique; femur + patella of leg IV 2.85 (♂) x longer than broad; tarsal tactile seta of leg IV situated distally, 0.76 (♂) of tarsus length (Fig. 7G); arolium slightly shorter than claws.

Abdomen (Fig. 7D): first four tergites entire, the others with faint medial suture. Tergal chaetotaxy: ♂, 12: 14: 13: 18: 18: 19: 19: 18: 19: 15: 6: 2, ; all setae foliate; mostly uniseriate but some tergites with a few setae placed anteriorly. All sternites entire, except

for the last three with a faint medial suture. Sternal chaetotaxy: ♂, 10: (1) 6 (1): (1) 8 (1): 14: 12: 8 + 13/13 gls: 8 + 18/16 gls: 8: 8 : 6 (including 2 clavate setae): 2; sternites VII–VIII of ♂ with two small patch circles of glandular setae each (Fig. 8F, Fig. 9B-D), paratypes with one circular patch of glandular setae each (Fig. 8E); setae uniseriate and acuminate; ♂without paired invaginations on anterior margins of sternites.

Genitalia: simple structure with most of the components extremely reduced to chitinized lateral apodemes that do not merge with the dorsal apodemes. The level of sclerotization of the ejaculatory channel is weak, however it is projected from the lateral apodemes, which allow us to classify *Oligowithius* as a Cacodemoniini (Fig 10).

Dimensions (mm): male: holotype (followed by male paratype): body length 2.18 (2.10). Pedipalps: trochanter 0.328/0.160 (0.272/0.152), femur 0.576/0.152 (0.544/0.152), patella 0.536/0.176 (0.496/0.160), chela (with pedicel) 0.940/0.232 (0.880/0.216), chela (without pedicel) 0.880 (0.840), hand (without pedicel) length 0.480 (0.432), movable finger length 0.448 (0.432). Chelicera 0.190, movable finger length 0.160. Carapace 0.736/0.576 (0.672/0.560) (width at medial area); eyespots diameter 0.080. Leg I: femur 0.144/0.144, patella 0.264/0.136, tibia 0.264/0.080, tarsus 0.264/0.056. Leg IV: femur + patella 0.456/0.160, tibia 0.336/0.112, tarsus 0.328/0.064, TS 0.756.

Female: allotype: body length 2.36. Pedipalps: trochanter 0.280/0.160, femur 0.536/0.152, patella 0.520/0.176, chela (with pedicel) 0.940/0.248, chela (without pedicel) 0.880, hand (without pedicel) length 0.480, movable finger length 0.440. Carapace 0.728/0.580.

Etymology.–This species is named after the indigenous people, original inhabitants of the Meta, Vichada and the Venezuelan Llanos, the Achaguas. The name should be treated as a noun in apposition.

3.1.5 Final remarks

In Withiidae, the presence of patches of abdominal glandular setae, especially in males, have been used as a diagnostic character since Weygoldt (1970) first elevated the Withiinae to family level. However, some taxa attributed to the family such as *Protowithius* Beier, 1955, *Juxtachelifer* Hoff, 1956 and *Termitowithius* lack glandular setae (Beier 1955; Harvey 2015; Hoff 1956; Muchmore 1990). The arrangement of the patches of glandular

setae has often been used to define withiid genera, but without a phylogenetic framework in which to test whether these patterns validly define clades. On the other hand, trichobothrial patterns seem to provide additional support for some genera, especially when combined with glandular setael patterns and other characters such as the presence or position of tactile setae on the posterior tarsi.

Male genitalia need to be addressed when defining withiid genera. As shown by Mahnert (1975) and Romero-Ortiz & Sarmiento (2021), there is morphological correspondence between their structure and proposed genera, and Harvey (2004) suggested that the triangular dorsal apodeme shape found in many withiids likely represented a monophyletic group, the Cacodemoniini. Romero-Ortiz & Sarmiento (2021) were able to define individual Neotropical genera using the morphology of the male genitalia, which is here extended to *Oligowithius*, which is clearly defined based on its genitalia, among other characters. In future, an assessment of the female genitalia may provide further diagnostic features.

Finally, we are aware that a fully resolved phylogeny is needed to support the new clades and the assessment of the old ones, however, as for the new genus *Paciwithius*, and for the new genus rank of *Oligowithius*, we presented a unique and exclusive combination of morphological characters that allow us to conclude and support the taxonomic changes presented in this work.

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FIGURES

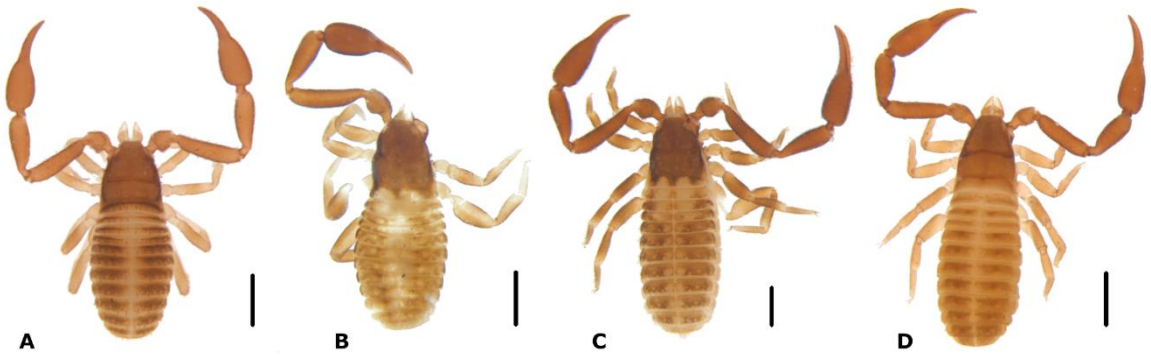

Figure 1. Habitus of the new species: A. *Paciwithius chimbilacus* sp. nov.; B. *Cystowithius florezi* sp. nov.; C. *Parawithius bromelicola* sp. nov.; D. *Oligowithius achagua* sp. nov.

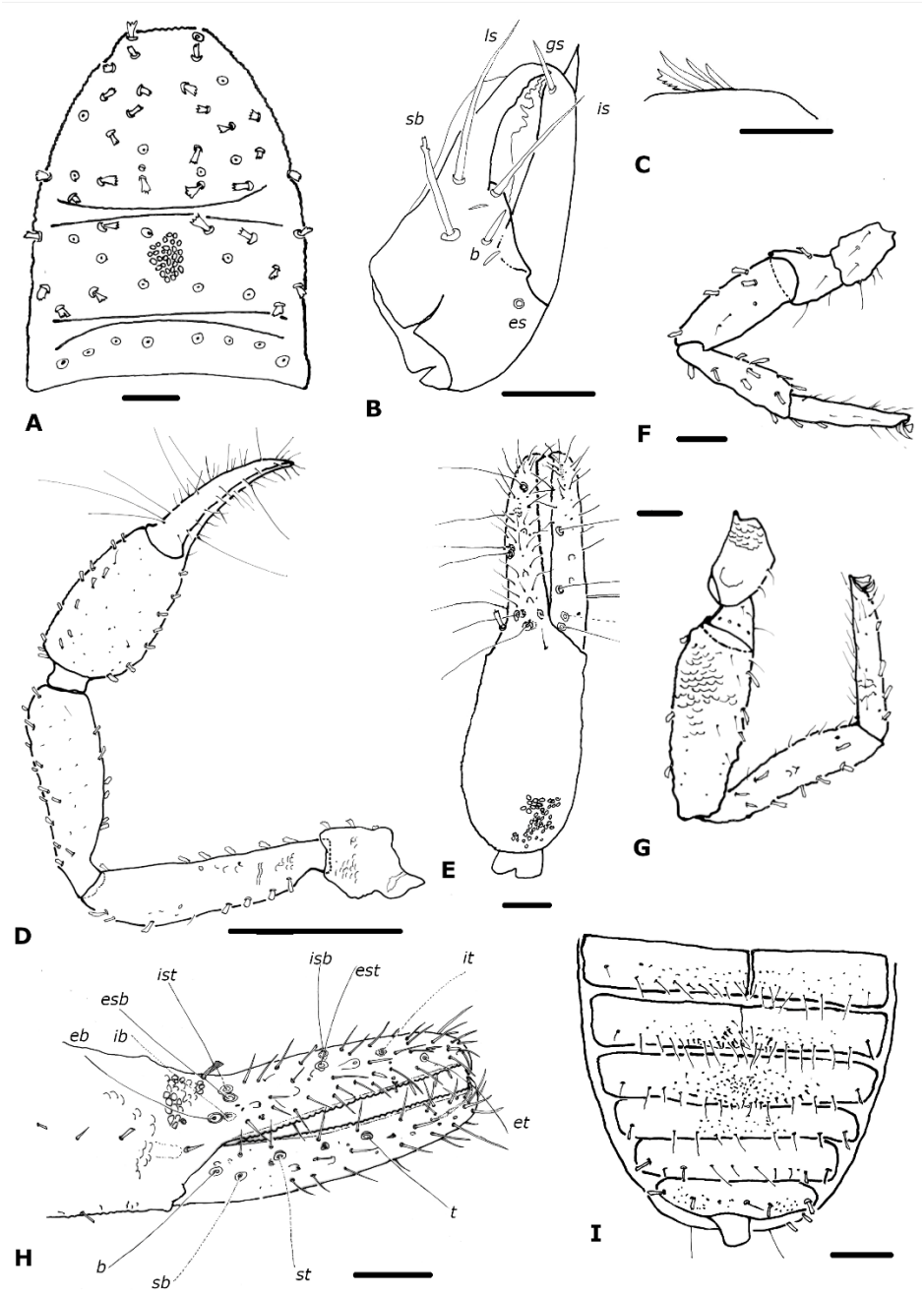

Figure 2.–*Paciwithius chimbilacus* sp. nov. Male holotype (ICN-Aps-076): A. Carapace; B. Chelicera; C. Rallum; D. Left pedipalp; E. Right chela; F. Left leg I; G. Left leg IV; H. Right chela fingers; I. Abdomen, sternites. Scale lines = 0.5 mm (Figure D); 0.1 mm (Figures A, E-I); 0.05 mm (Figure B); 0.0125 mm (Figure C).

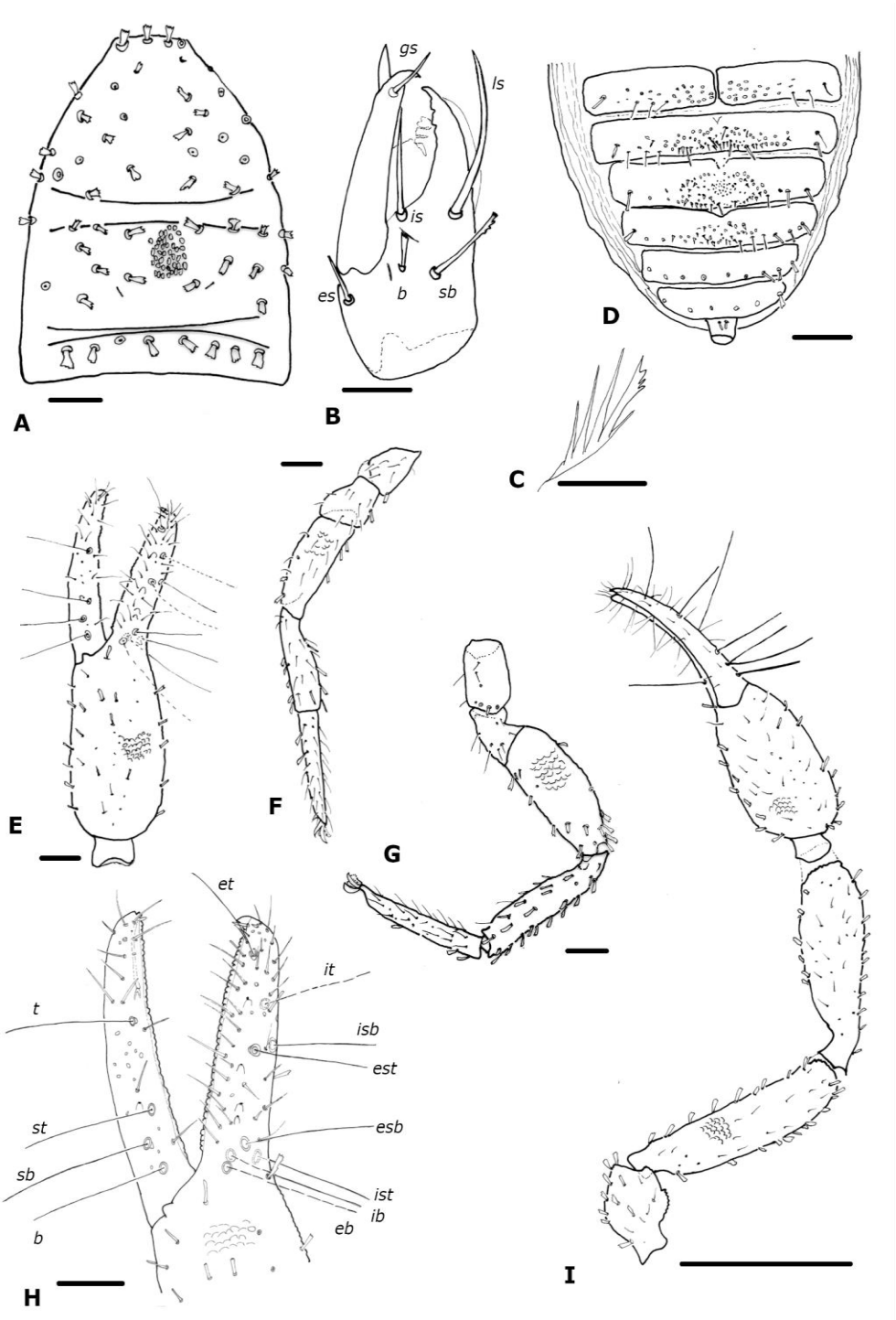

Figure 3.–*Paciwithius valduparensis* sp. nov. Male holotype (ICN-Aps-597): A. Carapace; B. Chelicera; C. Rallum; D. Abdomen, sternites; E. Right chela; F. Left leg I; G. Left leg IV;

H. Right chelal fingers; I. Left pedipalp. Scale lines = 0.5 mm (Figure I); 0.1 mm (Figures A, D-H); 0.05 mm (Figure B); 0.0125 mm (Figure C).

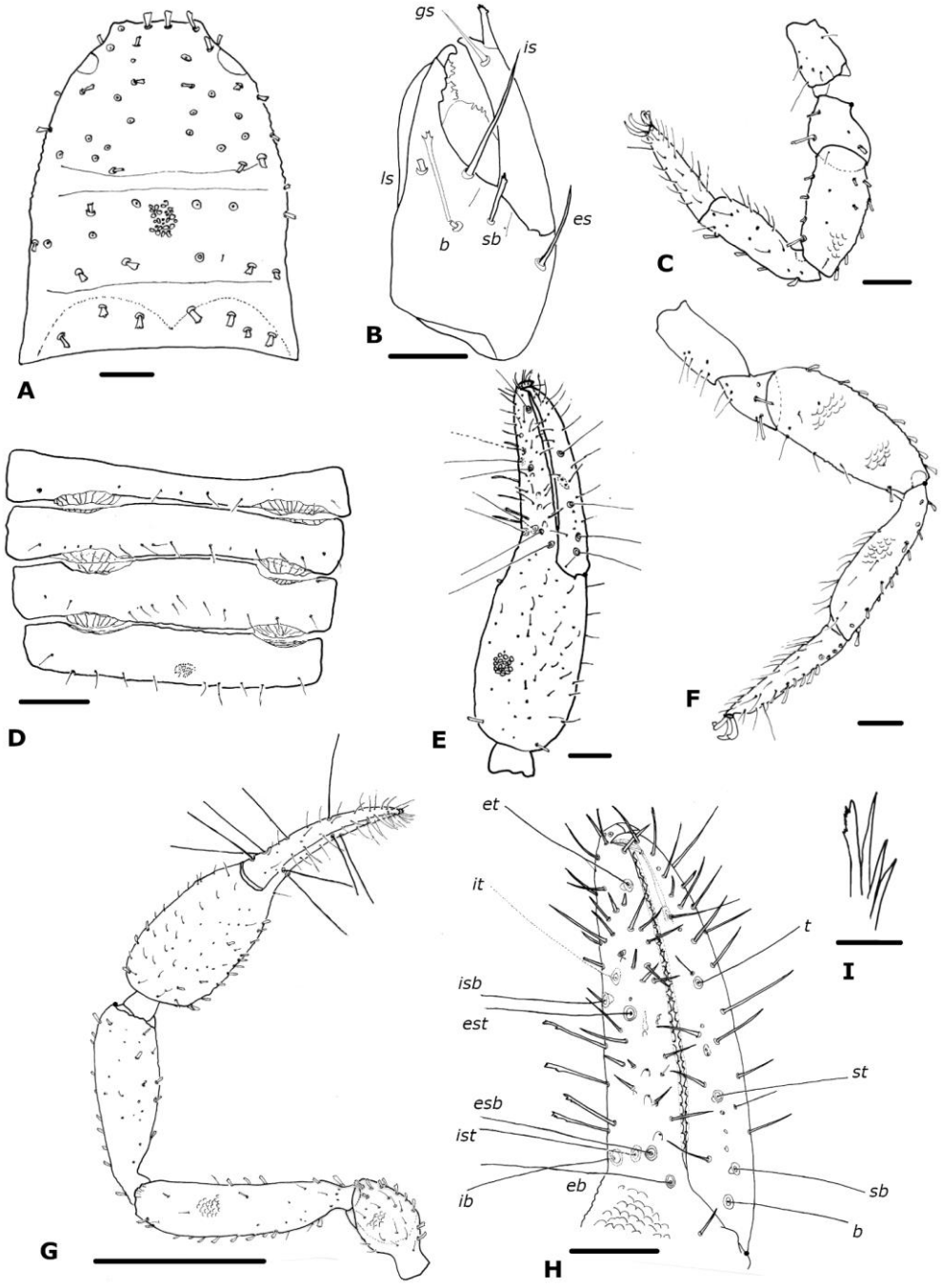

Figure 4.–*Cystowithius florezi* sp. nov. Male holotype (ICN-Aps-077): A. Carapace; B. Chelicera; C. Left leg I; D. Abdomen, sternites; E. Right chela; F. Left leg IV; G. Left

pedipalp; H. Right chelal fingers; I. Rallum. Scale lines = 0.5 mm (Figure G); 0.1 mm (Figures A, C-F, H); 0.05 mm (Figure B); 0.0125 mm (Figure I).

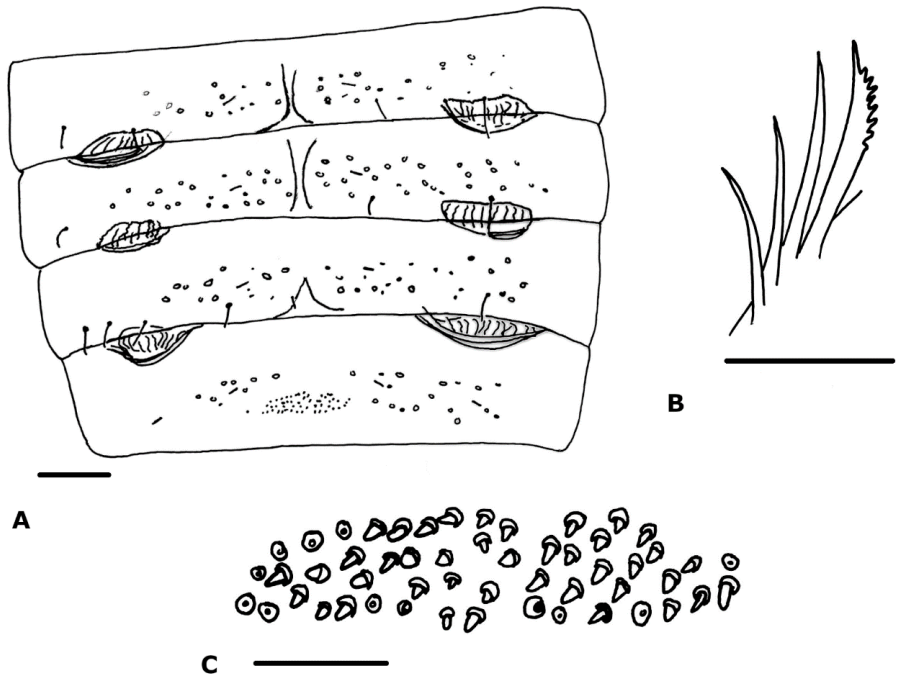

Figure 5.–*Cystowithius ankeri* García & Romero-Ortiz, 2021. Male (ICN-Aps-298): A. Abdomen, sternites; B. Rallum; C. Patch of glandular setae on sternite VIII. Scale lines = 0.1 mm (Figure A); 0.0125 mum (Figures B and C).

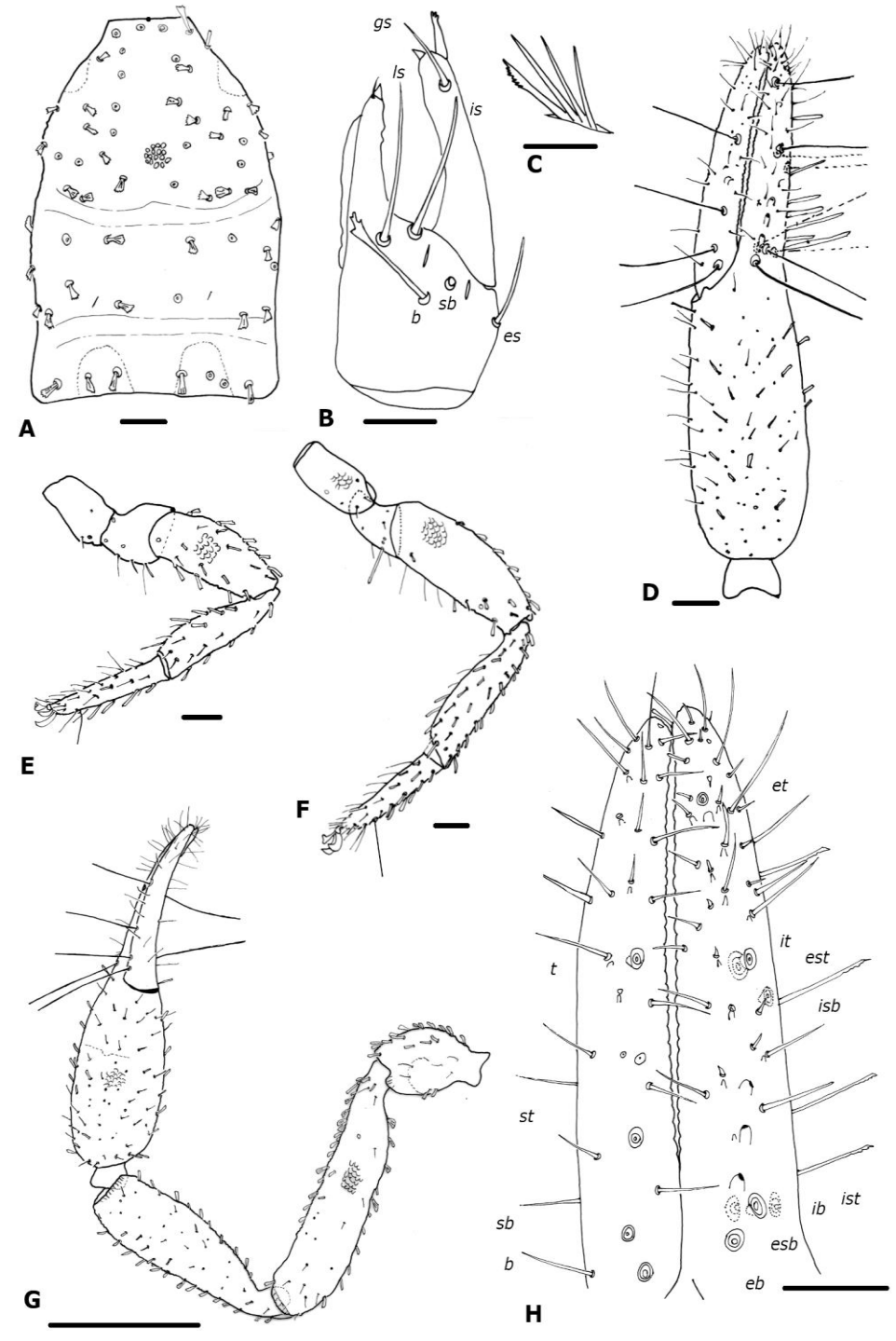

Figure 6.–*Parawithius bromelicola* sp. nov. Male holotype (ICN-Aps-082): A. Carapace; B. Chelicera; C. Rallum; D. Right chela; E. Left leg I; F. Left leg IV ; G. Left pedipalp; H. Right chelal fingers. Scale lines = 0.5 mm (Figure G); 0.1 mm (Figures A, C-F, H); 0.05 mm (Figure B); 0.0125 mm (Figure C).

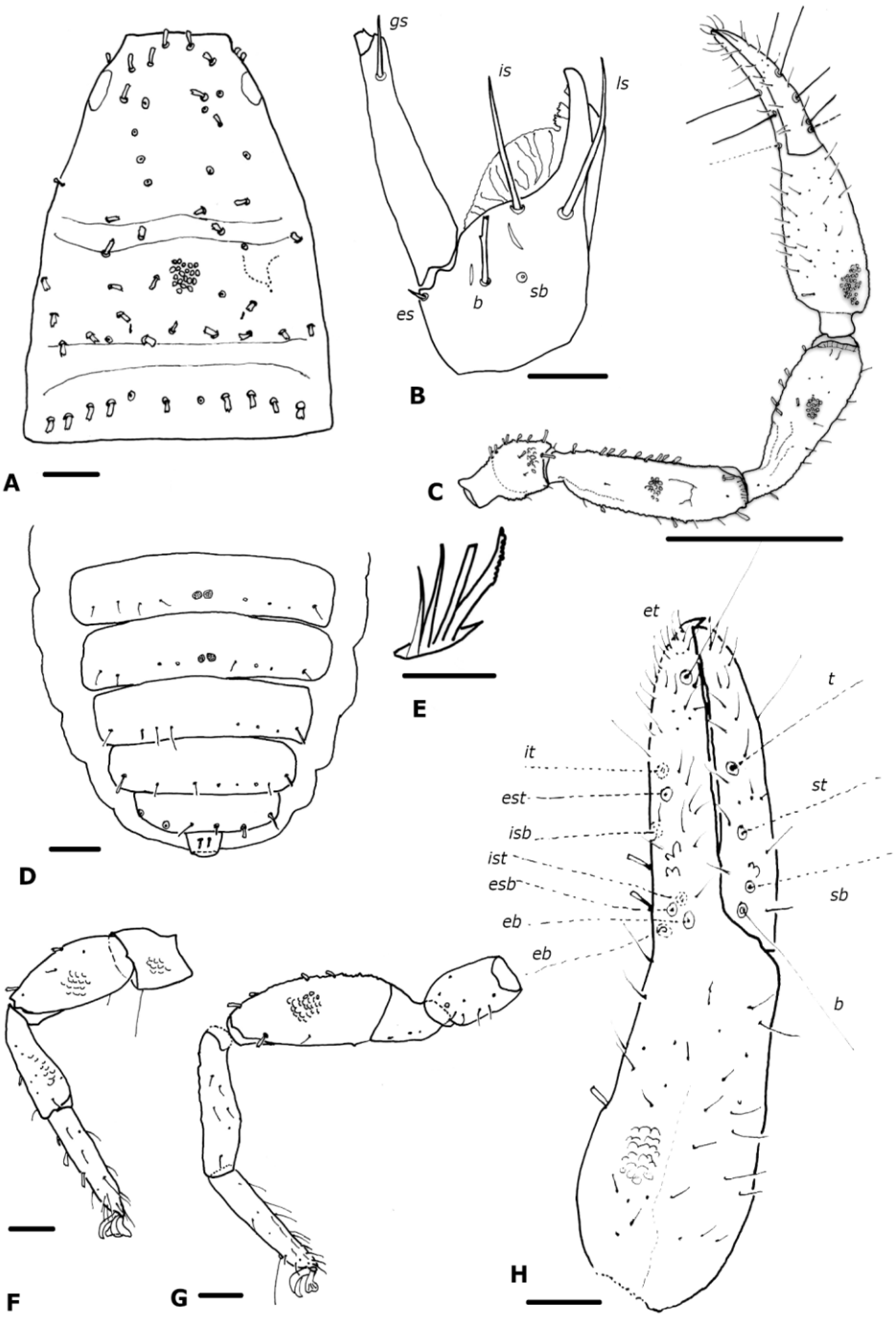

Figure 7.–*Oligowithius achagua* sp. nov. Male holotype (ICN-Aps-388): A. Carapace; B. Chelicera; C. Left pedipalp; D. Abdomen, sternites; E. Rallum; F. Left leg I; G. Left leg IV; H. Right chela. Scale lines = 8 um (Figures A, C, D, F-H); 2 um (Figures B, E). Granulation is shown in detail on each segment in a small section but is meant to cover all the structure.

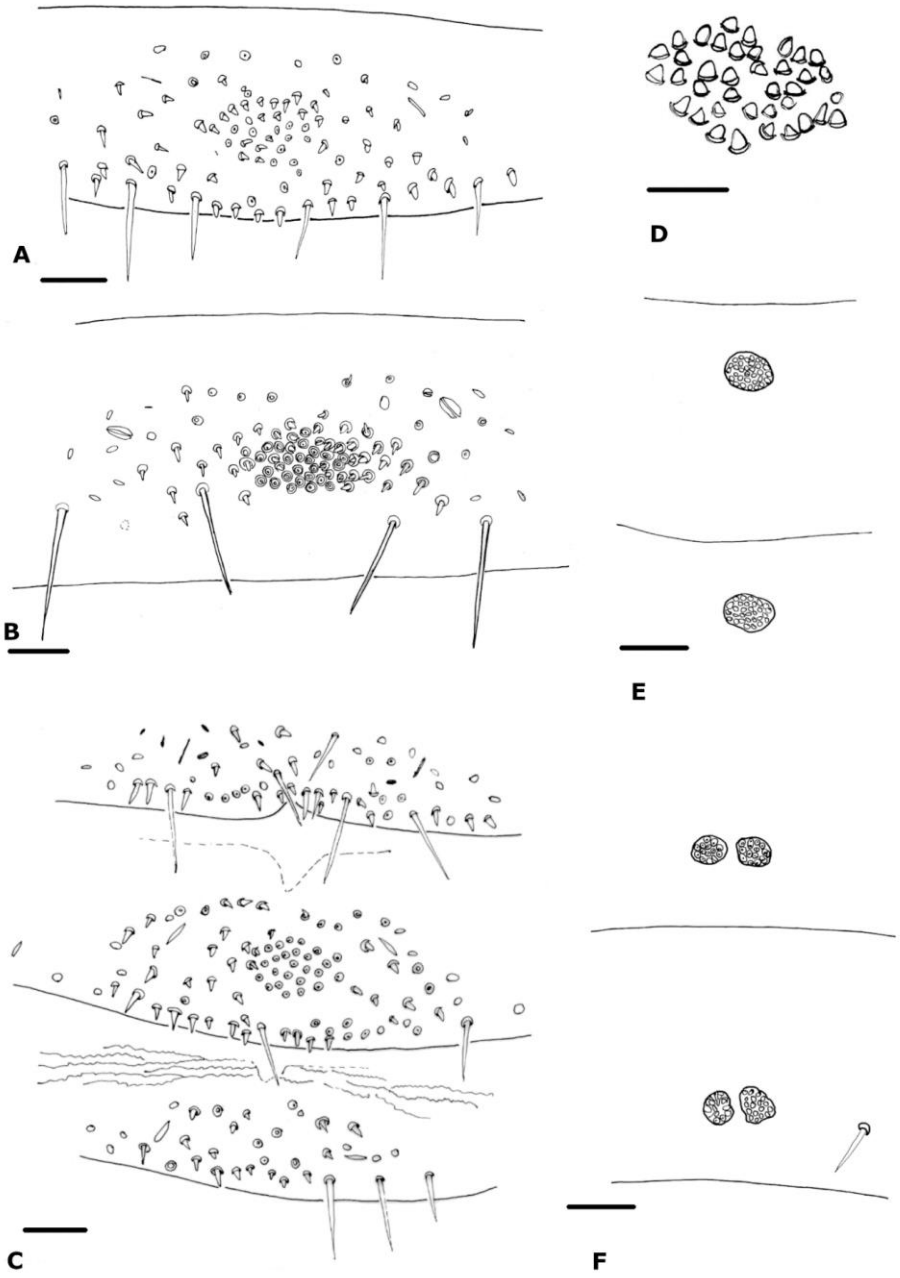

Figure 8.– Glandular setae patches of the new species A. *Cacodemonius chimbilacus* sp. nov. holotype (ICN-Aps-076); B. *Parawithius bromelicola* sp. nov. holotype (ICN-Aps-082); C. *Cacodemonius valduparensis* sp. nov. holotype (ICN-Aps-597); D. *Cystowithius florezi* sp. nov. holotype (ICN-Aps-077); E-F *Oligowithius achagua* sp. nov. E-Male paratype, F Male holotype (ICN-Aps-388). Scale lines = 0.1 mm (Figures A, C, D, E, F); 0.05 mm (Figure D).

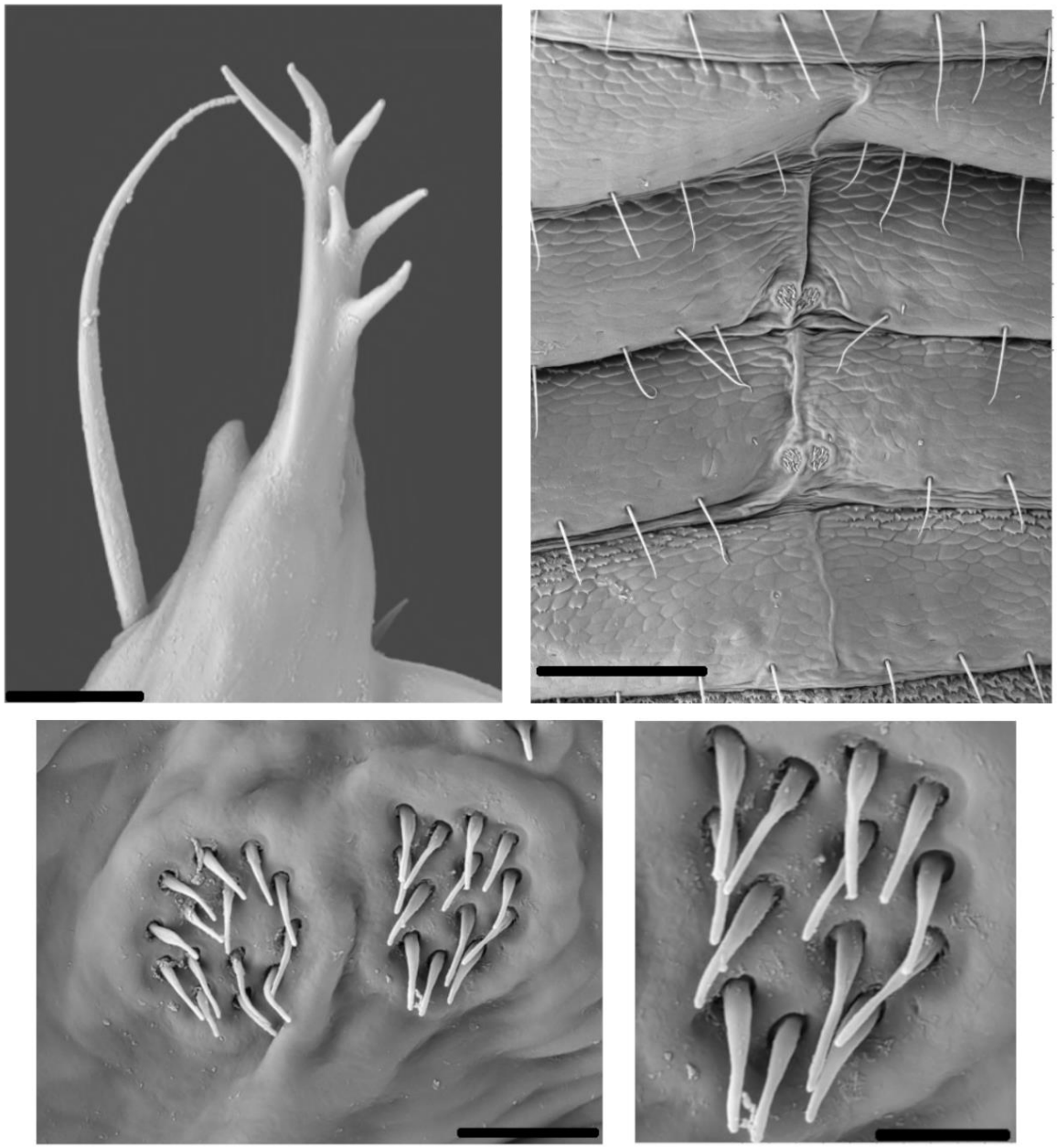

Figure 9.–*Oligowithius achagua* sp. nov. Male paratype (ICN-APs-388) A. Right galea, B. Sternites VII-VII, C. Patch of glandular setae on sternite VIII, D. Detail of the glandular setae on sternite VIII. Scale lines = A-B, 100 um; C, 10 um; D, 5 um.

Figure 10.–Male genitalia of *Oligowithius achagua* n. sp. holotype (ICN-Aps-388). Scale line = 100 um

3.2 The pseudoscorpion genus *Verrucachernes* (Pseudoscorpiones: Chernetidae) in the Indian region*

3.2.1 Abstract

The pseudoscorpion genus *Verrucachernes* Chamberlin, 1947 is widely distributed in the Old World tropics, with three named Australasian and West Pacific species and two from Africa. A review of some pseudoscorpions described from India has revealed that *Withius parvus* Beier, 1930 (currently in the genus *Metawithius*) and *Pselaphochernes indicus* Beier, 1974 are misplaced and actually belong to the genus *Verrucachernes* Chamberlin, 1947, forming the new combinations *V. parvus* (Beier, 1930) comb. nov. and *V. indicus* (Beier, 1974) comb. nov., respectively. Both species possess the single, large, rounded spermatheca and other features typical of *Verrucachernes*.

3.2.2 Introduction

Pseudoscorpiones, a mesodiverse arachnid order, is widely distributed around the world (Harvey 2013). The Indian fauna currently comprises 161 named species in 19 families, thus being the eighth country in pseudoscorpion diversity (e.g. Murthy & Ananthakrishnan 1977; Harvey 2013). However, recent research indicates that there are still many species yet to be described (Johnson et al. in press), and that some previously described species are poorly known.

Beier (1930) described *Withius parvus* from Travancore, southern India, from a female lodged in the Carl F. Roewer collection. Beier (1932) transferred the species to the genus *Metawithius* Chamberlin, 1931, a monotypic genus previously represented only by *M. murrayi* (Pocock, 1900) from Christmas Island. Although the genus *Metawithius* was reviewed by Harvey (2015b), he excluded *M. parvus* without explanation.

Recently, Johnson et al. (in press) described a new Indian species of the genus *Metawithius* and studied the type specimen of *M. parvus*, noting that it lacked the typical withiid characters. Most withiids can be distinguished from other pseudoscorpion families by the

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presence of a patch of glandular setae on the abdominal sternites, although these setae are lacking in the genera *Juxtachelifer* Hoff, 1956 from south-western U.S.A., *Protowithius* Beier, 1955 from the Juan Fernandez Islands, Chile and *Termitowithius* Muchmore, 1990 from east Africa (Harvey 2015a), and by the slightly oblique or perpendicular junction between the femora and patella of legs I and II (Harvey 1992). Most withiids have venom glands in both chelal fingers, although these are vestigial in *Termitowithius* (Muchmore 1990; Harvey 2015a). The holotype of *Metawithius parvus* was found to have strongly oblique junctions between the femora and patellae of the anterior legs, a fully developed venom duct only in the movable finger, and the sternites lacked any glandular setae. As these characteristics are consistent with members of the family Chernetidae, Johnson et al. (in press) provisionally excluded it from the Withiidae. The specimen also showed several similarities to *Pselaphochernes* indicus Beier, 1974, which was also described from southern India (Beier 1974), and our examination of the type material confirmed that it shared few of the features that define the genus *Pselaphochernes* Beier, 1932. The aim of the present paper is to provide redescriptions of *W. parvus* and *P. indicus*, and to assess their generic position.

3.2.3 Material and methods

The specimens examined for this study are lodged in the Muséum d'Histoire Naturelle de Genève, Switzerland (MHNG) and the Senckenberg Museum, Frankfurt, Germany (SMF). Specimens were studied by preparing temporary slide mounts by immersion in 75% lactic acid at room temperature for one to several days and mounting them on microscope slides with 10 mm coverslips supported by small sections of 0.25 mm or 0.50 mm diameter nylon fishing line. The holotype of *Withius parvus* was cleared by digestion in an enzyme solution, as described by (Álvarez-Padilla & Hormiga 2007). Specimens were examined with a Leica MZ16 dissecting microscope, and an Olympus BH–2 compound microscope, and illustrated with the aid of a drawing tube. Measurements were taken at the highest possible magnification using an ocular graticule, taken to the nearest 0.005 mm. After study the specimens were rinsed in water and returned to 75% ethanol, with the body and dissected portions placed in 12 × 3 mm glass genitalia microvials (BioQuip Products, Inc.).

Terminology and measurements largely follow Chamberlin (1931), with the exception of the nomenclature of the pedipalps, legs and with some minor modifications to the terminology

of the trichobothria (Harvey 1992), chelicera (Harvey & Edward 2007; Judson 2007) and faces of the appendages (Harvey et al. 2012).

Family Chernetidae Menge, 1855

Verrucachernes Chamberlin, 1947

Verrucachernes Chamberlin 1947: 312–313.
Microchernes Beier 1951: 91 (synonymised by Beier 1957: 40).

Type species: *Verrucachernes*: *Verrucachernes oca* Chamberlin, 1947, by original designation.

Microchernes: Microchernes orientalis Beier, 1951, by original designation.

Diagnosis: The genus *Verrucachernes* is the only known genus of Chernetidae with a single round spermatheca (Figs. 10, 18).

Remarks: The genus *Verrucachernes* is widely distributed in the tropics of the Old World, and currently comprises five species: V. oca from tropical Asia and islands in the western Pacific (Bhutan, Cambodia, China, Federated States of Micronesia, Guam, Indonesia, Marshall Islands, Nepal, Northern Mariana Islands; Papua New Guinea, Solomon Islands, Sri Lanka, Thailand, and Vietnam) (Harvey 2013), *V. montigenus* Beier, 1965 from Papua New Guinea (Beier 1965), *V. sublaevis* Beier, 1965 from the Indonesian province of West Papua (Beier 1965), *V. spinosus* Beier, 1979 from Côte d'Ivoire (Beier 1979), and *V. congicus* Beier, 1959 from the Democratic Republic of the Congo (Beier 1959). However, only V. oca has been well described, including the original description by Chamberlin (1947), a redescription by Harvey (1988), and two scanning electron micrographs by Schawaller (1994), using the name *Pselaphochernes indicus* Beier, 1974. The main diagnostic feature of *Verrucachernes* is the presence of a single, large, rounded spermathecae which is connected to the gonopore by a slender tube. This contrasts with the majority of other chernetids which have paired spermathecae (Chernetinae) or a single T-shaped spermatheca (Lamprochernetinae) (e.g. Muchmore 1975; Callaini 1986; Harvey 1995; Mahnert 2009; Harvey et al. 2012). Unfortunately, the spermathecae of *V. congicus*, *V. montigenus, V. sublaevis* and *V. spinosus* are unknown, and their inclusion in *Verrucachernes* must be considered to be provisional until their spermathecae are examined.

Our study of the type specimens of *Withius parvus* and *Pselaphochernes indicus* has demonstrated that they share the main features of *Verrucachernes*, and we transfer them to this genus and provide redescriptions of the type material.

The removal of P. indicus from *Pselaphochernes* makes better biogeographic sense, since all other species of the genus are restricted to cooler, temperate ecosystems of the Palaearctic and Nearctic (Harvey 2013). Indeed, the most easterly records include the widespread *P. scorpioides* (Hermann, 1804) from Uzbekistan (Redikorzev 1949, using the name *P. macrocheatus* Redikorzev, 1949) which was redescribed (Nassirkhani 2018), and *P. rybini* Scahwaller, 1986 from Kyrgyzstan (Schawaller 1986).

***Verrucachernes indicus* (Beier, 1974), comb. nov. (Figs. 1–10)**

Pselaphochernes indicus Beier 1974: 1011–1012, fig. 7.

Not *Pselaphochernes indicus* Beier: Schawaller 1991: 786, fig. 1 (misidentification; actually *V. oca* Chamberlin, 1947); Schawaller 1994: 754–755, figs 71–72 (misidentification; actually *V. oca* Chamberlin, 1947).

Type material: INDIA: Tamil Nadu: holotype male, 6 km E. of Coonoor, Nilgiri [11°21'N, 76°51'E], 1,600 m, “Fôret près d’une riviere”, 22 November 1972, C. Bésuchet, I. Löbl, collection number 42/77 (MHNG). Paratypes: allotype female, collected with holotype (MHNG). 5 males, 14 females, collected with holotype (MHNG); 1 male, 1 female, Coonoor [11°21’N, 76°48’E], 1,600 m, “Fôret dessous ville”, 22 November 1972, C. Besuchet, I. Löbl, collection number 43/83 (MHNG).

Diagnosis: *Verrucachernes* indicus differs from other species of the genus as follows: from V. oca by the coarsely granulate chela, which is smooth in *V. oca*; from *V. sublaevis* and *V. montigenus* in its small size, e.g. the pedipalpal femur of these two species exceeds 0.50 mm, whereas it reaches a maximum of 0.41 mm in V. indicus; from *V. parvus* by the cheliceral seta bs being acuminate (slightly denticulate in *P. parvus*); and from *V.*

congicus and *V. spinosus* by the shape of the pedipalpal femur which is tubular in V. indicus, but noticeably expanded in half basal part in *V. congicus* and *V. spinosus*.

Description (adults) (Figs. 1, 2): Colour: carapace and pedipalps pale red-brown, tergites and coxal region yellow-brown, and legs pale yellow-brown.

Carapace (Fig. 3): anterior section and lateral regions coarsely granulate, remainder smoother, with most of mesozone completely smooth; 1.02–1.35 (m#), 1.22–1.41 (f#) × longer than broad; without eyes or eye-spots; with 60 (m#), 66 (f#) setae, including 33 (m#), 32 (f#) setae in prozone including 6 near anterior margin, 16 (m#), 20 (f#) in mesozone and 11 (m#), 14 (f#) setae in metazone; with 2 furrows, anterior furrow deep, posterior furrow shallow, posterior furrow slightly closer to posterior margin than to anterior furrow; posterior margin slightly emarginate.

Chelicera (Fig. 4): with 5 setae on hand and 1 subdistal seta on movable finger; all setae acuminate; with 2 dorsal lyrifissures and 1 ventral lyrifissure; galea slender with ca. 5–6 rami; rallum (Fig. 5) of 3 blades, anterior blade with several distal serrations, other blades smooth; serrula exterior with 15 (m#, f#) blades; lamina exterior present.

Pedipalp (Fig. 6): trochanter, femur, patella and chelal hand coarsely granulate, chelal fingers mostly smooth; patella with several small sub-basal lyrifissures; all segments very robust, trochanter 1.80 (m#), 1.85 (f#), femur 2.69–2.92 (m#), 2.79–3.15 (f#), patella 2.28–2.51 (m#), 2.39–2.58 (f#), chela (with pedicel) 3.47–3.73 (m#), 3.50–3.76 (f#), chela (without pedicel) 3.24–3.44 (m#), 3.24–3.46 (f#), hand 1.71–1.84 (m#), 1.69–1.91 (f#) × longer than broad, movable finger 0.84–0.92 (m#), 0.80–0.97 (f#) × longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 7): eb and esb situated basally; est situated midway between esb and et; ib and ist situated sub-basally; it situated closer to isb than to tip of finger; isb midway between it and ist; st situated midway between sb and t, and sb situated much closer to b than to st. Fixed and movable fingers without pseudotactile setae. Venom apparatus present only in movable chelal finger, venom duct long, terminating in nodus ramosus slightly proximad to t. Most of chelal fingers teeth rounded, but distal teeth slightly pointed; fixed finger with 37 (m#), 40 (f#) teeth, plus 1 (m#, f#) subdistal prolateral accessory tooth; movable finger with 38 (m#), 42 (f#) teeth, without accessory tooth; fixed finger with 5 (m#, f#) retrolateral sense spots and movable finger with 1 (m#, f#) retrolateral sense spot.

Coxal region: maxillae smooth, except for granulate antero-lateral region; coxae smooth; manducatory process triangular, with 1 short apical and 1 long sub-apical acuminate setae, 1 small sub-oral seta, and 18 (m#), 24 (f#) additional setae; median maxillary lyrifissure rounded and situated submedially; posterior maxillary lyrifissure rounded. Chaetotaxy of coxae I–IV: m#: 11: 11: 18: 23; f#: 13: 15: 18: 29.

Legs (Fig. 8): junction between femora and patellae I and II strongly oblique to long axis; femoropatella of leg IV 3.38 (m#), 4.00 (f#) × longer than broad; patella and tibia III and IV without 'pseudotactile' setae (Fig. 7); tarsi III and IV with tactile seta (TS), situated slightly proximad of middle, TS = 0.36 (m#), 0.41 (f#); subterminal tarsal setae arcuate and acute; claws not modified; arolium about same length as claws.

Abdomen: tergites I–X and sternites IV–X with medial suture line. Tergal chaetotaxy: m#, 11: 10: 9: 14: 12: 13: 13: 16: 16: 13: 8 (including 4 tactile setae): 2; f#, 12: 12: 11: 12: 13: 16: 15: 17: 16: 16: 8 (including 4 tactile setae): 2; all setae clavate and dentate. Sternal chaetotaxy: m#, 18: (2) 6 [? + ?] (2): (2) 7 (2): 17: 18: 17: 18: 18: 14: 8 (including 2 tactile setae): 2; f#, 11: (2) 6 (2): (2) 6 (2): 14: 18: 18: 20: 17: 17: 8 (including 2 tactile setae): 2. Sternite II of f# with setae arranged in inverted U pattern (Fig. 9). Spiracles with helix. Pleural membrane stellate; without setae.

Genitalia: male: of typical chernetid morphology; female: with a single rounded spermatheca on a long thin duct (Fig. 10).

Dimensions (mm): holotype male, followed by 6 male paratypes in parentheses: Body length 1.27 (1.14–1.49). Carapace 0.450/0.390 (0.425–0.48/0.325–0.370). Chelicera 0.170/0.090, movable finger length 0.140. Pedipalps: trochanter 0.225/0.125, femur 0.350/0.120 (0.350–0.355/0.120–0.130), patella 0.345/0.145 (0.325–0.365/0.140-0.155), chela (with pedicel) 0.675/0.190 (0.660–0.700/0.180–0.195), chela (without pedicel) 0.620 (0.615–0.640), hand (without pedicel) length 0.335 (0.325–0.340), movable finger length 0.290 (0.285–0.310). Leg IV: femoropatella 0.270/0.080, tibia 0.210/0.060, tarsus 0.195/0.045, TS = 0.070.

Dimensions (mm): allotype female, followed by 15 female paratypes in parentheses: Body length 1.83 (1.42–1.82). Carapace 0.505/0.405 (0.460–0.505/0.335–0.415). Chelicera 0.175/0.095, movable finger length 0.140. Pedipalp: trochanter 0.250/0.135, femur 0.41/0.135 (0.305–0.410/0.120–0.140), patella 0.410/0.165 (0.340–0.415 /0.135–0.165),

chela (with pedicel) 0.800/0.220 (0.680–0.795/0.185–0.220), chela (without pedicel) 0.735 (0.640–0.735), hand (without pedicel) length 0.395 (0.340–0.410), movable finger length 0.350 (0.305–0.355). Leg IV: femoropatella 0.320/0.080 tibia 0.240/0.060, tarsus 0.195/0.045, TS = 0.080.

Remarks: *Pselaphochernes indicus* was described from numerous specimens collected from forest litter in southern India (Beier 1974), but the original description lacked important morphological details, most importantly the shape of the female spermatheca. This structure is of fundamental importance in chernetid taxonomy but is sadly lacking in many older descriptions. The spermatheca of *Pselaphochernes* consists of a single T-shaped receptaculum which is connected to the gonopore via a thickened tube (e.g. Vachon 1957; Callaini 1986). The T-shaped spermatheca has been used to define the subfamily Lamprochernetinae (Harvey 1995).

Pselaphochernes indicus lacks the T-shaped spermatheca that are characteristic of *Pselaphochernes*. Instead, it has a single rounded receptaculum that is only known from the genus *Verrucachernes* (Chamberlin 1947; Harvey 1988). We therefore transfer P. indicus to *Verrucachernes*.

Schawaller (1991, 1994) identified specimens from Nepal and Thailand as P. indicus but later treated them as *Verrucachernes* oca (Schawaller 1995), noting that P. indicus is likely to be a synonym of V. oca. We have found that Schawaller's suspicions were correct that P. indicus was a member of *Verrucachernes*, but there are sufficient morphological differences to retain them as distinct species.

Beier (1974) stated that the paratype vial from the type locality contained seven males, 17 females and one nymph, but we could only locate five males and 14 females, and the nymph was represented only by the abdomen with the remainder of the specimen absent. Likewise, the vial from Coonoor was stated by Beier (1974) contain one male, one female and one nymph, but only contained the adults when examined by us.

***Verrucachernes parvus* (Beier, 1930), comb. nov. (Figs. 11–18)**

Withius parvus Beier 1930: 293–294, fig. 5.
Metawithius parvus (Beier): Beier 1932: 201, fig. 206; Roewer 1937: 308.

Type material: INDIA: Kerala: holotype female, Travancore [ca. 8°30'N, 77°00'E] (SMF1940).

Diagnosis: *Verrucachernes parvus* differs from other species of the genus as follows: in general *V. parvus* is very small, e.g. the pedipalpal femur does not exceed 0.36 mm while in all other species it is larger than 0.41 mm; from V. oca by the coarsely granulate chela, which is smooth in V. oca, as well as its smaller size; from *V. sublaevis* and *V. montigenus* in its small size, e.g. the pedipalpal femur of these two species exceeds 0.50 mm, whereas it reaches 0.36 mm in *V. parvus;* from *V. indicus* by the cheliceral seta bs being slightly denticulate (acuminate in *P. indicus*); from *V. congicus* by the proportions of pedipalpal femur and patella which possess a slender femur and a stouter patella, e.g. femur 2.35 and patella 2.5 times longer than wide in *V. congicus* vs. femur 2.88 and patella 2.42 times longer than wide in *V. parvus*; and from *V. spinosus* by the shape of the pedipalpal femur which is slender in *V. parvus*, and stouter in *V. spinosus* e.g. 2.88 vs. 2.18 times longer than wide.

Description (adult female): Colour: carapace and pedipalps white-yellow, tergites and coxal region pale yellow, and legs white-yellow.

Carapace (Fig. 11): finely granulate; 1.43 × longer than broad; without eyes or eye-spots; with ca. 68 setae, including 38 in anterior section including 6 near anterior margin, 21 in median section and 9 setae in posterior section; with 2 furrows, just the anterior margin evident, posterior furrow weak, slightly closer to posterior margin than to anterior furrow; posterior margin straight.

Chelicera (Fig. 12): with 5 setae on hand and 1 subdistal seta on movable finger; seta bs slightly denticulate, and ls, is and es acuminate; with 2 dorsal lyrifissures and 1 ventral lyrifissure; galea with rami bifid at the base and bifid in one top; rallum of 3 blades, anterior blade with several distal serrations, other blades smooth; lamina exterior present.

Pedipalp (Fig. 13): trochanter, femur and patella coarsely granulate, chelal hand granulate on prolateral and retrolateral regions, nearly smooth elsewhere; patella with two small sub-basal lyrifissures; trochanter 1.66, femur 2.88, patella 2.42, chela (with pedicel) 3.62, chela (without pedicel) 4.43, hand 1.81 × longer than broad, movable finger 0.89 × longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 14): eb and esb situated basally; est situated midway between et and esb; ib and ist situated

basally; it situated closer to isb than to the tip of finger; isb slightly proximal to est; st situated much closer to t than to sb, and sb situated much closer to b than to st. Movable finger without pseudotactile setae. Venom apparatus only present in movable chelal finger, venom duct long, terminating in nodus ramosus near t. Chelal fingers teeth slightly blunt, basal teeth wider; fixed finger with ca. 52 teeth, without accessory teeth; movable finger with ca. 48 teeth, without accessory teeth; movable chelal finger with 2 retrolateral sense spots.

Coxal region: maxillae smooth, except for granulate anterior region; coxae smooth; manducatory process somewhat triangular, with 3 apical acuminate setae, 1 small sub-oral seta, and 18 additional setae; median maxillary lyrifissure rounded and situated submedially; posterior maxillary lyrifissure rounded. Chaetotaxy of coxae I–IV: 10: 13: 15: 28.

Legs (Figs. 15, 16): junction between femora and patellae I and II slightly oblique to long axis (Fig. 15); femur + patella of leg IV 4.06 × longer than broad; patella and tibia III and IV without ‘pseudotactile’ setae (Fig. 16); tarsi III and IV with tactile seta, situated proximal, TS = 0.12; claws not modified; arolium slightly shorter than claws.

Abdomen: tergites I–XI and sternites IV–XI with medial suture line. Tergal chaetotaxy: 9: 10: 10: 12: 13: 13: 13: 13: 15: 13: 6: 2; all slightly clavate. Sternal chaetotaxy: 12: (2) 6 (2): (2) 8 (2): 11: 16: 24: 18: 17: 14: 8 (including 2 short tactile setae): 2. Sternite II with setae arranged in inverted U pattern (Fig. 17). Pleural membrane finely longitudinally striate; without setae.

Genitalia: female: with a single rounded spermatheca on a long thin duct (Fig. 18).

Dimensions (mm): holotype female: Body length 1.48. Carapace 0.520/0.365. Chelicera 0.150/0.095, movable finger length 0.150. Pedipalp: trochanter 0.225/0.135, femur 0.360/0.125, patella 0.375/0.155, chela (with pedicel) 0.760/0.210, chela (without pedicel) 0.720, hand (without pedicel) length 0.380, movable finger length 0.340. Leg I: femur 0.095/0.060, patella 0.150/0.070, tibia 0.160/0.055, tarsus 0.180/0.035. Leg IV: femur + patella 0.325/0.080, tibia 0.215/0.060, tarsus 0.215/0.045, TS 0.120.

Remarks: Due to the age of the material and its preservation it was impossible to see the spermatheca using the standard clearing technique with lactic acid. We therefore cleared the specimen with an enzymatic digestion method (Álvarez-Padilla & Hormiga

2007). The lack of a venom gland in the fixed chelal finger and the presence of a single large ovoid spermathecal receptaculum indicates that this species is not a member of the Withiidae but should be placed in *Verrucachernes*.

3.2.4 Acknowledgements

We are very grateful to Peter Schwendinger (MHNG) and Peter Jäger (SMF) for access to specimens in their care, and to Erich Volschenk for his suggestions on the enzyme treatment of pseudoscorpion specimens. CRO acknowledges COLCIENCIAS and the Universidad Nacional de Colombia for support her through the “Convocatoria 727-Doctorados Nacionales” to allow her visit to the Western Australian Museum.

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3.2.6 Figures

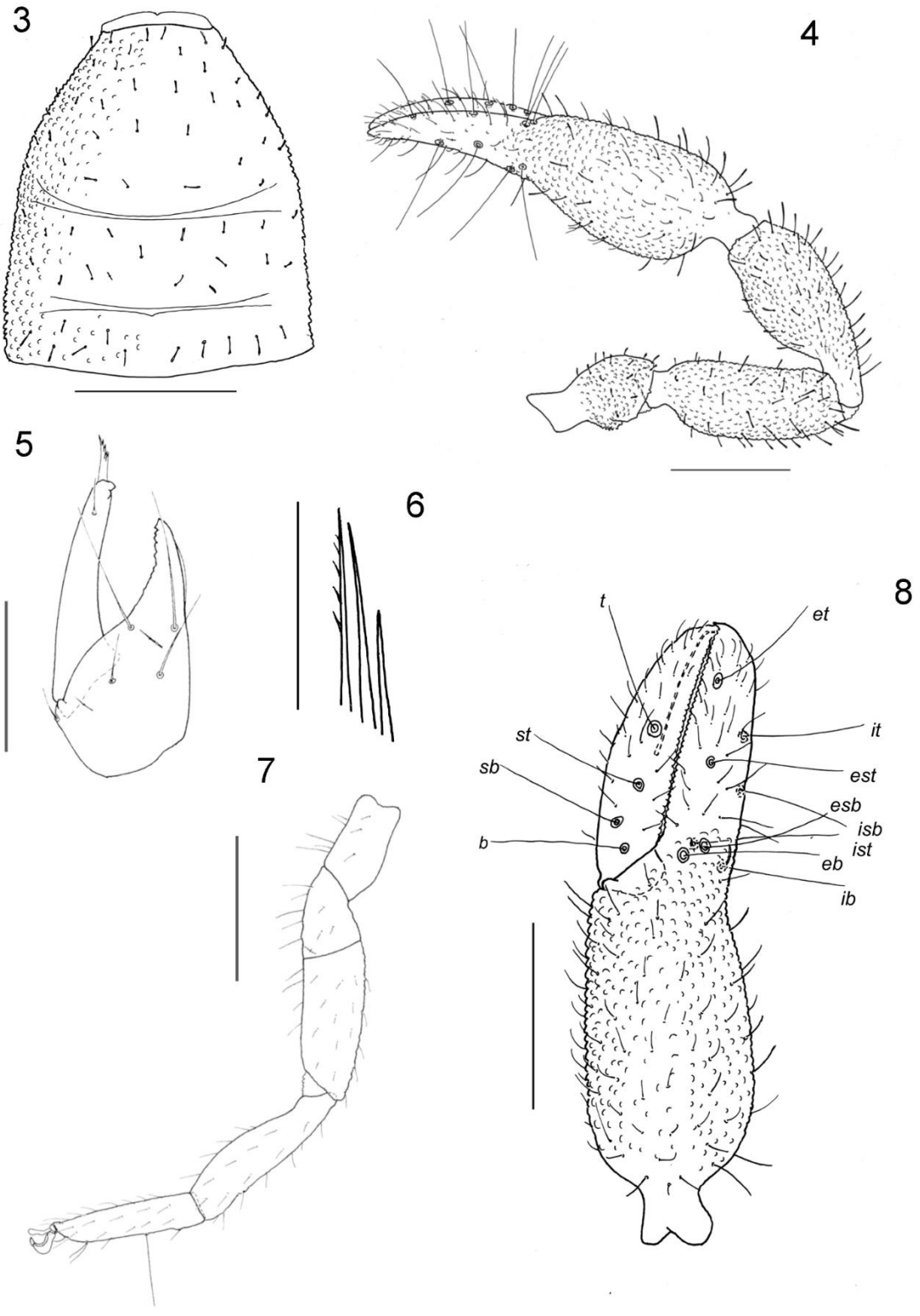
Figs. 1–2: *Verrucachernes indicus* (Beier, 1974), paratype male (MHNG): 1, dorsal view; 2, ventral view. Scale lines = 0.5 mm.

Figs. 3–8: *Verrucachernes indicus* (Beier, 1974), holotype male (MHNG), unless stated otherwise: 3, carapace; 4, right pedipalp, dorsal; 5, left chelicera, dorsal, allotype female; 6, left rallum, allotype female; 7, left leg IV, allotype female; 8, left chela, lateral. Scale lines: 0.2 mm (Figs. 3, 4, 7, 8); 0.1 mm (Fig. 5); 0.05 mm (Fig. 6).

Figs. 9–10: *Verrucachernes indicus* (Beier, 1974), allotype female (MHNG): 9, genital sternites, ventral; 10, spermatheca, ventral. Scale lines: 0.1 mm (Fig. 9); 0.05 mm (Fig. 10).

Figs. 11–16: *Verrucachernes parvus* (Beier, 1930), holotype female (SMF 1940): 11, carapace; 12, chelicera, dorsal; 13, right pedipalp, dorsal; 14, left leg I; 15, left leg IV; 16, left chela, lateral. Scale lines: 0.2 mm (Figs. 11, 13–16); 0.1 mm (Fig. 12).

Figs. 17–18: *Verrucachernes parvus* (Beier, 1930), holotype female (SMF 1940): 17, genital sternites, ventral; 18, spermatheca, ventral. Scale lines = 0.1 mm.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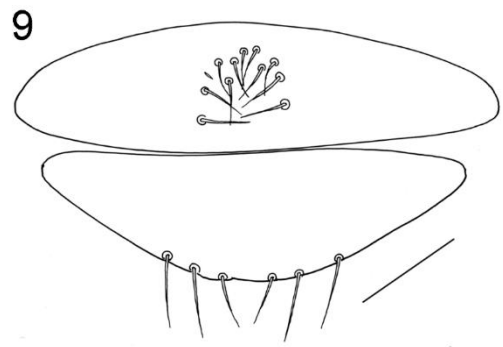
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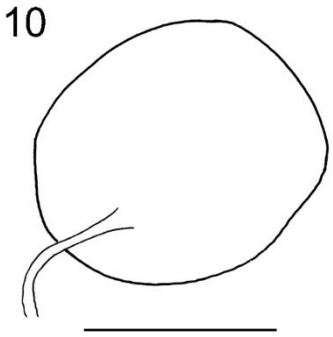
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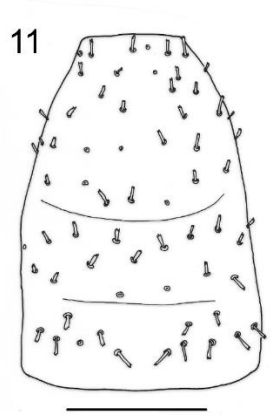
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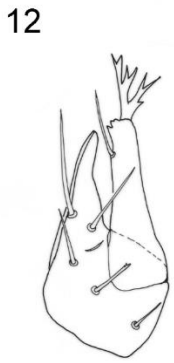
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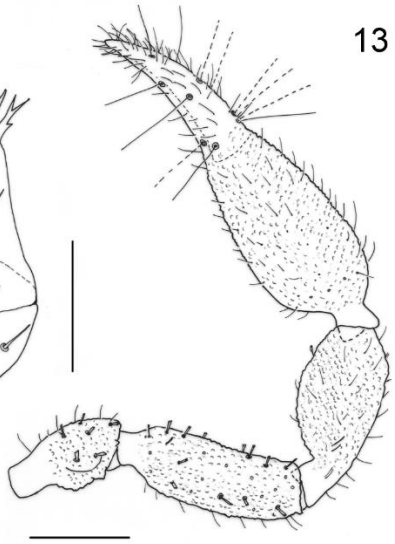
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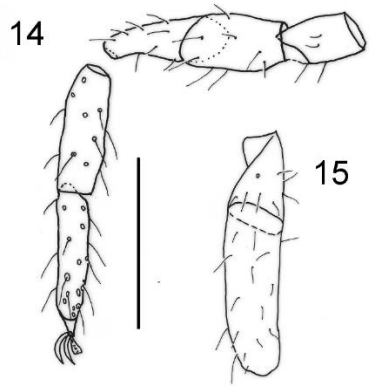
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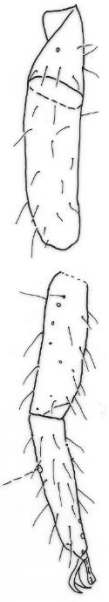
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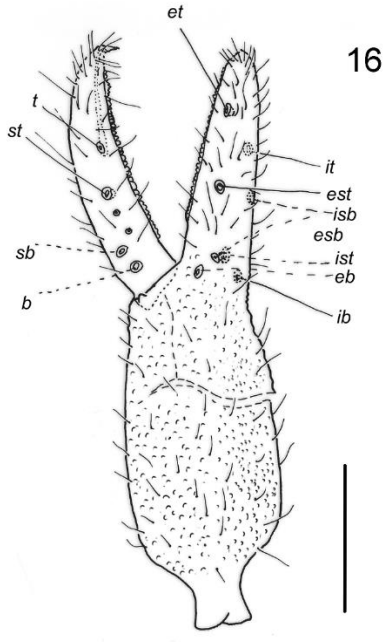
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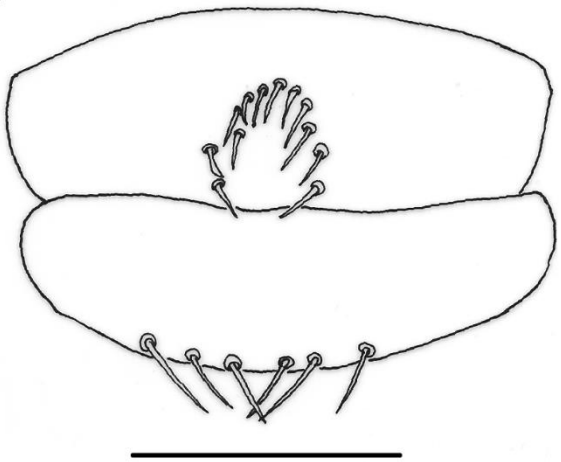

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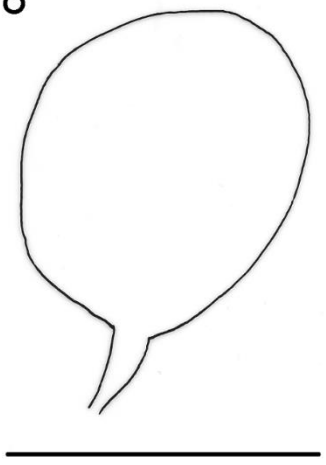

3.3 A review of the pseudoscorpion genus *Metawithius* (Pseudoscorpiones: Withiidae) from the Indian subcontinent*

As the second author of the paper, Catalina contributed to making the redescription of *Metawithius nepalensis* (Beier, 1974) including its corresponding figures.

3.3.1 Abstract

A new species of the pseudoscorpion genus *Metawithius* Chamberlin, 1931 is described from Kerala, India. Detailed morphological descriptions, diagnostic features and illustrations of *Metawithius keralensis* Johnson, Mathew, Sebastian & Joseph, sp. nov. are provided. Detailed redescription and illustrations of *M. nepalensis* (Beier, 1974) are also provided. The current distribution of all the known *Metawithius* species is mapped. *Metawithius parvus* (Beier, 1930) from Travancore, India is recognized as a species of Chernetidae.

3.3.2 Introduction

The arachnid order Pseudoscorpiones is a cosmopolitan group of small arthropods found in a variety of terrestrial habitats (Harvey 2013). There are 26 families currently recognized, including over 3,600 species in more than 440 genera (Harvey 2013). Withiidae is a nearly cosmopolitan family of pseudoscorpions with the highest diversity in the tropical and sub-tropical biotopes (Harvey 2015), with 170 recognized species in 37 genera. Although the family seems to represent a robust monophyletic group (Harvey 1992), the subfamilial and tribal classification may represent an artificial system that is in need of further research (Harvey 2015).

The genus *Metawithius* Chamberlin, 1931 is endemic to south-eastern Asia, with its distribution ranging from India and Nepal to the Indonesian archipelago (Harvey 2015). Chamberlin (1931a) erected this genus for *Chelifer murrayi* Pocock, 1900, collected from Christmas Island, Australia (Pocock 1900). *Metawithius* was later partially revised by

*Published: Johnson, J., Romero-Ortiz, C., Mathew, A.V., Sebastian, P.A., Joseph, M.M. and Harvey, M.S. 2019. A review of the pseudoscorpion genus *Metawithius* (Pseudoscorpiones: Withiidae) from the Indian subcontinent. *The Journal of Arachnology*, 47(1), pp.84-94. https://doi.org/10.1636/0161-8202-47.1.84

Harvey (2015), who redescribed and illustrated *M. murrayi* (Pocock, 1900), based on the type series and other material. The genus was primarily defined by the presence of a small patch of rugose cuticle on the internal surface of the maxilla of males and the sub-oral seta being born on a small protuberance.

Metawithius currently includes seven nominal species and two subspecies: *M. annamensis* (Redikorzev, 1938), *M. dawydoffi* (Beier, 1951), *M. murrayi*, *M. nepalensis* (Beier, 1974), *M. philippinus* Beier, 1937, *M. spiniventer* Redikorzev, 1938, *M. spiniventer spiniventer* Redikorzev, 1938, *M. spiniventer pauper* Beier, 1953, and *M. tonkinensis* (Beier, 1951) (Harvey 2015).

Although the Indian pseudoscorpion fauna is diverse, comprising 19 families and 153 nominal species (Harvey 2013), there are only two Indian representatives of *Metawithius*: *M. murrayi*, recorded from Nankovry and Car Nikobar of the Nicobar Islands chain (Harvey 2015), and *M. parvus* (Beier, 1930) from Travancore, India (Beier 1930). In this paper, we provide the description of a new *Metawithius* species collected from southern India and provide a redescription and new illustrations of *M. nepalensis* and recognize *M. parvus* as a species of Chernetidae.

3.3.3 Methods

The specimens used for this study are lodged in the following institutions: Senckenberg Museum, Frankfurt, Germany (SMF) and Division of Arachnology, Department of Zoology, Sacred Heart College, Thevara, Cochin, Kerala, India (ADSH). They were examined by preparing temporary slide mounts by immersing the specimens in 75% lactic acid at room temperature for several days and mounting them on microscope cavity slides with 18 mm coverslips. Small specimens were instead mounted in glycerol to avoid their being over-cleared. Permanent slide mounts were prepared, when necessary, by clearing the

specimens in clove oil and mounting them using Canada balsam. After study, the specimens were rinsed with distilled water and returned to 75% ethanol. The dissected portions such as leg, pedipalp, chelicera etc., were kept in microvials containing 75% ethanol. Terminology and mensuration follow Chamberlin (1931b), Harvey (1992, 2015), Harvey *et al.* (2012) and Judson (2007).

The specimens were examined using a Leica S8AP0 stereomicroscope and a Labovision AXL compound microscope (Kerala) or a Leica DM2500 compound microscope (Perth). Measurements are in millimeters (mm) and were taken with the aid of Leica DFC 295 digital camera mounted on Leica M205C stereomicroscope (at the highest possible magnification) using the measurement module of the software package Leica Application Suite (LAS), version 4.3.0 (Kerala) or using an ocular graticule (Perth). Microphotographs were taken using Leica DMC 2900 digital camera mounted on a Leica M205A stereomicroscope with the software package LAS, version 4.5.0. Scanning electron micrographs were taken using the Scanning Electron Microscope (JEOL Model JSM – 6390 LV) of the Sophisticated Test & Instrumentation Centre (STIC) facility of Cochin University of Science and Technology (CUSAT).

Abbreviations used: *b*—basal trichobothrium, *bs*—basal seta, *ca*—chitinized arch, *dd*—dorsal diverticulum, *eb*—exterior basal trichobothrium, *es*—exterior seta, *esb*—exterior sub-basal trichobothrium, *est*—exterior sub-terminal trichobothrium, *et*—exterior terminal trichobothrium, *gls*—glandular setae, *gs*—galeal seta, *ib*—interior basal, *is*—interior seta, *isb*—interior sub-basal trichobothrium, *ist*—interior sub-terminal trichobothrium, *it*—interior terminal trichobothrium, *la*—lateral apodeme, *lcp*—lateral cribriform plate, *ls*—laminal seta, *mcp*—median cribriform plate, *md*—median diverticulum, *ts*—tactile seta, *rc*—rugose cuticle, *sb*—sub-basal trichobothrium, *sbs*—sub-basal seta,

sos—sub-oral seta, *sp*—spermatheca, *ga*—genital atrium, *st*—sub-terminal trichobothrium, *t*—terminal trichobothrium.

3.3.4 Systematics

Family Withiidae Chamberlin, 1931

Subfamily Withiinae Chamberlin, 1931

Metawithius Chamberlin, 1931

Metawithius Chamberlin 1931a: 293.

Hyperwithius Beier 1951: 99–100. Synonymized by Harvey (2015).

Type species.—*Metawithius: Chelifer murrayi* Pocock, 1900, by original designation.

Hyperwithius: *Sundowithius annamensis* Redikorzev, 1938, by original designation.

Diagnosis & description.—See Harvey (2015).

Remarks.—With (1906) noted the occurrence of densely placed transverse lines/small patch of rugose cuticle in the internal surface of the male maxillae of *C. murrayi* (With 1906: plate III, fig. 8g). However, the significance of this was not noticed by subsequent authors (Beier 1930; Chamberlin 1931b). Later Harvey (2015) recognised its taxonomic importance and proposed it as a major feature for separating *Metawithius* from other withiid genera. The only pseudoscorpion genus with a similar feature is *Rugowithius* Harvey, 2015, where this patch is situated on the external surface of the male maxillae (Harvey 2015: figs 16–17). Another feature diagnostic for *Metawithius* is the excavated mesal margin of the male maxilla, where the sub-oral seta is born on a small protuberance (Harvey 2015: fig. 5; also this paper Figs 5–6).

Metawithius parvus (Beier, 1930) was described from a single female from Travancore, in the same region of southern India as *M. keralensis* (Beier 1930). The specimen is lodged in SMF and has been examined by CRO and MSH. It was found to belong to the family Chernetidae. A more detailed description will be presented in a forthcoming paper.

Distribution.—*Metawithius* species are currently known to occur throughout the Asian region, and have been recorded from Cambodia, China, Christmas Island, India, Indonesia, Malaysia, Myanmar, Nepal, Nicobar Islands, Philippines, Thailand and Vietnam (Harvey 2015; present records) (Figs 26, 42).

Metawithius keralensis Johnson, Mathew, Sebastian & Joseph, sp. nov.
http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:C3A4AD8F-4099-4A34-AE47-F96148FDF3DA

(Figs 1–26, 42)

Type material.—*Holotype male.* INDIA: *Kerala:* Alappuzha, Pathiramanal Island, 9°37'N, 76°23'E, 2 m, under bark of *Mangifera indica*, by hand, 30 January 2014, A. Joseliph (ADSH PS0001).

Paratypes. INDIA: *Kerala:* 3 males, 1 female, same data as holotype (ADSH PS0002), except 23 January 2014 (1 male) and 30 January 2014 (2 males, 1 female).

Other material examined.—INDIA: *Kerala*: 2 males, 1 female, Kottayam, Changanassery, Paippad, 9°25'34"N, 76°35'22"E, 20 m, under bark of *Hevea brasiliensis*, by hand, 22 September 2017, M.V. Aneesh (ADSH PS0003); 1 male, Pathanamthitta, Thiruvalla, Mundiyappally, 9°25'40"N, 76°37'16"E, 59 m, under bark of *Hevea brasiliensis*, by hand, 22 September 2017, M.V. Aneesh (ADSH PS0004); 1 male, Thiruvalla,

Kunnanthanam, 9°25'36"N, 76°35'44"E, 49 m, under bark of *Hevea brasiliensis*, by hand, 16 February 2017, M.V. Aneesh (ADSH PS0005); 4 males, 1 female, Alappuzha, Pathiramanal Island, 9°37'N, 76°23'E, 2 m, under bark of *Anacardium occidentale*, by hand, 17 January 2018, J. Johnson (ADSH PS0006); 2 males, 1 female, Ernakulam, Thrippunithura, Hill Palace area, 9°57'09"N, 76°21'49"E, 30 m, under bark of *Garcinia gummi-gutta*, by hand, 17 January 2018, J. Johnson (ADSH PS0007); 1 male, Thrissur, Irinjalakuda, Thumboor, 10°18'35"N, 76°15'42"E, 20 m, under bark of *Artocarpus heterophyllus*, by hand, 10 March 2018, J. Johnson (ADSH PS0008).

Diagnosis.—*Metawithius keralensis* sp. nov. appears to be most similar to *M. nepalensis* but differs in the reduced number of glandular setae on the male sternites (ca. 32: 39: 44: 46: 40 vs ca. 50: 71: 74: 64: 40 in *M. nepalensis*). The pedipalpal chela of *M. keralensis* is less slender than that of *M. nepalensis* [chela (with pedicel) 3.23–3.69 (♂), 2.83 (♀) and chela (without pedicel) 2.8–3.43 (♂), 2.73 (♀) x longer than broad, respectively], whereas in *M. nepalensis*, it is slender [chela (with pedicel) 3.75 (♂), 3.28 (♀) and chela (without pedicel) 3.51 (♂), 3.07 (♀) x times longer than broad, respectively]. The pedipalpal chela of *M. keralensis* has ca. 33 (♂), 32 (♀) teeth on the fixed finger and ca. 38 (♂), 34 (♀) teeth on the movable finger [whereas, it is ca. 32 (♂), 27 (♀) on the fixed and ca. 34 (♂), 28 (♀) on the movable finger of *M. nepalensis*]. Furthermore, the presence of only 16 (♂), 14 (♀) blades in the serrula exterior of *M. nepalensis* also distinguishes it from the new species [18 (♂), 19–20 (♀)].

Description.—Adults (Figs 1–4) with sclerotized portions generally dark reddish-brown in colour; dorsal setae mostly denticulate; setae on sternites acicular (Fig. 12).

Chelicera: movable finger with rasp-like ornamentation (Figs 10, 25). Hand with 5 setae, *sbs* slightly denticulate (with serrated edge), *bs*, *es*, *ls* and *is* smooth and acuminate;

movable finger with 1 sub-distal seta (*gs*); galea of ♂ with 2–3 small terminal rami and 2–4 sub-terminal rami, ♀ with 2–3 terminal and 3–4 sub-terminal rami; rallum of 4 blades, the most distal blade with a few serrations on anterior edge, other blades smooth and acuminate (Fig. 9); serrula exterior with 18 (♂), 19–20 (♀) blades; lamina exterior present.

Pedipalp: trochanter, femur and patella granulate, chela smooth; dorsal setae denticulate; trochanter with dorsal tubercle, 1.39–1.61 (♂), 1.71 (♀), femur 2.79–3.10 (♂), 2.79 (♀), patella 3.22–3.64 (♂), 2.80 (♀), chela (with pedicel) 3.23–3.69 (♂), 2.83 (♀), chela (without pedicel) 2.8–3.43 (♂), 2.73 (♀), hand 1.65–1.95(♂), 1.72 (♀) x longer than broad, movable finger 0.75–0.83 (♂), 0.65 (♀) x longer than hand**.** Male femur with unexpanded basal region (Fig. 20). Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 21): trichobothria *ib*, *ist*, *isb* and *it* grouped in basal half of finger; *b* and *sb* situated near one another; *st* slightly closer to *sb* than to *t*. Venom apparatus present in both chelal fingers, venom ducts long, nodus ramosus slightly basal to *et* in fixed finger, proximal to *t* in movable finger. External margin of fixed finger with 3–6 sense-spots situated midway between *esb* and *est*; external margin of movable finger with 2–3 sense-spots situated between *sb* and *st* (Fig. 21). Chelal teeth rounded; fixed finger with ca. 33 (♂), 32 (♀) teeth; movable finger with 38 (♂), 34 (♀) teeth; accessory teeth absent.

Carapace: 1.13–1.19 (♂), 1.43 (♀) x longer than broad; lateral margins convex, not posteriorly widened, with 2 non-corneate eyes, with ca. 37–41 (♂), 45–60 (♀) number of setae, including 6 (♂, ♀) near anterior margin and 6–8 (♂), 8 (♀) near posterior margin; with 2 furrows, distinct anterior and indistinct posterior (Fig. 7), posterior furrow slightly closer to posterior carapaceal margin than anterior furrow; carapaceal metazone without any paired pale spots.

Coxal region: maxilla with 2 apical setae and 1 very small internal, sub-oral seta; patch of rugose cuticle/parallel ridges (ca. 6–7 in number) anterior to median maxillary lyrifissure of males (Figs 5–6); sub-oral seta of male maxilla on a 'hooked' mound (Figs 5–6). Coxal chaetotaxy: ♂, 9–13: 9–14: 8–16: 14–31; ♀, 9–17: 9–18: 10–15: 22–35.

Legs: yellowish; monotarsate; junction between femora and patellae I and II only slightly oblique (Fig. 19); femur + patella of leg IV 2.69–2.91 (♂), 3.19 (♀) x longer than deep; tarsal tactile seta of leg IV situated medially or sub-medially; 0.68–0.78 (♂), 0.63–0.83 (♀) of tarsus length (Fig. 18); sub-terminal tarsal setae arcuate and acute; arolium slightly shorter than claws.

Abdomen: tergites and sternites with faint medial suture. Tergal chaetotaxy: ♂, 8: 8–9: 10: 11–12: 10–13: 12–13: 11–14: 13: 10–11: 10: 10 (including two tactile setae): 2, ♀, 8–10: 8–9: 10–12: 10–12: 10–13: 12–13: 10–12: 11–13: 11: 10: 10 (including two tactile setae): 2; setae uniseriate with a few setae placed anteriorly; all setae foliate (Fig. 8) (except tergites XI–XII with denticulate). Sternal chaetotaxy: ♂, 9: 8–9: 9: 12–15 + ca. 16/16 *gls*: 15 + ca. 22/17 *gls*: 15 + ca. 25/19 *gls*: 13–15 + ca. 23/23 *gls*: 13–15 + ca. 22/18 *gls*: 11 (including two tactile setae): 11–12 (including four tactile setae): 2, ♀, 8–17: (3) 8–10 (3): (3) 10 (3): 15–19: 19–20: 19–21: 16–19 + 2 *gls*: 18–19 + 2 *gls*: 12 (including two tactile setae): 11–14 (including four tactile setae): 2; sternal setae mostly uniseriate and acicular (Fig. 12), becoming progressively denticulate on posterior sternites X–XII (except for the tactile setae); sternites V–IX of male with patches of glandular setae (Figs 11, 22); sternites VIII–IX of female with 1 pair of glandular setae; glandular setae ca. 0.02 (♂), 0.03 (♀) in length, stout and conical in males (♂) (Fig. 14, 23–24), slender in female (♀) (Fig. 15); male without paired invaginations on anterior margins of sternites.

Genitalia: males with elongated and posteriorly tapering lateral apodemes (Fig. 17). Females with a circular genital atrium and 2 long, tubular, slightly coiled spermathecae (Fig. 16), with sclerotized lateral apodemes, with a single median and paired lateral cribriform plates.

Dimensions: Males: holotype followed by 3 paratypes (where applicable): body length 2.19 (2.30–2.56). Pedipalps: trochanter 0.33/0.21 (0.28–0.36/0.21–0.22), femur 0.64/0.20 (0.61–0.66/0.20–0.22), patella 0.73/0.20 (0.67–0.74/0.18–0.22), chela (with pedicel) 1/0.33 (1.13/0.39), chela (without pedicel) 0.92 (0.87–0.95), hand length 0.55 (0.50–0.58), movable finger length 0.45 (0.39–0.46). Chelicera 0.20/0.10 (0.19/0.23), movable finger length 0.17 (0.16–0.19). Carapace 0.67/0.56 (0.65–0.72/0.56–0.64); eye diameter 0.07 (0.06–0.08). Leg I: femur 0.12/0.11 (0.11–0.16/0.11–0.13), patella 0.29/0.11 (0.28–0.30/0.11–0.13), tibia 0.26/0.07 (0.25–0.27/0.07–0.12), tarsus 0.19/0.04 (0.17–0.25/0.04–0.05). Leg IV: femur + patella 0.51/0.17 (0.45–0.57/0.17–0.20), tibia 0.40/0.10 (0.37–0.42/0.09–0.11), tarsus 0.25/0.06 (0.24–0.32/0.06–0.06), TS 0.44 (0.44–0.49).

Females: Paratype (ADSH PS0002) followed by 1 other paratype (where applicable): body length 2.93 (2.52). Pedipalps: trochanter 0.37/0.22 (0.28**/**0.17), femur 0.64/0.23 (0.42/0.19), patella 0.69/0.24 (0.42/0.11), chela (with pedicel) 1.13/0.39, chela (without pedicel) 1.06, hand length 0.68, movable finger length 0.44. Chelicera 0.22/0.14, movable finger length 0.20. Carapace 0.81/0.57; eye diameter 0.07. Leg I: femur 0.56/0.14, patella 0.30/0.13, tibia 0.27/0.08, tarsus 0.23/0.05. Leg IV: femur + patella 0.59/0.18, tibia 0.33/0.11, tarsus 0.23/0.06 (0.32/0.08), TS 0.49 (0.44–0.51).

Remarks.—The new species can be differentiated from all other *Metawithius* species by the following combination of characters: morphometry, shape of the chela, chelal dentition and glandular setae on the sternites. *Metawithius keralensis* resembles *M.*

nepalensis in having glandular setae on the male sternites V–IX, but differs in the reduced number of glandular setae (ca. 32: 39: 44: 46: 40 in the new species vs ca. 50: 71: 74: 64: 40 in *M. nepalensis*). The males of *M. keralensis* also differs from *M. murrayi*, *M. annamensis* and *M. spiniventer* in the presence of glandular setae on the sternites V–IX (whereas, it is on the sternites V–X, IV–XI and IV–X in the above-mentioned three species respectively). It further differs from *M. murrayi* in the number of coils in the female genital atria (2 coils each in *M. keralensis* vs 1 in *M. murrayi*) and the shape of the male genitalia. The new species differs from *M. annamensis*, *M. dawydoffi* and *M. tonkinensis* in the shape of the pedipalpal femur, which have a slightly expanded basal region, which is lacking in *M. keralensis*. It differs from *M. philippinus* in the shape of the chelal hand, which is more slender in *M. keralensis* and the pedipalpal tibia is longer in *M. keralensis*.

Distribution.—*Metawithius keralensis* has been collected from a variety of localities in the southern Indian state of Kerala: Alappuzha, Ernakulam, Kottayam, Pathanamthitta and Thrissur districts (Figs 26, 42).

Etymology.—This species is named after the Indian state of Kerala, from where the species has so far been reported.

Metawithius **nepalensis (Beier, 1974)**

(Figs 27–42)

Withius nepalensis Beier 1974:277–278, fig. 11.

Metawithius nepalensis (Beier): Harvey 2015:358–359.

Type material.—*Holotype male*. NEPAL: *Central*: Daman, Mahabarat region [27°41'N, 85°07'E], 2500 m, under bark of *Rhododendron arboreum*, February 1970, J. Martens (SMF 28969).

Allotype. NEPAL: *Central*: female, collected with holotype (SMF 28969).

Description.—Adults (Figs 27–30): *Colour*: with sclerotized portions generally light yellow-brown; carapaceal metazone without paired pale spots.

Chelicera (Fig. 32): with 5 setae on hand, *sbs* denticulate, all others including *bs* acuminate; movable finger with 1 subdistal seta; galea of male with 3 very small terminal rami, of female with 2 sub-terminal and 3 terminal rami; rallum of 4 blades, the most distal blade with several serrations on leading edge, other blades smooth (Fig. 33); serrula exterior with 16 (♂), 14 (♀) blades; lamina exterior present; 2 lyrifissures on dorsal and 1 on ventral side.

Pedipalp (Fig. 34): trochanter, femur and patella granulate, chela smooth; dorsal setae clavate and denticulate; trochanter 2.00 (♂), 1.79 (♀), femur 3.06 (♂), 3.15 (♀), patella 3.26 (♂), 2.92 (♀), chela (with pedicel) 3.75 (♂), 3.28 (♀), chela (without pedicel) 3.51 (♂), 3.07 (♀), hand 2.11 (♂), 1.81 (♀) x longer than broad, movable finger 0.74 (♂), 0.76 (♀) x longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria, although only 3 in the left chela of ♂ (Figs 37–38): *eb* and *esb* situated basally; *ib*, *ist*, *isb* and *it* grouped in basal half of finger; *b* and *sb* situated near one another; *st* slightly closer to *sb* than to *t*. Venom apparatus present in both chelal fingers, venom ducts not visible in ♂; nodus ramosus distal to *t* on movable finger, distal to *est* on fixed finger in ♀. External margin of fixed finger with 6 sense-spots situated midway between *esb* and *est*. Chelal teeth rounded; fixed finger with 32 (♂), 27 (♀) teeth; movable finger with 34 (♂), 28 (♀) teeth; accessory teeth absent.

Carapace (Fig. 31): 1.15 (♂), 1.27 (♀) x longer than broad; lateral margins convex, not posteriorly widened; with 2 non-corneate eyes; with ca. 40 (♂), 44 (♀) setae, including 4 (♂), 6 (♀) near anterior margin and 6 (♂, ♀) near posterior margin; with 2 distinct furrows; posterior furrow slightly closer to posterior carapaceal margin than to median furrow (Fig. 31).

Coxal region: coxal chaetotaxy: ♂, 9: 7: 9: 18, ♀, 7: 9: 8: 19; maxilla with 4 apical setae and 1 very small internal, sub-oral seta (Fig. 39); interno-median region of male maxilla with rugose area located anterior to the median maxillary lyrifissure; sub-oral seta of male maxilla on 'hooked' mound (Fig. 39).

Legs: junction between femora and patellae I and II only slightly oblique; femur + patella of leg IV 3.06 (♂), ? (♀) x longer than deep; tarsal tactile seta of leg IV situated medially, 0.54 (♂), 0.51 (♀) of tarsus length (Fig. 35–36); subterminal tarsal setae arcuate and acute; arolium slightly shorter than claws.

Abdomen: tergites and sternites with faint medial suture. Tergal chaetotaxy: ♂, 6: 10: 8: 10: 10: 12: 10: 12: 10: 10: 8 (including 2 tactile setae): 2; ♀, 10: 10: 12: 11: 12: 14: 12: 14: 14: 11: 10 (including 2 tactile setae): 2; mostly uniseriate but some tergites with a few setae placed anteriorly; all setae foliate. Sternal chaetotaxy: ♂, 10: (0) 6 (0): (1) 10 (1): 17 + 26/24 gls: 13 + 35/36 gls: 12 + 40/34 gls: 14 + 32/32 gls: 9 + 23/17 gls: 10 : 10 (including 4 tactile setae): 2; ♀, 16: (0) 12 (0): (1) 12 (1): 19: 19: 17: 16 + 1/1 gls: 14 + 1/1 gls: 14: 10: 2; sternites V–IX of ♂ with patches of glandular setae (Fig. 41); glandular setae ca. 21 µm in length, stout and conical (Fig. 40); sternites VIII–IX of female with 1 pair of glandular setae; setae uniseriate and acuminate; ♂ without paired invaginations on anterior margins of sternites.

Genitalia: male with elongated lateral apodemes, although other structures not visible; female genitalia not visible.

Dimensions: Male: holotype: body length 1.64. Pedipalps: trochanter 0.290/0.145, femur 0.505/0.165, patella 0.570/0.175, chela (with pedicel) 0.880/0.235, chela (without pedicel) 0.825, hand length 0.370, movable finger length 0.495. Chelicera 0.195/0.120, movable finger length 0.165. Carapace 0.625/0.545 (width at medial area); eye diameter 0.075. Leg I: femur 0.135/0.105, patella 0.255/0.120, tibia 0.285/0.075, tarsus 0.295/0.050. Leg IV: femur + patella 0.505/0.165, tibia 0.410/0.085, tarsus 0.360/0.060, TS 0.195.

Female: Allotype: body length 1.78. Pedipalps: trochanter 0.295/0.165, femur 0.535/0.170, patella 0.570/0.195, chela (with pedicel) 0.935/0.285, chela (without pedicel) 0.875, hand length 0.515, movable finger length 0.390. Chelicera 0.275/0.130, movable finger length 0.175. Carapace 0.695/0.545 (width at medial area); eye diameter 0.095. Leg I: femur 0.130/0.120, patella 0.270/0.110, tibia 0.285/0.075, tarsus 0.350/0.055. Leg IV: femur + patella ?/0.165, tibia 0.425/0.085, tarsus 0.365/0.065, TS 0.185.

Distribution.—*Metawithius nepalensis* is currently known only from the type locality in Nepal (Fig. 42).

3.3.5 Acknowledgments

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3.3.7 Figures

Figures 1–6.–*Metawithius keralensis* sp. nov., holotype male (ADSH PS0001), unless otherwise stated: 1. Habitus, dorsal; 2. Habitus, ventral; 3. Female habitus, dorsal (ADSH PS0002); 4. Female habitus, ventral (ADSH PS0002); 5. Maxillae, ventral; 6. Maxillae, ventral view, illustrating rugose cuticle and sub-oral setae, ventral view. Scale bars: 1 mm (Figs 1–4); 0.1 mm (Figs 5–6).

Figures 7–21.–*Metawithius keralensis* sp. nov., holotype male (ADSH PS0001), unless otherwise stated: 7. Carapace, dorsal; 8. Foliate seta; 9. Rallum, paratype male (ADSH PS0005); 10. Right chelicera, dorsal, paratype male (ADSH PS0005); 11. Sternites IV–XII; 12. Acicular seta; 13. Glandular setae on sternite V; 14. Glandular seta; 15. Glandular seta, paratype female (ADSH PS0005); 16. Female genitalia, ventral (ADSH PS0002); 17. Genitalia, ventral, paratype male (ADSH PS0005); 18. Right leg IV (paratype male); 19. Right leg I (trochanter omitted; paratype male); 20. Right pedipalp, dorsal; 21. Right chela, retrolateral. Scale bars: 0.2 mm (Figs 7, 11, 18–21); 0.1 mm (Figs 16–17); 0.05 mm (Figs 10, 13); 0.01 mm (Figs 8, 9, 12, 14–15).

Figures 22–25.–*Metawithius keralensis* sp. nov., paratype male (ADSH PS0003), scanning electron micrographs: 22. Sternites V–IX with glandular setae, ventral; 23. Glandular setae on sternite IX; 24. Glandular setae; 25. Right chelicera, dorsal.

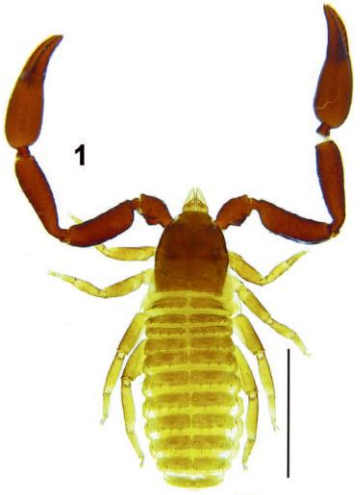
Figure 26. Collecting localities of *Metawithius keralensis* sp. nov.

Figures 27–30.–*Metawithius nepalensis* (Beier, 1974), holotype male (SMF 28969): 27. Habitus, dorsal; 28. Habitus, ventral; 29. Female habitus, dorsal; 30. Female habitus, ventral. Scale bars: 1 mm (Figs 27–30).

Figures 31–38.–*Metawithius nepalensis* (Beier, 1974): 31–37. Holotype male (SMF 28969): 31. Carapace; 32. Chelicera; 33. Rallum; 34. Left pedipalp; 35. Left leg I; 36. Left leg IV; 37. Right chela; 38. Allotype female (SMF 28969): Right chela. Scale bars: 0.5 mm (Fig. 34); 0.2 mm (Figs 31, 35–38); 0.1 mm (Figs 32–33).

Figures 39–41.–*Metawithius nepalensis* (Beier, 1974), holotype male (SMF 28969): 39. Maxillae; 40. Glandular setae, sternite VII, left hemi-sternite; 41. Abdominal segments V-XII, ventral. Scale bars: 0.5 mm (Fig. 41); 0.2 mm (Fig. 39); 0.1 mm (Fig. 40).

Figure 42.–Geographic distribution of known *Metawithius* spp.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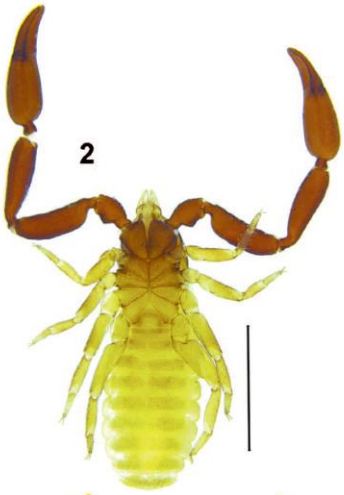
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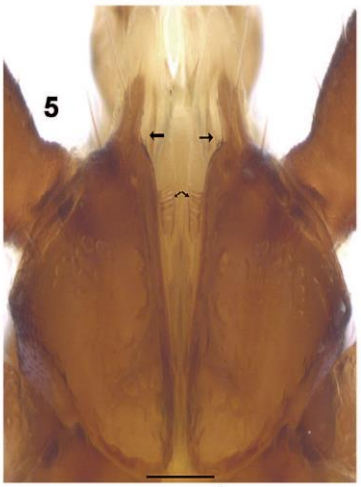
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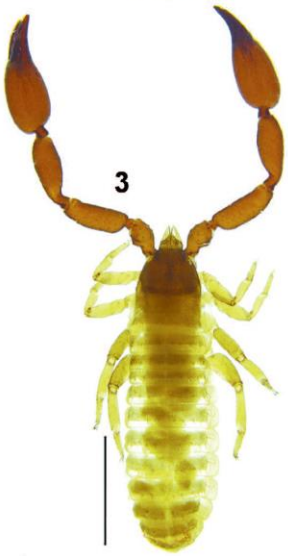
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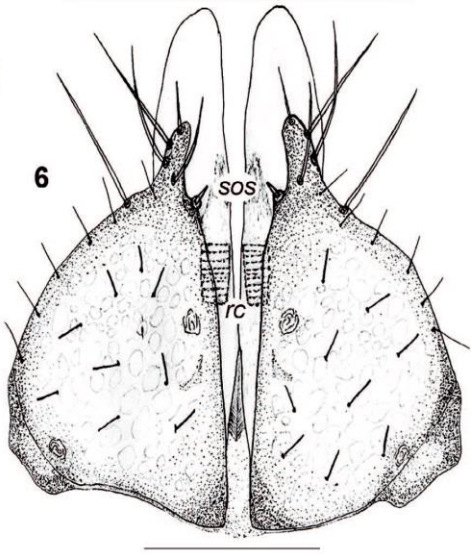
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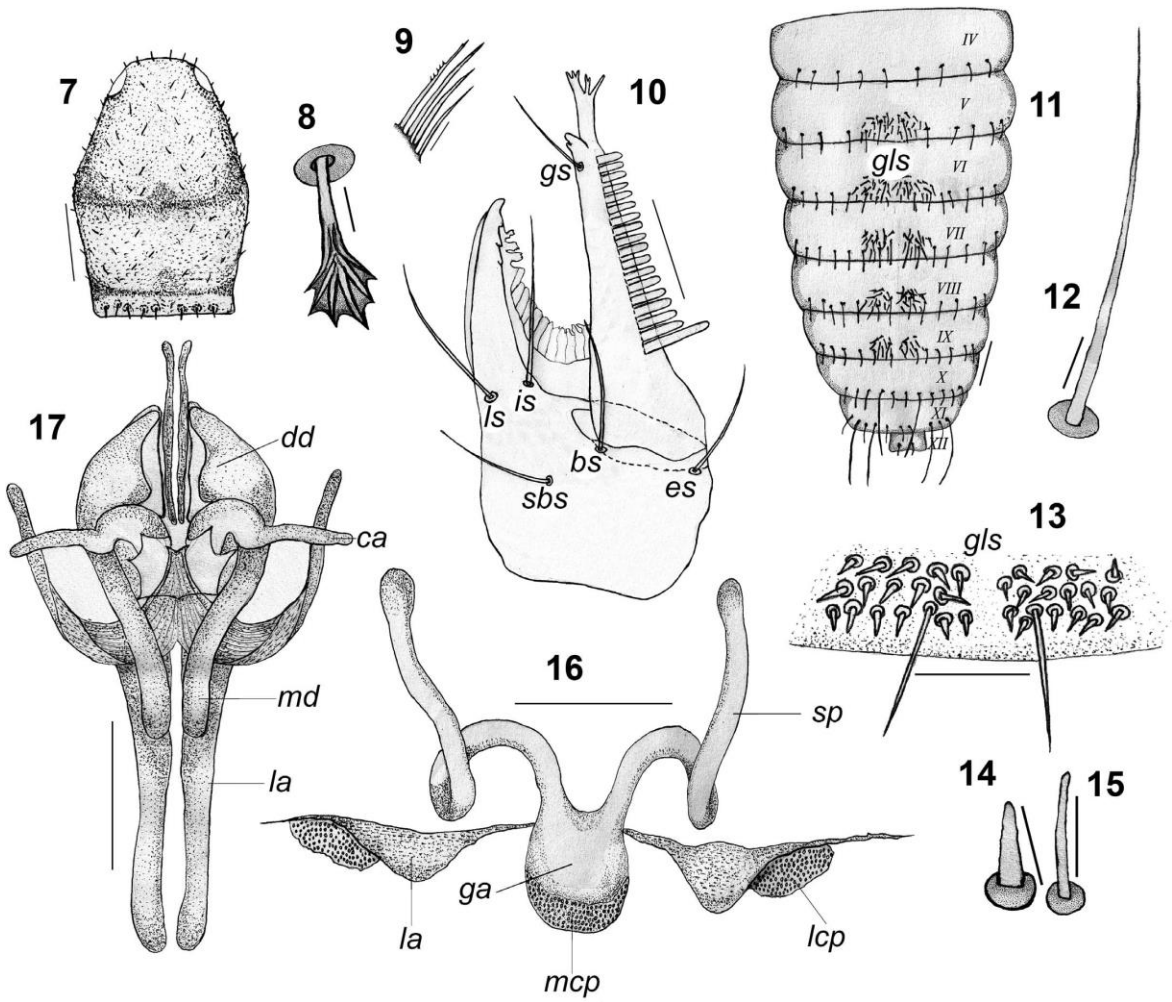
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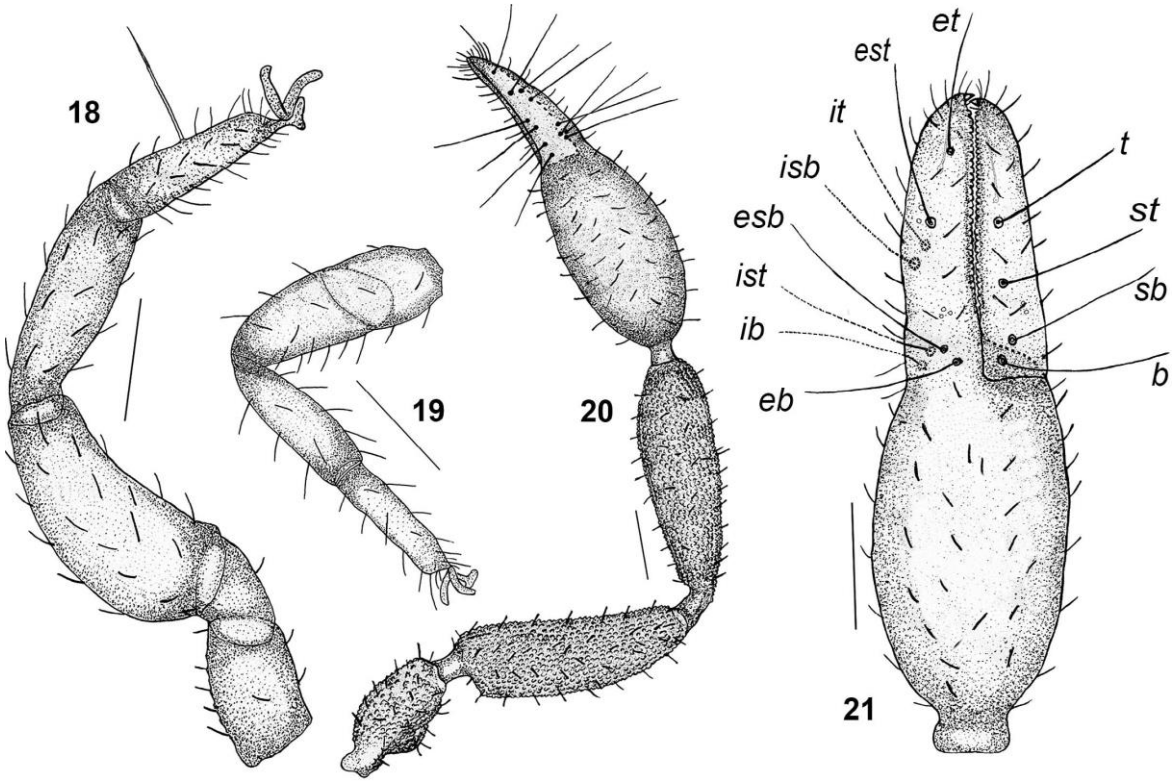
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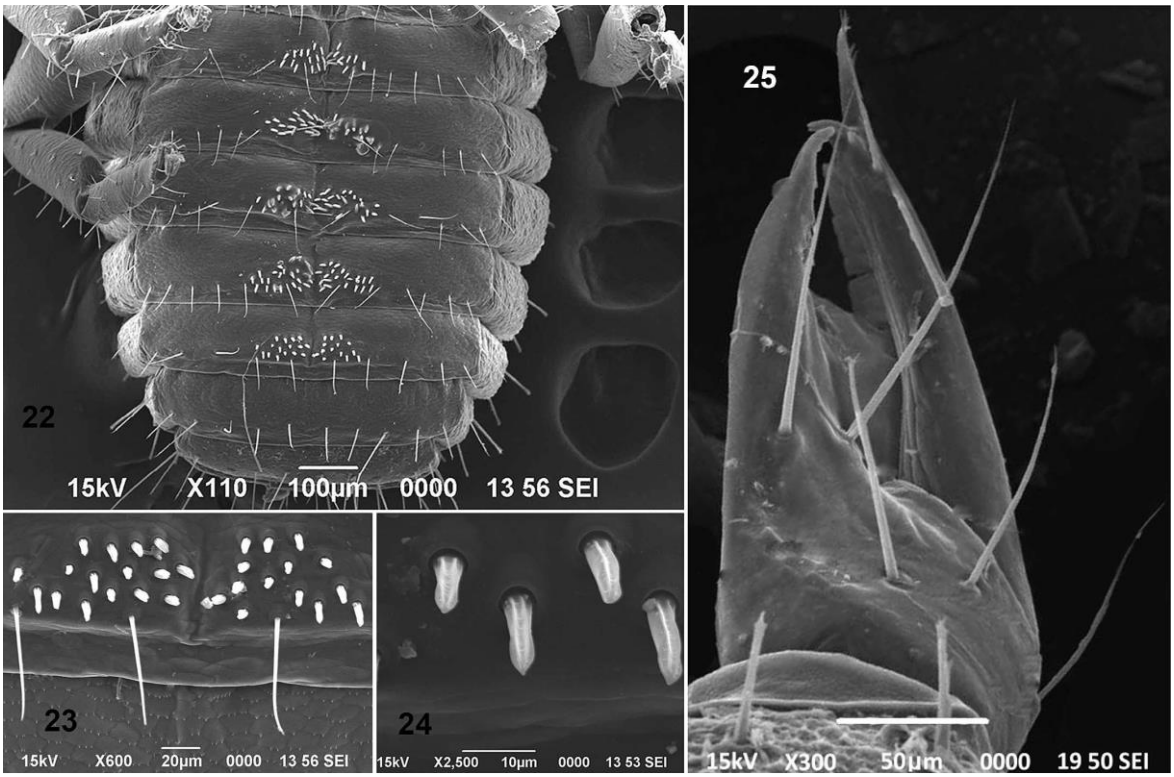
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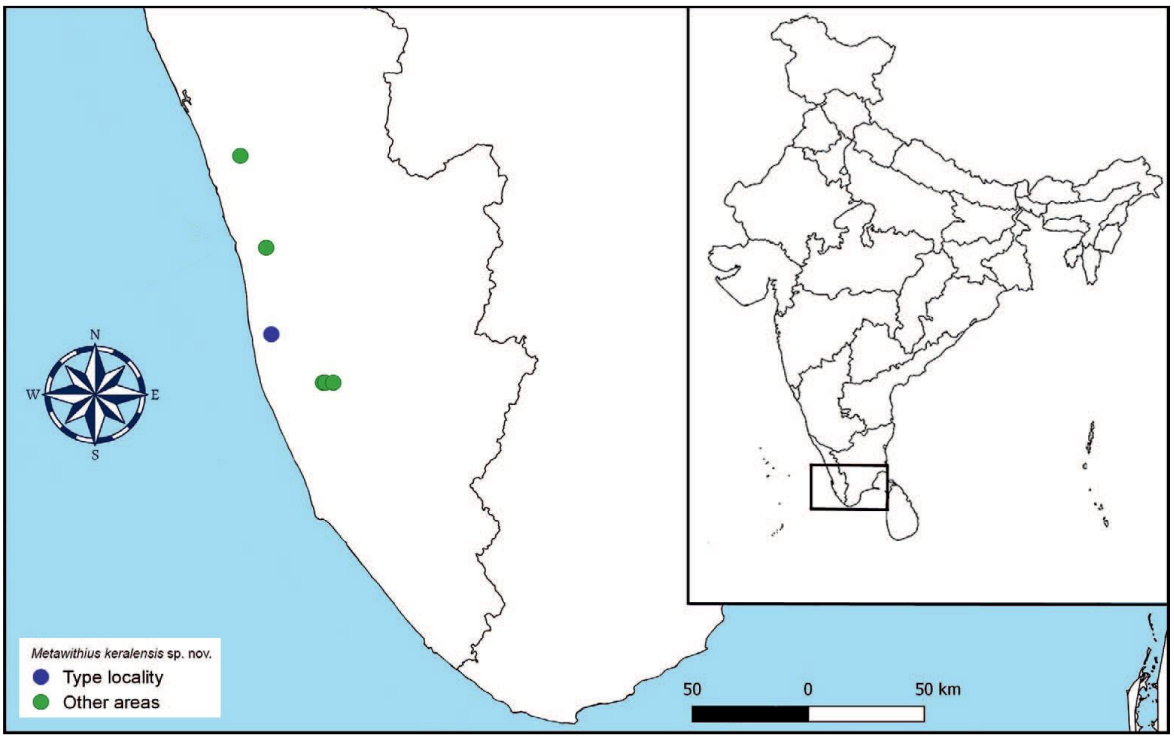
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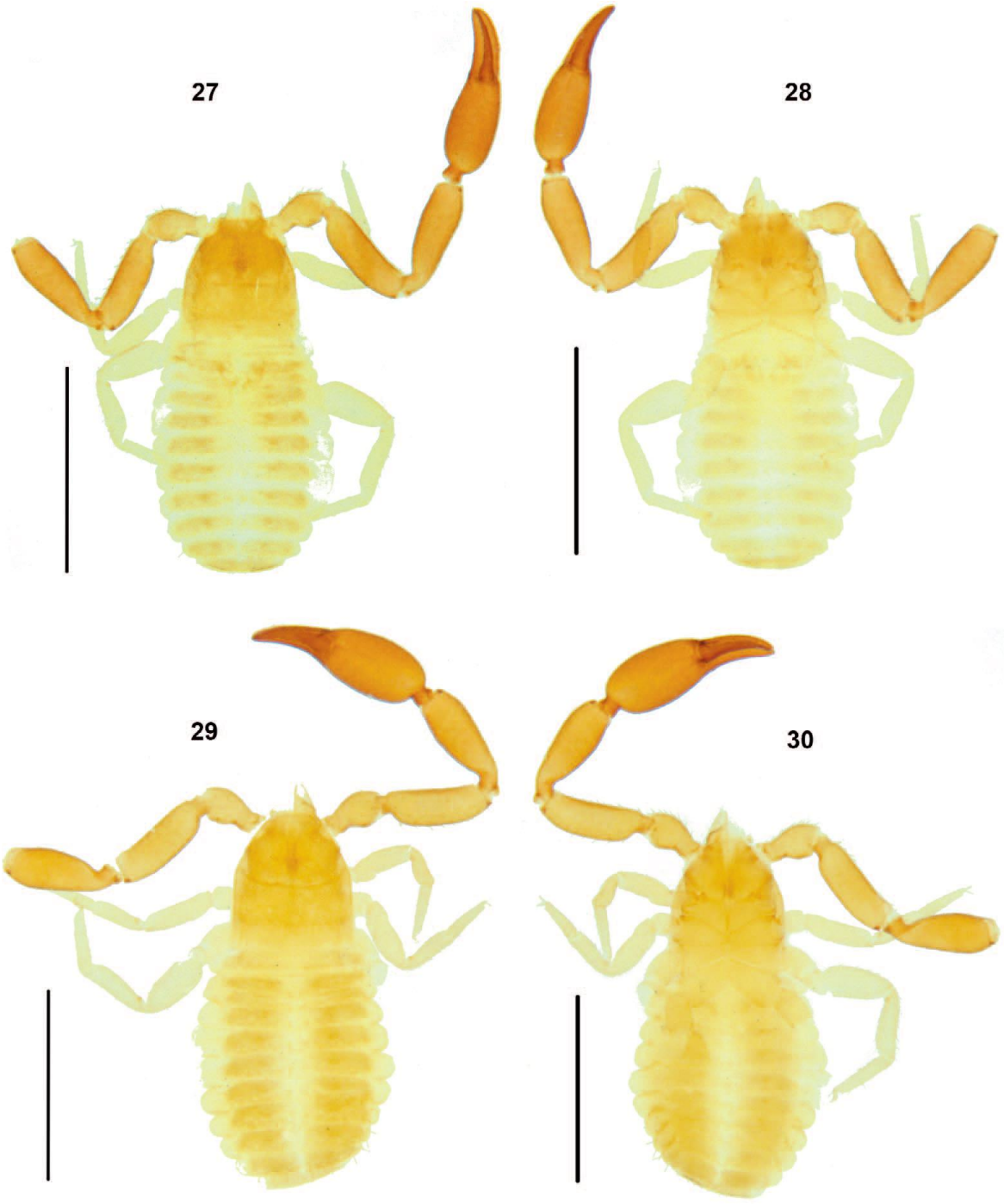
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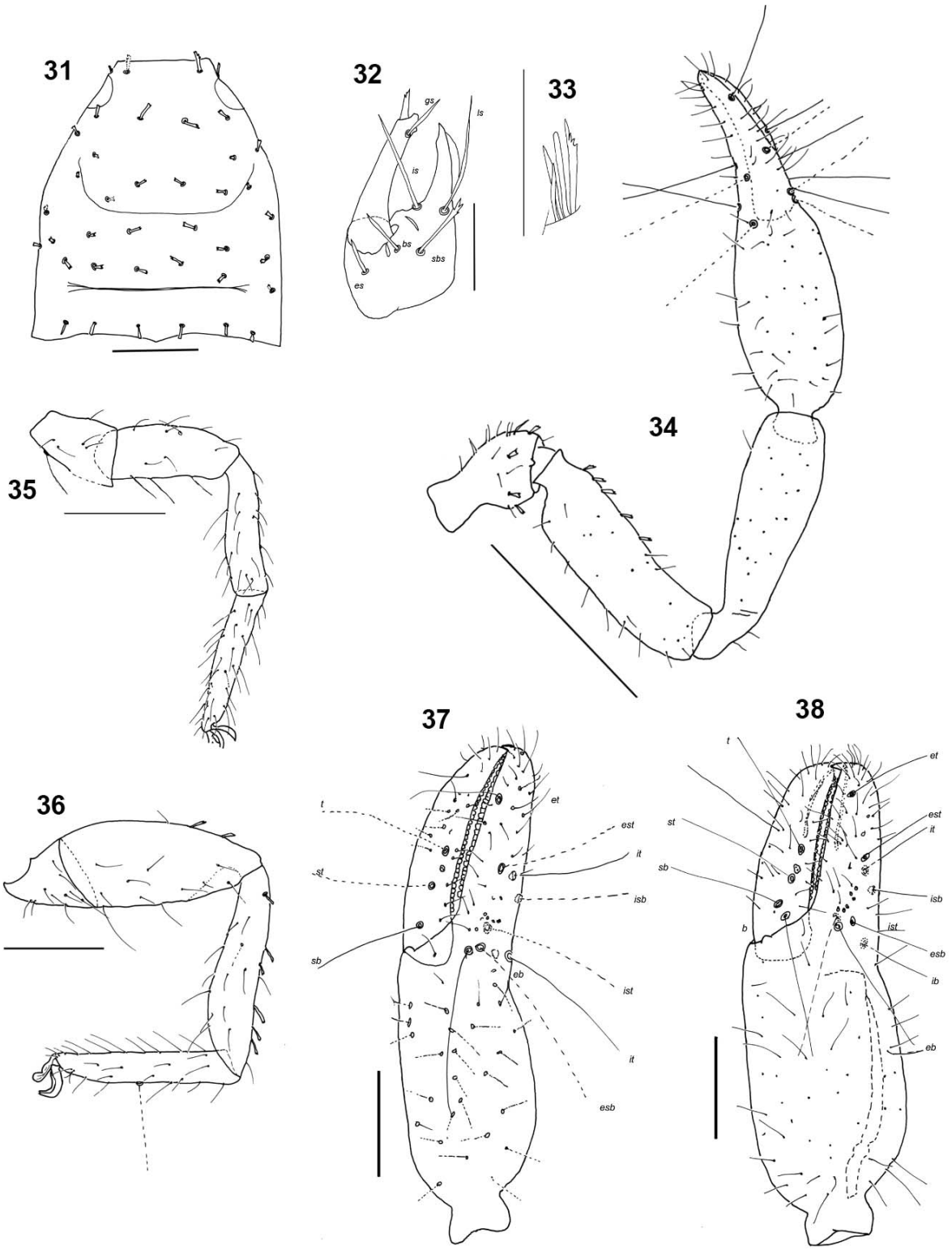
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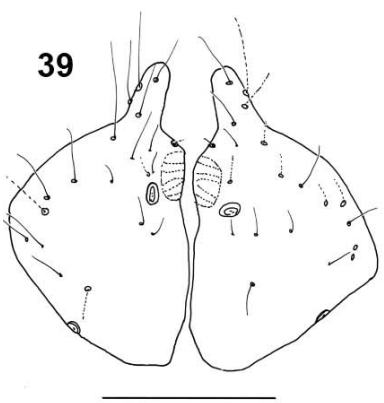
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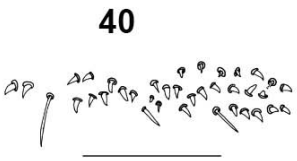
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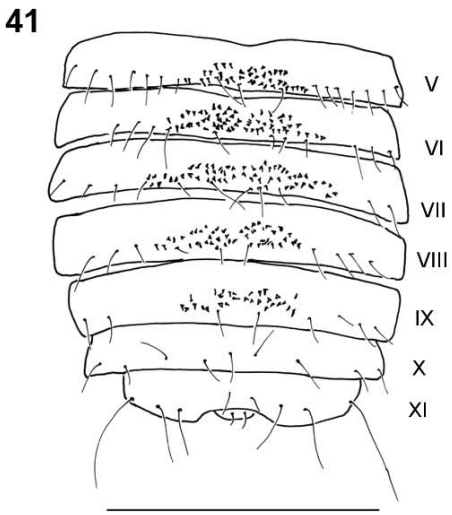
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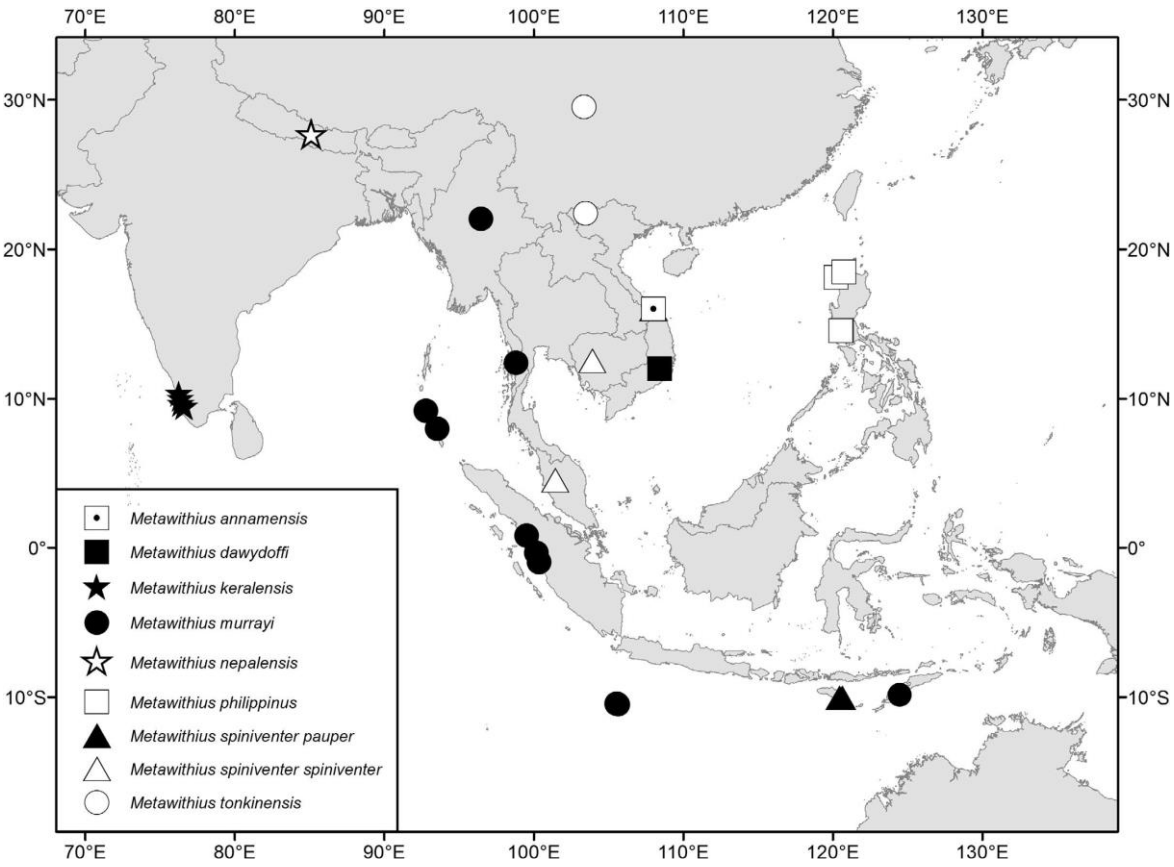
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Metawithius keralensis
Metawithius murrayi
Metawithius nepalensis
Metawithius philippinus
Metawithius spiniventer pauper
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4. Conclusions

As stated in the introduction, sexual selection can shape genital evolution (Eberhard 1985, Hosken & Stockley 2004). With pseudoscorpions of the family Withiidae as our model, we explored the expectation of a higher evolutionary speed for genitalia structures compared to other structural characters as a consequence of sexual selection; to achieve this we: 1) describe and propose homology statements for Cacodemoniini (Withiidae) genitalia (Romero-Ortiz & Sarmiento 2021) (chapter 1), and 2) assess the evolutionary rate of change of genitalia and sensorial characters on a phylogenetic framework and compare these rates with those exhibited by no sexually related characters (chapter 2). Moreover, as a result of specimen revision, we proposed several taxonomic novelties for the group (chapter 3).

Since homology statements are crucial in evolutionary studies, a clear and rigorous definition of each homology proposal is an essential step in the process of inferring phylogenies. Additionally, phylogenies, where many other characters are included, can put those statements to test and support or refute them. We proposed homology statements for four genital structures: the ejaculatory canal atrium (*ejca)*, the ejaculatory canal (*ejc)*, the lateral apodemes (*la)*, and the lateral rods (*lr)*. We characterized those four structures for the eight genera of the Cacodemoniini and proposed homology with the genitalia of members of the family Atemnidae as sister of Withiidae. We did an extensive examination of specimens and descriptions to fulfil the first approach. As proposed by Ochoterena et al. (2019) these homology proposals could only be proven as such, after a phylogenetic analysis.

Consequently, we built a phylogenetic framework for testing the support of the homology propositions and test the role of proposed sexual selection consequences on the evolution of Withiidae genitalia, looking for the ancestral states of the genital atrium components (male genitalia) and assessing a possible correlation between evolutionary rates of those characters with non-sexual characters, all of these, based on the homology statements

proposed in the first chapter. We used three molecular markers, two nuclear (18S and 28S) and one mitochondrial (COI), and searched for a phylogenetic tree under three approaches, Parsimony, Maximum Likelihood, and Bayesian reconstruction. We found that Withiidae is monophyletic and that there are two strongly supported groups, one with all specimens from the Neotropics, and another with all other withiids. We have not samples of the African subfamily Paragoniochernetinae mainly due to absence of those in biological collections and the difficulty to access the areas where they inhabit. It is important to include this subfamily in future studies, as it could give us a more complete idea of the evolution of genitalia and other structures. Yet, we consider that the monophyly of the family is a reliable proposal since support values for this group are strong, as showed by the parsimony, ML and BI analyses with the concatenated molecular matrix (MOL) (Br 11, SH-aLRT and UFBoot 100%, pp1), and by the combined dataset that includes morphological and molecular data (pp 1, bs 99).

We also inferred that the family appeared in the Jurassic and that the splitting between the neotropical and the non-neotropical clades coincides with Gondwana breaking, in the Cretaceous. Also, the correlation analysis between evolutionary rates of a sexual (length of ejaculatory canal atrium, *ejca),* and two non-sexual (position of trichobothria *ib* and *isb* and eye morphology) traits, indicated that changes in the *ejca,* the sexual trait, preceded changes in the eye morphology; this means that the *ejca* changes were given before eye morphology changes, suggesting a role of a differential selective pressure over this character. Moreover, comparing the rate of change of the characters, the fastest one occurs in the position of the trichobothria *ib* and *isb*, followed by the length of *ejca*, and lastly, the change on the eye morphology. These results do not fit our expectation of sexual selection acting over male genitalia as this does not change at a higher rate than other character types. We suggest that this variation could be due to other selective pressures concerning sensorial adaptations over the position of the trichobothria. Other reasons may be considered to understand the structural complexity of the male genitalia.

Finally, because of specimen revision and phylogenetic analysis we propose four sets of taxonomic novelties. First, we redescribed *Metawithius nepalensis*, a species which description was outdated and was included in the revision of the genus (Johnson et al. 2019); second, we transferred two species originally described under the genus *Metawithius* (Withiidae) to the genus *Verrucachernes* (Chernetidae) (Romero-Ortiz & Harvey 2019), third, we describe five new species of the genera *Cystowithius*, *Parawithius*,

and the new genus *Paciwithius*, and give a new record of *Cystowithius ankeri* as well as an identification key for the species of *Cystowithius*, and fourth we raised the subgenus *Oligowithius,* to the genus level (Chapter 3).

To conclude, although we started our research with the hypothesis that sexual selection is acting upon male genitalia due the high structural complexity of this structure in the family, we found no evidence that this is happening. The fastest rate of change occurred in the trichobothria position giving us a hint that there is a selective pressure upon sensorial characters more than any other. As such, pseudoscorpions as a model gave us an opportunity to explore how evolution could work in a phylogenetic context.

Nowadays, the assessment of selective pressures and rates of morphological change is carried on over macroevolutionary studies with multiple characters considering that there are many other evolutionary forces acting upon morphological traits. In this sense, our results should include a more complete taxonomic sampling and a wider set of morphological and molecular data. We think that with the fast development of genomics in phylogenetic analyses, and the development of new computational tools for evaluating multiple characters over big phylogenies, more hints about this intriguing matter can be reached in the near future.

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