RESEARCH ARTICLE



# Revision of the freshwater crabs of the genus *Tehuana* Rodríguez & Smalley in Smalley 1970 (Decapoda, Pseudothelphusidae), with the descriptions of two new species

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| Academic editor: Célio Magalhães   Received 15 April 2022   Accepted 19 May 2022   Published 11 August 202 |
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| http://zoobank.org/CF280A34-D24A-4B8F-8BF5-5FB43D9B5C29  |

**Citation:** Moreno-Juárez EG, Villalobos JL, Álvarez F (2022) Revision of the freshwater crabs of the genus *Tehuana* Rodríguez & Smalley in Smalley, 1970 (Decapoda, Pseudothelphusidae), with the descriptions of two new species. ZooKeys 1117: 1–35. https://doi.org/10.3897/zookeys.1117.85362

# Abstract

The freshwater crab genus *Tehuana* Rodríguez & Smalley in Smalley, 1970 includes eight species distributed in southeastern Mexico. A recent review of organisms belonging to this genus uncovered new variations in the male gonopod morphology. A phylogenetic analysis based on molecular characters using three genes (H3, 16S, and COI) resulted in the identification of two new species which are described herein: *Tehuana ayotzintepecensis* **sp. nov.** from Oaxaca and *Tehuana col* **sp. nov.** from Veracruz. New diagnoses are provided for those species that had very brief descriptions lacking the treatment of important taxonomic characters and an identification key for all the species in the genus is also given. A discussion of the distribution of all the species in *Tehuana* in the Isthmus of Tehuantepec is presented.

# Keywords

Molecular phylogeny, Neotropical region, southeastern Mexico, *Tehuana ayotzintepecensis* sp. nov., *Tehuana col* sp. nov.

# Introduction

The genus *Tehuana* Rodríguez & Smalley in Smalley, 1970, comprises eight species of freshwater crabs of the family Pseudothelphusidae, which are distributed throughout the oriental slope of the states of Veracruz, Oaxaca, and Chiapas, Mexico (Fig. 1). They can be distinguished from other members of the subfamily Pseudothelphusinae (sensu Álvarez et al. 2020) by the morphology of the male first gonopod (G1) which is characterized by an evident meso-distal conical prominence, a strong semicircular carina on the internal surface of the proximal lobe of the caudo-marginal projection and a medial constriction on the lateral surface of the gonopod's principal axis (Villalobos and Álvarez 2010).

Rodríguez and Smalley (in Smalley 1970) were the first authors to recognize a different and discrete group of species inside the genus *Pseudothelphusa* de Saussure, 1857 with a characteristic morphology of the male G1, and erected the new subgenus *Pseudothelphusa* (*Tehuana*) to accommodate three species: *Pseudothelphusa* (*T.) cordobensis* Rodríguez & Smalley, 1972, *P.* (*T.) veracruzana* Rodríguez & Smalley in Smalley, 1970, and *P.* (*T.) lamellifrons* (Rathbun, 1893). Pretzmann (1972) also recognized *Tehuana* as a subgenus of *Pseudothelphusa* including in his monograph: *Pseudothelphusa* (*T.) lamellifrons* lamellifrons (Rathbun, 1893), *P.* (*T.) lamellifrons gruneri* Pretzmann, 1972 and *P.* (*T.) veracruzana*. Pretzmann (1978) subsequently presented two more new subspecies, *Pseudothelphusa* (*T.) lamellifrons poglayenorum* and *P.* (*T.) lamellifrons diabolis*, from Los Tuxtlas region, Veracruz, Mexico. Later, Pretzmann (1980) presented the same two subspecies with an extended diagnosis. Türkay (1978) revised the nomenclatural status of *T. complanata* (Rathbun, 1905) (= *Pseudothelphusa complanata* Rathbun, 1905), described for first time the male G1 of the holotype deposited in the Natural History Museum of Paris, and concluded that it belonged in the subgenus *Pseudothelphusa (Tehuana*).

Rodriguez (1982), in his revision of the freshwater crabs of America, raised *P. (Tehuana)* to the genus level, and considered that the new genus was closely related to *Pseudothelphusa* and *Epithelphusa* Rodríguez & Smalley in Smalley, 1970 (= *Ehecatusa* Ng & Low, 2010). Furthermore, he placed three species in *Tehuana* and synonymized *T. cordobensis* to *T. complanata*. The two subspecies described by Pretzmann (1980), are mentioned in the Addendum to the monograph without any further analysis, but it is assumed that at that point *Tehuana* included five species.

Alvarez and Villalobos (1994) assigned *Pseudothelphusa guerreroensis* (Rathbun, 1933) to *Tehuana*, although the authors later reassigned this species back to *Pseudothelphusa* based on a morphological phylogeny (Villalobos and Álvarez 2010), and described *T. lamothei* Alvarez & Villalobos, 1994 from Chiapas, Mexico. Villalobos and Alvarez (2003) described *T. chontalpaensis* and *T. jacatepecensis*, from the states of Tabasco and Oaxaca, Mexico, respectively. Villalobos and Alvarez (2010) presented a phylogeny of the tribe Pseudothelphusini (= subfamily Pseudothelphusinae) confirming the close relationship of *Pseudothelphusa* and *Tehuana*. Álvarez et al. (2020) in their revision of the superfamily Pseudothelphusoidea recovered *Tehuana* in the subfamily Pseudothelphusinae, however it appears more closely related to *Disparithelphusa* Smalley & Adkison, 1984 than to *Pseudothelphusa*, as was always considered.



Figure 1. Distribution of the species of the genus Tehuana in southeastern Mexico.

We present a revision of all the material of *Tehuana* deposited in the Colección Nacional de Crustáceos (CNCR) of the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). We present a revised diagnosis of the genus, the description of two new species, updated diagnoses for four species that lacked sufficient detail in the original descriptions, and an identification key for the species of *Tehuana*. All descriptions and illustrations correspond to the left first male gonopod (G1). In addition, a phylogeny for *Tehuana* and closely related genera based on partial sequences of three genes, two mitochondrial (16S and COI) and one nuclear (H3), is presented. We discuss the relationships among several related genera distributed in southern Mexico and northern Central America.

# Materials and methods

#### Taxon sampling and morphological characters

A total of 18 crabs belonging to the genus *Tehuana* was studied. All the specimens are deposited in the Colección Nacional de Crustáceos (**CNCR**) of the Instituto de Biología, Universidad Nacional Autónoma de México (Table 1). The terminology

used to describe the male G1 follows Villalobos and Alvarez (2010) (Fig. 2). Photographs of the G1 were taken with a Leica DFC490 camera mounted on a Leica Z16 APOA microscope.

Updated diagnoses are provided for *Tehuana diabolis* (Pretzmann, 1978), *T. lamellifrons* (Rathbun, 1893) and *T. poglayenorum* (Pretzmann, 1978), whose original descriptions are too short and do not include relevant taxonomic characters (Fig. 2A). Abbreviations used are **CL** for carapace length, **CW** for carapace width, and **G1** for the male first gonopod.

# DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the gill tissue and muscle of pereopods of males preserved in 70–80% ethanol. Extraction was performed with the Animal and fungi DNA preparation kit from Jena Bioscience, following the manufacturer's protocol. Concentration of DNA was measured with a NanoDrop 2000 spectrophotometer, and the integrity assessed using an agarose gel (1%). Three genes were partially sequenced, two mitochondrial: COX 1 (ChelF1 5'-TAC TCT ACT AAT CAT AAA GAC ATT GG-3'; ChelR1 5'-CCT CCT CCT GAA GGG TCA AAA AAT GA-3'; Barret and Hebert 2005) and 16S (16Sa 5'- ACT TGA TAT ATA ATT AAA GGG CCG-3'; 16Sb (5'-CTG GCG CCG CTC TGA ACT CAA ATC-3'; Palumbi and Benzie 1991); and one nuclear: H3 (H3AF 5'- ATG GCT CGT ACC AAG CAG ACV GC-3, H3AR 5'- ATA TCC TTR GGC ATR ATR GTG AC-3'; Colgan et al. 1998). The polymerase chain reaction (PCR) was performed with MyTaq Kit from Bioline; following the manufacturer's protocol. The PCR thermic profiles were as follows: COX 1 and H3, with an initial step of 5 min at 95 °C; 35 cycles of 45 s of 94 °C, 45 s at 50 °C and 1 min at 72 °C; with a final extension of 10 min at 72 °C. For the 16S gene: an initial step of 5 min at 95 °C; 35 cycles of 45 s of 94 °C, 45 s at 48 °C and 1 min at 72 °C; with a final extension of 10 min at 72 °C. PCR products were visualized on agarose gel (1%). The sequencing of samples was performed with the reaction kit ABI Prism 3100 Genetic Analyzer, Applied Biosystems automated sequencer.

# Phylogenetic analysis

The consensus sequences were manually obtained using MEGA v. 7.0 (Kumar et al. 2016) and Finch T.V. v. 1.4.0 (Geospiza Inc.). The presence of stop codons was reviewed with MESQUITE v. 3.6.1 (Maddison and Maddison 2019). Finally, the sequences were compared with online libraries of BLAST and deposited on GenBank (Table 1). The alignment was performed in MAFFT v. 7 (Katoh et al. 2019) with default parameters. Partition for protein sequences genes were performed as in Álvarez et al. (2020), one partition for each gene. The best fit model was obtained in jModelTest v. 2.1.10 (Darriba et al. 2012), with the Akaike Information Criterion

(AIC) (Hurvich and Tsai 1989). The final concatenated matrix of 1,368 base pairs was analyzed with two phylogenetic inference methods, maximum likelihood (ML) and Bayesian inference (BI). The maximum likelihood tree was performed in RAxML-HPC BlackBox v.8.2.12 (Stamatakis 2014) in CIPRES (Miller et al. 2010). The optimal number of pseudoreplicates was calculated by the program and the tree with the best bootstrap values was chosen. We only presented the concatenated tree and

**Table 1.** Specimens used for the phylogenetic analysis of the genus *Tehuana* including taxon name, catalog number in the Colección Nacional de Crustáceos (CNCR), locality, sequenced genes, and GenBank accession numbers.

| Species  | CNCR  | Locality   | COI      | 165      | H3       |
|--|-------|--|----------|----------|----------|
| Subfamily Pseudothelphusinae                                       |       |  |          |          |          |
| Tehuana poglayenorum (Pretzman, 1978)                              | 33931 | Río Basura, San Andrés Tuxtla, Veracruz<br>18°31'55"N, 95°03'30"W                          | OK165442 | OK256890 | OK188918 |
| Tehuana diabolis (Pretzman, 1978)                                  | 34488 | Río Las Margaritas, Catemaco, Veracruz<br>18°22'06"N, 95°01'00"W                           | OK165444 | OK256892 | OK188920 |
| <i>Tehuana veracruzana</i> Rodríguez<br>& Smalley, in Smalley 1970 | 33932 | Terracería, Zapoapan de Cabañas, Veracruz<br>18°20'32"N, 95°04'13"W                        | OK165443 | OK256891 | OK188919 |
| Tehuana col sp. nov.   | 33928 | Río Col, San Andrés Tuxtla, Veracruz<br>18°38'29"N, 95°09'00"W                             | OK165445 | OK256893 | OK188921 |
| Tehuana lamellifrons (Rathbun, 1893)                               | 33939 | Nizanda, Asunción Ixtaltepec, Oaxaca<br>16°41'24" N, 95°22'53" W                           | OK165446 | OK256894 | OK188922 |
| Tehuana complanata (Rathbun, 1905)                                 | 11957 | Amatlán de Los Reyes, Córdoba, Veracruz<br>18°51'23"N, 96°54'19"W                          | OK165447 | OK256896 | OK188924 |
| <i>Tehuana ayotzintepecensis</i> sp. nov.                          | 34628 | Arroyo tributario, Río<br>Cajone, Ayotzintepec, Oaxaca<br>17°39'46"N, 96°07'51"W           | OK165448 | OK256895 | OK188923 |
| <i>Tehuana jacatepecensis</i><br>Villalobos & Alvarez, 2003        | 11920 | Río Santo Domingo, Santa<br>María Jacatepec, Oaxaca<br>17°51'37"N, 96°12'36"W              | -        | OK256897 | OK188925 |
| <i>Tehuana chontalpaensis</i><br>Villalobos & Alvarez, 2003        | 25445 | Arroyo Frio, Cerro Cola de Sapo;<br>Reserva de la Biósfera Selva El Ocote,<br>Ocozocoautla | MT852948 | MT871970 | MT860380 |
| <i>Tehuana lamothei</i><br>Alvarez & Villalobos, 1994              | 8812  | Arroyo cerca de Tapilula, Tapilula, Chiapas<br>18°16'05"N, 93°01'33"W                      | OK165449 | OK256898 | OK188926 |
| <i>Pseudothelphusa americana</i><br>de Saussure, 1857              | 25527 | Río Ajajalpa, Zacatlán, Puebla<br>19°52'19"N, 97°58'52"W                                   | MT852944 | MT871966 | MT860376 |
| Pseudothelphusa doenitzi Bott, 1968                                | 26190 | La Lobera, Zaachila, Oaxaca<br>16°56'55"N, 96°50'10"W                                      | OK165451 | OK256900 | OK188928 |
| Pseudothelphusa belliana Rathbun, 1898                             | 19228 | Chautipan, Chilpancingo, Guerrero<br>17°30'28"N, 99°44'30"W                                | MT860377 | MT871967 | MT852945 |
| Ehecatusa mixtepensis<br>(Rodríguez & Smalley, 1972)               | 309   | San Gabriel Mixtepec, Oaxaca<br>16°05'33"N, 97°04'53"W                                     | MT852943 | -        | MT860375 |
| Smalleyus tricristatus Alvarez, 1989                               | 7034  | Sierra de Santa Marta, Los Tuxtlas, Veracruz<br>18°26'00"N, 94°57'00"W                     | MT852947 | MT871969 | MT860379 |
| <i>Disparithelphusa pecki</i><br>Smalley & Adkinson, 1984          | 34625 | Cerro Cangrejo, San Juan<br>Bautista Valle Nacional, Oaxaca<br>17°48'04"N, 96°19'06"W      | OK165450 | OK256899 | OK188927 |
| Subfamily Raddausinae  |       |  |          |          |          |
| <i>Odontothelphusa lacandona</i><br>Alvarez & Villalobos, 1998     | 11204 | Ocosingo, Chiapas<br>16°25'00"N, 90°30'00" W   | MT852048 | MT871956 | MT860366 |
| <i>Odontothelphusa toninae</i><br>Alvarez & Villalobos, 1991       | 5770  | Ruinas de Toniná, Chiapas<br>16°54'08"N, 92°00'33"W  | MT852049 | MT871957 | MT860367 |

report clades with branch support above 50%. The Bayesian inference analysis was run in MrBayes v. 3.2.7 (Ronquist and Huelsenbeck 2003) in CIPRES, with the previously inferred substitution models. The parameters were as follows: two independent runs with four Monte Carlo Markov chains, a temperature of 0.1, 10,000,000 generations sampling every 1,000 generations, and a burn-in of 25%. A majority consensus tree was obtained, only clades with a branch support greater than 50% are reported. The convergence of the chains and the optimal ESS values were corroborated in Tracer v. 1.7.1 (Rambaut et al. 2018), as well as the optimal PSRF values were visualized in Figtree v. 4.3 (Rambaut et al. 2018).



**Figure 2.** Schematic representation of the terminology used to describe the male first gonopod (G1) of some *Tehuana* species using as examples the G1 of **A** *T. lamellifrons* **B** *T. veracruzana* **C** *T. lamotheii* **D** *T. jacatepecensis* **E** *T. poglayenorum* **F** *T. chontalpaensis.* **A–C** mesial view **D, E** distal view **F** lateral view. Scale bars: 1 mm (**A, B, C, F**); 0.5 mm (**D, E**). Abbreviations: ac, apical cavity; cc, central crest; cmp, caudo-marginal projection; ccmp, distal crest of the caudo-marginal projection; cdc, caudal distal crest; cpl, cephalic border of the proximal lobe; dl, distal lobe; dmp, distal border of the mesial process; hc, internal carena height; hdl, distal lobe height; hpl, proximal lobe height; ic, internal carena of the proximal lobe; imp, internal angle of the mesial process; ldc, lateral distal crest; ldl, distal lobe length; lmp, lateral border of the mesial process; lpl, proximal lobe length; ls, lateral spine; mc, medial constriction; mdc, mesial distal crest; mdl, meso-distal lobe; mp, mesial process; mpl, marginal plate; ms, marginal suture; nmp, notch of the caudo-marginal projection; pl, proximal lobe; sf, spine field; sp, spermatic pore.

# Results

# Phylogenetic analysis

For the ML analysis in RAxML-HPC BlackBox v. 8.2.12, each gene was analyzed under the GTR model (Tavaré 1986), assuming the following parameters: COI nucleotide frequencies: A = 0.3236; C = 0.1771; G = 0.1498; T = 0.3493; substitution model: A/C: 1.34; A/G: 4.84; A/T: 2.34; C/G: 0.33; C/T: 20.33: G/T: 1.00. 16S nucleotide frequencies: A = 0.3581; C = 0.0990; G = 0.1787; T = 0.3640; substitution model: A/C: 0.65; A/G: 15.21; A/T: 2.30; C/G: 0.00; C/T: 4.32; G/T: 1.00. H3 nucleotide frequencies: A = 0.2065; C = 0.3160; G = 0.2787; T = 0.1986; substitution model: A/C: 0.02; A/G: 0.11; A/T: 0.02; C/G: 0.00; C/T: 0.15; G/T: 1.00. For the BI analysis in MrBayes v. 3.2.7 the following models were assumed COI: GTR + G with a gamma distribution of 0.15 and nucleotide frequencies of A = 0.3157, C = 0.1729, G = 0.1550, 0.3564; 16S, GTR + I with an invariable site frequency of 0.6780 and nucleotide frequencies of A = 0.3729, C = 0.0935, G = 0.1689, T = 0.3647; and H3, F81 (Felsenstein 1981), with nucleotide frequencies of A = 0.2076, C = 0.3126, G = 0.2723, T = 0.2075.

The phylogenetic analysis resolved the genus *Tehuana* as monophyletic with an internal organization of three main clades (Fig. 3, clades a, b, c), which are consistent with the male G1 morphology. Species in clade a have a well-developed laminar mesial process in longitudinal position, laterally or caudally oriented with a wide distal border and a strong and acute lateral median spine. Species of clade b have a transversal, reduced, cylindrical with the distal surface of mesial process excavated, without lateral ornamentation and with the internal angle developed as a triangular projection. Species in clade c have a moderately developed mesial process longitudinal, oriented laterally, ornamented with a small lateral median spine.

The arrangement of the species of *Tehuana* within the tree is also concordant with their geographical distribution: clade a, *Tehuana lamothei* and *T. chontalpaensis* from Chiapas and Tabasco, respectively; clade b, *T. jacatepecensis, T. ayotzintepecensis* sp. nov., *T. lamellifrons*, and *T. complanata*, from the southwestern margins of the Papaloapan River basin along the Sierra Norte, Oaxaca; and clade c, *T. diabolis, T. veracruzana, T. poglayenorum*, and *T. col* sp. nov., from Los Tuxtlas region, Veracruz (Figs 1, 3).

#### Taxonomy

# Family Pseudothelphusidae Ortmann, 1893 Subfamily Pseudothelphusinae Ortmann, 1893

# Genus Tehuana Rodríguez & Smalley in Smalley, 1970

Pseudothelphusa (Tehuana) Rodríguez & Smalley in Smalley, 1970: 106 (in key).— Pretzmann 1971: 22.—Pretzmann 1972: 107; 1980: 660.—Rodríguez and Smalley 1972: 77.—Türkay 1978: 144. Tehuana.—Rodriguez, 1982: 129; 1986: 66.—Villalobos-Hiriart et al. 1993: 285 (list).—Alvarez and Villalobos 1994: 730.—Alvarez et al. 1996: 129.—Villalobos and Alvarez 2003: 228.—Álvarez et al. 2005: 191.—Ng et al. 2008: 177 (list).—Villalobos Hiriart and Álvarez 2008: 279.—Villalobos and Alvarez 2010: 474.—Álvarez et al. 2011b: 289.—Mejía-Ortíz et al. 2011: 19 (list).—Guinot and Hendrickx 2014: 477, 478, tab. 1.—Alvarez and Villalobos 2016: 244.—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).—Álvarez et al. 2020: 12, tab. 4, 20 (list).

# Type species. Pseudothelphusa lamellifrons Rathbun, 1893 [by original designation].

Diagnosis. Carapace with dorsal surface flat, smooth, punctate, with small granulations adjacent to antero-lateral margin. Front vertically deflexed, smooth, superior border formed by low rounded tubercles, medially divided by V-shaped notch reaching inferior margin; inferior margin not distinguishable dorsally, in ventral view projected, widely bilobed. Third maxillipeds with ischium trapezoidal, slightly longer than wide; merus smaller than ischium, anterolateral margin rounded, with anterior shallow concavity; ratio exopod/ischium length 0.6 to 0.8. G1 slender, proximal half cylindrical, compressed distally, with a meso-distal lobe conical, well developed on mesial surface; principal axis with medial constriction on lateral surface and twisted towards medial suture of ventral sternites (Fig. 2). In mesial view, proximal half with marginal plate closing spermatic channel, border facing marginal suture with proximal fringe of long and stout setae; distal half inclined towards cephalic surface; caudal surface fused with marginal plate and expanded cephalically to form the bilobed caudo-marginal projection, distal lobe well-developed (except in T. lamothei where it is reduced), subrectangular with cephalic border rounded, distal portion can be curved proximally and separated from proximal lobe by an incision; proximal lobe well developed or reduced, trapezoidal, circular, ax-shaped or elongated proximally to reach and sometimes overlap gonopod principal axis (Fig. 2B). Meso-distal lobe arising from distocaudal angle of mesial surface, conical, subacute, or flattened caudo-cephalically, distally rounded. In cephalic view, mesial process in longitudinal position, oval shaped, closing apical cavity, distal margin widely rounded, lateral margin straight, armed with strong median spine (T. chontalpaensis, T. lamothei), or with moderate or reduced triangular tooth (T. diabolis, T. col sp. nov., T. poglayenorum, T. veracruzana); or in transversal position, reduced, spoon shaped, with lateral margin smooth without spine or tooth (T. ayotzintepecensis sp. nov., T. complanata, T. jacatepecensis, T. lamellifrons). Caudo-marginal projection with the two lobes directed cephalically, distal one slightly curved mesially; proximal one, ax-shaped or elongated proximally, and inclined in different gradations; carina of inner surface partially visible. Meso-distal lobe arise from caudal corner of G1 mesial surface, commonly is well developed, conical, with subacute or rounded apex. In lateral view, distal half inclined cephalically. Caudo-marginal projection with distal lobe (partially or totally visible; inner surface of proximal lobe with circular or semicircular strong carina, extending to different extents over proximal surface. In caudal view, distal third straight, apical cavity distally directed, caudal surface



**Figure 3.** Phylogenetic tree obtained for the genus *Tehuana* based on the concatenated analysis of three genes (H3, 16S, COI), using maximum likelihood and Bayesian inference. Branch supports values are posterior probability/bootstrap. Only clades with branch support above 50% indicated. Clades a, b, and c are labelled for discussion purposes.

ending distally in a wide and shallow concavity. Caudo-marginal projection with the distal crest of mesial surface higher or at the same level as lateral. Meso-distal lobe well developed, conical, with the apex rounded or subacute rounded, its position with respect the distal crest of mesial surface, could variate through different species. Mesial process as a longitudinal apical plate or transversal reduced with the distal surface excavated, in the first case, oval shaped, the distal margin could be widely rounded, and the lateral margin straight and armed with a strong median spine, or with a moderate or reduced triangular tooth; in the second, only partially visible. In distal view, apical cavity U-shaped, opening cephalically. Field of apical setae with 20 to 60 setae; aperture

of spermatic channel in caudal position; central crest ending cephalically in acute and triangular or rounded internal angle of mesial process (Fig. 2D, E).

**Remarks.** The phylogeny presented shows *Tehuana* to be closely related to *Disparithelphusa* (Fig. 2); however, there are strong differences in the G1 morphology: in *Tehuana* it is stouter, the lateral notch on the main shaft is in the middle, the internal surface of the proximal lobe of the caudo-marginal projection is marked with a strong carina, and the meso-distal lobe arises from the distocaudal corner of the mesial surface. Álvarez et al. (2020) in their phylogenetic analysis of the family also recovered *Tehuana* and *Disparithelphusa* as sister taxa.

**Distribution.** The species of *Tehuana* are distributed in southeastern Mexico covering the Isthmus of Tehuantepec region (Fig. 1) in Chiapas, Oaxaca, Tabasco, and Veracruz.

**Species included.** *Tehuana ayotzintepecensis* sp. nov.; *T. col* sp. nov.; *T. complanata* (Rathbun, 1905); *T. chontalpaensis* Villalobos & Alvarez, 2003; *T. diabolis* (Pretzmann, 1978); *T. jacatepecensis* Villalobos & Alvarez, 2003; *T. lamellifrons* (Rathbun, 1893); *T. lamothei* Alvarez & Villalobos, 1994; *T. poglayenorum* (Pretzmann, 1978); *Tehuana veracruzana* (Rodríguez & Smalley in Smalley, 1970).

# Key to the species of *Tehuana* based on the GI morphology

| 1 | Mesial process well developed with lateral spine (Fig. 9A)5                           |
|---|---|
| _ | Mesial process reduced without lateral spine (Fig. 9B)2                               |
| 2 | Mesial process cephalad oriented (Fig. 9H) T. jacatepecensis                          |
| _ | Mesial process distally oriented (Fig. 9F)  |
| 3 | Proximal lobe of CMP with rounded margins (Fig. 8F) T. lamellifrons                   |
| _ | Proximal lobe of caudo-marginal projection with internal margin straight (Fig. 8A, D) |
| 4 | Distal lobe of CMP as long as proximal one (Fig. 8A)                                  |
|   |   |
| _ | Distal lobe of CMP shorter than proximal one (Fig. 8D T. complanata                   |
| 5 | Lateral spine on lateral border of mesial process large, in proximal third            |
|   | (Fig. 9B, G) <b>6</b>   |
| _ | Lateral spine on lateral border of mesial process small or incipient in distal        |
|   | half (Fig. 9C, I, J)  |
| 6 | Mesial process oriented laterally, distal lobe of caudo-marginal projection well      |
|   | developed (Figs 8B, 9B) T. chontalpaensis   |
| _ | Mesial process oriented caudally, caudally, distal lobe of caudo-marginal pro-        |
|   | jection reduced (Figs 8G, 9G)   |
| 7 | Apex of gonopod strongly inclined cephalically, lobes of caudo-marginal pro-          |
|   | jection overlapping (Fig. 8C, I)  |
| _ | Apex of gonopod slightly inclined cephalically, lobes of caudo-marginal pro-          |
|   | jection not overlapping (Fig. 8E, J)  |

| 8 | Mesial process as widely rounded plate with a proximal triangular tooth   |
|---|---|
|   | (Fig. 9I)   |
| _ | Mesial process irregular in shape with a sinuous lateral margin (Fig. 9C) |
|   | <i>T. col</i> sp. nov.  |
| 9 | Proximal lobe of caudo-marginal projection 2.5× as high as wide (Fig. 8J) |
|   |   |
| _ | Proximal lobe of caudo-marginal projection 1.5× as high as wide (Fig. 8E) |
|   |   |

#### Tehuana ayotzintepecensis sp. nov.

http://zoobank.org/0B1975C1-09E6-4BFC-87CB-F487DAB0AF87 Figs 4, 5

**Type material.** *Holotype*: MEXICO – Oaxaca • 1 Å, CL 42.6 mm, CW 67.3 mm; Municipality of Ayotzintepec, Cajone River, south of Ayotzintepec town, stream tributary of Cajone River; 17°39'46"N, 96°07'51"W; alt. 128 m; 5 Mar. 2018; J.L. Villalobos, I.A. Toledano, E.G. Moreno leg; CNCR 34628. *Paratype*: 1 Å, CL 43.4 mm, CW 67.4 mm; same as for holotype; CNCR 36323.

Additional material examined. MEXICO – Oaxaca • 2 ♂, CL 26.5–28.2 mm, CW 40.6–42.4 mm; same data as for holotype; CNCR 36324.

Description. Carapace with dorsal surface flat, finely punctate, with small black granulations on frontal and anterolateral areas; gastric and branchial regions little inflated; postfrontal portion lightly depressed, almost horizontal, reaching anteriorly superior frontal border. Postfrontal lobes low, but evident, delimited anteriorly by shallow depressions, separated by narrow and deep median groove. Gastric pits deep, wide. Cardiac region discernible. Cervical groove shallow, curved, deep posteriorly, straight anteriorly, becoming obsolete near anterolateral margin, forming shallow notch. Anterolateral margin prominent, armed with 22-24 rounded granules of similar size; granulated between orbit and cervical groove, shallow notch next to orbit. Posterior margin straight (Fig. 4A). In frontal view, superior frontal border straight, formed by low tubercles, divided by deep, V-shaped median notch, external angle internally projected almost touching internal orbital tooth; inferior frontal border thin, granulated, sinuous, more projected than superior one (Fig. 4B). Orbits with external angle slightly granulated, with deep basal notch; internal orbital tooth triangular, well developed, extending to interior of orbit floor as high keel. Basal article of antennal peduncle separated from front by orbital hiatus. Antennules and antennular fossae partially visible, slightly widening in middle portion by an undulation of inferior frontal border; interantennular septum concealed by inferior frontal border. Operculum of antennal gland as ovoidal, flat plate, with middle constriction and tuft of short bristles on lateral third. Epistome devoid of setae; pterygostomian region with low granules; epistomal tooth triangular, directed downwards. Opening

of efferent branchial channel subrectangular, longer than wide, width/length ratio 0.68. Third maxilliped with trapezoidal ischium, slightly longer than wide; merus anterior margin rounded with shallow rounded notch in palp articulation; ratio exopod/ischium length 0.70.

Chelipeds distinctly heterochelous in males, subequal size in females. Major chela right, merus subtriangular in cross section, superior margin rounded with short transversal rows of low granules; lower inner margin with longitudinal row of conical tubercles increasing in size distally. Carpus proximal half with row of small conical tubercles, distal with scattered tubercles, ending in short acute spine, median spine obtuse. Fingers moderately gaping, punctate, cutting margins with rounded teeth; fixed finger with row of variable sized subtriangular teeth, median ones larger; tips slightly crossing when closed. Palm slightly swollen (length/width ratio 1.34), inner surface smooth, rest of palm with scattered black granules (Fig. 4C). Dactylus moderately arched, slightly longer than palm (dactylus/palm ratio 1.05).

G1 slender, proximal half cylindrical, becoming compressed distally, meso-distal lobe conical, well developed; principal axis with medial constriction on lateral surface, twisted mesially. In mesial view (Fig. 5B), distal half inclined towards cephalic surface. Caudomarginal projection with distal lobe well developed, subrectangular with cephalic margin rounded, separated from proximal lobe by an incision; proximal lobe well developed, ax-shaped, 1.24 as higher than long. Meso-distal lobe arise from caudal corner of mesial surface, well developed, conical, with rounded apex. In caudal view (Fig. 5C), distal third straight, apical cavity distally directed, caudal surface ending distally in wide, shallow concavity. Caudo-marginal projection distal crest of mesial surface higher than lateral one. Meso-distal lobe well developed, conical, apex rounded. Mesial process reduced, spoon shaped, only partially visible. In cephalic view (Fig. 5A), mesial process reduced, with the distal surface excavated, transversal to principal axis of G1, without spine on lateral margin, laying over carina of inner surface of proximal lobe of caudo-marginal projection; cephalic border bilobed, internal lobe pyramidal, rounded not touching internal face of caudo-marginal projection distal lobe, lateral lobe rounded, subcylindrical, projected anteriorly more than internal one. Field of apical setae visible, cephalo-caudally elongated, setae brownish, shorter than distal crest of lateral surface. Caudo-marginal projection lobes cephalically directed, separated, distal one slightly curved mesially; proximal one oval shaped, lateromesially inclined; carina of inner surface not visible. Distal crest of lateral surface sharp, with some tufts of short setae; subdistal circular scar on lateral face of principal axis partially visible. In lateral view (Fig. 5D), distal half inclined cephalically. Caudomarginal projection with the distal lobe partially visible, separated from the proximal lobe by incision, as long as lobe; inner surface of proximal lobe with semicircular strong carina, which extends over basal third of proximal surface. Mesial process lateral lobe subcylindrical, superior surface excavated, lateral border smooth, developing proximally rounded margin ending in subcircular scar. In distal view (Fig. 5E), apical cavity U-shaped, opening cephalically. Field of apical setae delimited by central crest and internal surface of lateral surface; 20-60 apical setae; aperture of spermatic channel in caudal position; central crest ending cephalically in acute, triangular internal lobe of mesial process, close to internal



**Figure 4.** *Tehuana ayotzintepecensis* sp. nov., male holotype **A** total dorsal view **B** frontal view of carapace **C** left chela. Scale bars: 4 cm (**A**); 2 cm (**B**, **C**).

surface of distal lobe of caudo-marginal projection. Mesial process with distal surface excavated, as an anterior continuation of the field of setae, raised border delimit the lateral and internal lobes. Meso-distal lobe well developed, conical, apex rounded.

Distribution. Only known from the type locality in northern Oaxaca, Mexico.

**Etymology.** The specific epithet is taken from the town where the specimens were collected: near town of Ayotzintepec. The word is Náhuatl "Ayotlzin-tepec", and means "hill of the little turtles" ("en el cerro de las tortuguitas" in Spanish).

**Remarks.** *Tehuana ayotzintepec* sp. nov. has the typical morphology of the species distributed throughout the Isthmus of Tehuantepec, with a reduced mesial process, cylindrical or spoon shaped, in a transversal position relative to the G1 principal axis

and laying over the proximal lobe of the caudo-marginal projection. These characters, make the new species similar to *T. complanata*, *T. jacatepecensis*, and *T. lamellifrons*; however, in *T. ayotzintepecensis* sp. nov. the two lobes of the caudo-marginal projection are of the same length in mesial view, the proximal one has a subacute inner angle and the distal one is completely rounded. Geographically, *T. ayotzintepecensis* sp. nov.



**Figure 5.** *Tehuana ayotzintepecensis* sp. nov., left G1 of male holotype **A** cephalic view **B** mesial view **C** caudal view **D** lateral view **E** distal view. Abbreviations: dl, distal lobe; ic, interal carena of the proximal lobe; imp, internal angle of the mesial process; ldc, lateral distal crest; mc, middle constriction; mdc, mesial distal crest; mdl, meso-distal lobe; mp, mesial process; ms, marginal suture; pl, proximal lobe; sf, spine field; sp, spermatic pore. Scale bars: 1 mm (**A–D**), 0.5 mm (**E**).

and *T. jacatepecensis* occur in the same general area along the Gulf of Mexico versant of the Sierra de Juárez in northern Oaxaca. In contrast, *T. lamellifrons* is distributed along the Pacific versant of the Sierra Madre Occidental in southern Oaxaca, and *T. complanata* occurs in central Veracruz (Fig. 1). The phylogenetic tree is consistent with the morphological similarity as it shows a close relationship between the four species (Fig. 3).

#### Tehuana col sp. nov.

http://zoobank.org/27434075-2C03-403B-89EE-0A0A412034EE Figs 6, 7

**Type material.** *Holotype*: MEXICO – Veracruz • 1 ♂, CL 30.4 mm, CW 50.3 mm; Municipality of San Andrés Tuxtla, Col River at Cascadas Park; 18°38'29"N, 95°09'00"W; alt. 416 m; 25 Apr. 2017; J.L. Villalobos, I.A. Toledano, E.G. Moreno leg; CNCR 33928. Paratype: 1 ♂, CL 13.6 mm, CW 20.8 mm; same data as for holotype; CNCR 36325.

Additional material examined. MEXICO – Veracruz • 1  $\bigcirc$ , CL 19.6 mm, CW 29.5 mm; same data as for holotype; CNCR 36325.

Description. Carapace dorsal surface slightly concave, finely punctate, frontal and anterolateral surfaces with minute granulations; gastric and branchial regions slightly inflated; postfrontal portion depressed, almost horizontal, continued anteriorly to reach superior frontal border. Postfrontal lobes low, delimited anteriorly by shallow depressions, separated by narrow, deep median groove. Cardiac region hardly discernible. Cervical groove shallow, curved posteriorly, anterior 1/4 straight, becoming obsolete before anterolateral margin, not reaching it. Anterolateral margin prominent, armed with 21–23 conical granules of similar size; portion between orbit and cervical groove granulated, with shallow notch next to orbit. Posterolateral area of carapace with short setae; posterior margin widely concave (Fig. 6A). In frontal view, superior frontal border inclined towards central portion, formed by low tubercles, divided by moderately deep, narrow V-shaped, median notch; inferior frontal border continuous, sinuous, thinner, more projected than superior one (Fig. 6B). Orbits with external angle slightly granulated, with shallow basal notch; internal orbital tooth triangular, well developed, extending to interior of orbit floor as high keel. Basal article of antennal peduncle separated from front by orbital hiatus. Antennules and antennular fossae partially visible, slightly wider in the middle; interantennular septum concealed by inferior frontal border. Operculum of antennal gland as ovoidal, flat plate, with middle constriction, tuft of short bristles on lateral third. Epistome, area around buccal cavity and pterygostomian region with short setae; epistomal tooth covered by patch of short setae, triangular, directed downwards. Opening of branchial efferent channel subcircular, longer than wide, width/length ratio 0.68. Third maxilliped with ischium subrectangular, slightly longer than wide, anterior margin of merus rounded; ratio exopod/ischium length 0.81.



**Figure 6.** *Tehuana col* sp. nov., male holotype **A** total dorsal view **B** frontal view of carapace **C** left chela. Scale bars: 4 cm (**A**); 3 cm (**B**); 2 cm (**C**).

Chelipeds moderately heterochelous in both sexes, more evident in males. Major chela right, merus subtriangular in cross section, superior margin rounded with short transversal rows of low granules; lower inner margin with longitudinal row of conical tubercles increasing in size distally (Fig. 6C).

G1 slender, proximal half cylindrical, becoming compressed distally, meso-distal lobe on mesial surface conical, well developed; principal axis with medial constriction on lateral surface, twisted mesially. In mesial view (Fig. 7B), distal half inclined towards cephalic surface. Caudo-marginal projection with distal lobe well developed, subrectangular, cephalic margin rounded, separated from proximal lobe by an incision; proximal lobe well developed, ax-shaped, higher than long. Distal crest of MP, partially visible, some rounded and higher than apical cavity. Meso-distal lobe arising from caudal angle of mesial surface, well developed, conical, with subacute apex. In caudal view (Fig. 7C), distal



**Figure 7.** *Tehuana col* sp. nov., left G1 of male holotype **A** cephalic view **B** mesial view **C** caudal view **D** lateral view **E** distal view. Abbreviations: cdc, caudal distal crest; dl, distal lobe; dmp, distal border of mesial process; ic, internal carena of the proximal lobe; imp, internal angle of the mesial process; mc, middle constriction; mdl, meso-dital lobe; mp, mesial process; ms, marginal suture; pl, proximal lobe; sp, spermatic pore. Scale bars: 1 mm (**A–D**); 0.5 mm (**E**).

third straight, apical cavity distally directed, caudal surface ending distally in a wide and shallow concavity. Caudo-marginal projection with distal crest of mesial surface as high or higher than lateral one. Mesial process as a longitudinal plate, caudally undulated, partially visible; subdistal circular scar on the base of the plate partially visible. In cephalic view (Fig. 7A), mesial process in longitudinal position relative to principal axis of G1, with an incipient rounded tooth on lateral margin, laying over carina of inner surface of proximal lobe of caudo-marginal projection; cephalic border bilobed, internal lobe pyramidal, rounded not touching internal face of the distal lobe of caudo-marginal projection, lateral lobe rounded, semicylindrical, projected anteriorly beyond internal one. Field of apical setae visible, cephalo-caudally elongated, setae brownish, shorter than distal crest of lateral surface. Lobes of caudo-marginal projection cephalically directed, separated, slightly curved mesially; proximal one oval shaped, lateromesially inclined, inner surface carina not visible. Distal crest of lateral surface sharp, smooth, with few short setae; subdistal circular scar on principal axis partially visible. In lateral view (Fig. 7D), distal lobe of caudo-marginal projection partially visible, separated from proximal one by long incision; inner surface of proximal lobe with circular or semicircular strong carina, extending over basal third of proximal surface. Mesial process as a longitudinal plate that close the apical cavity; lateral margin of smooth, superior angle rounded, median portion with an incipient rounded tooth, continued proximally to end in a subcircular scar. In distal view (Fig. 7E), Apical cavity U-shaped, opening cephalically, field of apical setae delimited by central and lateral crests; 20-60 apical setae; aperture of spermatic channel in caudal position; central crest ending cephalically in acute, triangular internal lobe of mesial process. Meso-distal lobe (MDL) well developed, conical, distal tip curved mesocephalically.

Distribution. Only known from type locality.

**Etymology.** The name of this species is taken from the River Col, Los Tuxtlas region of Veracruz, where the specimens were collected. We declare the specific epithet as noun in apposition.

**Remarks.** *Tehuana col* sp. nov. is morphologically similar to *T. poglayenorum* which occurs in the same area in Los Tuxtlas region, with both exhibiting partially overlapping lobes of the caudo-marginal projection; however, they can be easily separated by the mesial process, irregular shape with a sinuous lateral margin in the former, versus a widely rounded plate with a proximal triangular tooth in the latter. Consistent with the morphology, *T. col* sp. nov. and *T. poglayenorum* are also genetically closely related (Fig. 3), and in turn they are related to *T. diabolis* and *T. veracruzana*. It is interesting to highlight that four clearly defined species of *Tehuana* together with *Smalleyus tricristatus* Alvarez, 1989 and *Pseudothelphusa parabelliana* Alvarez, 1989 occur in Los Tuxtlas region which is small mountain range occupying an 80 × 33 km area in the coastal plain of southern Veracruz.

# Tehuana complanata (Rathbun, 1905)

Figs 8D, 9D

- ? Pseudothelphusa bocourti.—Rathbun, 1898: 512 (in key), 513, 533 (list) [not Boscia bocourti A. Milne-Edwards, 1866].
- Pseudothelphusa complanata Rathbun, 1905: 303, fig. 3.—Coifmann 1939: 107 (list). "Pseudothelphusa" complanata.—Pretzmann, 1965: 10 (list).

- Pseudothelphusa (Pseudothelphusa) lamellifrons gruneri.—Pretzmann, 1968: 7.— Pretzmann 1971: 22 (list).
- Pseudothelphusa (Pseudothelphusa) americana lamellifrons.—Bott, 1970: 334, pl. 2, figs 11–13.
- Potamocarcinus (Raddaus) bocourti complanata.—Pretzmann, 1971: 20.(list).
- Potamocarcinus (Raddaus) bocourti complanatus.—Pretzmann, 1972: 78, text fig. 18, fig. 542.
- Pseudothelphusa (Tehuana) lamellifrons gruneri.—Pretzmann, 1972: 108, figs 621–623, 674–677.

*Pseudothelphusa (Tehuana) cordobensis* Rodríguez & Smalley, 1972: 77, fig. 8, pl. 5. *Pseudothelphusa (Tehuana) complanata* Türkay, 1978: 145, figs 2a, b, 3.

*Tehuana complanata* Rodriguez, 1982: 131, fig. 85.—Villalobos 1982: 221 (list).— Villalobos-Hiriart et al. 1993: 284 (list).—Alvarez and Villalobos 1994: 730 .— Álvarez et al. 1999: 20, fig. 3 (map), 23, box 3 (list).—Villalobos and Alvarez 2003: 2003 (in key) .—Villalobos Hiriart and Álvarez 2008: 280, 298 (list).—Ng et al. 2008: 177 (list).—Villalobos and Alvarez 2010: 474, 477, fig. 11 (map).— Álvarez et al. 2011a: appx. VIII.20, p. 13 (list).—Mejía-Ortíz et al. 2011: 97, 136 (map 2).—Álvarez et al. 2012: 1078, box 1 (list).—Cumberlidge et al. 2014: 144, tab. 3 (list).—Alvarez and Villalobos 2016: 254, tab.8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).

**Material examined.** MEXICO – Veracruz • 1 ♂, holotype of *Pseudothelphusa (Tehuana) cordobensis* Rodríguez and Smalley (1972); LC 38.2 mm, AC 59.0 mm; Municipality of Córdoba Ojo de Agua, Paraje Nuevo, 18°52'35"N, 96°51'49"W; alt. 661 m; 2 May 1953; A. Villalobos leg.; CNCR 311. 1 ♂; LC 30.0 mm, AC 45.4 mm; Municipality of Amatlán de Los Reyes, Lourdes River Cave; 18°47'00"N, 96°54'00"W; alt. 439 m; 13 Jun. 1996; J. Herrera, E. Ramírez leg.; CNCR 11958. 3 ♂, 3 ♀; LC 15.4–27.9 mm, AC 22.9–42.7 mm; Municipality of Amatlán de Los Reyes, Amatlán II Power Station; 18°51'23"N, 96°54'19"W; alt. 730 m; 6 Jun. 1992; J. Herrera, E. Ramírez leg.; CNCR 11957. 1 ♀, LC 26.2 mm, AC 18.9 mm; Motzorongo, Municipality of Tezonapa, Motzorongo River; 18°38'30"N, 96°43'53"W; alt. 271 m; C. Pedraza, L. García leg.; CNCR 34618.

**Diagnosis.** G1, in cephalic view, with three protuberances on lateral surface, proximal one being the most developed. In mesial view, meso-distal lobe conical, with round and slender apex. In caudal view, median constriction forming a large lobe oriented proximally. Caudo-marginal projection with distal lobes separated by linear notch without leaving space in between. Distal lobe with rounded cephalic edge, shorter than proximal lobe. Proximal lobe ax-shaped, higher than distal (1.5×); cephalic border circular, caudal border straight; distal crest slightly laterally oriented, lobe with sloping appearance. Internal carina well marked, circular, its length covering at least <sup>1</sup>/<sub>3</sub> of internal surface. Mesial process reduced, in transversal position, without lateral spine; distal edge oriented cephalad. Internal angle developed in form of lobe, two-thirds as high as mesial process, wide, touching the internal surface of distal lobe



Figure 8. Mesial view of the apical portion of the G1 of the species of *Tehuana* A *T. ayotzintepecensis* sp. nov., CNCR 34628 B *T. chontalpaensis*, CNCR 17093 C *T. col* sp. nov., CNCR 33928 D *T. complanata*, CNCR 11957 E *T. diabolis*, CNCR 12056 F *T. lamellifrons*, CNCR 33939 G *T. lamothei*, CNCR 8812 H *T. jacatepecensis*, CNCR 11920 I *T. poglayenorum*, CNCR 33931 J *T. veracruzana*, CNCR 33934. Scale bars: 1 mm.

(DL) of CMP. In distal view, mesial process concave, internal angle hidden below the proximal lobe (PL) of caudo-marginal projection.

**Distribution.** *Tehuana complanata* is distributed around the city of Cordoba, Veracruz, Mexico (Fig. 1).

**Remarks.** The recognition of the type locality of *Tehuana complanata* has been problematic since Rathbun (1905) cited "Coban, Alta Vera Paz, Guatemala" as the type locality of *Pseudothelphusa complanata*. Later, Rodríguez and Smalley (1972) described *Pseudothelphusa (Tehuana) cordobensis* from "Paraje Nuevo, Córdoba, Veracruz" which fits the description of *T. complanata*. Türkay (1978) discussed this situation concluding that it was a labelling error by Bocourt who placed crabs from Veracruz, Mexico in a jar with specimens from Coban, Guatemala. Rodriguez (1982) synonymized *P. (T.) cordobensis* under *T. complanata* and designated the male from "Paraje Nuevo, Córdoba, Veracruz" (CNCR 311) as the holotype.

Morphologically, the G1 of *T. complanata* is similar to that of *T. jacatepecensis*; both species have a broadly rounded to semicircular proximal lobe of the CMP, although in the former the distal and proximal lobes are subequal in length, whereas in the latter the proximal lobe is clearly shorter (Fig. 8D). *Tehuana complanata* is also similar to *T. lamellifrons* even when they are geographically distant within the genus range (Fig. 1); however, in the obtained phylogeny they appear as sister species (Fig. 3).



Figure 9. Cephalic view of the apical portion of the G1 of the species of *Tehuana* A *T. ayotzintepecensis* sp. nov., CNCR 34628 B *T. chontalpaensis*, CNCR 17093 C *T. col* sp. nov., CNCR 33928 D *T. complanata*, CNCR 11957 E *T. diabolis*, CNCR 12056 F *T. lamelliftons*, CNCR 33939 G *T. lamothei*, CNCR 8812 H *T. jacatepecensis*, CNCR 11920 I *T. poglayenorum*, CNCR 33931 J *T. veracruzana*, CNCR 33934. Scale bars: 1 mm (A, C, H, J); 0.5 mm (B, D, E, F, G, I).

# Tehuana chontalpaensis Villalobos & Alvarez, 2003

Figs 8B, 9B

*Tehuana chontalpaensis* Villalobos & Alvarez, 2003: 224, 228 (in key), figs 2, 4A.— Álvarez et al. 2005: 191.—Rodríguez and Magalhães 2005: 356, tab. 1 (list).— Villalobos Hiriart and Álvarez 2008: 280, 298 (list).—Ng et al. 2008: 177 (list).— Villalobos and Alvarez 2010: 474, 477, fig. 11 (map).—Alvarez and Villalobos 2016: 254, tab.8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).—Álvarez et al. 2020: 979, tab. 1 (list).

**Material examined.** MEXICO – Tabasco • 1  $\Diamond$ , *holotype*; LC 35.1 mm, AC 57.3 mm; Municipality of Huimanguillo, Carlos A. Madrazo, Pueblo Viejo Stream; 17°23'45"N, 93°39'45"W; alt. 135 m; 8 May 1997; J.L. Villalobos leg.; CNCR 18952. 2  $\Diamond$ , paratypes; LC 17.3–24.3 mm, AC 27.7–37.8 mm; same data as for holotype; 12 Jun. 1997; J.L. Villalobos, R. Robles leg.; CNCR 17093. 1  $\Diamond$ , 1  $\bigcirc$ , paratypes; LC 11.0– 14.8 mm, AC 17.0–23.0 mm; same collection data as for holotype; 12 Jun. 1997; J.L. Villalobos, R. Robles leg.; CNCR 17171. 1  $\Diamond$ , 2  $\heartsuit$ ; LC 10.0–22.0 mm, AC 14.2–34.0 mm; Municipality of Huimanguillo, 3 km E of Carlos A. Madrazo, small tributary of Pedregal-Tonala River; 17°23'52"N, 93°40'51"W; alt. 116 m; 22 Jan. 1998; J.L. Villalobos, R. Robles leg.; CNCR 17290. Chiapas 1 ♂; LC 25 mm, AC 42 mm; Municipality of Ocozocoautla, Reserva de la Biósfera Selva el Ocote, Cerro Cola de Sapo, Frio Stream; 18 Dic 2008; A. García and M. Anzueto leg.; CNCR 25445.

Diagnosis. As in Villalobos and Alvarez (2003).

Distribution. Only known from the type locality and surroundings (Fig. 1).

**Remarks.** As noted by Villalobos and Alvarez (2003) *Tehuana chontalpaensis* is morphologically similar to *T. lamothei*, a similarity that is consistent with their being sister species as shown in the molecular phylogeny (Fig. 3).

#### Tehuana diabolis (Pretzmann, 1978)

Figs 8E, 9E

- Pseudothelphusa (Tehuana) lamellifrons diabolis Pretzmann, 1978: 3.—Pretzmann 1980: 660, pl. 13, figs 56–60, pl. 17, fig. 77.—Rodriguez 1982: 210.
- *Tehuana diabolis.*—Villalobos-Hiriart et al. 1993: 284 (list) .—Alvarez and Villalobos 1994: 730, 735.—Álvarez and Villalobos 1997a: 416, 417, box 4.17 (list).—Álvarez and Villalobos 1997b: 438, appx. 4.23 (list).—Álvarez et al. 1999: 20, fig. 3 (map), 23, box 3 (list).—Villalobos and Alvarez 2003: 228 (in key).—Villalobos and Álvarez 2008: 281, 298 (list).—Ng et al. 2008: 177 (list).—Villalobos and Alvarez 2010: 474, 477, fig. 11 (map).—Álvarez et al. 2011a: appx. VIII.20, p. 13 (list); 2012: 1078, box 1 (list).—Alvarez and Villalobos 2016: 254, tab. 8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).—Álvarez et al. 2020: 979, tab. 1 (list).

Material examined. MEXICO – Veracruz • 2 ♀, CL 8.2–13.5 mm, CW 12.4–20.2 mm; Municipality of Catemaco, Veracruz, Catemaco Lake, Playa Hermosa; 18°26'00"N, 95°04'60"W; alt. 351 m; 31 Aug. 1966; L. Holthuis, J. Cabrera leg.; CNCR 333. 3 Å, CL 12.1–16.4 mm, CW 18.1–25.7 mm; Municipality of Catemaco, Catemaco Lake, El Zapotal; 18°25'00"N, 95°05'60"W; alt. 335 m; 18 Sep. 1954; A. Villalobos leg.; CNCR 334. 4 ♂, 6 ♀, CL 12.7–27.1 mm, CW 20.1–42.3 mm; Municipality of Catemaco, Catemaco Lake, Las Margaritas Stream; 18°22'04"N, 95°01'01"W; alt. 345 m; 4 Aug. 1994; M.E. Camacho leg.; CNCR 12956. 4 Å, CL 12.1-23.4 mm, CW 17.7-38.1 mm; same locality as previous record; 6 Aug. 1994; F. Álvarez leg.; CNCR 12965. 1 Å, CL 26.4 mm, CW 43.6 mm; same locality as previous record; 20 Apr. 2016; E. Moreno leg.; CNCR 34488. 1 3, 1 2, CL 12.9-74.6 mm, CW 11.9-44.7 mm; Municipality of Catemaco, 1 km S from Coyame; 18°25'50.6"N, 95°01'16"W; alt. 364 m; 6 Aug. 1994; J.L. Villalobos leg.; CNCR 12952. 2 Q, CL 11.7-18.2 mm, CW 17.7-23.2 mm; same locality as previous record; 1 Aug. 1994; M.E. Camacho leg.; CNCR 12966. 2 Q, CL 12.8-15.7 mm, CW 18.8-22.5 mm; Municipality of Catemaco, Catemaco Lake, La Agayota; 18°24'02"N, 95°00'06"W; alt. 545 m; 1 Jul. 1986; F. Álvarez leg.; CNCR 12907. 1 Å, 4 Q, CL 11.3–24.6 mm, CW 16.2–38.2 mm; same locality as previous record; 18 Jul. 1986; J.L. Villalobos leg.; CNCR 12911. 1 Å, CL 13.1 mm, CW 20.1 mm; same locality as previous record;

4 Aug. 1994; F. Álvarez leg.; CNCR 12954. 1 ♀, CL 26.3 mm, CW 42.4 mm; same locality as previous record; 18 Jul. 1986; F. Álvarez leg.; CNCR 13125.

**Diagnosis.** G1 slender, proximal half cylindrical, distal half becoming compressed. In caudal view, apical crest widely concave, mesial crest higher than lateral one, Meso-distal lobe conical, tip rounded. In mesial view, distal third of gonopod slightly inclined cephalically, caudo-marginal projection bilobed, lobes separated by V-shaped notch, distal lobe shorter than proximal one, rounded; proximal lobe axshaped, cephalic margin broadly rounded, internal margin straight. In cephalic view, caudo-marginal projection slightly curved mesially, meso-distal lobe prominent; mesial process as a widely rounded plate, distal margin rounded, lateral margin with triangular tooth, cephalic margin with rounded projection closing the apical cavity. In lateral view, mesial process oblique relative to apical cavity longitudinal axis, mesial crest clearly higher than lateral one. In apical view, apical cavity U-shaped, opening of sperm channel in caudal position, field of apical pore setae on lateral portion of cavity, caudal crest thicker than the rest.

**Type material.** The holotype (NHMW 4068) and paratypes (NHMW 4069) are deposited in the Natural History Museum in Vienna, Austria.

**Type locality.** México, Veracruz, Municipality of Catemaco, Catemaco Lake; 18°25'00"N, 95°06'00"W; alt. 325 m (Pretzmann 1980).

**Distribution.** Only known from the north and eastern shores of Catemaco Lake, Veracruz, Mexico (Fig. 1).

**Remarks.** A diagnosis, based on the description of G1 is provided for *T. diabolis* since Pretzmann (1980) description and subsequent mentions of the species by other authors omitted important morphological details of G1. In *Tehuana diabolis* G1 the proximal lobe of the caudo-marginal projection has an intermediate shape between those of *T. veracruzana* and *T. poglayenorum* (Fig. 8), and in the phylogenetic tree the three species appear also closely related (Fig. 3). Geographically, *T. diabolis* distribution around Lake Catemaco is also between that of *T. poglayenorum* to the northeast and that of *T. veracruzana* to the south of Catemaco Lake in the town of Zapoapan de Cabañas.

#### Tehuana jacatepecensis Villalobos & Alvarez, 2003

Figs 8H, 9H

Tehuana jacatepecensis Villalobos & Alvarez, 2003: 226, 228 (in key), figs 3, 4B.—Rod-ríguez and Magalhães 2005: 356, tab. 1 (list).—Villalobos Hiriart and Álvarez 2008: 280, 298 (list).—Ng et al. 2008: 177 (list).—Villalobos and Alvarez 2010: 475, 477, fig. 11 (map).—Mejía-Ortíz et al. 2011: 99, 136 (map 2).—Alvarez and Villalobos 2016: 254, tab. 8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).

Material examined. MEXICO – Oaxaca • 1 ♂, *holotype*; CL 30.5 mm, CW 48.0 mm; Municipality of Santa María Jacatepec, Santo Domingo River in Santa María Jacatepec; 17°51'37"N, 96°12'36"W; alt. 54 m; 23 May 1992; L. Huidobro, C. Rosas, D. Becerril, R. Palma leg.; CNCR 11920. 2 3, 1 2, CL 11.9–25.9 mm, CW 17.6–39.9 mm; Municipality of San Juan Bautista Tuxtepec, km 165 highway Tuxtepec-Palomares, El Zapote stream; 17°09'51"N, 95°09'35" W; alt. 167 m; 27 Sep. 1981; R. Lamothe leg.; CNCR 8817. 3 Å, 2 ♀, CL 15.9–24.4 mm, CW 24.1–37.2 mm; Municipality of Santa María Jacatepec, San Isidro El Naranjal, El Mazate waterfall; 17°53'41"N, 96°08'01"W; alt. 103 m; 3 Mar. 2018; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 34620. 5 ♂, 3 ♀, CL 11.4–28.1 mm, CW 16.6–43.7 mm; Municipality of Santa María Jacatepec, stream in San Isidro El Naranjal; 17°53'32"N, 96°07'46"W; alt. 84 m; 3 Mar. 2018; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 34622. 3 ♂, 7 ♀, CL 7.4–11.9 mm, CW 10–17 mm; Municipality of San José Chiltepec, Arroyo de Pueblo Viejo; 17°54'26"N, 96°03'12"W; alt. 79 m; 3 Mar. 2018; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 34640. 1 ∂, 1 ♀, CL 16.7–35.8 mm, CW 24.6–43.3 mm; Municipality of San Juan Bautista Tuxtepec, Tuxtepec-Palomares highway; 17°09'00"N, 95°06'00"W; alt. 96 m; collection data unknown; CNCR 8806. Veracruz • 2 3, 3 2, CL 9–43 mm, CW 12.8–67.3 mm; Municipality of Playa Vicente, Nueva Era; 1 km from Santa Rosa; 17°41'22"N, 95°48'55"W; alt. 111 m; 7 Mar. 2018; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 34624. 3 ♂, 3 ♀, CL 11.3–21.6 mm, CW 15.6–31.2 mm; Municipality of Playa Vicente, El Tomate, El Manantial Ranch, Manzo River; 17°41'52.3"N, 95°51'51"W; alt. 43 m; 6 Mar. 2018; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 34626.

Diagnosis. As in Villalobos and Alvarez (2003).

**Distribution.** This species is distributed in and around the town of Santa María Jacatepec in northern Oaxaca, Mexico (Fig. 1).

**Remarks.** As noted by Villalobos and Alvarez (2003), the G1 of *T. jacatepecensis* is morphologically similar to those of *T. complanata* and *T. lamellifrons* (Figs 8, 9), and coincidentally the three species together with *T. ayotzintepecensis* sp. nov. are also closely related genetically forming a separate clade in the phylogenetic tree presented herein (Fig. 3).

# Tehuana lamellifrons (Rathbun, 1893)

Figs 8F, 9F

*Pseudothelphusa lamellifrons* Rathbun, 1893: 654, pl. 75, figs 2–5; 1898: 534, 537.— Young 1900: 221.—Rathbun 1905: 304.—Coiffman, 1939: 108.

Potamocarcinus lamellifrons.—Ortmann, 1897: 317 (key).

Pseudothelphusa (Pseudothelphusa) lamellifrons.—Pretzmann, 1965: 4.

- Pseudothelphusa (Tehuana) lamellifrons lamellifrons.—Pretzmann, 1971: 22 (list).— Pretzmann 1972: 107, figs 612–614, 662–664.
- Pseudothelphusa (Tehuana) lamellifrons.—Rodríguez & Smalley, 1972: 79, fig. 10.— Türkay 1978: 144.

Tehuana lamellifrons.—Rodriguez, 1982: 129, fig. 84.—Villalobos 1982: 220 (list).—
Villalobos-Hiriart et al. 1993: 284 (list).—Alvarez and Villalobos 1994: 730.—
Álvarez and Villalobos 1997a: 416, 417, fig. 4.17 (list).—Álvarez and Villalobos 1997b: 438, appx. 4.23 (list).—Álvarez et al. 1999: 20, fig. 3 (map).—Villalobos

and Alvarez 2003: 228 (in key).—Villalobos Hiriart and Álvarez 2008: 282, 298 (list).—Ng et al. 2008: 177 (list).—Villalobos and Alvarez 2010: 475, 477, fig. 11 (map).—Guinot and Hendrickx 2014: 477.—Alvarez and Villalobos 2016: 254, tab. 8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).

**Material examined.** MEXICO – Oaxaca • 1  $\Diamond$ , CL 50.4 mm, CW 80.6 mm; Municipality of Asunción Ixtaltepec, Nizanda, Cerro del Naranjo, Naranjo stream; 16°41'14.8"N, 95°02'09"W; alt. 293 m; 15 Apr. 1999; D. Barreto, V.H. Reynoso leg.; CNCR 16875. 1  $\Diamond$ , CL 31.2 mm, CW 43.4 mm; Municipality of Asunción Ixtaltepec, Naranjo stream; 16°41'24"N, 95°22'53"W; alt. 639 m; 15 Sep. 1997; V.H. Reynoso leg.; CNCR 18951. 3  $\Diamond$ , 2  $\heartsuit$ , CL 8.9–40.8 mm, CW 12.4–65 mm; Municipality of Asunción Ixtaltepec, Nizanda, stream; 16°39'30"N, 95°00'37"W; alt. 186 m; 26 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33939.

**Diagnosis.** G1 slender, almost straight. Meso-distal lobe conical, in mesial view somewhat compressed caudo-cephalically. Mesial process in transversal position relative to G1 main axis, laying over proximal lobe of caudo-marginal projection, reduced, rounded, superior margin projected distally, without lateral tooth; in cephalic view internal angle triangular, pointing towards distal lobe of caudo-marginal projection (Figs 8F, 9F). Lobes of CMP separated by long incision, lobes not gaping; in cephalic view proximal lobe oblique relative to main axis of gonopod, distal lobe slightly curved laterally. Distal lobe of caudo-marginal projection simple, rounded, projected cephalically; proximal lobe broadly rounded extending proximally, with internal semicircular carina. Apical cavity with elongated field of setae next to lateral margin; opening of spermatic channel in caudal position.

**Type material.** The syntypes are deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM 3289).

**Type locality.** Mexico, Oaxaca, Municipality of Santo Domingo Tehuantepec, Tehuantepec, Tehuantepec, Tehuantepec River; 16°18'60"N, 95°13'60"W; alt. 60 m (Rathbun, 1893).

**Distribution.** Along the Pacific versant of the Isthmus of Tehuantepec, in the drainage systems of the Tehuantepec and Zanatepec rivers, in SW Oaxaca, Mexico (Fig. 1).

**Remarks.** A new diagnosis for *T. lamellifrons* is here presented since those of Rathbun (1893) and Rodríguez and Smalley (1972) are too short omitting important characters of the G1. Other remarks see those for *T. jacatepecensis*.

#### Tehuana lamothei Alvarez & Villalobos, 1994

Figs 8G, 9G

Tehuana lamothei Alvarez & Villalobos, 1994: 732, figs 2, 4c.—Álvarez and Villalobos 1995: 93.—Villalobos and Alvarez 2003: 228 (in key).—Rodríguez and Magalhães 2005: 356, tab. 1 (list).—Villalobos Hiriart and Álvarez 2008: 282, 298 (list).—Ng et al. 2008: 177 (list).—Villalobos and Alvarez 2010: 474, 477, fig. 11 (map).— Álvarez et al. 2011b: 289.—Cumberlidge et al. 2014: 147, tab. 7 (list).—Alvarez and Villalobos 2016: 254, tab. 8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).

**Material examined.** Mexico – Chiapas • 1 ♂, *holotype*; CL 18.2 mm, CW 27.5 mm; Municipality of Ixtacomitán, 1 km from Ixtacomitán, La Piedra stream; 17°24'00"N, 93°06'00"W; alt. 232 m; 4 Apr. 1986; J.L. Villalobos, J.C. Nates, A. Cantú, D. Valle; CNCR 5604. 2 ♂, CL 21–24 mm, CW 32–37.2 mm; Municipality of Tapilula, stream near Tapilula; 17°16'05"N, 93°01'33"W; alt. 780 m; 20 Apr. 1981; R. Lamothe leg.; CNCR 8812.

**Diagnosis.** As in Alvarez and Villalobos (1994).

**Distribution.** Restricted to a small area in NE Chiapas, Mexico (Fig. 1). **Remarks.** As in *T. chontalpaensis*.

# Tehuana poglayenorum (Pretzmann, 1978)

Figs 8I, 9I

- Pseudothelphusa (Tehuana) lamellifrons poglayenorum Pretzmann, 1978: 3; 1980: 660, pl. 12, figs 51–55.
- Tehuana poglayenorum.—Villalobos-Hiriart et al. 1993: 284 (list).—Álvarez and Villalobos 1997a: 416, 417, box 4.17 (list); 1997b: 338, appx. 4.23 (list).—Álvarez et al. 1999: 20, fig. 3 (map), 23, box 3 (list).—Villalobos and Alvarez 2003: 228 (in key).—Villalobos Hiriart and Álvarez 2008: 283, 298 (list).—Villalobos and Alvarez 2010: 475, 477, fig. 11 (map).—Álvarez et al. 2011a: appx. VIII.20, p. 13 (list); 2012: 1078, box 1 (list).—Cumberlidge et al. 2014: 147, tab. 7 (list).—Alvarez and Villalobos 2016: 254, tab. 8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).

*Tehuana poglayenora.*—Ng et al. 2008: 177 (list) [error]. *Tehuana lamellifrons.*—Poettinger et al. 2016: 1722, tab. I (list).

Material examined. Mexico – Veracruz • 4 Å, CL 11.4–26.0 mm, CW 17.0–41.2 mm; Municipality of San Andrés Tuxtla, Basura River; 18°31'55"N, 95°03'30"W; alt. 33 m; 19 Jul. 1998; R. Robles, C. Graham leg.; CNCR 17422. 1 2, CL 15.8 mm, CW 22.9 mm; same locality as previous record; 18 Jul. 1986; J.L. Villalobos, F. Álvarez leg.; CNCR 13140. 3 Å, 5 ♀, CL 8.9–28.8 mm, CW 13.1–45.2 mm; same locality as previous record; 4 Oct. 1994; J.L. Villalobos, F. Álvarez leg.; CNCR 13187. 7 Å, 1 Q, CL 9.6-21.8 mm, CW 14.7-36.4 mm; same locality as previous record; 24 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33931. 4 ♂, 11 ♀, CL 11.8–19.5 mm, CW 12.8-33.2 mm; Municipality of Santiago Tuxtla, Tapalapan River; 18°32'00"N, 95°18'00"W; alt. 393 m; 17 Apr. 1957; H. Hobbs, A. Villalobos leg.; CNCR 336. 5 ♀, CL 25.1–36.1 mm, CW 40.6–59.1 mm; same locality as previous record; 23 May 1955; A. Villalobos leg.; CNCR 338. 4 3, 4 2, CL 7.3–14 mm, CW 10.8–20.3 mm; same locality as previous record; 17 April 1957; H. Hobbs, A. Villalobos leg.; CNCR 386. 2 Å, CL 10.9–11.9 mm, CW 12.4–18.8 mm; Municipality of San Andrés Tuxtla, Otapan River; 18°26'00"N, 95°12'00"W; alt. 379 m; 22 Sep. 1955; G. Pérez leg.; CNCR 337. 2 Å, CL 11.9–20.7 mm, CW 21.9–30.3 mm; Municipality of San Andrés Tuxtla,

Laguna Escondida; 18°35'00"N, 95°05'00"W; alt. 76 m; 1 Aug. 1985; J.L. Villalobos, M.D. Valle, P. Schmidtsdorf leg.; CNCR 4473. 15 3, 10 9, CL 7.4–21.7 mm, CW 10.4-34.9 mm; same locality as previous record 13 Jun. 1985; C. Nates, J.L. Villalobos leg.; CNCR 4709. 1 3, 3 9, CL 8.2-25.6 mm, CW 11.6-41 mm; same locality as previous record; 11 Jul. 1994; J.L. Villalobos, F. Álvarez leg.; CNCR 5303. 4 3, 3  $\mathcal{Q}$ , CL 25.4–32.3 mm, CW 42.2–53.2 mm; same locality as previous record; 24 Feb. 1989; M. Santiago leg.; CNCR 10220. 2 3, 2 2, CL 11–19.4 mm, CW 15.5–30.5 mm; same locality as previous record; 17 Jul. 1985; J.L. Villalobos, F. Álvarez leg.; CNCR 12908. 6 ♂, 4 ♀, CL 11.7–18.7 mm, CW 17.7–45.9 mm; same locality as previous record; 5 Aug. 1994; J.L. Villalobos leg.; CNCR 12964. 2 3, 2 2, CL 6.8–11.3 mm, CW 9.3– 17.0 mm; same locality as previous record; 10 Jul. 1986; F. Álvarez leg.; CNCR 13138. 1 Å, CL 20.0 mm, CW 32.3 mm; Municipality of San Andrés Tuxtla, Playa Escondida; 18°35'00"N, 95°03'00"W; alt. 6 m; 28 Feb. 1986; A. Cantú leg.; CNCR 5782. 3 3, 5 ♀, CL 13.3–17.2 mm, CW 20.0–27.0 mm; same locality as previous record; 28 Feb. 1986; A. Cervantes, J. García, A. Cantú leg.; CNCR 5788. 1 3, 4 2, CL 9.2–20.9 mm, CW 13.1-32.1 mm; same locality as previous record; 28 Feb. 1986; R. Lamothe leg.; CNCR 8821. 6 ♂, 11 ♀, CL 6.3–20.8 mm, CW 9.1–32.8 mm; Municipality of San Andrés Tuxtla, trail to Laguna Escondida; 18°35'00"N, 95°04'00"W; alt. 108 m; 24 Feb. 1989; M. Santiago leg.; CNCR 10222. 1 &, 2 Q, CL 11.8–21.7 mm, CW 17.3– 35.1 mm; same locality as previous record; 3 Aug. 1994; F. Álvarez, J.L. Villalobos leg.; CNCR 12967. 9 ♂, 5 ♀, CL 9.7–28.6 mm, CW 14.3–49.7 mm; same locality as previous record; 23 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33927. 13 ♂, 21 ♀, CL 9.9–30.0 mm, CW 14.7–50.4 mm; Municipality of San Andrés Tuxtla, Lázaro Cárdenas;18°34'00"N, 95°06'00"W; alt. 359 m; 9 Jul. 1991; A. Cruz leg.; CNCR 12302. 1 Å, CL 20.3 mm, CW 32.3 mm; Municipality of San Andrés Tuxtla, Cuetzalapan River; 18°24'00"N, 95°00'00"W; alt. 220 m; 13 Jul. 1986; F. Álvarez leg.; CNCR 12555. 2 ♂, 1 ♀, CL 11.3–23.6 mm, CW 18.0–37.7 mm; Municipality of San Andrés Tuxtla, La Palma River; 18°33'00"N, 95°03'00"W; alt. 77 m; 12 Jul. 1986; F. Álvarez leg.; CNCR 12896. 1 ♂, 1 ♀, CL 21.1–23.0 mm, CW 35.0–38.5 mm; Balzapote River; 18°36'00"N, 95°04'00"W; alt. 54 m; Municipality of San Andrés Tuxtla, Veracruz; 14 Jul. 1986; F. Álvarez leg.; CNCR 12906. 7 ♂, 3 ♀, CL 13.0–20.3 mm, CW 20.0-32.5 mm; Municipality of San Andrés Tuxtla, Máquinas River; 18°37'00"N, 95°05'00"W; alt. 143 m; 1 Aug. 1994; F. Álvarez, J.L. Villalobos leg.; CNCR 12961. 1 ♂, 2 ♀, CL 10.1–18.3 mm, CW 16.1–21.6 mm; Municipality of San Andrés Tuxtla, Zacatal Lagoon; 18°35'00"N, 95°06'00"W; alt. 263 m; 14 Oct. 1994; F. Álvarez, J.L. Villalobos leg.; CNCR 13188. 4 3, CL 11.8–23.4 mm, CW 17.5–37.2 mm; Municipality of Santiago Tuxtla, Simapan River; 18°27'00"N, 95°21'00"W; alt. 336 m; 3 May 1995; J.L. Villalobos leg.; CNCR 13346.

**Diagnosis.** G1 slender, in mesial view distal third inclined cephalically. Mesodistal lobe conical, tip rounded, large relative to size of other apical structures (Fig. 9I). Mesial process well developed; lateral margin with triangular, acute tooth; distal margin broadly rounded; internal angle with triangular projection in cephalic position. Lobes of the caudo-marginal projection elongated, overlapping along most of their length; proximal lobe curved cephalically, distal lobe straight, oriented forward or with the tip moderated curved proximally, slightly longer than proximal one. In mesial view, distal lobe with superior margin inclined proximally; distally, falling over proximal lobe. Internal carina of proximal lobe rounded, prominent, separated from lobe. Apical cavity with elongated field of setae, opening of spermatic channel in caudal position.

**Type material.** The holotype (NHMW 4066) and paratypes (NHMW 4067) are deposited in the Natural History Museum in Vienna, Austria.

**Type locality.** Mexico, Veracruz, Municipality of San Andrés Tuxtla, Basura River; 18°32'00"N, 95°03'00"W; alt. 33 m (Pretzmann, 1978).

**Distribution.** *Tehuana poglayenorum* is distributed in the north-central section, of the Los Tuxtlas region.

**Remarks.** *Tehuana poglayenorum* is the most widely distributed and abundant freshwater crab in the whole Los Tuxtlas Mountain Range. It belongs to clade c, where all the species from Los Tuxtlas are grouped (Fig. 1). We noted there small morphological variations through its distribution range: in the carapace with setae or without them, and in the G1 in the caudo-marginal projection and mesial process.

Poettinger et al. (2016) presented the sequence of the16S mitochondrial gene (KU578859) as belonging to *T. lamellifrons*; however, the two females in the lot NHMW 4067 (from Río Basura, state of Veracruz) are the paratypes of *T. poglayenorum* designated by Pretzman (1978, 1980).

# *Tehuana veracruzana* (Rodríguez & Smalley in Smalley 1970)

Figs 8J, 9J

Pseudothelphusa (Tehuana) veracruzana Rodríguez & Smalley in Smalley, 1970: 100, fig. 11. Pretzmann 1972: 108.—Rodríguez and Smalley 1972: 77, fig. 9.—Türkay 1978: 148, fig. 4.—Guinot and Hendrickx 2014: 478, tab. 1 (list).

Tehuana veracruzana.—Rodriguez, 1982: 131, fig. 86.—Villalobos 1982: 221 (list).—
Rodríguez 1986: 59, fig. 10l, 61, fig.12—Villalobos-Hiriart et al. 1993: 284.—
Álvarez and Villalobos 1995: 93; 1997a: 416, 417, box 4.17 (list); 1997b: 338, appx. 4.23 (list).—Álvarez et al. 1999: 20, fig. 3 (map), 23, box 3 (list).—Villalobos and Álvarez 2003: 228 (in key).—Villalobos Hiriart and Álvarez 2008: 284, 298 (list).—Ng et al. 2008: 177 (list).—Villalobos and Alvarez 2010: 474, 477, fig. 11 (map).—Álvarez et al. 2011a: appx. VIII.20, p. 13 (list); 2012: 1078, box 1 (list).—Guinot and Hendrickx 2014: 477.—Cumberlidge et al. 2014: 147, tab. 7 (list).—Alvarez and Villalobos 2016: 254, tab. 8.1 (list).—Villalobos-Hiriart et al. 2019: 156, tab.1 (list).

**Material examined.** MEXICO – Veracruz • 1 ♂, *bolotype*; CL 48.0 mm, CW 28.2 mm; Municipality of Catemaco, Zapoapan de Cabañas Stream; 18°20'00"N, 95°05'48"W; alt. 518 m; 15 Apr. 1957; A. Villalobos, H.H. Hobbs leg.; CNCR 335. 1 ♂, 1 ♀, *paratypes*; CL 23.8–28.2 mm, CW 37.2–47.5 mm; same data as for holotype; CNCR 335. 1 Å, 1 Q, CL 15.7–27.8 mm, CW 24.8–45.2 mm; Municipality of Catemaco, stream near Zapoapan de Cabañas; 18°20'09"N, 95°02'22"W; alt. 629 m; 25 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33934. 1 Å, 3 Q, CL 12.1–20.5 mm, CW 18.4–32.5 mm; Municipality of Catemaco, stream near Zapoapan de Cabañas; 18°20'32"N, 95°04'13"W; alt. 364 m; 25 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33932. 1 Å, 2 Q, CL 9.2–12.5 mm, CW 14.5–21.6 mm; Municipality of Catemaco, stream near Zapoapan de Cabañas; 18°19'18"N, 95°03'02"W; alt. 364 m; 25 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33937. 1 Å, 1 Q, CL 9.8–20.5 mm, CW 14.4–32.2 mm; Municipality of Catemaco, road Zapoapan de Cabañas–Zoteapan; 18°17'52"N, 94°58'06"W; alt. 848 m; 25 Apr. 2017; J.L. Villalobos, I.A. Toledano, E. Moreno leg.; CNCR 33955.

Diagnosis. As in Rodríguez and Smalley (1972).

**Distribution.** This species occurs in the southeastern portion of the Los Tuxtlas Mountain Range, in an area that starts sloping towards the coastal plain of southern Veracruz.

**Remarks.** *Tehuana veracruzana* is easily distinguishable from the rest of its congeners due to the very large proximal lobe of the CMP of the G1 (Fig. 8). Although it exhibits an extreme form of the G1 it is genetically closely related to the other species from Los Tuxtlas (Fig. 1).

### Discussion

The ten species of the genus *Tehuana* are distributed in the Isthmus of Tehuantepec, from central Veracruz to northern Chiapas (Fig. 1). The Isthmus of Tehuantepec has a complex geologic history being the area where three tectonic plates interact: North American, Cocos, and Caribbean (Barrier et al. 1998). As a result, the region has an intricate geography that has deeply influenced the evolution of many biological groups (e.g., Mendonça et al. 2022). It has also acted as a barrier for dispersal, promoting speciation and lineage differentiation (Gutiérrez-García and Vázquez-Domínguez 2013). Many examples from mammals and birds to plants, insects, and amphibians (Ornelas et al. 2013; Mendoza et al. 2019), show that the region harbors a high diversity and several areas of endemism. Interestingly, the Isthmus has acted as a barrier at different times for different groups of organisms.

For pseudothelphusid crabs, the Isthmus of Tehuantepec is a region where 16 genera belonging to three subfamilies occur, representing the highest diversity of lineages in the whole range of the family (Villalobos and Álvarez 2010; Álvarez et al. 2020). The involved genera represent major lineages, one with Central American affinity (subfamily Potamocarcininae), a second one that encompasses southern Mexico and northern Central America (subfamily Raddausinae) and a third one from central and northwestern Mexico (subfamily Pseudothelphusinae) (Álvarez et al. 2020).

The genus *Tehuana* is distributed entirely within the Isthmus of Tehuantepec, forming three groups, as indicated in the phylogenetic tree (Fig. 1). A salient feature is that they occupy both the Gulf of Mexico and Pacific slopes. In contrast to other

groups of species, especially in the genus *Pseudothelphusa*, where progressive variation of the G1 morphology can be seen, in *Tehuana* the morphological variation of the CMP and MP do not follow a geographic gradient. The phylogenetic analysis groups species that are not morphologically the most similar, one example is the clade formed by the species from Los Tuxtlas region which show significant variation in the CMP.

According to Álvarez et al. (2020) *Tehuana* is one of the most recent groups to appear within the subfamily Pseudothelphusinae; the age estimate presented by these authors (2.5–0.5 mya) explains the relative short branches obtained in our phylogenetic tree (Fig. 3). The branching pattern within *Tehuana* we obtained has a geographic correlation with clade a, being the oldest one in Tabasco and Chiapas, Clade b, occurring in a diagonal band the stretches from central Veracruz to Oaxaca, and clade c, restricted to Los Tuxtlas region in Veracruz (Fig. 3). This progression suggests that the species in the genus radiated from southwest to northeast within the Isthmus of Tehuantepec.

# Acknowledgements

We thank Laura Márquez, Andrea Jiménez, and Nelly López from the Biodiversity National Laboratory (LaNaBio) of the Institute of Biology, UNAM, for their assistance in the DNA sequencing. Susana Guzmán of the Institute of Biology, UNAM offered technical assistance while taking the photographs used in this study. Eric Moreno gratefully acknowledges the Posgrado en Ciencias Biológicas, UNAM, and the financial support received through a CONACYT graduate scholarship (register-607746, scholarship 448293). We thank Celio Magalhães for his interest in this work and his valuable comments.

# References

- Alvarez F (1989) Smalleyus tricristatus, new genus, new species, and Pseudothelphusa parabelliana, new species (Brachyura: Pseudothelphusidae) from Los Tuxtlas, Veracruz, Mexico. Proceedings of the Biological Society of Washington 102(1): 45–49. https://www. biodiversitylibrary.org/page/34606636#page/65/mode/1up
- Alvarez F, Villalobos JL (1991) A new genus and two new species of freshwater crabs from México, Odontothelphusa toninae and Stygothelphusa lopezformenti (Crustacea: Brachyura: Pseudothelphusidae). Proceedings of the Biological Society of Washington 104(2): 288–294. https://www.biodiversitylibrary.org/page/34808980#page/310/mode/1up
- Alvarez F, Villalobos JL (1994) Two new species and one new combination of freshwater crabs from Mexico (Crustacea: Brachyura: Pseudothelphusidae). Proceedings of the Biological Society of Washington 107(4): 729–737. https://www.biodiversitylibrary.org/ page/35515374#page/763/mode/1up

- Álvarez F, Villalobos JL (1995) Lista Anotada de los Tipos de la Colección de Crustáceos (Decapoda: Brachyura) del Instituto de Biología, de la Universidad Nacional Autónoma de México. Revista de la Sociedad Mexicana de Historia Natural 46: 86–96. https://research. nhm.org/pdfs/31883/31883.pdf
- Álvarez F, Villalobos JL (1997a) Pseudothelphusidae y Trichodactylidae (cangrejos). In: González E, Dirzo R, Vogt RC (Eds) Historia Natural de Los Tuxtlas. Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, 415–418.
- Álvarez F, Villalobos JL (1997b) Decapoda. In: González E, Dirzo R, Vogt RC (Eds) Historia Natural de Los Tuxtlas. Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, 433–438.
- Alvarez F, Villalobos JL (1998) Six new species of fresh-water crabs (Brachyura: Pseudothelphusidae) from Chiapas, Mexico. Journal of Crustacean Biology 18(1): 187–198. https://doi.org/10.2307/1549533
- Alvarez F, Villalobos JL (2016) Chapter 8: Freshwater decapod diversity and conservation in México. In: Kawai T, Cumberlidge N (Eds) A global overview of the conservation of freshwater decapod crustaceans. Springer, Cham, 237–266. https://doi.org/10.1007/978-3-319-42527-6\_8
- Álvarez F, Villalobos JL, Lira E (1996) Decapoda. In: Llorente J, García, AN, Gónzalez E (Eds) Biodiversidad, taxonomía y biogeografía de artrópodos de México: Hacia una síntesis de su conocimiento. Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, 103–129.
- Álvarez F, Villalobos JL, Rojas Y, Robles R (1999) Listas y comentarios sobre los crustáceos decápodos de Veracruz, México. Anales del Instituto de Biologia Universidad Nacional Autónoma de México 70(1): 1–27. http://www.ejournal.unam.mx/zoo/070-01/ZOO70101.pdf
- Álvarez F, Villalobos JL, Robles R (2005) Crustáceos, Cap. 8. In: Bueno J, Álvarez F, Santiago S (Eds) Biodiversidad del Estado de Tabasco. Instituto de Biología, Universidad Nacional Autónoma de México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, 177–195.
- Álvarez F, Villalobos JL, Cházaro-Olvera S (2011a) Camarones y cangrejos dulceacuícolas y marinos (Crustacea: Decapoda). In: Cruz-Angón A, Lorea-Hernández FG, Hernández-Ortiz V, Morales-Mavil JE (Eds) La biodiversidad en Veracruz, estudio de estado, diversidad de especies: conocimiento actual. Vol. 2. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Gobierno del Estado de Veracruz, Universidad Veracruzana, Instituto de Ecología, 287–294.
- Álvarez F, Villalobos JL, Elías-Guiérrez M, Rivera G (2011b) Crustáceos dulceacuícolas y terrestres de Chiapas. In: Álvarez F (Coord) Chiapas: estudios sobre su diversidad biológica. Universidad Nacional Autónoma de México, México, 209–297.
- Álvarez F, Villalobos JL, Armendariz G, Hernández C (2012) Relación biogeográfica entre cangrejos dulceacuícolas y acociles a lo largo de la zona mexicana de transición: Revaluación de la hipótesis de Rodríguez (1986). Revista Mexicana de Biodiversidad 83(4): 1073–1083. https://doi.org/10.22201/ib.20078706e.2012.4.1268

- Álvarez F, Ojeda JC, Souza-Carvalho E, Villalobos JL, Magalhães C, Wehrtmann IS, Mantelatto FL (2020) Revision of the higher taxonomy of Neotropical freshwater crabs of the family Pseudothelphusidae, based on multigene and morphological analyses. Zoological Journal of the Linnean Society 193(3): 973–1001. https://doi.org/10.1093/zoolinnean/zlaa162
- Barret RDH, Hebert PDN (2005) Identifying spiders through DNA barcodes. Canadian Journal of Zoology 83(3): 481–449. https://doi.org/10.1139/z05-024
- Barrier E, Velasquillo L, Chavez M, Gaulon R (1998) Neotectonic evolution of the Isthmus of Tehuantepec (southeastern Mexico). Tectonophysics 287(1–4): 77–96. https://doi. org/10.1016/S0040-1951(98)80062-0
- Bott R (1968) Fluß-Krabben aus dem östlichen Mittel-Amerika und von den Großen Antillen (Crustacea, Decapoda). Senckenbergiana biologica 49(1): 39–49. https://decapoda.nhm. org/pdfs/28147/28147.pdf
- Bott R (1970) Betrachtungen über die Entwicklungsgeschichte und Verbreitung der Süßwasserkrabben nach der Sammlung des Naturhistorischen Museums in Genf/Schweiz. Revue Suisse de Zoologie 77: 327–344. https://doi.org/10.5962/bhl.part.75900
- Coifmann I (1939) Potamonidi dell R. Museo Zoologico di Torino. Archivo Zoologico Italiano 27: 93–116.
- Colgan DJ, McLauchlan C, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone 3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46(5): 419–437. https://doi.org/10.1071/ ZO98048
- Cumberlidge N, Álvarez F, Villalobos JL (2014) Results of the global conservation assessment of the freshwater crabs (Brachyura, Pseudothelphusidae and Trichodactylidae): The Neotropical region, with an update on diversity. ZooKeys 45: 122–137. https://doi. org/10.3897/zookeys.457.6598
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): e772. https://doi.org/10.1038/nmeth.2109
- de Saussure H (1857) Diagnoses de quelques Crustacés nouveaux des Antilles et du Mexique. Revue et Magasin de Zoologie pure et appliquée, série 2, 9: 304–308. https://decapoda. nhm.org/pdfs/15439/15439.pdf
- Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. Journal of Molecular Evolution 17(6): 368–376. https://doi.org/10.1007/BF01734359
- Gelman A, Ribun D (1992) Inference from iterative simulation using multiple sequences. Statistical Science 7(4): 457–511. https://doi.org/10.1214/ss/1177011136
- Guinot D, Hendrickx ME (2014) Correct date and authorship of taxa of Middle American freshwater crabs described by Rodríguez and Smalley (1972) (not 1969) and included in Smalley (1970) (Crustacea: Decapoda: Brachyura: Pseudothelphusidae). Zootaxa 3821(4): 476–484. https://doi.org/10.11646/zootaxa.3821.4.6
- Gutiérrez-García A, Vázquez-Domínguez E (2013) Consensus between genes and stones in the biogeographic and evolutionary history of Central America. Quaternary Research 79(3): 311–324. https://doi.org/10.1016/j.yqres.2012.12.007
- Hurvich CM, Tsai CL (1989) Regression and time series model selection in small samples. Biometrika 76(2): 297–307. https://doi.org/10.1093/biomet/76.2.297

- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054
- Maddison WP, Maddison DR (2019) Mesquite: a modular system for evolutionary analysis. Version 3.61. http://www.mesquiteproject.org
- Mejía-Ortíz L, López-Mejía M, Martínez MI (2011) Crustáceos decápodos del Río Papaloapan, Veracruz. Universidad de Quintana Roo, Unidad Cozumel, México, 155 pp.
- Mendonça P, Dias C, Aleixo A, Carneiro LS, Araripe J, Rego PS (2022) Diversification across the Isthmus of Tehuantepec explains the phylogeographic arrangement of the widespread brightrumped Attila (*Attila spadiceus*; Tyrannidae) and reveals the existence of two major lineages. Journal of Ornithology 163(1): 327–332. https://doi.org/10.1007/s10336-021-01928-3
- Mendoza AM, Bolívar-García W, Vázquez-Domínguez E, Ibañez R, Parra-Olea G (2019) The role of Central American barriers in shaping the evolutionary history of the northernmost glassfrog, *Hyalinobatrachium fleischmanni* (Anura: Centrolenidae). PeerJ 7: e611. https://doi.org/10.7717/peerj.6115
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 1–8. https://doi.org/10.1109/GCE.2010.5676129
- Milne-Edwards A (1866) Descriptions de trois nouvelles species du genre Boscia, Crustacés Brachyures de la tribu des Tepheusiens. Annales de la Société Entomologique de France 6(4): 203–205. https://www.biodiversitylibrary.org/item/107112#page/209/mode/1up
- Ng PKL, Low MEY (2010) On the generic nomenclature of nine brachyuran names, with four replacement names and two nomina protecta (Crustacea: Decapoda). Zootaxa 2489(1): 34–46. https://doi.org/10.11646/zootaxa.2489.1.2
- Ng PKL, Guinot D, Davie PJF (2008) Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. The Raffles Bulletin of Zoology 17: 1–286. https://lkcnhm.nus.edu.sg/wp-content/uploads/sites/10/app/uploads/2017/04/s17rbz.pdf
- Ornelas JF, Sosa V, Soltis DE, Daza JM, González C, Soltis PS, Gutiérrez-Rodríguez C, Espinosa de los Monteros A, Castoe TA, Bell C, Ruiz-Sanchez E (2013) Comparative phylogeographic analysis illustrate the complex evolutionary history of threatened cloud forests of northern Mesoamerica. PLoS ONE 8(2): e56283. https://doi.org/10.1371/journal.pone.0056283
- Ortmann AE (1893) Die Decapoden-Krebse des Strassburger Museums, mit besonderer Berücksichtigung der von Herrn Dr. Döderlein bei Japan und bei den Liu-Kiu-Inseln gesammelten und zur Zeit im Strassburger Museum aufbewahrten Formen. VII. Theil. Abtheilung: Brachyura (Brachyura genuina Boas) II. Unterabtheilung: Cancroidea, 2. Section: Cancrinea, 1. Gruppe: Cyclometopa. Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Thiere 7(3): 411–495. https://doi.org/10.5962/bhl.part.24064
- Ortmann AE (1897) Carcinologische Studien. Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Thiere 10(3): 258–372. https://decapoda.nhm.org/ pdfs/28108/28108.pdf

- Palumbi SR, Benzie J (1991) Large mitochondrial DNA differences between morphologically similar penaeid shrimp. Molecular Marine Biology and Biotechnology 1: 27–34.
- Poettinger T, Klaus S, Schubart CD (2016) Phylogenetic relationships among neotropical freshwater crabs of the Pseudothelphusidae (Decapoda, Brachyura) and the taxonomic position of *Ptychophallus* and related genera. Crustaceana 89(14): 1717–1728. https://doi. org/10.1163/15685403-00003627
- Pretzmann G (1965) Vorläufiger Bericht über die Familie Pseudothelphusidae. Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Klasse der Österreichischen Akademie der Wissenschaften, Wien 1: 1–11. https://decapoda.nhm.org/pdfs/27647/27647. pdf
- Pretzmann G (1968) Mexicanische S
  üßwasserkrabben der Gattung Pseudothelphusa (Vorl
  äufige Mitteilung). Entomologisches Nachrichtenblatt, Wien 15(2): 6–8. https://decapoda. nhm.org/pdfs/28117/28117.pdf
- Pretzmann G (1971) Fortschritte in der Klassifizierung der Pseudothelphusidae. Sitzungsberichte der Mathematisch-naturwissenschaftlichen Klasse der Österreichischen Akademie der Wissenschaften, Wien 179: 15–24. https://decapoda.nhm.org/pdfs/27495/27495.pdf
- Pretzmann G (1972) Die Pseudothelphusidae (Crustacea, Brachyura). Zoologica 42(1): [pt 120] 15–182. https://decapoda.nhm.org/pdfs/30682/30682.pdf
- Pretzmann G (1978) Neue Potamocarcini, Poglayen-Neuwald leg. 1975 (vorläufige Mitteilung). Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Klasse der Österreichischen Akademie der Wissenschaften, Wien 187(2): 51–54. https://decapoda. nhm.org/pdfs/28121/28121.pdf
- Pretzmann G (1980) Von Dr Ivo Poglayen-Neuwall 1975 in Mittelamerika gesammelte Krabben. Annalen des Naturhistorichen Museums in Wien 83: 651–666. https://decapoda. nhm.org/pdfs/31219/31219.pdf
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi. org/10.1093/sysbio/syy032
- Rathbun MJ (1893) Descriptions of new species of American Freshwater crabs. Proceedings of the United States National Museum 16(959): 649–661. https://doi.org/10.5479/ si.00963801.16-959.649
- Rathbun MJ (1898) A contribution to a knowledge of the fresh-water crabs of America. The pseudothelphusinæ. Proceedings of the United States National Museum 21(1158): 507–537. https://doi.org/10.5479/si.00963801.21-1158.507
- Rathbun MJ (1905) Les Crabes d'eau douce (Potamidae). Nouvelles Archives du Museum d'Histoire naturelle. Paris, series 4 7: 159–321.
- Rathbun MJ (1933) A new species of *Pseudothelphusa* from Mexico. Journal of the Washington Academy of Sciences 23(7): 360–360. https://decapoda.nhm.org/ pdfs/27792/27792.pdf
- Rodriguez G (1982) Les crabes d'eau douce d'Amérique. Famille des Pseudothelphusidae. Faune Tropicale, ORSTOM, Paris 22: 1–224. https://decapoda.nhm.org/pdfs/11760/11760.pdf
- Rodríguez G (1986) Centers of radiation of freshwater crabs in the Neotropics. In: Gore RH, Heck KL (Eds) Crustacean Biogeography. Crustacean Issues. Vol. 4. Rotterdam: A.A. Balkema Press, 51–67. https://doi.org/10.1201/9781315140674-3

- Rodríguez G, Magalhães C (2005) Recent advances in the biology of the neotropical freshwater crab family Pseudothelphusidae (Crustacea, Decapoda, Brachyura). Revista Brasileira de Zoologia 22(2): 354–365. https://doi.org/10.1590/S0101-81752005000200009
- Rodríguez G, Smalley AE (1972) Los cangrejos de agua dulce de México de la familia Pseudothelphusidae (Crustacea, Brachyura). Anales del Instituto de Biología, Universidad Nacional Autónoma de México, serie Ciencias del Mar y Limnología 40: 69–112. https://decapoda.nhm.org/pdfs/27336/27336.pdf
- Ronquist F, Huelsenbeck JD (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, applications note 19(12): 1572–1574. https://doi.org/10.1093/ bioinformatics/btg180
- Smalley AE (1970) A new genus of freshwater crabs from Guatemala, with a key to the Middle American genera (Crustacea, Decapoda, Pseudothelphusidae). American Midland Naturalist 83(1): 96–106. https://doi.org/10.2307/2424009
- Smalley AE, Adkison DL (1984) Disparithelphusa pecki, a new genus and species of fresh water crab from Mexico (Brachyura: Pseudothelphusidae). Journal of Crustacean Biology 4(1): 127–133. https://doi.org/10.2307/1547901
- Stamatakis A (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics (Oxford, England) 30(1): 1312–1313. https://doi. org/10.1093/bioinformatics/btu033
- Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17: 57–86.
- Türkay M (1978) Die identität von *Pseudothelphusa complanata* Rathbun, 1905 mit Bemerkugen zu *Pseudothelphusa (Tehuana)* Rodríguez & Smalley, 1969 (Crustacea: Decapoda: Pseudothelphusidae). Senckenbergiana Biologica 59(1/2): 143–150.
- Villalobos A (1982) Decapoda. In: Hulbert SH, Villalobos A (Eds) Aquatic Biota of Mexico, Central America and the West Indies, San Diego University, California, 215–239.
- Villalobos JL, Álvarez F (2003) Two new species of freshwater crabs of the genus *Tehuana* (Brachyura: Pseudothelphusidae) from southern Mexico. Journal of Crustacean Biology 23(1): 223–229. https://doi.org/10.1163/20021975-99990329
- Villalobos JL, Álvarez F (2010) Phylogenetic analysis of the Mexican freshwater crabs of the tribe Pseudothelphusini (Decapoda: Brachyura: Pseudothelphusidae). Zoological Journal of the Linnean Society 160(3): 457–481. https://doi.org/10.1111/j.1096-3642.2009.00606.x
- Villalobos Hiriart JL, Álvarez F (2008) Los cangrejos de agua dulce de la familia Pseudothelphusidae (Decapoda: Brachyura: Eubrachyura) de México. Con un apéndice de las especies citadas para América hasta el 2006. In: Álvarez F, Rodríguez G (Eds) Crustáceos de México: Estado actual de su conocimiento. UANL-PROMEP-SEP. Dirección General de Publicaciones, Universidad Autónoma de Nuevo León, México, D.F., 239–299.
- Villalobos-Hiriart JL, Díaz-Barriga A, Lira-Fernández E (1993) Los crustáceos de agua dulce de México. Revista de la Sociedad Mexicana de Historia Natural 44: 267–290.
- Villalobos-Hiriart JL, Kamanli SA, Álvarez F, Garbout A, Clark PF (2019) Lobithelphusa mexicana Rodriguez, 1982 (Decapoda: Pseudothelphusidae): a reassessment of key characters and systematics. Zootaxa 4586(1): 151–161. https://doi.org/10.11646/zootaxa.4586.1.8
- Young CG (1900) The stalk-eyed Crustacea of British Guiana, West Indies, and Bermuda. London, 514 pp. https://doi.org/10.5962/bhl.title.10670
RESEARCH ARTICLE



# Two new species of the genus *Paradexamine* (Crustacea, Amphipoda, Dexaminidae) from Korean Waters

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| Academic editor: Alan Myers   Received 21 April 2022   Accepted 28 June 2022   Published 11 August 2 | 2022 |
|--|------|
| https://zoobank.org/2BCB4390-CD80-4A32-B4AB-3139F92FC6FF   |      |

**Citation:** Zhang X, Kim K-W, Kim Y-H (2022) Two new species of the genus *Paradexamine* (Crustacea, Amphipoda, Dexaminidae) from Korean Waters. ZooKeys 1117: 37–52. https://doi.org/10.3897/zooKeys.1117.85644

#### Abstract

Two dexaminid amphipod species belonging to the genus *Paradexamine* were collected from Korean waters. After observation and identification compared with related congeners, these two species are revealed to be new to science. In comparative identification, one of the new species, *P. acuta* **sp. nov.** is similar to *P. houtete* in having an acutely rounded lobe and posteroventrally pointed coxa 7. However, this new species is distinguished from *P. houtete* in having a larger number of medial setae on the propodus of gnathopod 1 and a rounded basis of pereopod 6. This new species is also very similar to *P. marlie* s.l. Hirayama from Japanese waters, and *P. marlie* s.l. might be re-established or synonymized with *P. acuta* **sp. nov.** in the future. The other new species, *P. rotundogena* **sp. nov.** is similar to *P. tafunsaka* in having a rounded eye lobe; however, it is distinguished from *P. tafunsaka* in having an elongate carpus on gnathopod 2 and the differently shaped basis of pereopod 7. A key to the five Korean species of *Paradexamine*, including the two new species, is also provided.

#### Keywords

Crustacea, Paradexamine acuta sp. nov., Paradexamine rotundogena sp. nov., taxonomy

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# Introduction

Twelve genera of the family Dexaminidae have been reported worldwide (Lowry and Myers 2017; Horton et al. 2022). Among these genera, the genus *Paradexamine* Stebbing, 1899 is the largest genus of the family and is easily distinguished from other genera in having a distinct cephalic lobe and dorsal teeth on the pleonites. In *Paradexamine*, the number of dorsal pleon teeth increases with growth of juveniles, but the teeth remain fairly stable in their count in adults (Barnard 1972a). *Paradexamine* is divided into two groups based on characteristics of the cephalic lobe, which is either rounded or acute. Of the 46 paradexaminid species in the world, there are approximately three times as many species with the cephalic lobe rounded as those with the cephalic lobe acute.

Dexaminids occur in various habitats but are most common among algae, sand, gravel, or rubble habitats in relatively shallow water (Myers and LeCroy 2009). Paradexaminids mainly live in algae habitats and have phototactic (light preference) responses, so often appear in shallow Korean waters in night surveys using light traps.

Hitherto, three *Paradexamine* species with an acute cephalic lobe have been recorded from Korea: *P. fraudatrix* Tzvetkova, 1976, *P. gigas* Hirayama, 1984, and *P. jindoensis* Kim & Lee, 2008 (Kim et al. 2006; Kim and Lee 2008). In addition, we add two new species, *P. acuta* and *P. rotundogena* to the Korean dexaminid fauna. Furthermore, a paradexaminid with a rounded cephalic lobe is recorded for the first time in Korean waters. A key to the Korean *Paradexamine* species is also given.

## Materials and methods

Materials for this study were obtained by scuba diving from Baengnyeongdo, Chujado, Geomundo, and Jejudo Islands located off Korea's west and south coasts from 2018-2022 (Fig. 1). The specimens were fixed with 95% ethanol and dissected in glycerol on Cobb's aluminum hollow slides. Permanent mounts were made using polyvinyl lactophenol with lignin pink added. Pencil drawings were made and measurements were taken using a drawing tube mounted on an Olympus SZX 12 stereomicroscope and an Olympus BX 51 interference contrast compound microscope. Line drawings were produced using the program 'Graphic'. Body length was measured from the tip of the rostrum to the posterior end of the urosome, along the dorsal parabolic line of the body. In this study, we followed the scheme of Barnard (1972a), who counted dorsal pleonal teeth from the rear to the front, commencing with pleonite 4 and progressing forward to pereonite 6. Number "3" means the segment has 1 mediodorsal and 2 dorsolateral teeth; "1" means the only mediodorsal tooth is present. Type specimens are deposited at the Marine Biodiversity Institute of Korea (MABIK) in Seocheon, Korea and the Department of Biological Science, Dankook University (DKU), Cheonan, Korea.



**Figure 1.** Distribution of the *Paradexamine acuta* sp. nov. (red circle) and *P. rotundogena* sp. nov. (blue square): 1 = Baengnyeongdo Island, 2 = Chujado Island, 3 = Geomundo Island, 4 = Jongdal Port, Jejudo Island.

## Taxonomy

# Order Amphipoda Latreille, 1816 Suborder Amphilochidea Boeck, 1871 Family Dexaminidae Leach, 1814

## Genus Paradexamine Stebbing, 1899

Species composition. Paradexamine acuta sp. nov.; P. aequiserrata (Myers & LeCroy, 2009); P. alkoomie (Barnard, 1972a); P. barnardi (Sheard, 1938); P. bisetigera (Hirayama, 1984); P. churinga (Barnard, 1972a); P. dandaloo (Barnard, 1972a); P. echuca (Barnard, 1972a); P. excavata (Ledoyer, 1984); P. exilis (Myers & LeCroy, 2009); P. fissicauda (Chevreux, 1906); P. flindersi (Stebbing, 1888); P. fraudatrix (Tzvetkova, 1976); P. frinsdorfi (Sheard, 1938); P. gigas (Hirayama, 1984); P. goomai (Barnard, 1972a); P. houtete (Barnard, 1972b); P. indentata (Ledoyer, 1978); P. jindoensis (Kim & Lee, 2008); P. lanacoura (Barnard, 1972a); P. harlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. marlie (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. massa (Myers & LeCroy, 2009); P. linga (Barnard, 1972a); P. massa (Myers & LeCroy, 2

2009); P. maunaloa (Barnard, 1970); P. micronesica (Ledoyer, 1979); P. miersi (Haswell, 1885); P. moorehousei (Sheard, 1938); P. mozambica (Ledoyer, 1979); P. muriwai (Barnard, 1972b); P. nana (Stebbing, 1914); P. narluke (Barnard, 1972a); P. orientalis (Spandl, 1923); P. otichi (Barnard, 1972a); P. pacifica (Thomson, 1879); P. quadratus (Myers & LeCroy, 2009); P. quarallia (Barnard, 1972a); P. rewa (Myers, 1985); P. rotundogena sp. nov.; P. ronngi (Barnard, 1972a); P. saxeta (Myers & LeCroy, 2009); P. serraticra (Walker, 1904); P. setigera (Hirayama, 1984); P. sexdentata (Schellenberg, 1931); P. tafunsaka (Myers, 1995); P. thadalee (Barnard, 1972a); P. windarra (Barnard, 1972a).

#### Paradexamine acuta sp. nov.

https://zoobank.org/4FCC0F2F-0AFE-4200-94FF-F8418E61342B Figs 2A, 3–5 Korean name: Ppyo-jok-yeop-ga-si-but-eun-kko-ri-yeop-sae-u, new

**Type material.** *Holotype:* female, 5.5 mm, MABIK CR00250813, Korea, Incheon, Baengnyeongdo Island, Dumujin, 37°58'36"N, 124°37'09"E, 13 August 2020, scuba collection in red alga *Gelidium* sp., depth 5–10 m, S.G. Lee & Y.H. Kim leg. *Paratypes:* 3 females, 4.6 mm, 5.1 mm, and 5.4 mm, DKUAMP202201, same station data as holotype.

Additional material examined. 22 females, DKUAMP202202, Korea, Incheon, Baengnyeongdo Island, 37°58'26"N, 124°38'39"E, Y.H. Kim leg., 12 August 2020. 1 female, Korea, Chujado Island, 33°57'13"N, 126°18'08"E, Z. Xin, K.W. Kim, & Y.H. Kim leg., 27 August 2021. 7 females, Korea, Jejudo Island, Jongdal Port, 33°29'49"N, 126°54'41"E, Y.H. Kim leg., 5 February 2022.

**Diagnosis.** Lateral cephalic lobe acute. Eye small. Dorsal pleonites tooth formulae 1-3-3-3-0, rear to front. Outer lobe of lower lip with two corns. Maxilla 1, inner plate with two setae. Maxilliped, inner plate lacking lateral setae. Antenna 1, peduncular article 2 1.25 times article 1. Gnathopod 1, propodus subovate, medial side with oblique row of 11 or 12 setae; palm oblique. Coxa 7, posterodistal corner acutely produced. Telson deeply cleft, with lateral and apical spines.

**Description.** *Holotype*, female, MABIK CR00250813. Body (Fig. 3A) length about 5.5 mm. Ocular lobe acutely produced. Eye small, subovate. Pereonites smooth.

Pleonites 1–3 (Fig. 3B) dorsal tooth formulae 1-3-3-3-0, rear to front; pleonal epimera 1–3 each with posteroventral tooth, gradually enlarging distally; epimeron 1 with oblique row of 5 setae ventrally; epimeron 2 similar to pleonal epimeron 1 but with two clusters of setae anteroventrally; epimeron 3 excavate posteriorly, with five clusters of setae ventrally.

Antenna 1 (Fig. 3C) slightly longer than half as long as body length; peduncular articles rectangular, length ratio of peduncular articles 1-3 = 1.00: 1.25: 0.25; accessory flagellum small, with three apical setules; flagellum subequal in length to peduncle, 15-articulate.

Antenna 2 (Fig. 3D) 0.78 times as long as antenna 1; gland corn well developed; peduncular articles 3–5 setaceous facially, length ratio = 1.00: 5.00: 2.90; flagellum 1.13 times as long as peduncle, 12-articulate.

Lower lip (Fig. 3E), inner lobe subovate, coalescent proximally, rounded apically; outer lobe with two corns, mandibular process upturned and acute.



**Figure 2.** A *Paradexamine acuta* sp. nov., adult female, habitus **B** *Paradexamine rotundogena* sp. nov., adult female, habitus. Scale bar: 1.0 mm (**A**, **B**).

Left mandible (Fig. 3F), incisor produced forward, with five blunt teeth; lacinia mobilis bifid, upper part with five teeth, lower part with four teeth; three accessory spines placed between lacinia mobilis and molar process; molar process massive, developed.

Right mandible (Fig. 3G) similar to left mandible, except two accessory spines placed between lacinia mobilis and molar process.

Maxilla 1 (Figs 3H), inner plate with two simple setae apically; outer plate six denticulate and five bifid tooth-like spines apically; palp broad, with 11 simple setae.

Maxilla 2 (Fig. 3I), inner plate shorter and narrower than outer plate, with nine setae; outer plate with 14 setae overall.

Maxilliped (Fig. 3J), inner plate elongate, about one-third as long as outer plate, with three apical setae; outer plate elongate-ovate, slightly extending beyond end of palp article 3; inner margin crenulate, with 12 conical teeth which gradually increase in size toward distal end; distal half of outer margin with a row of 11 simple setae; palp 4 articulate, rather slender, inner margin setaceous, extending outer plate.

Gnathopod 1 (Fig. 4A), coxa trapezoidal, ventral margin rounded, with unequal setae; basis slender, about half as long as gnathopod 1, with nine simple setae posteriorly; ischium small, subrectangular, 0.75 times as long as merus; carpus 1.17 times as long as propodus; propodus subovate, with oblique row of 11 setae medially, palm oblique, with a row of short setae, delimited by a group of four spines; dactylus falcate, fitting palm.

Gnathopod 2 (Fig. 4B) similar to gnathopod 1, but longer and slenderer than gnathopod 1.

Pereopod 3 (Fig. 4C), coxa subrectangular, one-third as wide as long, ventral margin setose; length ratio of articles 2-7 = 1.00: 0.18: 0.62: 0.50: 0.67: 0.35.

Pereopod 4 (Fig. 4D) similar to pereopod 3.

Pereopod 5 (Fig. 5A), coxa subquadrate, bilobate, anterior rounded lobe protruding downward, with short setae; basis with longish ovate form, posteroventral lobe rounded downward, reaching somewhat near distal margin of ischium, with several clusters of long to short spines along anterior margin; ischium to dactylus slender, setose; length ratio of articles 2-7 = 1.00: 0.18: 0.90: 0.68: 0.66: 0.20.



**Figure 3.** *Paradexamine acuta* sp. nov., holotype, adult female, MABIK CR00250813, 5.0 mm **A** habitus **B** pleonal epimera 1–3 **C** antenna 1 **D** antenna 2 **E** lower lip **F** left mandible **G** right mandible **H** maxilla 1 **I** maxilla 2 **J** maxilliped. Scale bars: 0.5 mm (**A**); 0.2 mm (**B**); 0.1 mm (**C–J**).

Pereopod 6 (Fig. 5B), coxa 6 bilobate, similar to coxa 5, but shallower than coxa 5; basis ovate, posterior margin rounded and finely serrulate; length ratio of articles 2-7 = 1.00: 0.20: 1.10: 1.02: 1.15: 0.59.

Pereopod 7 (Fig. 5C), coxa subquadrate, with acutely produced posteroventrally; basis subrectangular, narrow, width 0.57 times length; length ratio of articles 2-7 = 1.00: 0.24: 0.71: 1.07: 0.71: 0.36.



**Figure 4.** *Paradexamine acuta* sp. nov., holotype, adult female, MABIK CR00250813, 5.0 mm **A** gnathopod 1 **B** gnathopod 2 **C** pereopod 3 **D** pereopod 4. Scale bars: 0.2 mm (**A**, **B**), 0.1 mm (**C**, **D**).

Uropod 1 (Fig. 5D), peduncle subrectangular, subequal to outer ramus, with six dorsolateral, four medial, three basofacial, and one apicolateral large spines; inner ramus slightly longer than outer ramus.

Uropod 2 (Fig. 5E) 0.64 times as long as uropod 1; peduncle subequal to outer ramus, with two dorsolateral and one apicomedial spines; inner ramus slightly longer than outer ramus, apical portion broken.

Uropod 3 (Fig. 5F) longer and broader than uropod 2; peduncle 0.69 times as long as outer ramus, with three dorsolateral, five medial, and one apicolateral large spines; both rami subequal in length.

Telson (Fig. 5G) longish, 2.37 times as long as wide, thoroughly cleft, lateral margin with a row of unequal spines, apical margin truncate, with serrulation and one spine.

Male. Unknown.

**Immature female**, 3.0 mm, DKUAMP202202. Gnathopod 1, propodus subovate, with oblique row of eight setae medially; pereopod 6, basis ovate, posterior margin rounded and finely serrulate; coxa 7 pointed posteroventrally; telson, lateral margin with a row of seven spines.

**Etymology.** The species name is derived from the Latin *acutus* (= sharp, pointed), referring to the acute cephalic lobe and posteroventral acute projection on coxa 7.



Figure 5. *Paradexamine acuta* sp. nov., holotype, adult female, MABIK CR00250813, 5.0 mm A pereopod 5 B pereopod 6 C pereopod 7 D uropod 1 E uropod 2 F uropod 3 G telson. Scale bar: 0.2 mm (A–G).

**Remarks.** The new species *Paradexamine acuta* sp. nov. resembles *P. houtete* J.L. Barnard, 1972b from New Zealand, *P. jindoensis* Kim & Lee, 2008 from Jindo Island, Korea, *P. gigas* Hirayama, 1984, *P. marlie* s.l., and *P. micronesica* Ledoyer, 1978 from Tomioka Bay, Japan, in having acute ocular lobe and dorsal pleonites tooth formulae 1-3-3-3-0, rear to front (Table 1). However, this new species is distinguished from its congeners in the following characteristics (compared with the characteristics of congeners in parentheses): 1) inner plate of maxilla 1 with five lateral setae (vs without setae

in *P. gigas*, *P. houtete*); 2) maxilliped, inner plate without lateral setae (vs seven lateral setae in *P. jindoensis*); 3) gnathopod 1 having medial setae on propodus with 10 or 11 setae (vs four or five setae in *P. gigas*, *P. houtete*, *P. micronesica*); 4) pereopod 6 with basis ovate, roundly produced posteriorly and with serrulations (vs tapering posterodistally in *P. gigas*, *P. houtete*, *P. micronesica*); 5) pereopod 7 with coxa pointed posteroventrally (vs rounded posteroventrally in *P. gigas*, *P. jindoensis*); 6) pereopod 7, basis subrectangular and narrow (vs subovate in *P. gigas*, elongate-ovate and moderate in *P. micronesica*).

In general, *Paradexamine acuta* sp. nov. is very similar to *P. marlie* s.l. from Japanese waters as described and figured by Hirayama (1984). *Paradexamine marlie* s.l. from Japan differs from the original description of *P. marlie* from Australia (Barnard 1972a)

| Species Characters                            | <b>P. gigas</b> (♂)         | <i>P. houtete</i> $(\bigcirc)$ | <i>P. jindoensis</i> $(\bigcirc$ ) | <i>P. marlie</i> s.l. (♀)    | P. micronesica<br>(♂?)               | <i>P. acuta</i> sp. nov. (♀)    |
|---|-----------------------------|--------------------------------|------------------------------------|------------------------------|--------------------------------------|---------------------------------|
| Body length                                   | 3.5 mm                      | 3.7 mm                         | 5.5 mm                             | 3.5 mm                       | 3.0 mm                               | 5.5 mm                          |
| Pleonites tooth<br>formulae                   | 1-3-3-3-0                   | 1-3-3-3-0                      | 1-3-3-3-0                          | 1-3-3-3-0                    | 1-3-3-3-0<br>1-3-3-1                 | 1-3-3-3-0                       |
| Ocular lobe                                   | acute                       | acute                          | acute                              | acute                        | acute                                | acute                           |
| Lower lip, corn                               | 1 cone                      | 2 cones                        | 2 unequal cones                    | 2 cones                      | 1 cone                               | 2 cones                         |
| Mandibular process                            | blunt, weakly<br>developed  | apically hooked                | apically hooked                    | apically hooked              | blunt, weakly<br>developed           | apically hooked                 |
| Mandible, accessory<br>spines                 | no spine                    | 2 spines                       | 2 or 3 tiny spines                 | ?                            | no spine                             | 2 or 3 spines                   |
| Maxilla 1, palp                               | 5 apical setae              | 8 setae                        | 6 apical setae                     | 7 apical setae               | 5 apical setae                       | 11 apical setae                 |
| Maxilla 2, inner<br>margin of inner<br>plate  | no seta                     | no seta                        | 5 setae                            | 4 setae                      | 4 setae                              | 5 setae                         |
| Maxilliped,<br>inner plate                    | no lateral seta             | no lateral seta                | 7 lateral setae                    | no lateral seta              | no lateral seta                      | no lateral seta                 |
| Maxilliped,<br>outer plate                    | 11 conical teeth            | 12 conical teeth               | 16 conical teeth                   | 12 conical teeth             | 11 conical teeth                     | 17 conical teeth                |
| Antenna 1, articles<br>1 & 2 ratio            | 1.0 : 1.5                   | 1.0 : 1.1                      | 1.0 : 1.5                          | 1.0 : 1.1                    | 1.0 : 2.0                            | 1.0 : 1.3                       |
| Gnathopod 1,<br>interior setae of<br>propodus | 4 setae                     | 5 setae                        | 8 setae                            | 9 setae                      | 4 setae                              | 10 or 11 setae                  |
| Gnathopod 1,<br>carpus & propodus<br>ratio    | 1.0 : 0.9                   | 1.0:0.9                        | 1.0:0.9                            | 1.0:1.3                      | 1.0 : 1.0                            | 1.0:0.9                         |
| Gnathopod 2,<br>carpus & propodus<br>ratio    | 1.0 : 0.7                   | 1.0 : 0.8                      | 1.0:0.9                            | 1.0:0.8                      | 1.0:0.5                              | 1.0:0.9                         |
| Pereopod 6, basis                             | tapering<br>posterodistally | excavated posterodistally      | roundly produced posteriorly       | roundly produced posteriorly | slightly tapering<br>posterodistally | roundly produced<br>posteriorly |
| Coxa 7  | rounded posteroventrally    | pointed posteroventrally       | rounded posteroventrally           | pointed posteroventrally     | pointed posteroventrally             | pointed posteroventrally        |
| Pereopod 7, basis                             | subovate                    | subrectangular                 | subrectangular,                    | subrectangular,              | elongate-ovate,                      | subrectangular,                 |
|   |                             |                                | narrow                             | narrow                       | moderate                             | narrow                          |
| Telson  | cleft, roundish             | slender and                    | slender and                        | elongate-ovate, 7            | slender and long-                    | slender and longish, 7          |
|   | marginally, 1               | longish, 4 or 5                | longish, 14 or 15                  | lateral spines               | ish, 2 lateral spines                | or 9 lateral spines             |
|   | lateral spine               | lateral spines                 | lateral spines                     |                              |                                      |                                 |
| Distribution                                  | Tomioka bay,<br>Japan       | New Zealand                    | Jindo Island,<br>Korea             | Tomioka bay,<br>Japan        | Tomioka bay,<br>Japan                | Korea                           |
| References                                    | Hirayama, 1984              | Barnard, 1972b                 | Kim & Lee, 2008                    | Hirayama, 1984               | Hirayama, 1984                       | Present study                   |

Table 1. Morphological characters of *Paradexamine acuta* sp. nov. and related species.

in having the dorsal pleonites tooth formulae 1-3-3-3-0 and coxa 7 posteroventrally pointed. In many ways, including these two major characteristics, *P. marlie* s.l. from Japan is more similar to *P. acuta* sp. nov. However, as we have not had the opportunity to examine Hirayama's (1984) type material, we cannot confidently determine if they are the same species. Nevertheless, *P. marlie* s.l. Hirayama could be re-established or synonymized with *P. acuta* sp. nov. in the future.

Distribution. South Korea (Baengnyeongdo Island, Chujado Island, Jejudo Island)

#### Paradexamine rotundogena sp. nov.

https://zoobank.org/BE364F87-4E67-4023-9F95-B094D345F835 Figs 2B, 6, 7 Korean name: Dung-geun-ppyam-yeop-ga-si-but-eun-kko-ri-yeop-sae-u, new

**Type material.** *Holotype:* female, 5.0 mm, MABIK CR00250814, Korea, Gyeongsangnam-do, Geomundo Island, Guroba, 34°00'52"N, 127°17'41"E, 09 July 2019, scuba collection, depth 10–15 m, S.G. Lee & Y.H. Kim leg. *Paratypes:* two females, 3.4 mm and 4.5 mm, DKUAMP202203, same station data as holotype.

Additional material examined. 1 female, Korea, Gyeongsangnam-do, Geomundo Island, 34°00'43"N, 127°18'05"E, 06 June 2018, S.H. Kim leg. 4 females, Korea, Chujado Island, 33°56'43"N, 126°18'42"E, Z. Xin, K.W. Kim, & Y.H. Kim leg., 28 August 2021.

**Diagnosis.** Lateral cephalic lobe rounded. Eye medium-sized. Dorsal pleonites tooth formulae 3-3-3-3, rear to front. Antenna 1, peduncular article 2 slightly longer than article 1. Maxilla 1, inner plate without apical seta. Maxilliped, inner plate without lateral setae. Gnathopod 1, propodus broad, palm steeply angled. Pereopods 3–7 spinose. Pereopod 7, basis subquadrate, with irregular serrations posteriorly. Telson deeply cleft nearly to the base.

**Description.** *Holotype*, female, MABIK CR00250814. Body (Fig. 6A) length about 5.0 mm. Cephalic lobe rounded. Eye medium-sized, subround. Pereonites smooth.

Pleonites 1–3 (Figs 6B), dorsal tooth formulae 3-3-3-3, rear to front; pleonal epimera 1–3 each with posteroventral tooth, its gradually getting bigger distally and posterior margins with irregularly crenulated; urosomite 1 with one dorsodistal carinate tooth and a pair of dorsolateral teeth with a spine.

Antenna 1 (Fig. 6C) slightly longer than half as long as body length; peduncular articles rectangular, length ratio of peduncular articles 1-3 = 1.00: 1.19: 0.41; accessory flagellum small, with three apical setules; flagellum about 1.3 times as long as peduncle, 15-articulate.

Antenna 2 (Fig. 6D) one-third of the body length and two-thirds of antenna 1 length. peduncular articles 1-3 short, peduncular articles 4 and 5 elongated, length ratio of peduncular articles 3-5 = 1.00: 4.14: 4.42; flagellum 7-articulate, 1.4 times as long as peduncular article 5.

Lower lip (Fig. 6E) inner lobe elongate-ovate, covered with patch of pubescence; outer lobe with three or four cusps; mandibular process produced subacutely.



**Figure 6.** *Paradexamine rotundogena* sp. nov., holotype, adult female, MABIK CR00250814, 5.5 mm **A** habitus **B** pleonal epimera 1–3 **C** antenna 1 **D** antenna 2 **E** lower lip **F** left mandible **G** right mandible **H** maxilla 1 **I** maxilla 2 **J** maxilliped. Scale bars: 0.5 mm (**A**); 0.2 mm (**B–D**); 0.1 mm (**E–J**).

Left mandible (Fig. 6F) incisor produced forward, with eight blunt teeth; lacinia mobilis bifid, upper part with six teeth, lower part with two blunt teeth; three accessory spines placed between lacinia mobilis and molar process; molar process developed, truncate. Right mandible (Fig. 6G) similar to left mandible, except two accessory spines placed between lacinia mobilis and molar process.

Maxilla 1 (Fig. 6H) inner plate small, elongate, without apical seta; outer plate with 11 tooth-like spines (simple, bifid, and denticulate) apically; palp slender, not reaching end of outer plate, with a long apical seta.

Maxilla 2 (Fig. 6I) inner plate much shorter than outer one, with four apical setae; outer plate with four subapical and three apical setae.

Maxilliped (Fig. 6J) inner plate small, with one apical setae and without lateral setae; outer plate large, elongate-ovate, slightly extending beyond end of palp article 3, inner margin with 15 conical teeth and five simple setae apically; palp 4 articulate, rather slender, inner margin setaceous, slightly extending outer plate.

Gnathopod 1, coxa (Fig. 7A) trapezoidal, anterior margin rounded, with 13 unequal simple setae, posterior margin straight, unarmed; carpus (Fig. 7B) subtriangular, subequal to propodus, with six ventral setae; propodus broad, gradually widening distally, without oblique row of setae medially, palm steeply angled, with a row of short setae, delimited by a group of four spines; dactylus falcate, fitting palm.

Gnathopod 2 (Fig. 7C) similar to gnathopod 1, but coxa narrowly rectangular and carpus elongate, 1.36 times as long as propodus.

Pereopod 3 (Fig. 7D) slender, spinose; coxa tapering distally; length ratio of articles 2–7 = 1.00: 0.27: 0.70: 0.53: 0.87: 0.40.

Pereopod 4 (Fig. 7E) similar to pereopod 3, except coxa 4 wider than coxa 3 ventrally; length ratio of articles 2-7 = 1.00: 0.25: 0.69: 0.53: 0.78: 0.38.

Pereopod 5 (Fig. 7F), coxa quadrate, bilobate, anterior rounded lobe protruding downward; basis longish ovate form, posteroventral lobe roundly downward, reaching somewhat distal margin of ischium, with several clusters of long to short spines along anterior margin, posterior margin straight, unarmed; ischium to dactylus slender, setose; length ratio of articles 2-7 = 1.00: 0.18: 0.70: 0.58: 0.50: 0.34.

Pereopod 6 (Fig. 7G) coxa 6 bilobate, similar to coxa 5, but shallower than coxa 5; basis ovate, posterior margin rounded, weakly serrate, slightly excavate posterodistally; length ratio of articles 2-7 = 1.00: 0.15: 0.85: 1.08: 0.77: 0.30.

Pereopod 7 (Fig. 7H) coxa small, semicircular; basis subquadrate, width 0.91 times length, produced posteriorly, anterior margin straight, posterior margin with irregular serrations; length ratio of articles 2–7 = 1.00: 0.26: 0.60: 1.29: 0.72: 0.29.

Uropod 1 (Fig. 7I) peduncle subrectangular, with five dorsolateral, three medial, and one apicolateral large spines; inner ramus slightly shorter than peduncle, with two longitudinal rows of 10 and three apical spines; outer ramus broken.

Uropod 2 (Fig. 7J) about half length of uropod 1, with four dorsolateral and one apicomedial spines; outer ramus 0.75 times as long as peduncle, with two rows of four and three apical spines; inner ramus broken.

Uropod 3 unknown.

Telson (Fig. 7K) longish, 2.00 times as long as wide, thoroughly cleft, lateral margin with two spines, apical margin truncate with acute cusp, serrulation, and one spine.

**Etymology.** The species name is derived from the Latin *rotundus* (= round) and *gena* (= cheek), referring to the rounded cephalic lobe.



**Figure 7.** *Paradexamine rotundogena* sp. nov., holotype, adult female, MABIK CR00250814, 5.5 mm **A** coxa 1 **B** gnathopod 1 **C** gnathopod 2 **D** pereopod 3 **E** pereopod 4 **F** pereopod 5 **G** pereopod 6 **H** pereopod 7 **I** uropod 1 **J** uropod 2 **K** telson. Scale bars: 0.1 mm (**A–C**); 0.2 mm (**D–K**).

**Remarks.** The new species *Paradexamine rotundogena* sp. nov. resembles *P. tafunsaka* Myers, 1995 distributed in Kosrae, Micronesia, *P. levitelson* Myers & LeCroy, 2009 from Queensland, Australia, and *P. bisetigera* Hirayama, 1984 from Tomioka Bay, Japan, in

| Species                                 | <i>P. tafunsaka</i> (♀) | P. bisetigera (♂)     | <i>P. levitelson</i> (♀) | <i>P. rotundogena</i> sp. nov. $(\bigcirc)$ |
|---|-------------------------|-----------------------|--------------------------|---|
| Characters                              |                         |                       |                          |   |
| Body length                             | 2.7 mm                  | 6.3 mm                | 3.0 mm                   | 5.0 mm                                      |
| Pleonites tooth formulae                | 3-3-3-3-0               | 3-3-3-3               | 3-3-3-3-0                | 3-3-3-3-0                                   |
| Ocular lobe                             | round                   | round                 | round                    | round                                       |
| Lower lip, corn                         | 2 cones                 | 2 cones               | ?                        | 3 or 4 cones                                |
| Mandibular process                      | subacutely produced     | subacutely produced   | ?                        | subacutely produced                         |
| Mandible, accessory spines              | 2 spines                | 2 or 3 spines         | 2 spines                 | 2 or 3 spines                               |
| Maxilla 1, palp                         | 2 apical setae          | 2 apical setae        | 6 apical setae           | 1 apical seta                               |
| Maxilla 2, inner margin of inner plate  | ?                       | 3 setae               | ?                        | no seta                                     |
| Maxilliped, inner plate                 | no lateral seta         | no lateral seta       | ?                        | no lateral seta                             |
| Maxilliped, outer plate                 | 12 conical teeth        | 21 conical teeth      | ?                        | 15 conical teeth                            |
| Antenna 1, articles 1 & 2 ratio         | 1.0:1.3                 | 1.0:1.5               | 1.0:1.5                  | 1.0:1.2                                     |
| Gnathopod 1, interior setae of propodus | 5 or 6                  | 6                     | ?                        | 3   |
| Gnathopod 1, palm                       | steeply angled          | steeply angled        | transverse               | steeply angled                              |
| Gnathopod 2, carpus & propodus ratio    | 1.0:1.1                 | 1.0:1.3               | 1.0:1.3                  | 1.0:0.7                                     |
| Coxa 7                                  | ?                       | semicircular          | semicircular             | semicircular                                |
| Pereopod 7, basis                       | broad, regular          | broad, no serrations  | broad, regular           | broad, irregular serrations                 |
|   | serrations posteriorly  | posteriorly           | serrations posteriorly   | posteriorly                                 |
| Telson                                  | 3 or 4 lateral spines   | 5 or 6 lateral spines | 2 or 3 lateral spines    | 2 lateral spines                            |
| Distribution                            | Kosrae, Micronesia      | Tomioka bay, Japan    | Queensland, Australia    | Korea                                       |
| References                              | Myers, 1995             | Hirayama, 1984        | Myers & LeCroy, 2009     | Present study                               |

Table 2. Morphological characters of Paradexamine rotundogena sp. nov. and related species.

having a rounded ocular lobe and dorsal pleonite tooth formulae of 3-3-3-3, rear to front (Table 2). However, the new species is distinguished from its congeners in the following characteristics (compared with the characteristics of congeners in parentheses): 1) gnathopod 2 with carpus longer than propodus (vs subequal in length in *P. bisetigera*, shorter than in *P. tafunsaka* and *P. levitelson*); 2) pereopod 7 with basis broad, with irregular serrations posteriorly (vs regular serrations posteriorly in *P. bisetigera*, *P. levitelson*, and *P. tafunsaka*); 3) maxilla 1 with palp having one apical seta (vs two apical setae in *P. tafunsaka* and *P. bisetigera*, six apical setae in *P. levitelson*); 4) maxilla 2 with inner plate without medial seta (vs with three setae in *P. bisetigera*); 5) telson with two lateral spines (vs five or six lateral spines in *P. bisetigera*, three or four lateral spines in *P. tafunsaka*).

Distribution. South Korea (Chujado Island, Geomundo Island).

## Key to Korean species of Paradexamine

| 1 | Ocular lobe rounded P. rotundogena sp. nov.                                |
|---|--|
| _ | Ocular lobe acute  |
| 2 | Dorsal pleonite tooth formulate 1-3-3-0 P. fraudatrix Tzvetkova, 1976      |
| _ | Dorsal pleonite tooth formulate 1-3-3-3                                    |
| 3 | Antenna 1, peduncular article 2 1.3× article 1; coxa 7 pointed posteroven- |
|   | trally   |
| _ | Antenna 1, peduncular article 2 1.5× article 1; coxa 7 rounded posteroven- |
|   | trally4  |

| 4 | Pereopod 7, basis subrectangular and narrow; telson with a row of lateral |
|---|---|
|   | spines P. jindoensis Kim & Lee, 2008                                      |
| _ | Pereopod 7, basis ovate and broad; telson with one lateral spine          |
|   | <i>P. gigas</i> Hirayama, 1984  |

## Acknowledgements

We greatly appreciate the suggestions and comments given from reviewers: Dr Azman Abdul Rahim of the Marine Ecosystem Research Centre (EKOMAR), Malaysia, and Dr Karaon Wongkamhaeng of Kasetsart University, Thailand. Their helpful comments greatly improved the manuscript. This research was supported by National Marine Biodiversity Institute of Korea (2022M01100).

## References

- Barnard JL (1970) Sublittoral Gammaridea (Amphipoda) of the Hawaiian Islands. Smithsonian Contributions to Zoology 34(34): 1–296. https://doi.org/10.5479/si.00810282.34
- Barnard JL (1972a) Gammaridean Amphipoda from Australia, Part I. Smithsonian Contributions to Zoology 103: 1–333. https://doi.org/10.5479/si.00810282.103
- Barnard JL (1972b) The marine fauna of New Zealand: Algae-living littoral Gammaridea (Crustacea Amphipoda). Memoir of the New Zealand Oceanographic Institute 62: 7–216.
- Chevreux E (1906) Crustaces amphipodes. In: JOUBIN L. (Ed.). Expedition Antarctique Francaise (1903–1905) commandee par le Dr Jean Charcot. Sciences naturelles: documents scientifi ques. Crustaces. Masson et Cie, Paris. 1–100.
- Haswell WA (1885) Notes on the Australian Amphipoda. Proceedings of the Linnean Society of New South Wales 10: 95–114. https://doi.org/10.5962/bhl.part.17903
- Hirayama A (1984) Taxonomic studies on the shallow water gammaridean Amphipoda of west Kyushu, Japan. III. Dexaminidae (*Polycheria* and *Paradexamine*). Publications of the Seto Marine Biological Laboratory 29(5): 187–230. https://doi.org/10.5134/176094
- Horton T, Lowry J, De Broyer C, Bellan-Santini D, Coleman CO, Corbari L, Costello MJ, Daneliya M, Dauvin JC, Fišer C, Gasca R, Grabowski M, Guerra-García JM, Hendrycks E, Hughes L, Jaume D, Jazdzewski K, Kim YH, King R, Krapp-Schickel T, LeCroy S, Lörz AN, Mamos T, Senna AR, Serejo C, Sket B, Souza-Filho JF, Tandberg AH, Thomas JD, Thurston M, Vader W, Väinölä R, Vonk R, White K, Zeidler W (2022) World Amphipoda Database. World Register of Marine Species. https://www.marinespecies.org/amphipoda/ [Accessed on: 2022-7-6]
- Kim YH, Lee KS (2008) A new species of the genus *Paradexamine* (Crustacea: Amphipoda: Dexaminidae) from Korea. Animal Cells and Systems 12(3): 157–163. https://doi.org/10 .1080/19768354.2008.9647169
- Kim YH, Eun Y, Lee KS (2006) Two new records of Dexaminidae (Crustacea: Amphipoda) from Korea. Animal Systematics, Evolution and Diversity 22(1): 37–49.

- Ledoyer M (1978) Amphipodes gammariens (Crustacea) des biotopes cavitaires organogènes récifaux de l'Ile Maurice. The Mauritius Institute Bulletin 8(3): 197–332.
- Ledoyer M (1979) Les gammariens de la pente externe du Grand Récif de Tuléar (Madagascar) (Crustacea Amphipoda). Memorie del Museo Civico di Storia Naturale, Verona (Serie 2) 2: 1–149.
- Ledoyer M (1984) Les gammariens (Crustacea, Amphipoda) des herbiers de phanérogames marines de Nouvelle Calédonie (région de Nouméa). Mémoires du Museum national d'Histoire naturelle (Série A) 129: 1–113.
- Lowry JK, Myers AA (2017) A phylogeny and classification of the Amphipoda with the establishment of the new order Ingolfiellida (Crustacea: Peracarida). Zootaxa 4265(1): 1–89. https://doi.org/10.11646/zootaxa.4265.1.1
- Myers AA (1985) Shallow-water, coral reef and mangrove Amphipoda (Gammaridea) of Fiji. Records of the Australian Museum 5: 1–143. https://doi.org/10.3853/j.0812-7387.5.1985.99
- Myers AA (1995) Marine Amphipoda of Micronesia: Kosrae. Records of the Australian Museum 47(1): 27–38. https://doi.org/10.3853/j.0067-1975.47.1995.4
- Myers AA, LeCroy SE (2009) Dexaminidae. Zootaxa 2260(1): 393–424. https://doi. org/10.11646/zootaxa.2260.1.20
- Ren X (2006) Fauna Sinica, Invertebra. Vol. 41, Crustacea, Amphipoda, Gammaridea (I). Science Press, Beijing, 588 pp.
- Schellenberg A (1931) Gammariden und Caprelliden des Magellangebietes, Sudgeorgiens und der Westantarktis. Further Zoological Results of the Swedish Antarctic Expedition 1901–1903 2(6): 1–290.
- Sheard K (1938) The amphipod genera *Euonyx, Syndexamine* and *Paradexamine*. Records of the South Australian Museum 6(2): 169–186.
- Spandl H (1923) Amphipoden der 'Pola'-Expeditionen in das Rote Meer. Akademie Wissenschaftlichen Wien, Anzeiger 60: 17-20, 87-89, 111-112.
- Stebbing TRR (1888) Report on the Amphipoda collected by H.M.S. *Challenger* during the years 1873–1876. Report of the Scientific Results of the Voyage of H.M.S. *Challenger* During the Years 1873–76. Zoology 29(67): [i–xxiv] 1–1737.
- Stebbing TRR (1914) Crustacea from the Falkland Islands collected by Mr. Rupert Vallentin, F.L.S. - Pt. 11. Proceedings of the Zoological Society of London, 1914, 1: 341–378. https://doi.org/10.1111/j.1469-7998.1914.tb07042.x
- Thomson GM (1879) New Zealand Crustacea, with descriptions of new species. Transactions and Proceedings of the New Zealand Institute 11: 230–248.
- Tzvetkova NL (1976) Novye vidy amfipod semejstv Dexaminidae, Phliantidae, i Biancolinidae—Teplovodnye elementy fauny zaliva Pos'eta (Japonskoe more). Zoologicheskij Zhurnal 55: 684–695. [New species of Dexaminidae, Phliantidae and Biancolinidae, warmwater elements of the Possjet Bay fauna (Japan Sea).] [in Russian]
- Walker AO (1904) Report on the Amphipoda collected by Professor Herdman, at Ceylon, in 1902. Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar, with Supplementary Reports upon the Marine Biology of Ceylon. Part II. The Royal Society, London, 229–300.

RESEARCH ARTICLE



# A new species of *Gammarus* (Crustacea, Amphipoda, Gammaridae) from South Korea

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| Academic editor: Charles Oliver Coleman   Received 28 June 2022   Accepted 18 July 2022   Published 11 August 2022 |
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| https://zoobank.org/76184F9B-BAB5-43F7-B4B4-0A45F6AC326F   |
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Citation: Ahn Y-U, Lee C-W, Min G-S (2022) A new species of *Gammarus* (Crustacea, Amphipoda, Gammaridae) from South Korea. ZooKeys 1117: 53–69. https://doi.org/10.3897/zooKeys.1117.89610

#### Abstract

A new species of freshwater gammarid, *Gammarus somaemulensis* **sp. nov.**, was collected from Somaemuldo Island, South Korea. This new species is morphologically characterised by the absence of calceoli in antenna 2, absence of anteroproximal setae on bases in pereopods 6 and 7, outer ramus in uropod 3 with plumose setae on both margins, and a small terminal article of the outer ramus, which is shorter than adjacent spines. A description of the new species and morphological differences from related species are provided in the text. The new species was also compared to related species using partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Genetic distances of COI sequences between the new species and related species, consisting of 21.5–26.3% difference, support *Gammarus somaemulensis* **sp. nov.** as a valid species. Additionally, a key to identifying *Gammarus* species in South Korea is provided.

#### Keywords

COI, freshwater, gammarid, Korea, morphology, new species, taxonomy

## Introduction

The genus *Gammarus* Fabricius, 1775 is one of the most speciose genera of Amphipoda, comprising more than 200 species (Väinölä et al. 2008). *Gammarus* inhabit various environments across the Northern Hemisphere, including freshwater, brackish and littoral marine waters, and 80% of these species inhabit freshwater (Väinölä et al. 2008; Hou et al. 2018). The freshwater *Gammarus* is an essential component of freshwater ecosystems and is often used as a bioindicator for water quality assessment (Gerhardt et al. 2011). However, it is well known that morphological identification of species in this genus is difficult because of the high occurrence of convergent characteristics (Karaman and Pinkster 1977).

Freshwater *Gammarus* was first reported in Korea by Uéno (1940). Since then, 11 species of freshwater *Gammarus* have been reported and described in South Korea: *G. baengnyeongensis* Kwon, Kim, Heo & Kim, 2020; *G. gageoensis* Kim, Lee & Min, 2010; *G. galgosensis* Lee & Kim, 1980; *G. hoonsooi* Lee, 1986; *G. kyonggiensis* Lee & Seo, 1990; *G. longisaeta* Lee & Seo, 1992; *G. odaensis* Lee & Kim, 1980; *G. sobaegensis* Uéno, 1966; *G. soyoensis* Lee & Kim, 1980; *G. wangbangensis* Lee & Seo, 1992; and *G. zeongogensis* Lee & Kim, 1980 (Uéno 1940, 1966; Lee and Kim 1980; Lee 1986; Lee and Seo 1990; 1992; Kim et al. 2010; Kwon et al. 2020). Islands are known to have high levels of endemism due to geographic isolation and limited interchange with mainland biota (Whittaker and Fernández-Palacios 2007), three *Gammarus* species (*G. baengnyeongensis*, *G. gageoensis*, and *G. galgosensis*) are already known to be endemic to certain islands of South Korea. The coast of South Korea is composed of thousands of South Korea.

In the present study, a new species of the genus *Gammarus*, collected from the island of South Korea is described, based on morphological differences and mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence analyses. In addition, a key to species of *Gammarus* in South Korea is provided.

## Materials and methods

#### Sampling and morphological observations

*Gammarus* specimens were collected using hand-nets from three localities in South Korea (Fig. 1). The collected specimens were immediately fixed in 95% ethanol and deposited in a -20 °C refrigerator. Body length was measured along the dorsal margin of the body from the base of fist antenna to the base of the telson. Specimens were dissected under a stereomicroscope (SZX12, Olympus, Japan). All dissected appendages were mounted with glycerol on microscope slides and drawn using an optical microscope (DM2500, Leica, Germany) equipped with a drawing tube. The terminology of the setae in article 3 of mandibular palp followed Cole (1980). All the specimens were deposited at the Nakdonggang National Institute of Biological Resources (**NNIBR**), South Korea.



Figure 1. Sampling localities of *Gammarus* specimens for this study 1 *Gammarus* somaemulensis sp. nov. 2 *G. wangbangensis* 3 *G. soyoensis*. The details of localities are shown in Table 1.

## Molecular data

Genomic DNA was extracted from muscle tissue of abdomen using LaboPass Tissue Mini Kit (Cosmo Genetech, Seoul, South Korea), according to the manufacturer's instructions. COI sequences were obtained using the primer sets, LCO1490-JJ (5'-TAY-TCHACYAAYCAYAAAGAYATYGG-3') and HCO2198-JJ (5'-AWACTTCVG-GRTGVCCAAARAATCA-3') (Astrin and Stüben 2011). Polymerase chain reaction amplification was performed under the following conditions: initial denaturation at 98 °C for 1 min, followed by 5 cycles of 10 s at 98 °C, 30 s at 43 °C, and 60 s at 72 °C. This was followed by 30 cycles of 10 s at 98 °C, 60 s at 48 °C, 60 s at 72 °C, and a 5 min extension at 72 °C. The obtained sequences were aligned using Geneious 8.1.9 (Biomatters Ltd., Auckland, New Zealand). The uncorrected *p*-distance of COI sequences was calculated using MEGA X (Kumar et al. 2018). The details of the sequences obtained in this study and those downloaded from GenBank are listed in Table 1.

| Species                | Locality (numbers in parentheses are those in Fig. 1)       | Coordinates       | COI       | Reference        |
|------------------------|---|-------------------|-----------|------------------|
| Gammarus               | Somaemuldo-Island, Hansan-                                  | 34°37'23"N,       | ON980527- | This study       |
| somaemulensis sp. nov. | myeon, Tongyeong-si, South                                  | 128°32'57.1"E     | ON980532  |                  |
|                        | Korea (1)   |                   |           |                  |
| G. wangbangensis       | Kiji-ri, sinbuk-myeon, Pocheon-si,                          | 37°54'58"N,       | ON980560  | This study       |
| 0 0                    | South Korea (2)   | 127°14'9.4"E      |           |                  |
| G. soyoensis           | Sangbongam-dong,  | 37°56'39.5"N,     | ON980559  | This study       |
|                        | Dongducheon-si, South Korea (3)                             | 127°5'17"E        |           |                  |
| G. sobaegensis         | Sannae-myeon, Namwon-si,                                    | 35°53'28"N,       | AB893337  | Tomikawa et al.  |
| 0                      | South Korea   | 127°47'24"E       |           | 2014             |
| G. baengnyeongensis    | Baengnyeongdo Island,                                       | 37°55'37.5"N,     | MW291608  | Kwon et al. 2020 |
| 0, 0                   | Baengnyeong-myeon, Incheon,<br>South Korea                  | 128°38'33.8"E     |           |                  |
| G. zeongogensis        | Cheongsan-myeon, Yeoncheon-                                 | 38°00'46"N.       | MW353844  | Kwon et al. 2020 |
| 8.8                    | gun, Gyeonggi-do, South Korea                               | 127°07'35"E       |           |                  |
| G. gageoensis          | Gageodo Island, Heuksan-myeon,<br>Jeollanam-do, South Korea | 34°03'N, 125°07'E | GU270652  | Kim et al. 2010  |

Table 1. Species information and GenBank accession numbers used in this study.

## Results

## Order Amphipoda Latreille, 1816 Family Gammaridae Leach, 1814 Genus *Gammarus* Fabricius, 1775

Gammarus somaemulensis sp. nov.

http://zoobank.org/8374BC9A-4008-4BAA-A20C-47552C679353 Figs 2–7 New Korean name: so-mae-mul-yeop-sae-u

**Material examined.** *Holotype*: male, dissected on 14 slides (NNIBRIV92290), 9.0 mm, Maejuk-ri (34°37'23"N, 128°32'57.1"E), Somaemuldo Island, Hansan-myeon, Tongyeong-si, Gyeongsangnam-do, South Korea, October 7, 2021, collected by Y. U. Ahn. *Paratypes*: male, dissected on 10 slides (NNIBRIV92291), 8.4 mm; male, dissected on 10 slides (NNIBRIV92292), 8.8 mm; male, dissected on 10 slides (NNIBRIV92293), 8.2 mm; male, dissected on 11 slides (NNIBRIV92294), 8.4 mm; male, dissected on 11 slides (NNIBRIV92295), 8.9 mm; female, dissected on 10 slides (NNIBRIV92296), 8.2 mm; female, dissected on 10 slides (NNIBRIV92297), 7.8 mm; 11 males and 3 females in ethanol vials (NNIBRIV92298–NNIBRIV92311); all other data same as holotype.

**Etymology.** The specific name *somaemulensis* is derived from the name of the type locality, Somaemuldo Island.

**Diagnosis.** Antenna 2 with four clusters of long setae on posterior margin of peduncular article 4, calceoli absent; pereopods 3 and 4 with long straight setae on posterior margins of merus and carpus; pereopods 6 and 7 without anteroproximal setae on basis; inner ramus of uropod 3 reaching approximately 0.8 × as long as outer ra-



Figure 2. Gammarus somaemulensis sp. nov., male, paratype (NNIBRIV92298), habitus. Scale bar: 1.0 mm.

mus, outer ramus with plumose setae on both margins, terminal article of outer ramus shorter than adjacent spines.

**Description of male.** *Head* (Fig. 2): rostrum short; inferior antennal sinus deep; eyes reniform.

*Antenna 1* (Fig. 3A): peduncular articles 1–3 in length ratio 1.0: 0.7: 0.4, bearing distal setae clusters on each peduncular article; main flagellum 33-articulate, each article with short distal setae; accessory flagellum four-articulate, article 4 very short.

**Antenna 2** (Fig. 3B): peduncular article 1 with three short setae distally; gland cone tapering distally; anterior, posterior and interior margins of peduncular article 4 with four, four and five clusters of setae, respectively, length of longest seta on posterior margin  $1.6 \times$  the width of peduncular article 4; peduncular article 5 slightly longer than article 4, anterior, posterior and interior margins with six clusters of setae, respectively, length of longest seta on posterior than article 4, anterior, posterior and interior margins with six clusters of setae, respectively, length of longest seta on posterior margin  $2.3 \times$  the width of peduncular article 4; flagellum 11-articulate, calceoli absent.

Upper lip (Fig. 3C): rounded, ventral margin with numerous minute setae.

*Lower lip* (Fig. 3D): inner lobes absent, outer lobes broad.

*Mandible* (Fig. 3E, F): incisor of left mandible with five teeth; lacinia mobilis of left mandible with four teeth; molar triturative, bearing one plumose seta; palp three-articulate in length ratio 1.0: 3.1: 2.2, article 1 unarmed, article 2 with 19 marginal



**Figure 3.** *Gammarus somaemulensis* sp. nov., male, holotype (NNIBRIV92290) **A** antenna 1, omitted from main flagellar article 7 **B** antenna 2 **C** upper lip **D** lower lip **E** left mandible **F** incisor and lacinia mobilis of right mandible **G** right maxilla 1 **H** palp of left maxilla 1 **I** maxilla 2 **J** maxilliped. Scale bars: 0.5 mm (**A–D**); 0.2 mm (**E, G–J**); 0.1mm (**F**).



**Figure 4.** *Gammarus somaemulensis* sp. nov., male, holotype (NNIBRIV92290) **A** gnathopod 1 **B** palm of propodus and dactylus in gnathopod 1, setae omitted **C** gnathopod 2 **D** palm of propodus and dactylus in gnathopod 2, setae omitted **E** pereopod 3 **F** pereopod 4 **G** coxal plate of pereopod 5 **H** basis to dactylus of pereopod 5 **I** dactylus of pereopod 5 **J** dactylus of pereopod 3. Scale bars: 0.5 mm (**A**, **C**, **E**–**H**); 0.2 mm (**B**, **D**); 0.1 mm (**I**, **J**).

setae, article 3 bearing eight B-setae on inner surface, six A-setae on outer surface, 28 D-setae on posterior margin and five E-setae apically; right mandible incisor with four teeth; lacinia mobilis of right mandible bifurcate, with small teeth.

*Maxilla 1* (Fig. 3G, H): inner plate with 17 plumose setae; outer plate with 11 serrated spines apically; palp two-articulate and asymmetrical, right palp shorter and stouter than left palp, article 2 of right palp with five stout spines, one slender spine and one seta apically; article 2 of left palp with five slender spines and eight setae apically.

*Maxilla 2* (Fig. 3I): inner plate bearing 17 plumose setae in an oblique row; outer plate broader than inner plate; both plates with numerous long setae apically.

*Maxilliped* (Fig. 3J): inner plate bearing three stout spines apically; outer plate with a row of blade-like spines and two plumose setae; palp four-articulate, article 1 unarmed, inner margin of article 2 with numerous setae, article 3 curved, with numerous setae on posterior margin and a row of subapical setae, article 4 hooked, with three setae at hinge of unguis.

**Gnathopod 1** (Fig. 4A, B): coxal plate with two setae on both anterodistal and posterodistal corners; basis with long setae on both anterior and posterior margins; length of carpus  $1.4 \times$  as long as width,  $0.8 \times$  as long as propodus, bearing two clusters of setae on anterior margin; propodus pyriform in shape, palm oblique, with one medial palmar spine and 11 spines on posterior margin; dactylus exceeding near half of propodus, outer margin with one seta.

**Gnathopod 2** (Fig. 4C, D): coxal plate with three setae on anterodistal corner and one seta on posterodistal corner; basis similar to that of gnathopod 1; length of carpus  $1.7 \times as$  long as width,  $0.8 \times the$  length of propodus, with four clusters of setae on anterior margin; propodus subrectangular in shape, palm concave, with one medial palmar spine and four spines on posterodistal corner; dactylus curved beyond the palmar margin, bearing one seta on outer margin.

**Pereopod 3** (Fig. 4E, J): coxal plate with two setae on anterodistal corner and one seta on posterodistal corner; basis with long setae on both anterior and posterior margins; merus bearing two spines accompanied by setae on anterior margin, eight clusters of long straight setae on posterior margin, the longest seta of them approximately  $2.0 \times$  as long as width of merus, anterodistal corner bearing one spine accompanied by setae; carpus with five clusters of long straight setae on posterior margin, one spine accompanied by setae; on both anterodistal and posterodistal corners; propodus with three spines accompanied by clusters of setae on posterior margin, one spine on posterodistal corner; dactylus bearing one plumose seta on anterior margin, two setae at hinge of unguis.

**Pereopod 4** (Fig. 4F): coxal plate with posterior excavation, bearing two setae on anterodistal corner and four setae on posterior margin; basis similar to that of pereopod 3; merus with one spine accompanied by setae on anterior margin, four clusters of long straight setae on posterior margin, the longest seta of them approximately  $1.4 \times as$  long as width of merus, anterodistal corner bearing one spine accompanied by setae; carpus with three clusters of long straight setae on posterior margin, one spine accompanied by setae on both anterodistal and posterodistal corners; propodus with



**Figure 5.** *Gammarus somaemulensis* sp. nov., male, holotype (NNIBRIV92290) **A** coxal pate of pereopod 6 **B** basis to dactylus of pereopod 6 **C** coxal pate of pereopod 7 **D** basis to dactylus of pereopod 7 **E** inner surface near posterodistal corner of basis in pereopod 7 **F** pleopod 1 **G** inner distal corner of peduncle in pleopod 1 **H–J** epimeral plates 1–3, respectively **K–M** pleonites 1–3, respectively. Scale bars: 0.5 mm (**A–D, F, H–M**); 0.2 mm (**E**); 0.05 mm (**G**).

three spines accompanied by clusters of setae on posterior margin; dactylus similar to that of pereopod 3.

**Pereopod 5** (Fig. 4G–I): coxal plate bilobed, posterior lobe with three setae on posterior margin; basis with two anteroproximal setae and six small spines on anterior margin, anterodistal corner bearing two spines accompanied by setae, posterior margin with 11 short setae, posterodistal lobe developed; merus with five clusters of setae on anterior margin, one spine on posterior margin, one and two spines accompanied by setae on anterodistal and posterodistal corners, respectively; carpus with three clusters of setae and two spines on anterior margin, two spines accompanied by setae on posterior margin; propodus with four groups of spines accompanied by setae on anterior margin; dactylus bearing one plumose on posterior margin, two setae at hinge of unguis.

**Pereopod 6** (Fig. 5A, B): coxal plate bilobed, posterior lobe with three setae on posterior margin; basis with five small spines on anterior margin and without anteroproximal setae, posterior margin with 14 short setae, posterodistal lobe not developed; merus with six clusters of setae and two spines on anterior margin, two spines on posterior margin, one and two spines accompanied by setae on anterodistal and posterodistal corners, respectively; carpus with three groups of spines accompanied by setae on posterior margin; propodus with four groups of spines accompanied by setae on anterior margin; by setae on anterior margin; by setae on anterior margin; by setae on anterior margin, two groups of spines accompanied by setae on anterior margin; by setae on a

**Pereopod** 7 (Fig. 5C–E): coxal plate shallowly concave ventrally, four setae on posterior margin; anterior margin of basis with five small spines and without anteroproximal setae, posterior margin with 15 short setae, inner surface near posterodistal corner with four short setae, posterodistal lobe not developed; merus with five clusters of setae and two spine on anterior margin, one spine on posterior margin, two spines accompanied by setae on both anterodistal and posterodistal corners; carpus with three groups of spines accompanied by setae on anterior margin, one spine and one cluster of setae on posterior margin; propodus with four groups of spines accompanied by setae on anterior margin, one spine and one cluster of setae on anterior margin; dactylus similar to those of pereopods 5 and 6.

*Coxal gills* present on gnathopod 2 and percopods 3–7.

*Pleonites 1–3* (Fig. 5K–M): posterodorsal margins of pleonites 1–3 with four, four and five setae, respectively.

*Epimeral plates 1–3* (Fig. 5H–J): plate 1 with three long setae on anteroventral margin and four short setae on posterior margin; plate 2 with two spines on ventral margin and six short setae on posterior margin; plate 3 with three spines on ventral margin and four short setae on posterior margin.

*Pleopods* (Fig. 5F, G): peduncle with two retinacula accompanied by one seta; inner ramus slightly longer than outer ramus, both rami fringed with plumose setae.

*Urosomites 1–3* (Fig. 6F–H): dorsally flat; urosomites 1 and 2 with one-one-one-one spines accompanied by setae on dorsal margins from left to right, respectively;



**Figure 6.** *Gammarus somaemulensis* sp. nov., male, holotype (NNIBRIV92290) **A** uropod 1 **B** uropod 2 **C** uropod 3 **D** terminal article of outer ramus in uropod 3, distal setae omitted **E** telson **F–H** urosomites 1–3, respectively. Scale bars: 0.5 mm (**A–C**); 0.05 mm (**D**); 0.2 mm (**E–H**).

urosomite 3 with two spines accompanied by setae on left and right sides each, and three setae on dorsal margin.

**Uropod 1** (Fig. 6A): peduncle bearing two basofacial spines, two and three spines on inner and outer margins, respectively, with one spine on both inner and outer distal corners; inner ramus approximately  $0.7 \times$  the length of peduncle and almost the same length as outer ramus, with two and one spines on inner and outer margins, respectively; outer ramus with two and three spines on inner and outer margins, respectively; both rami with five distal spines.

**Uropod 2** (Fig. 6B): peduncle with one spine on inner margin and two spines on outer margin, one spine on both inner and outer distal corners; inner ramus approximately  $0.9 \times$  the length of peduncle and  $1.3 \times$  as long as outer ramus, with two and one spines on inner and outer margins, respectively; outer ramus with two spines on outer margin; both rami with five distal spines.

**Uropod 3** (Fig. 6C, D): peduncle with several spines and setae on distal margin; inner ramus approximately  $2.0 \times$  as long as peduncle, reaching  $0.8 \times$  the length of outer ramus, bearing one distal spine, both inner and outer margins with plumose and simple setae; outer ramus two-articulate, proximal article with three spines on outer margin, bearing three distal spines, both inner and outer margins with plumose and simple setae, terminal article shorter than adjacent spines.

**Telson** (Fig. 6E): cleft nearly to base, width  $0.9 \times$  as long as length, each lobe with one cluster of setae and two single setae on surface, bearing one distal spine accompanied by five setae.

**Descrption of female.** General appearance similar to male. Observed sexual dimorphism as follows:

*Antenna 2* (Fig. 7A): setae of peduncular articles 4 and 5 longer than those of male, the longest seta on article 4 posterior margin 1.9 × as long as width of article 4, the longest seta of article 5 posterior margin 2.9 × as long as width of article 5.

*Gnathopod 1* (Fig. 7B, C): palm not as oblique as that of male, with six spines posterior margin, medial palmar spine absent; dactylus not exceeding half of propodus.

**Gnathopod 2** (Fig. 7D, E): carpus more elongate than that of male, length  $1.2 \times as$  long as propodus; palm with two spines on posterodistal corner, medial palmar spine absent.

**Oostegites:** present on gnathopod 2 (Fig. 6D) and pereopods 3–5, with numerous marginal setae.

**Uropod 3** (Fig. 7F): both rami shorter than those of male, inner ramus  $1.3 \times as$  long as peduncle length, and  $0.7 \times the length of outer ramus.$ 

Habitat. The specimens were collected from a small brook flowing along a cliff on Somaemuldo Island.

**Molecular analysis.** The COI sequences of *Gammarus somaemulensis* sp. nov. (GenBank accession numbers: ON980527–ON980532) were obtained from six individuals. Additionally, the sequences of *G. wangbangensis* (GenBank accession number:



**Figure 7.** *Gammarus somaemulensis* sp. nov., female, paratype (NNIBRIV92296) **A** antenna 2, omitted from flagellar article 3 **B** gnathopod 1 **C** palm of propodus and dactylus in gnathopod 1, setae omitted **D** gnathopod 2 **E** palm of propodus and dactylus in gnathopod 2, setae omitted **F** uropod 3. Scale bars: 0.5 mm (**A**, **B**, **D**, **F**); 0.2 mm (**C**, **E**).

|   | Species                         | 1     | 2     | 3     | 4     | 5     | 6     |
|---|---------------------------------|-------|-------|-------|-------|-------|-------|
| 1 | Gammarus somaemulensis sp. nov. |       |       |       |       |       |       |
| 2 | G. soyoensis                    | 0.215 |       |       |       |       |       |
| 3 | G. sobaegensis                  | 0.219 | 0.228 |       |       |       |       |
| 4 | G. baengnyeongensis             | 0.243 | 0.248 | 0.256 |       |       |       |
| 5 | G. gageoensis                   | 0.251 | 0.235 | 0.230 | 0.210 |       |       |
| 6 | G. wangbangensis                | 0.256 | 0.245 | 0.246 | 0.245 | 0.281 |       |
| 7 | G. zeongogensis                 | 0.263 | 0.217 | 0.240 | 0.230 | 0.206 | 0.282 |

Table 2. A matrix of the uncorrected *p*-distance of the COI sequence of this study.

ON980560) and *G. soyoensis* (GenBank accession number: ON980559) were determined in this study. The intraspecific variation of the COI sequence of the new species ranged between 0.0-0.2%. The interspecific variation between new species and the related species ranged between 21.5-26.3% (Table 2).

**Remarks.** Gammarus somaemulensis sp. nov. is most similar to *G. wangbangensis* Lee & Seo, 1990 in the following features: 1) antenna 2 peduncular article 4 with few clusters of long setae, calceoli absent, 2) percopods 3 and 4 with long straight setae on posterior margins of merus and carpus, 3) percopods 5–7 with short setae on posterior margins of basis, and 4) outer ramus of uropod 3 with plumose setae on both margins, setae length of outer margin longer than width of proximal article. However, the new species differs from *G. wangbangensis* in the following features (features of *G. wangbangensis* in parentheses): 1) article 3 of mandibular palp with one group of B-setae (two groups of B-setae), 2) bases of percopods 6 and 7 without anteroproximal setae (with long anteroproximal setae), 3) terminal article of outer ramus in uropod 3 shorter than adjacent spines (longer than adjacent spines), and 4) anteroventral margin of epimeral plate 1 with three or four setae (six or more setae).

*Gammarus somaemulensis* sp. nov. is also similar to *G. sobaegensis* Uéno, 1966 in the following features: 1) antenna 2 with long setae on peduncular articles, calceoli absent, 2) percopods 3 and 4 with long straight setae on posterior margins of merus and carpus, and 3) inner ramus of uropod 3 reaching  $0.8 \times$  the length of outer ramus. However, the new species can be distinguished from *G. sobaegensis* by the following features (features of *G. sobaegensis* in parentheses): 1) posterior margin of peduncular article 4 in antenna 2 with four clusters of long setae (six or more clusters of long setae), 2) bases of percopods 6 and 7 without anteroproximal setae (with anteroproximal setae), and 3) outer ramus of uropod 3 with plumose setae on both margins (outer margin without plumose setae).

*Gammarus soyoensis* Lee & Kim, 1980 also share the following features with the new species: 1) antenna 2 calceoli absent and 2) uropod 3 outer margin of outer ramus with plumose setae, terminal article shorter than adjacent spines. However, the new species can be distinguished from *G. soyoensis* by following features (features of *G. soyoensis* in parentheses): 1) male gnathopods 1 and 2 with medial palmar spine, each (without medial palmar spine), 2) setae on posterior margin of merus in pereopod 4 longer than width of merus (shorter than width of merus), and 3) setae on outer

margin of outer ramus in uropod 3 longer than width of proximal article (shorter than width of proximal article).

The interspecific variation within the COI sequence ranged from 21.5–26.3% for *G. somaemulensis* sp. nov. and related species (Table 2). Previous studies have reported similar or lower levels of COI sequence divergences among *Gammarus* species. Hou et al. (2009) suggested that the mean inter-specific divergence of the COI sequence among Chinese *Gammarus* species was 21.9%. Copilaş-Ciocianu et al. (2019) reported a 13.3% between *G. hamaticornis* and *G. kischineffensis*. Similarly, Zhang et al. (2022) reported 16.6% difference between *G. zhouqiongi* and *G. takesensis*. Therefore, COI sequence divergence, which is 21.5–26.3% among related species, supports *G. somaemulensis* sp. nov. as a new species.

## Key to the genus Gammarus in South Korea (adult males only)

| 1  | Antenna 2 caceoli present   |
|----|---|
| _  | Antenna 2 caceoli absent  |
| 2  | Posterior margins of pereopod 3 merus and carpus with long straight setae3        |
| _  | Posterior margins of pereopod 3 merus and carpus with long curled setae           |
|    | G. gageoensis   |
| 3  | Length ratio of uropod 3 inner/outer ramus ~ 0.7G. baengnyeongensis               |
| _  | Length ratio of uropod 3 inner/outer ramus ~ 0.5 G. zeongogensis                  |
| 4  | Inner ramus of uropod 3 with plumose setae on outer margin                        |
| _  | Inner ramus of uropod 3 without plumose setae on outer margin G. hoonsooi         |
| 5  | Peduncular articles 4 and 5 of antenna 2 with long setae                          |
| _  | Peduncular articles 4 and 5 of antenna 2 with short setae G. galgosensis          |
| 6  | Setae on outer margin of outer ramus in uropod 3 short and sparse7                |
| _  | Setae on outer margin of outer ramus in uropod 3 long and numerous8               |
| 7  | Gnathopods 1 and 2 with medial palmar spine on propodus G. odaensis               |
| _  | Gnathopods 1 and 2 without medial palmar spine on propodus G. soyoensis           |
| 8  | Pereopods 5–7 with long setae on posterior margin of basis9                       |
| _  | Pereopods 5-7 with short setae on posterior margin of basis10                     |
| 9  | Peduncular article 4 of antenna 2 with three or four setal clusters on posterior  |
|    | margin G. kyonggiensis  |
| _  | Peduncular article 4 of antenna 2 with seven or eight setal clusters on posterior |
|    | margin  |
| 10 | Outer ramus of uropod 3 with plumose setae on outer margin11                      |
| -  | Outer ramus of uropod 3 without plumose setae on outer margin                     |
|    | G. sobaegensis  |
| 11 | Terminal article of outer ramus in uropod 3 longer than adjacent spines           |
|    | G. wangbangensis  |
| _  | Terminal article of outer ramus in uropod 3 shorter than adjacent spines          |
|    | G. somaemulensis sp. nov.   |

#### Acknowledgements

We greatly appreciate Dr. Charles O. Coleman, Emeritus professor Alan A. Myers, and Professor Zhonge Hou giving constructive comments and suggestions to the improvement of manuscript. This work was supported by grants from the Nakdonggang National Institute of Biological Resources (NNIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR201901203, NNIBR202201101).

## References

- Astrin JJ, Stüben PE (2011) Molecular phylogeny of Echinodera and Ruteria (Coleoptera: Curculionidae: Cryptorhynchinae) and the parallel speciation of Canary Island weevils along replicate environmental gradients. Invertebrate Systematics 24(5): 434–455. https:// doi.org/10.1071/IS10021
- Cole GA (1980) The mandibular palps of North American freshwater species of Gammarus. Crustaceana (Supplement 6): 68–83. https://www.jstor.org/stable/25027515
- Copilaş-Ciocianu D, Zimţa AA, Petrusek A (2019) Integrative taxonomy reveals a new Gammarus species (Crustacea, Amphipoda) surviving in a previously unknown southeast European glacial refugium. Journal of Zoological Systematics and Evolutionary Research 57(2): 272–297. https://doi.org/10.1111/jzs.12248
- Gerhardt A, Bloor M, Mills CL (2011) Gammarus: Important taxon in freshwater and marine changing environments. International Journal of Zoology 2011: e524276. https://doi. org/10.1155/2011/524276
- Hou ZE, Li Z, Li SQ (2009) Identifying Chinese species of *Gammarus* (Crustacea: Amphipoda) using DNA barcoding. Current Zoology 55(2): 158–164. https://doi.org/10.1093/ czoolo/55.2.158
- Hou Z, Zhao S, Li S (2018) Seven new freshwater species of Gammarus from southern China (Crustacea, Amphipoda, Gammaridae). ZooKeys 749: 1–79. https://doi.org/10.3897/ zookeys.749.23165
- Karaman GS, Pinkster S (1977) Freshwater *Gammarus* Species from Europe, North Africa and Adjacent Regions of Asia (Crustacea-Amphipoda): Part I. *Gammarus* Pulex-Group and Related Species. Bijdragen tot de Dierkunde 47(1): 1–97. https://doi.org/10.1163/26660644-04701001
- Kim MS, Lee KS, Min GS (2010) Finding of a new freshwater gammarid (*Gammarus ga-geoensis*) from South Korea. Animal Cells and Systems 14(1): 59–71. https://doi.org/10.1080/19768351003765194
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
- Kwon SY, Kim MS, Heo JH, Kim YH (2020) A new Gammarus species (Crustacea, Amphipoda, Gammaridae) from Northwestern Islands, South Korea. Zootaxa 4896(4): 535–546. https://doi.org/10.11646/zootaxa.4896.4.5

- Lee KS (1986) Systematics study of Amphipoda (Crustacea) in Korea: VI. *Gammarus hoonsooi*, a new species of freshwater Gammarid (Gammaridae) from the South Korea. Tongmul Hakhoe Chi 29(3): 165–170.
- Lee KS, Kim HS (1980) On the geographical distribution and variation of freshwater Gammarus in Korea, including descriptions of four new species. Crustaceana (Supplement 6): 44–67. https://www.jstor.org/stable/25027514
- Lee KS, Seo IS (1990) Two new species of freshwater *Gammarus* (Crustacea, Amphipoda, Gammaridae) from South Korea. Korean Journal of Systematic Zoology 6(2): 219–230.
- Lee KS, Seo IS (1992) One new species of freshwater *Gammarus* (Crustacea, Amphipoda, Gammaridae) from South Korea. Animal Systematics, Evolution and Diversity (nspc3): 93–100.
- Tomikawa K, Soh HY, Kobayashi N, Yamaguchi A (2014) Taxonomic relationship between two *Gammarus* species, *G. nipponensis* and *G. sobaegensis* (Amphipoda: Gammaridae), with description of a new species. Zootaxa 3873(5): 451–476. https://doi.org/10.11646/ zootaxa.3873.5.1
- Uéno M (1940) Some freshwater amphipods from Manchoukuo, Korea and Japan. Nihon Seibutsu Chiri Gakkai Kaiho 10(4): 63–85.
- Uéno M (1966) Results of the speleological survey in South Korea 1966 II. Gammarid Amphipoda found in subterranean waters of South Korea. Bulletin of the National Science Museum, Tokyo 9(4): 501–535.
- Väinölä R, Witt JDS, Grabowski M, Bradbury JH, Jazdzewski K, Sket B (2008) Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. Hydrobiologia 595(1): 241–255. https://doi.org/10.1007/s10750-007-9020-6
- Whittaker RJ, Fernández-Palacios JM (2007) Island biogeography: ecology, evolution, and conservation. Oxford University Press, New York. 402 pp.
- Zhang K, Wang J, Ge Y, Ma J, Zhou Q (2022) A new *Gammarus* species from Xinjiang Uygur Autonomous Region (China) with a key to Xinjiang freshwater gammarids (Crustacea, Amphipoda, Gammaridae). ZooKeys 1090: 129–147. https://doi.org/10.3897/zookeys.1090.78834

RESEARCH ARTICLE



# One new genus and two new species of the spider family Phrurolithidae (Arachnida, Araneae) from Xishuangbanna Tropical Botanical Garden, Southwest China

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| Academic editor: Zhiyuan Yao   Receiv | red 17 June 2022   Accepted | 29 July 2022 | Published 11 August 2022 |
|---------------------------------------|-----------------------------|--------------|--------------------------|
| https://zoobank.                      | org/6D5ED7FE-C48B-4239-92C  | E-CB61657089 | 39                       |

**Citation:** Liu K, Ying Y, Li S (2022) One new genus and two new species of the spider family Phrurolithidae (Arachnida, Araneae) from Xishuangbanna Tropical Botanical Garden, Southwest China. ZooKeys 1117: 71–94. https://doi.org/10.3897/zookeys.1117.89211

#### Abstract

*Edelithus* gen. nov. is described based on the discovery and description of two new species from Xishuangbanna, Yunnan Province, China: *E. puer* sp. nov. and *E. shenmiguo* sp. nov. Both species are described in detail and illustrated. Types are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing, China.

#### Keywords

Eurasia, phrurolithid, taxonomy, types, Yunnan

# Introduction

The spider family Phrurolithidae Banks, 1892 includes 20 genera and 313 species from America, Australia, and Eurasia (WSC 2022). In China, 173 phrurolithid species are known belonging to 12 genera (Liu et al. 2022): *Abdosetae* Fu, Zhang & MacDermott, 2010, *Acrolithus* Liu & Li, 2022, *Aculithus* Liu & Li, 2022, *Alboculus* Liu, 2020,

*Bosselaerius* Zamani & Marusik, 2020, *Corealithus* Kamura, 2021, *Grandilithus* Liu & Li, 2022, *Otacilia* Thorell, 1897, *Pennalithus* Kamura, 2021, *Phrurolithus* C.L. Koch, 1839, *Phrurotimpus* Chamberlin & Ivie, 1935, and *Plynnon* Deeleman-Reinhold, 2001. It is clear that China has the most species- and genus-rich phrurolithid fauna (Wang et al. 2020; Li et al. 2021; Yao et al. 2021; Hong et al. 2022; Zhu et al 2022).

While studying the phrurolithid species from Xishuangbanna Tropical Botanical Garden in Yunnan Province, China (XTBG; Li 2020), a new genus and two new species are found. The goal of this paper is to describe the new genus and species from XTBG.

#### Materials and methods

Specimens were examined using a SZ6100 stereomicroscope. Both male and female copulatory organs were dissected and examined in 80% ethanol using an Olympus CX43 compound microscope with a KUY NICE CCD camera. The epigynes were cleared with pancreatin solution. Specimens, including dissected male palps and epigynes, were preserved in 75% ethanol after examination. For SEM photographs, the specimens were kept under natural dry conditions, coated with gold with a small ion-sputtering apparatus ETD-2000, and photographed with a Zeiss EVO LS15 scanning electron microscope. Types are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing, China.

The measurements were taken using a stereomicroscope (Axio Vision SE64 rel. 4.8.3) and are given in millimetres. The body lengths of all specimens exclude the chelicerae and spinnerets. Terminology of the male and female genitalia follows Ramírez (2014) and Liu et al. (2022).

Leg measurements are given as total length (femur, patella, tibia, metatarsus, tarsus). Promarginal and retromarginal teeth on the chelicerae are given as the fproximal, median and distal and counted from the base of the fang to the distal groove. Leg spines are documented by dividing each leg segment into four aspects: dorsal (d), prolateral (p), and retrolateral (r), and indicating the ventral (v) spines as single (1) or paired (2), e.g., femur I d2, pv1111; tibia d1, I v2222. The abbreviations used in the figures are as follows:

#### Eyes

| ALE<br>AME<br>MOA      | anterior lateral eye;<br>anterior median eye;<br>median ocular area;                     | PLE<br>PME            | posterior lateral eye;<br>posterior median eye.                          |
|------------------------|--|-----------------------|--|
| Legs                   |  |                       |  |
| CS<br>CTC<br>LO<br>MPB | chemsensory seta;<br>claw tuft clasper;<br>lyriform organ;<br>metatarsal preening brush; | MTS<br>Sc<br>SS<br>TS | metatarsal dorsal stopper;<br>scale;<br>slit sensillum;<br>tenent setae. |
# Chelicerae

| PES<br>PRS<br>RES             | promarginal escort seta;<br>promarginal rake seta;<br>retromarginal escort seta;   | SS<br>WS                | slit sensillum;<br>whisker seta.   |
|-------------------------------|--|-------------------------|--|
| Male pal                      | р  |                         |  |
| dTA<br>DTA<br>Em<br>FA<br>rTA | distal tegular apophysis;<br>dorsal tibial apophysis;<br>embolus;<br>femoral apophysis;<br>retrolateral tegular apophysis; | RTA<br>SD<br>sTA<br>VTA | retrolateral tibial apophysis;<br>sperm duct;<br>subdistal tegular apophysis;<br>ventral tibial apophysis. |
| Epigyne                       |  |                         |  |
| Bu<br>CD<br>CO<br>CT          | bursa;<br>copulatory duct;<br>copulatory opening;<br>connecting tube;  | FD<br>GA<br>MS<br>Spe   | fertilization duct;<br>glandular appendage;<br>median septum;<br>spermathecae.                             |

# Taxonomy

Family Phrurolithidae Banks, 1892

## Edelithus Liu & Li, gen. nov.

https://zoobank.org/59555B23-B0D4-4DD9-B5C9-3BCF0E6C98EE

## Type species. Edelithus shenmiguo Liu & Li sp. nov. by designation herein.

**Diagnosis.** The new genus differs from *Labialithus* Kamura, 2021 (see Kamura 2021: figs 9F–J, 10B, C) by the small PME with indistinct black pigment around the eye cup (vs large PME with clear pigment around the eye cup in *Labialithus*) (Figs 1D, 4C, 6D, 11D), the femora I with one dorsal spine (vs absent in *Labialithus*) and three prolateral spines (vs one in *Labialithus*) (Figs 1F, 4F, 6F, 8A, 11F) and the metatarsi III–IV lacking ventral spines (vs usually with two pairs in *Labialithus*), the male scutum covering nearly 1/2 of abdomen (vs more than 2/3 in *Labialithus*) and by the palpal tibia with a dorsal apophysis (vs absent in *Labialithus*). It can be separated from *Otacilia* (see Wang et al. 2015: fig. 14A; Liu et al. 2022: suppl. 2, figs 72, 74, 75, 77–79, 81, 82, 84, 85, 87, 88, 90, 91, 93–96, 98, 99, 101–105, 107–109, 111, 113, 114, 116–118, 120, 124, 137, 141) by the light abdomen lacking dark stripes (vs present in *Otacilia*) (Figs 1A, 4A, 6A, 11A), femora II lacking prolateral spine (in most specimens) or with one prolateral spine (in the few specimens) (vs 2–4 spines in *Otacilia*) (Figs 1G, 4G, 6G, 8C, 11G), the palpal femur with a weakly protruded ventral apophysis (vs moder-

ately or strongly protruded in *Otacilia*) (Figs 2, 3, 9, 10) and the small, short embolus (vs relatively large hook-shaped or spine-like embolus) (Figs 2, 3, 9, 10). Male of this genus can be easily distinguished from *Phrurolithus* (see Wang et al. 2015: fig. 15C–E; Zamani and Marusik 2020: figs 4A–C, E, F, 7A–E) by the scutum covering nearly 1/2 of abdomen (vs nearly entire abdomen in *Phrurolithus*) (Figs 1A, 6A) and by the palpal tibia with a dorsal apophysis (vs absent *Phrurolithus*) (Figs 2D, 3H, 9E, 10I). Females of this genus can be separated from the genus *Labialithus* by the very small, widely separated copulatory openings without atrium (vs relatively large, slightly separated copulatory openings with distinct atrium) (Figs 5, 12). Furthermore, *Edelithus* spp. differ from some phrurolithus and *Aculithus* Liu & Li, 2022 with three teeth, in *Alboculus* with two teeth, and in *Grandilithus* and *Otacilia* with four teeth (see Liu et al. 2020a: fig. 5J; Liu et al. 2022: figs 4C, D, G, H, L, P, 38D, E, H, K, O, 122B, C, E, I, M), but in *Phrurolithus* only with degenerated and inconspicuous blunt teeth (Ramírez 2014: fig. 75E).

**Etymology.** The name is a combination of the first three letters of "*edentatus*" (referring to the tarsal claws lacking tooth) and the latter half of *Phrurolithus*. The gender is masculine.

**Description.** Small, body length 1.0–2.5. Eyes (Figs 1D, 4C, 6D, 7A, 11D): AER straight and PER procurved in dorsal view, AME clearly smaller than other eyes, PME with indistinct black pigment around eye cups, smaller than ALE and PLE, nearly separated by their diameter. Chelicera (Figs 1D, 4A, 6D, 7A, B, 11D) with one frontal strong spine, three promarginal and two retromarginal teeth. Legs without annulations and stripes. Femora I–IV with one dorsal spine each (Figs 1F, G, 4F, G, 6F, G, 8A, C, 11F, G), femur I with three prolateral spines, and femur II with one prolateral spine or none, tibiae I and II with six pairs of ventral spines; metatarsi I and II with tour pairs of ventral spines. Scutum (Figs 1A, 6A) covers nearly 1/2 of abdomen in males, but absent in females (Figs 4A, 11A).

*Male palp* (Figs 2, 3, 9, 10): femur with a weak ventral extension; tibia with two welldeveloped apophyses, retrolateral apophysis very thick, as long as or shorter than tibia, dorsal apophysis hook-shaped, shorter than the retrolateral one; tegulum with a leaf-shaped subdistal apophysis and a blunt retrolateral apophysis; embolus short, shorter than subdistal tegular apophysis, with a round sperm pore, touching subdistal tegular apophysis.

*Epigyne* (Figs 5, 12) with a pair of small copulatory openings, located posteriorly or subposteriorly; median septum absent or located posteriorly; bursae large, covering nearly 1/2 of epigynal plate, anteriorly located.

**Composition.** *Edelithus puer* sp. nov. and *E. shenmiguo* sp. nov. **Distribution.** China (Yunnan Province).

### Edelithus puer Liu & Li, sp. nov.

https://zoobank.org/73417E12-1129-408E-9A01-046FC40865FF Figs 1–5

**Material examined.** *Holotype* ♂ (Phu-147), 21°54.607'N, 101°17.005'E, elevation ca 633 m, XTBG, Menglun Township, Mengla County, Xishuangbanna, Yunnan Province, China, 4–11.IV.2007, G. Zheng leg. *Paratypes* 1 ♂, 2 ♀, the same data

as holotype;  $1 \bigcirc$ , 4–11.IV.2007, other data as holotype (JSIII-2-18);  $1 \bigcirc$ , 10–20. VI.2007, other data as holotype (ISIII-1-20); 1 , 1--15.VIII.2007, other data as holotype (JSIII-3-23); 3 Å, 16–31.III.2007, other data as holotype (JSIII-5-16); 1 ♀, 10-20.VI.2007, other data as holotype (JSIII-2-20); 1 ♀, 16-31.V.2007, other data as holotype (JSIII-1-20); 2 3, 1–15.IV.2007, other data as holotype (JSIII-5-17); 5  $\emptyset$ , 1  $\Im$ , 1–15.IV.2007, other data as holotype (JSIII-2-17); 2  $\emptyset$ , 1–15.IV.2007, other data as holotype (JSIII-4-17); 3 Å, 2 juveniles, 1–15.IV.2007, other data as holotype (JSIII-3-17); 1 2, 19-26.V.2007, other data as holotype (JSIII-2-17); 1 ♀, 16–31.VI.2007, other data as holotype (JSIII-5-22); 3 ♀, 16–31. IV.2007, other data as holotype (JSIII-5-18);  $2 \stackrel{\bigcirc}{_{+}}$ , 4–11.V.2007, other data as holotype (JSIII-3-18);  $1 \, \bigcirc$ , 4–11.V.2007, other data as holotype (JSIII-1-19);  $1 \, \bigcirc$ , 19– 26.V.2007, other data as holotype (JSIII-2-17); 3 ♀, 16–31.IV.2007, other data as holotype (JSIII-3-22);  $1 \, \bigcirc$ , 4–11.V.2007, other data as holotype (JSIII-1-18);  $1 \, \bigcirc$ , 19–26.IV.2007, other data as holotype (JSIII-3-17); 1 ♀, 1–15.V.2007, other data as holotype (JSIII-5-19); 1 ♀, 10–20.VI.2007, other data as holotype (JSIII-3-20); 1 ♂, 16–31.IV.2007, other data as holotype (JSIII-4-18); 1 ♀, 16–31.V.2007, other data as holotype (ISIII-3-20);  $1 \, \bigcirc$ , 19–26.IV.2007, other data as holotype (ISIII-4-17); 2  $\bigcirc$ , 19–26.V.2007, other data as holotype (JSIII-2-19); 6  $\bigcirc$ , 1  $\bigcirc$ , 16–31.IV.2007, other data as holotype (JSIII-1-18); 1 , 19-26.V.2007, other data as holotype (JSI-II-4-19); 6  $(3, 1 \ 2, 16-31.III.2007, other data as holotype (ISIII-1-16); 3 <math>(3, 16-31.1II.2007, other data as holotype (ISIII-1-16); 3 (3, 16-31.1II.2007$ 31.III.2007, other data as holotype (JSIII-1-16); 2 3, 16–31.III.2007, other data as holotype (JSIII-3-16);  $1 \stackrel{\bigcirc}{\downarrow}$ , 1–15.V.2007, other data as holotype (JSIII-2-19);  $1 \, \bigcirc, 19-25. \text{XI.} 2007$ , other data as holotype (JSIII-3-03);  $4 \, \bigcirc, 2 \, \bigcirc, 1-15. \text{IV.} 2007$ , other data as holotype (JSIII-1-17); 2  $\stackrel{\scriptstyle \circ}{\scriptstyle \circ}$ , 1–15.III.2007, other data as holotype (JSI-II-3-15); 1 ♂, 16–31.IV.2007, other data as holotype (JSIII-3-18); 1 ♂, 1 ♀, 16–31. IV.2007, other data as holotype (JSIII-2-18); 2 ♀, 16–31.VI.2007, 21°55.428'N, 101°16.441'E, elevation ca 598 m, other data as holotype (CZI-3-22);  $1 \stackrel{\bigcirc}{_{-}}, 16-31$ . VI.2007, other data as holotype (CZI-5-22); 1 ♀, 16–31.VI.2007, other data as holotype (CZI-2-22); 4 3, 16–31.VI.2007, 21°54.984'N, 101°16.982'E, elevation ca 656 m, other data as holotype (JSIII-5-18); 1 Q, 4-11.V.2007, other data as previous (JSII-3-18); 1  $\bigcirc$ , 10–20.VI.2007, other data as previous (JSII-2-20); 1  $\bigcirc$ , 16-31.VI.2007, other data as previous (JSIII-4-18); 2 3, 1-15.III.2007, other data as previous (JSII-5-15); 1  $\bigcirc$ , 1–15.V.2007, other data as previous (JSII-2-19); 3  $\bigcirc$ , 4-11.IV.2007, other data as previous (JSII-2-16); 5 Å, 19-26.III.2007, other data as previous (JSII-4-15); 7  $\bigcirc$ , 1–15.IV.2007, other data as previous (JSII-2-17); 2  $\bigcirc$ , 1–15.V.2007, other data as previous (JSII-5-19); 4 3, 16–31.III.2007, other data as previous (JSII-4-16); 2  $\stackrel{?}{\odot}$ , 1–15.III.2007, other data as previous (JSII-1-15); 2  $\stackrel{?}{\ominus}$ , 19-26.V.2007, other data as previous (JSII-4-19); 2 3, 16-31.III.2007, other data as previous (JSII-5-16);  $2 \stackrel{\circ}{\triangleleft}$ , 1–15.IV.2007, other data as previous (JSII-4-17);  $6 \stackrel{\circ}{\dashv}$ , 2  $\bigcirc$ , 16–31.IV.2007, other data as previous (JSII-3-18); 3  $\bigcirc$ , 1–15.IV.2007, other data as previous (JSII-1-17);  $2 \bigcirc$ , 4–11.V.2007, other data as previous (JSII-2-18); 1  $\bigcirc$ , 16–31.IV.2007, other data as previous (JSII-5-22); 3  $\bigcirc$ , 4–11.V.2007, other data as previous (JSII-4-18); 1 , 19-26.V.2007, other data as previous (JSII-1-19); 2 ♀, 1–15.V.2007, other data as previous (JSII-1-19); 2 ♀, 16–31.IV.2007, other

data as previous (JSII-4-22); 6 Å, 16–31.III.2007, other data as previous (JSII-1-16); 2 3, 1 9, 16–31.IV.2007, other data as previous (JSII-2-18); 4 3, 16–31. III.2007, other data as previous (JSII-3-16); 6 3, 1–15.IV.2007, other data as previous (JSII-3-17); 3 ♀, 19–26.IV.2007, other data as previous (JSII-4-17); 2 ♀, 4–16. IV.2007, other data as previous (JSII-4-16); 1 , 10-20.VI.2007, other data as previous (JSII-4-20); 1  $\bigcirc$ , 16–31.V.2007, other data as previous (JSII-3-20); 3  $\bigcirc$ , 1  $\bigcirc$ , 1–15.IV.2007, other data as previous (JSII-5-17); 1 ♀, 16–31.V.2007, other data as previous (JSII-5-20); 1 , 19-26.IV.2007, other data as previous (JSII-1-17); 1 , 1919–26.IV.2007, other data as previous (JSII-2-17); 5 Å, 16–31.III.2007, other data as previous (JSII-2-16);  $3 \stackrel{\circ}{\downarrow}$ , 1–15.VI.2007, other data as previous (JSII-5-21); 1  $\mathcal{Q}$ , 1–15.VII.2007, other data as previous (JSII-5-23); 2  $\mathcal{Q}$ , 1–15.VI.2007, other data as previous (JSII-3-21); 1 ♀, 1–15.VI.2007, other data as previous (JSII-2-21); 2  $\bigcirc$ , 1–15.VII.2007, other data as previous (JSII-2-23); 1  $\bigcirc$ , 16–31.III.2007, 21°54.718'N, 101°16.940'E, elevation ca 645 m, other data as holotype (JSI-4-16); 1 ♀, 19–26.IV.2007, other data as previous (JSI-3-17); 2 ♂, 1–15.III.2007, other data as previous (JSI-3-15); 1  $\bigcirc$ , 16–31.IV.2007, other data as previous (JSI-5-18); 4 ♂, 1–15.IV.2007, other data as previous (JSI-4-17); 4 ♀, 16–31. VII.2007, other data as previous (JSI-2-24); 2 (3, 10-20.VI.2007), other data as previous (JSI-3-20); 2, 1–15.V.2007, other data as previous (JSI-2-19); 1  $\bigcirc$ , 1–15.IV.2007, other data as previous (JSI-4-21); 1  $\bigcirc$ , 10–20.IV.2007, other data as previous (JSI-1-20); 2  $\bigcirc$ , 1–15.VI.2007, other data as previous (JSI-2-21); 2, 1–15.VII.2007, other data as previous (JSI-2-23); 5  $\stackrel{\circ}{\circ}$ , 16–31.III.2007, other data as previous (JSI-1-16); 1  $\stackrel{\circ}{\circ}$ , 1–15.IV.2007, other data as previous (JSI-3-17);  $2 \ \mathcal{Q}$ , 16–31.V.2007, other data as previous (JSI-5-20); 1  $\bigcirc$ , 16–24.X.2007, other data as previous (JSI-2-06); 3  $\bigcirc$ , 1  $\bigcirc$ , 16–31.V.2007, other data as previous (JSI-1-20); 1 ♀, 16–31.VII.2007, other data as previous (JSI-3-24); 1  $\bigcirc$ , 4–11.V.2007, other data as previous (JSI-2-18); 3  $\bigcirc$ , 1–15.IV.2007, other data as previous (JSI-5-17); 1 2, 16–31.VII.2007, other data as previous (JSI-5-24); 2  $\bigcirc$ , 2  $\bigcirc$ , 19–26.IV.2007, other data as previous (JSI-4-17);  $1 \, \bigcirc$ , 4–11.V.2007, other data as previous (JSI-3-18); 2  $\bigcirc$ , 1–15.III.2007, other data as previous (JSI-2-15);  $1 \triangleleft, 2 \heartsuit, 16-31.V.2007$ , other data as previous (JSI-4-20); 1, 1–15.V.2007, other data as previous (JSI-5-19); 2 , 4–11.IV.2007, other data as previous (JSI-1-16);  $1 \stackrel{\bigcirc}{\downarrow}$ , 19–26.IV.2007, other data as previous (JSI-2-17);  $1 \stackrel{\bigcirc}{\downarrow}$ , 19–26.V.2007, other data as previous (JSI-3-19); 1 ♂, 1 ♀, 16–31.V.2007, other data as previous (JSI-2-20);  $1 \, \bigcirc$ , 10–20.VI.2007, other data as previous (JSI-4-20);  $1 \, \bigcirc, 19-26.$  V.2007, other data as previous (JSI-2-19);  $1 \, \bigcirc, 1 \, \bigcirc, 1-15.$  V.2007, other data as previous (JSI-1-19);  $1 \stackrel{\bigcirc}{\downarrow}$ , 4–11.IV.2007, other data as previous (JSI-2-16); 1  $\bigcirc$ , 10–20.VI.2007, other data as previous (JSI-2-20); 1  $\bigcirc$ , 16–31.IV.2007, other data as previous (JSI-4-18); 6 ♂, 1 ♀, 16–31.IV.2007, other data as previous (JSI-3-18); 5 3, 4 9, 16-31. IV.2007, other data as previous (JSI-2-18); 8 3, 16-31.III.2007, other data as previous (JSI-2-16); 4 Å, 1–15.IV.2007, other data as previous (JSI-1-17); 5 ♂, 1 ♀, 16–31.III.2007, other data as previous (JSI-3-16); 10 ♂, 2  $\bigcirc$ , 1–15.IV.2007, other data as previous (JSI-2-17); 1  $\bigcirc$ , 16–31.V.2007, other data as previous (JSI-3-30); 1 , 19-26.V.2007, other data as previous (JSI-4-19);  $2 \stackrel{\wedge}{\triangleleft}, 2 \stackrel{\circ}{\subsetneq}, 16-31.$  IV.2007, other data as previous (JSI-1-18).



**Figure 1**. *Edelithus shenmiguo* sp. nov., male **A** habitus, dorsal view **B** same, ventral view **C** same, lateral view **D** carapace, dorsal view, white arrow to cheliceral spine, black arrow to oval posterior median eyes without black annulations **E** same, ventral view **F** leg I, prolateral view, black arrows to prolateral spines on femur **G** leg II, white arrow to prolateral spine on femur. Scale bars: 0.1 mm (**A**, **B**, **D**–**G**), 0.5 mm (**C**).

**Etymology.** The specific name refers to a famous tea from Xishuangbanna, Pu'er tea, which is planted on the mountainsides of Xishuangbanna and has a long history in China; noun in apposition.



**Figure 2.** *Edelithus shenmiguo* sp. nov., male palps **A** holotype, prolateral view **B** same, ventral view **C** same, retrolateral view **D** same, dorsal view **E** paratype, prolateral view **F** same, ventral view **G** same, retrolateral view **H**, **I** tegulum, retrolateral view. Abbreviations: DTA – dorsal tibial apophysis, Em – embolus, FA – femoral apophysis, rTA – retrolateral tegular apophysis, RTA – retrolateral tibial apophysis, SD – sperm duct, sTA – subdistal tegular apophysis. Scale bars: 0.1 mm.



**Figure 3.** SEM micrographs of *Edelithus shenmiguo* sp. nov., male palp **A** femur, prolateral view **B** ventral view, detail of tegular end **C** femur, ventral view **D** retrolateral view **E** retrolateral view, detail of retrolateral tibial apophysis **F** retrolateral view, detail of tegular **G** same, detail of tegular end **H** same, detail of tibial apophyses. Abbreviations: dTA – distal tegular apophysis, DTA – dorsal tibial apophysis, Em – embolus, FA – femoral apophysis, RTA – retrolateral tibial apophysis, SD – sperm duct, sTA – subdistal tegular apophysis.

**Diagnosis.** The new species can be distinguished from *E. shenmiguo* sp. nov. (Figs 9, 10, 12) by the retrolateral tegular apophysis with bent apex (vs straight) and the very short embolus lacking spine-like tip (vs the relatively long embolus with a spine-like tip) in male palp (Figs 2, 3) and the triangular median septum (vs absent), the stout copulatory ducts (vs slender) and the C-shaped spermathecae (vs oval) in female epigyne (Fig. 5).

**Description.** Male (holotype). Habitus as in Fig. 1A–C. Total length 1.95, carapace 0.99 long, 0.78 wide, abdomen 0.92 long, 0.65 wide. Eye sizes and interdistances (Fig. 1A, D): AME 0.04, ALE 0.06, PME 0.05, PLE 0.06; AME–AME 0.03, AME–ALE 0.01, PME–PME 0.04, PME–PLE 0.04, AME–PME 0.05, AME–PLE 0.09, ALE–ALE 0.13, PLE–PLE 0.21, ALE–PLE 0.03; PME separated by slightly less than their diameters. MOA 0.14 long, frontal width 0.11, posterior width 0.13. Chelicerae (Fig. 1B, D, E) with three promarginal (median largest, distal smallest) and two retromarginal teeth (distal larger). Endites (Fig. 1B, E) slightly oblique, brush shaped, anterolateral area of endite with row of thick serrula and six long, thick setae. Labium wider than long, anteriorly with 10–12 setae. Sternum (Fig. 1E), longer than wide, lateral margin thickened, with weak precoxal triangles and lacking intercoxal extensions, posteriorly triangular, blunt end. Legs (Fig. 1): measurements: I 3.29 (0.90, 0.35, 0.84, 0.76, 0.44); II 3.85 (0.73, 0.48, 0.97, 0.99, 0.68); III 2.53 (0.66, 0.32, 0.48, 0.60, 0.47); IV 3.74 (0.96, 0.37, 0.84, 0.95, 0.62); spination: femora I d1, pv111, II d1, III d1, IV d1; tibiae I v22222, II v22221, metatarsi I v2221, II v2221. Scutum (Fig.1A) nearly covering 1/2 of abdomen.

**Colouration** (Fig. 1A–C). Carapace yellow, with radial, irregular light yellowbrown stripes submarginally and arc-shaped dark stripes around margin. AME, ALE and PLE with dark layer of black pigment around the eye cup, but PME absent. Chelicerae, endites, and labium yellow. Sternum yellow, mottled around margin. Legs yellow, without dark stripes. Abdomen yellow-brown, mottled, with dark brown netshaped stripes; venter yellow.

**Palp** (Figs 2, 3). Femoral apophysis weak, with shallow groove and one strong dorsal spine near distal femur. Retrolateral tibial apophysis large, thick, finger-like, longer than tibia. Dorsal tibial apophysis longer than 1/2 length of retrolateral tibial apophysis, with broad base and a small hook-shaped tip, subdistal part with a strong constriction. Sperm duct V-shaped, reaching subposterior part of tegulum. Distal tegular apophysis lamellate, membranous, touching the base of embolus, covered by subdistal tegular apophysis in ventral view. Subdistal tegular apophysis gramineous leaf-shaped, membranous, slightly less than 1/2 of tegular length. Embolus very short, horn-like, less than 1/3 length of subdistal tegular apophysis, covered by subdistal tegular apophysis. Sperm opening round, located in subapical part.

**Female.** Habitus as in Fig. 4. Total length 2.21, carapace 0.92 long, 0.75 wide, abdomen 1.27 long, 0.83 wide. As in male, except as noted. Eye sizes and interdistances (Fig. 4A, D): AME 0.04, ALE 0.07, PME 0.04, PLE 0.06, AME–AME 0.02, AME–ALE 0.01, PME–PME 0.06, PME–PLE 0.04, AME–PME 0.04, AME–PLE 0.09, ALE–ALE 0.12, PLE–PLE 0.20, ALE–PLE 0.03. MOA 0.12 long, frontal width 0.10, posterior width 0.13. Leg (Fig. 4A, B) measurements: I 4.05 (1.07, 0.48, 1.04, 0.95, 0.51); II 2.61 (0.67, 0.35, 0.54, 0.60, 0.45); III 2.37 (0.63, 0.29, 0.45, 0.57, 0.43); IV 3.41 (0.89, 0.37, 0.74, 0.89, 1.060.52). Leg spination (Fig. 4): tibiae II v22222, metatarsi I v2222, II v2222.



**Figure 4.** *Edelithus shenmiguo* sp. nov., female **A** habitus, dorsal view **B** same, ventral view **C** same, lateral view **D** carapace, dorsal view, white arrow to cheliceral spine, black arrow to oval posterior median eyes without black annulations **E** same, ventral view **F** leg I, prolateral view, white arrows to prolateral spines on femur **G** Leg II, white arrow to prolateral spine on femur. Scale bars: 0.1 mm (**A**, **B**, **D**–**G**); 0.5 mm (**C**).



**Figure 5.** *Edelithus shenmiguo* sp. nov., female **A** epigyne, ventral view **B** same, dorsal view **C** same, ventral view **D** same, dorsal view. Abbreviations: Bu – bursa, CD – copulatory duct, CO – copulatory opening, CT – connecting tube, FD – fertilization duct, GA – glandular appendage, MS – median septum, Spe – spermatheca. Scale bars: 0.1 mm.

*Colouration* (Fig. 4A, B). Lighter than male.

*Epigyne* (Fig. 5). Epigynal plate slightly longer than wide, subposterolaterally with pair of round copulatory openings, posteriorly with triangular median septum. Copulatory ducts short and thick, slghtly shorter than spermathecae. Bursae large round, touching, covering nearly 1/2 of epigynal plate. Glandular appendages short, transversal, directed laterally, less than the length of copulatory ducts. Connecting tubes very short, nearly as long as glandular appendages. Spermathecae nearly C-shaped, widely separated by median septum. Fertilization ducts short, located posteriorly on spermathecae, directed anterolaterally.

**Comments.** The detailed study of a large number of these specimens revealed that most specimens (ca 9/10) lack prolateral spine on femora I, but a few specimens (ca 1/10) with one prolateral spine which locate at the distal part of femora I.

Distribution. Known only from the type locality in Yunnan Province, China.

#### Edelithus shenmiguo Liu & Li, sp. nov.

https://zoobank.org/C4A6AD1-0DEE-4CCF-82A6-F14ABA2A6B66 Figs 6–12

**Type material.** *Holotype*  $\mathcal{E}$  (Phu-145, GBII-4-10), 21°57.669'N, 101°11.893'E, elevation ca 790 m, XTBG, Menglun Township, Mengla County, Xishuangbanna, Yunnan Province, China, 5–12.I.2007, G. Zheng leg. **Paratype** 2 3, 1  $\stackrel{\circ}{\downarrow}$ , the same data as holotype (GBII-2-17); 11  $\bigcirc$ , 1  $\bigcirc$ , 16–31.III.2007, other data as holotype (GBII-1-16); 3 ♀, 4–11.IV.2007, other data as holotype (GBII-4-16); 5 ♂, 4 ♀, 16–31. III.2007, other data as holotype (GBII-2-16); 25 ♂, 3 ♀, 16–31.III.2007, other data as holotype (GBII-4-16); 2 Å, 16–31.III.2007, other data as holotype (GBII-4-12); 2 , 5-12. II.2007, other data as holotype (GBII-3-10); 1 , 9, 16-31. VII.2007, other data as holotype (GBII-4-24);  $1 \Diamond, 2 \heartsuit, 16-31.$  IV.2007, other data as holotype (GBII-4-18); 2 ♀, 1–15.V.2007, other data as holotype (GBII-1-19); 4 ♀, 10–20.VI.2007, other data as holotype (GBII-1-20); 3  $\mathcal{Q}$ , 1–15.VII.2007, other data as holotype (GBII-3-23); 2  $\bigcirc$ , 19–26.IV.2007, other data as holotype (GBII-4-17); 3  $\bigcirc$ , 1  $\bigcirc$ , 5–12.I.2007, other data as holotype (GBII-2-10);  $6 \, \bigcirc$ , 4–11.V.2007, other data as holotype (GBII-3-18); 9 3, 3 2, 1–15.III.2007, other data as holotype (GBII-2-15); 1 ♂, 5–12.II.2007, other data as holotype (GBII-4-12); 2 ♂, 2 ♀, 19–26.III.2007, other data as holotype (GBII-3-15); 2 3, 5–12.I.2007, other data as holotype (GBII-2-12); 1 ♂, 1–15.I.2007, other data as holotype (GBII-2-11); 1 ♀, 10–20.VII.2007, other data as holotype (GBII-4-21); 3 ♀, 19-26.IV.2007, other data as holotype (GBII-2-17); 2  $\mathcal{Q}$ , 1–15.I.2007, other data as holotype (GBII-1-23); 1  $\mathcal{Q}$ , 10–20. VII.2007, other data as holotype (GBII-1-21);  $3 \stackrel{<}{\supset}, 1 \stackrel{<}{\subsetneq}, 1-15$ .III.2007, other data as holotype (GBII-5-15); 7 9, 19–26.V.2007, other data as holotype (GBII-1-19); 1 3, 19-26.V.2007, other data as holotype (GBII-3-19); 2 3, 1-15.II.2007, other data as holotype (GBII-4-13); 9  $\stackrel{?}{\odot}$ , 1  $\stackrel{?}{\ominus}$ , 16–31.III.2007, other data as holotype (unspecified); 1 ♂, 16–31.III.2007, other data as holotype (GBII-2-20); 2 ♀, 5–12.III.2007, other data as holotype (GBII-4-14); 1 Q, 1–15.V.2007, other data as holotype (GBII-4-19); 1 ♀, 1–15.IV.2007, other data as holotype (GBII-2-21); 1 ♀, 1–15.IV.2007, other data as holotype (GBII-1-21); 1 ♀, 5–12.XII.2007, other data as holotype (GBII-1-08); 2 ♀, 1–15.IV.2007, other data as holotype (GBII-4-21); 4 ♂, 1–15.III.2007, other data as holotype (GBII-2-13); 3 Å, 1–15.III.2007, other data as holotype (GBII-3-15); 10 (3, 2, 2, 16-31.IV.2007), other data as holotype (GBII-1-18); 9 (2, 3), 9 (2, 3)4–11.V.2007, other data as holotype (GBII-1-18); 2 3, 1–15.IV.2007, other data as holotype (GBII-1-17); 2  $\bigcirc$ , 10–20.VI.2007, other data as holotype (GBII-4-20); 1  $\bigcirc$ , 10-14.VIII.2006, other data as holotype (GBII-4-01); 2 Q, 19-26.IV.2007, other



**Figure 6.** *Edelithus puer* sp. nov., male **A** habitus, dorsal view **B** same, ventral view **C** same, lateral view **D** carapace, dorsal view, white arrows to cheliceral spines, black arrow to oval posterior median eyes without black annulations **E** endites, labium and sternum, ventral view **F** left leg I, prolateral view, black arrows to prolateral spines on femur **G** left leg II, black arrow to prolateral spine on femur. Scale bars: 0.1 mm (**A**, **B**, **D**–**G**); 0.5 mm (**C**).

data as holotype (GBII-2-17); 7  $\Diamond$ , 1  $\heartsuit$ , 16–31.III.2007, other data as holotype (GBII-5-16); 1  $\Diamond$ , 1  $\heartsuit$ , 19–25.II.2007, other data as holotype (GBII-4-13); 4  $\Diamond$ , 1  $\heartsuit$ , 1–15. IV.2007, other data as holotype (GBII-5-17); 7  $\Diamond$ , 16–31.II.2007, other data as holotype (GBII-2-14); 2  $\Diamond$ , 1  $\heartsuit$ , 1–15.IV.2007, other data as holotype (GBII-4-17); 1  $\Diamond$ , 16–31.II.2007, other data as holotype (GBII-3-14); 1  $\heartsuit$ , 4–11.V.2007, other data as



**Figure 7.** *Edelithus puer* sp. nov., male palps **A** holotype, prolateral view **B** same, ventral view **C**, **D** same, retrolateral view **E** same, dorsal view **F** tegulum of paratype, retrolateral view. Abbreviations: DTA – dorsal tibial apophysis, Em – embolus, FA – femoral apophysis, rTA – retrolateral tegular apophysis, RTA – retrolateral tibial apophysis, SD – sperm duct, sTA – subdistal tegular apophysis. Scale bars: 0.1 mm.

holotype (GBII-2-18); 1  $\bigcirc$ , 19–26.IV.2007, other data as holotype (GBII-3-17); 1  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-1-16); 5  $\bigcirc$ , 19–26.V.2007, other data as holotype (GBII-4-19); 5  $\bigcirc$ , 4–11.V.2007, other data as holotype (GBII-4-18); 1  $\bigcirc$ , 2–12.III.2007, other data as holotype (GBII-3-14); 1  $\bigcirc$ , 5–12.III.2007, other data as holotype (GBII-2-14); 2  $\oslash$ , 1  $\bigcirc$ , 19–25.I.2007, other data as holotype (GBII-2-11); 7  $\oslash$ , 16–31.III.2007, other data as holotype (GBII-3-16); 5  $\bigcirc$ , 19–26.V.2007, other data as holotype (GBII-2-19); 2  $\bigcirc$ , 10–20.VI.2007, other data as holotype (GBII-2-20); 1  $\bigcirc$ , 10–20.VI.2007, other data as holotype (GBII-3-18); 2  $\bigcirc$ , 10–20.VI.2007, other data as holotype (GBII-3-20); 2  $\bigcirc$ , 16–31.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 2  $\bigcirc$ , 16–31.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, other data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, her data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, her data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, her data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007, her data as holotype (GBII-3-18); 4  $\bigcirc$ , 4–11.IV.2007,

2-16); 1 ♀, 16–31.IV.2007, other data as holotype (GBII-3-22); 1 ♂, 21°54.813'N, 101°12.634'E, elevation ca 876 m, 1–15.IV.2007, other data as holotype (GBII-4-17); 5 Å, 1–15.IV.2007, other data as previous (GBIII-3-17); 1 Å, 1–15.IV.2007, other data as previous (GBIII-5-17);  $5 \emptyset$ , 1–15.IV.2007, other data as previous (GBIII-2-17); 1  $\bigcirc$ , 1–15.VII.2007, other data as previous (GBIII-2-23); 1  $\bigcirc$ , 19–26.IV.2007, other data as previous (GBIII-4-17); 4 3, 16-31.IV.2007, other data as previous (GBI-II-3-18); 2 \overline, 4-11.V.2007, other data as previous (GBIII-3-18); 2 \overline, 19-26.IV.2007, other data as previous (GBIII-2-19); 1 3, 4–11.IV.2007, other data as previous (GBI-II-1-16); 2 ♂, 1–15.III.2007, other data as previous (GBIII-1-15); 1 ♀, 16–31.V.2007, other data as previous (GBIII-3-20); 1 (3, 16–31.IV.2007, other data as previous (GBI-II-4-18); 2 ♀, 10–20.IV.2007, other data as previous (GBIII-4-20); 3 ♀, 19–26.V.2007, other data as previous (GBIII-3-19);  $1 \triangleleft, 1 \supsetneq, 4-11.$  IV.2007, other data as previous (GBIII-4-16); 2  $\bigcirc$ , 16–26.V.2007, other data as previous (GBIII-4-19); 2  $\bigcirc$ , 4–11.V.2007, other data as previous (GBIII-4-18); 2 ♂, 1 ♀, 16–31.III.2007, other data as previous (GBIII-4-16); 4 3, 16-31.III.2007, other data as previous (GBI-II-5-16); 1 ♀, 10–20.VII.2007, other data as previous (GBIII-4-18); 5 ♀, 21°57.445'N, 101°12.997'E, elevation ca 744 m, 4–11.V.2007, other data as holotype (GBIII-1-18);  $1 \, \odot, \, 16-31.$ V.2007, other data as previous (GBIII-4-20);  $3 \, \odot, \, 19-26.$ IV.2007, other data as previous (GBIII-3-17); 3 ♀, 19-26.III.2007, other data as previous (GBI-II-4-15); 1  $\Diamond$ , 6  $\heartsuit$ , 4–11.V.2007, other data as previous (GBIII-3-18); 4  $\heartsuit$ , 4-11.V.2007, other data as previous (GBIII-2-18); 2 , 4-11.IV.2007, other data as previous (GBIII-3-16); 1  $\stackrel{?}{\bigcirc}$ , 5–12.II.2007, other data as previous (GBIII-4-12); 5  $\stackrel{?}{\ominus}$ , 4–11.V.2007, other data as previous (GBIII-4-18); 1 ♀, 16–31.V.2007, other data as previous (GBIII-1-20); 8 3, 1 9, 16–31.III.2007, other data as previous (GBIII-1-16); 2 ♀, 10–20.VI.2007, other data as previous (GBIII-1-20); 3 ♀, 16–31.VI.2007, other data as previous (GBIII-2-22); 1 , 1-15.V.2007, other data as previous (GBIII-2-19); 6 ♀, 19–26.IV.2007, other data as previous (GBIII-4-17); 2 ♀, 1–15.V.2007, other data as previous (GBIII-4-19); 1 3, 1–15.III.2007, other data as previous (GBI-II-5-15); 2 ♀, 19–26.IV.2007, other data as previous (GBIII-1-17); 8 ♀, 19–26.V.2007, other data as previous (GBIII-4-19); 1 3, 19-26.III.2007, other data as previous (GBIII-3-15); 7 Å, 1 Q, 16–31.III.2007, other data as previous (GBIII-3-16); 4 Å, 1-15.IV.2007, other data as previous (GBIII-1-17); 1 3, 5-12.I.2007, other data as previous (GBIII-4-10);  $1 \bigcirc , 5-12.$  VI.2006, other data as previous (GBIII-1-06);  $2 \bigcirc ,$ 19–26.III.2007, other data as previous (GBIII-2-15); 1 Å, 16–31.III.2007, other data as previous (GBIII-3-05);  $3 \stackrel{\bigcirc}{_{-}}$ , 4–11.IV.2007, other data as previous (GBIII-4-16); 1 ♀, 16–31.II.2007, other data as previous (GBIII-4-14); 6 ♂, 1–15.IV.2007, other data as previous (GBIII-2-17); 3 2, 19–26.V.2007, other data as previous (GBIII-1-19); 1  $\mathcal{Q}$ , 16–31.V.2007, other data as previous (GBIII-3-20); 1  $\mathcal{Q}$ , 1–15.IV.2007, other data as previous (GBIII-5-21);  $1 \, \bigcirc, 5-12.$ III.2007, other data as previous (GBIII-3-14); 1 ∂, 19–26.III.2007, other data as previous (GBIII-1-15); 1 ♀, 10–20.VI.2007, other data as previous (GBIII-3-20); 2 ♀, 10-20.VI.2007, other data as previous (GBI-II-4-20); 13 3, 16–31.III.2007, other data as previous (GBIII-4-16); 7 3, 3  $\bigcirc$ , 1–15. IV.2007, other data as previous (GBIII-4-17); 7  $\bigcirc$ , 3  $\bigcirc$ , 16–31.III.2007, other data as previous (GBIII-2-16);  $2 \ \bigcirc$ , 4–11.IV.2007, other data as previous (GBIII-2-16);  $1 \ \bigcirc$ , 10–20.VI.2007, other data as previous (GBIII-3-21);  $1 \ \bigcirc$ , 5–12.III.2007, other data as previous (GBIII-2-14);  $1 \ \bigcirc$ , 16–24.IX.2006, other data as previous (GBIII-3-04);  $2 \ \bigcirc$ , 1–15.IV.2007, other data as previous (GBIII-5-17);  $1 \ \bigcirc$ , 1–15.IV.2007, other data as previous (GBIII-3-17);  $3 \ \bigcirc$ ,  $8 \ \bigcirc$ , 5–12.III.2007, other data as previous (GBIII-4-14); 11 \ \bigcirc, 10–31.III.2007, other data as previous (GBIII-5-16);  $2 \ \bigcirc$ , 5–12. III.2007, other data as previous (GBIII-3-19);  $2 \ \bigcirc$ , 5–12.XI.2007, other data as previous (GBIII-2-06);  $1 \ \bigcirc$ , 16–31.IV.2006, other data as previous (GBIII-5-18);  $2 \ \bigcirc$ , 16–31.VI.2006, other data as previous (GBIII-4-22);  $1 \ \bigcirc$ ,  $4 \ \bigcirc$ , 21°55.035'N, 101°16.500'E, elevation ca 558 m, 16–31.V.2007, other data as holotype (GZI-4-20);  $1 \ \bigcirc$  (GBIII-4-12).



**Figure 8.** SEM micrographs of *Edelithus puer* sp. nov., male **A** eyes and chelicerae, dorsal view, white arrows to cheliceral spines **B** chelicerae, endites, and labium, ventral view **C** chelicera, prolateral view **D** same, ventral view **E** same, ventral view, close-up, white arrows to details of teeth. Abbreviations: PES – promarginal escort seta, PRS – promarginal rake setae, RES – retromarginal escort seta, SS – slit sensil-lum, WS – whisker setae.



**Figure 9.** SEM micrographs of *Edelithus puer* sp. nov., male **A** left leg I, white arrows to detail of prolateral spines, prolateral view **B** same, tarsal claws, prolateral view **C** left leg II, white arrow to detail of prolateral spine, prolateral view **D** same, detail of claw tuft setae **E** left leg III, detail of metatarsal preening brush, prolateral view **F** left leg IV, metatarsus-tarsus joint, prolateral view **G** same, detail of tarsal end, prolateral view **H** same, tarsus, detail of the tarsal organ, prolateral view, slightly dorsal **I** same, tarsal claw and claw tuft setae, prolateral view **J** left femur II, prolateral view. Abbreviations: CS – chemosensory seta, LO – lyriform organ, MPB – metatarsal preening brush, MTS – metatarsal dorsal stopper, SS – slit sensillum, TS – tenent setae.



**Figure 10.** SEM micrographs of *Edelithus puer* sp. nov., male palp **A** prolateral view, slightly ventral **B** same, detail of embolus **C** ventral view **D** same, detail of subdistal tegular apophysis **E** femur, retrolateral view **F** retrolateral view **G** same, detail of retrolateral tibial apophysis **H** same, detail of tegular end **I** dorsal view. Abbreviations: DTA – dorsal tibial apophysis, Em – embolus, FA – femoral apophysis, rTA – retrolateral tibial apophysis, sTA – subdistal tegular apophysis.

**Etymology.** The specific name refers to the Chinese name of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell, 1852, shenmiguo, which was introduced to XTBG from Ghana; noun in apposition.

**Diagnosis.** The new species can be distinguished from *E. puer* sp. nov. (Figs 2, 3, 5) by the ridge-shaped retrolateral tegular apophysis (vs bent) and the relatively long embolus with a spine-like tip (vs the very short embolus lacking spine-like tip) in male palp (Fig. 10B, H), and the epigynal plate lacking median septum (vs present), the relatively long, thin copulatory duct (vs very short and thick) and the oval spermathecae (vs C-shaped) (Fig. 12) in female epigyne (Fig. 12).

Description. Male (holotype). Habitus as in Fig. 6A-C. Total length 1.93, carapace 1.03 long, 0.82 wide, abdomen 1.00 long, 0.70 wide. Eye sizes and interdistances (Fig. 6A, D): AME 0.04, ALE 0.06, PME 0.05, PLE 0.06, AME-AME 0.03, AME-ALE 0.01, PME-PME 0.05, PME-PLE 0.03, AME-PME 0.04, AME-PLE 0.09, ALE-ALE 0.12, PLE-PLE 0.22, ALE-PLE 0.03. MOA 0.13 long, frontal width 0.10, posterior width 0.14. Chelicerae (Fig. 7) with three promarginal (median largest, distal smallest) and two retromarginal teeth (distal larger); promarginal and retromarginal escort setae present, longer than fang; promarginal cheliceral whisker setae in a line; promarginal rake setae in three lines, comb-shaped; promarginal and retromarginal base of fang with two slit sensilla. Endites (Fig. 6E, 7B) slightly oblique, brush shaped, anterolateral area of endite with a row of thick serrula and a row of eight long and thick setae. Labium (Figs 6E, 7B) wider than long, anteriorly with 12 setae. Sternum (Fig. 6E), longer than wide, laterally with weak precoxal triangles and lacking intercoxal extensions, posteriorly triangular, blunt end. Leg measurements (Figs 6, 8): I 3.29 (0.93, 0.38, 0.88, 0.73, 0.42); II 3.85 (0.76, 0.34, 0.59, 0.62, 0.44); III 2.53 (0.64, 0.28, 0.48, 0.61, 0.42); IV 3.74 (0.98, 0.36, 0.83, 0.92, 0. 54). Leg spination (Figs 6, 8): femora I d1, pv111, II d1, III d1, IV d1; tibiae I v222222, II v222221; metatarsi I v2221, II v2221; metatarsi III and IV with conspicuous preening brushes, lyriform organs, and dorsal stoppers distally; tarsi with abundant scales, several long trichobothria dorsally, and several chemosensory setae on ventro-posterior tarsi and base of claws, slit sensillum located subdistally on dorsal part, oval, labium-shaped; inferior tarsal claw smooth without tooth, with a ventral scopula of tenent setae. Scutum (Fig. 6A) nearly covering 1/2 of abdomen.

**Colouration** (Fig. 6A–E). Carapace yellow, with light yellow-brown spot in front of fovea, radial, irregular yellow-brown stripes submarginally and arc-shaped dark stripes around margin. AME, ALE and PLE with dark layer of black pigment around the eye cup, but PME absent. Chelicerae, endites, and labium yellow. Sternum yellow, mottled around margin. Legs yellow, without dark stripes. Abdomen yellow-brown, mottled, with three light yellow chevrons posteriorly and many yellow spots on surface; venter yellow.

**Palp** (Figs 9, 10). Femoral apophysis weak, with shallow groove and one strong dorsal spine near distal femur. Retrolateral tibial apophysis large, thick, longer than tibia in retrolateral view, with blunt apex. Dorsal tibial apophysis shorter than retrolateral tibial apophysis, with a strong hook-shaped tip, submedial part with a strong constriction. Sperm duct U-shaped, reaching subposterior part of tegulum. Retrolateral tegular apophysis, arising from retrolateral tegulum, with two parts, one lamellate, transversely directed, touching the base of embolus, arising from retrolateral tegulum, the other

ridge-like, anteriorly located in retrolateral view. Subdistal tegular apophysis fan-shaped, slightly less than 1/2 of tegular length. Embolus short, right-angled, with a spine-like tip, covered by subdistal tegular apophysis. Sperm pore round, located in the medial part of embolus, around the sharp turn, slightly less than the length of dorsal tibial apophysis.

**Female.** Habitus as in Fig. 11A–C. As in male, except as noted. Total length 2.20, carapace 0.99 long, 0.79 wide, abdomen 1.18 long, 0.92 wide. Eye sizes and interd-



**Figure 11.** *Edelithus puer* sp. nov., male **A** habitus, dorsal view **B** same, ventral view **C** same, lateral view **D** carapace, dorsal view, white arrow to cheliceral spine, black arrow to oval posterior median eyes without black annulations **E** same, ventral view **F** leg I, prolateral view, black arrows to prolateral spines on femur **G** leg II, prolateral view. Scale bars: 0.1 mm (**A**, **B**, **D**–**G**); 0.5 mm (**C**).

![](_page_91_Figure_1.jpeg)

**Figure 12.** *Edelithus puer* sp. nov., female. **A** epigyne, ventral view **B** same, dorsal view **C** same, ventral view **D** same, dorsal view. Abbreviations: Bu – bursa, CD – copulatory duct, CO – copulatory opening, CT – connecting tube, FD – fertilization duct, GA – glandular appendage, Spe – spermatheca. Scale bars: 0.1 mm.

istances (Fig. 11D): AME 0.04, ALE 0.06, PME 0.04, PLE 0.06, AME–AME 0.01, AME–ALE 0.01, PME–PME 0.05, PME–PLE 0.04, AME–PME 0.04, AME–PLE 0.08, ALE–ALE 0.12, PLE–PLE 0.20, ALE–PLE 0.02. MOA 0.13 long, frontal width 0.10, posterior width 0.13. Leg measurements (Fig. 11): I 2.99 (0.77, 0.36, 0.79, 0.67, 0.40); II 2.60 (0.72, 0.34, 0.57, 0.56, 0.41); III 2.39 (0.62, 0.30, 0.46, 0.56, 0.45); IV 3.50 (0.91, 0.34, 0.78, 0.88, 0.59). Leg spination (Fig. 11): femora II lacking prolateral spine; tibiae I v222221; metatarsi I v2222.

*Colouration* (Fig. 11A–C). Lighter than male.

*Epigyne* (Fig. 12). Epigynal plate longer than wide, posterolaterally with pair of slit-like copulatory openings. Copulatory ducts tube-shaped, longer than bursal diameter, submedially with a slight constriction. Bursae large oval, anteriorly located, slightly separated. Connecting tubes slender, less than length of copulatory ducts. Spermathecae oval, medially located, separated by half of their diameter. Spermathecal head parallel, posteromedially located, directed posteriorly, as long as spermathecal diameter, club-shaped. Fertilization ducts as long as spermathecal length, located at the center of spermathecae, directed laterally.

**Comments.** Prolateral spine on femora I same detail as in *E. puer* sp. nov. **Distribution.** Known only from the type locality in Yunnan Province, China.

## Acknowledgements

The manuscript benefited greatly from comments by Zhiyuan Yao, Francesco Ballarin, and an anonymous referee. Danni Sherwood and Robert Forsyth checked the English. Guo Zheng helped in fieldwork. This study was supported by the Natural Science Foundation of China (NSFC-31972869, 32000301) and the Science and Technology Foundation of Jiangxi Provincial Department of Education (GJJ211017).

## References

- Hong D, Zhuang W, Zhu M, Ma K, Wang X, Huang D, Zhang Y, Ren G, Bu W, Cai W, Ren D, Yang D, Liang A, Bai F, Zhang R, Lei F, Li S, Kong H, Cai L, Dai Y, Zhu C, Yang Q, Chen J, Sha Z, Jiang J, Che J, Wu D, Li J, Wang Q, Wei X, Bai M, Liu X, Chen X, Qiao G (2022) Positioning taxonomic research for the future. Zoological Systematics 47(3): 185–187. https://doi.org/10.11865/zs.2022301
- Kamura T (2021) Three new genera of the family Phrurolithidae (Araneae) from East Asia. Acta Arachnologica 70(2): 117–130. https://doi.org/10.2476/asjaa.70.117
- Li S (2020) Spider taxonomy for an advanced China. Zoological Systematics 45(2): 73–77. https://doi.org/10.11865/zs.202011
- Li J, Yan X, Lin Y, Li S, Chen H (2021) Challenging Wallacean and Linnean shortfalls: *Ectatosticta* spiders (Araneae, Hypochilidae) from China. Zoological Research 42(6): 791– 794. https://doi.org/10.24272/j.issn.2095-8137.2021.212
- Liu K, Li S, Zhang X, Ying Y, Meng Z, Fei M, Li W, Xiao Y, Xu X (2022) Unknown species from China: the case of phrurolithid spiders (Araneae, Phrurolithidae). Zoological Research 43(3): 352–355. [Suppl. I 1–5(Suppl. II): 1–223] https://doi.org/10.11646/zootaxa.4613.2.4
- Ramírez MJ (2014) The morphology and phylogeny of dionychan spiders (Araneae: Araneomorphae). Bulletin of the American Museum of Natural History 390: 1–374. https://doi. org/10.1206/821.1
- Wang L, Chen H, Zhou K, Zhang F, Zhang Z (2015) Diversity of spiders in Fanjing Mountain Nature Reserve, Guizhou, China, I: Six new species of Phrurolithidae (Araneae). Zootaxa 4012(3): 447–464. https://doi.org/10.11646/zootaxa.4012.3.2

- Wang C, Li S, Zhu W (2020) Taxonomic notes on Leptonetidae (Arachnida, Araneae) from China, with descriptions of one new genus and eight new species. Zoological Research 41(6): 684–704. https://doi.org/10.24272/j.issn.2095-8137.2020.214
- WSC (2022) World Spider Catalog. Natural History Museum Bern. Version 23.0. [accessed 25 May 2022] https://doi.org/10.24436/2
- Yao Z, Wang X, Li S (2021) Tip of the iceberg: Species diversity of *Pholcus* spiders (Araneae, Pholcidae) in Changbai Mountains, Northeast China. Zoological Research 42(3): 267– 271. https://doi.org/10.24272/j.issn.2095-8137.2021.037
- Zamani A, Marusik YM (2020) A survey of Phrurolithidae (Arachnida: Araneae) in southern Caucasus, Iran and Central Asia. Zootaxa 4758(2): 311–329. https://doi.org/10.11646/ zootaxa.4758.2.6
- Zhu C, Luo A, Bai M, Orr MC, Hou Z, Ge S, Chen J, Hu Y, Zhou X, Qiao G, Kong H, Lu L, Jin X, Cai L, Wei X, Zhao R, Miao W, Wang Q, Sha Z, Lin Q, Qu M, Jiang J, Li J, Che J, Jiang X, Chen X, Gao L, Ren Z, Xiang C, Luo S, Wu D, Liu D, Peng Y, Su T, Cai C, Zhu T, Cai W, Liu X, Li H, Xue H, Ye Z, Chen X, Tang P, Wei S, Pang H, Xie Q, Zhang F, Zhang F, Peng X, Zhang A, Gao T, Zhou C, Shao C, Ma L, Wei Z, Luan Y, Yin Z, Dai W, Wei C, Huang X, Liu J, Chen X, Yi T, Zhang Z, Aishan Z, Li Q, Hu H (2022) A joint call for actions to advance taxonomy in China. Zoological Systematics 47(3): 188–197. https://doi.org/10.11865/zs.2022302

RESEARCH ARTICLE

![](_page_94_Picture_2.jpeg)

# A new genus and new species of Ecuadorian Philopotamidae (Trichoptera)

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| Academic editor: Ana Previšić   Received 25 May 2022   Accepted 20 July 2022   Published 11 August 202 |  |  |  |
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| https://zoobank.org/C583CC7A-B2AD-4204-8FA0-83C49BB088EA   |  |  |  |
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**Citation:** Holzenthal RW, Blahnik RJ, Ríos-Touma B (2022) A new genus and new species of Ecuadorian Philopotamidae (Trichoptera). ZooKeys 1117: 95–122. https://doi.org/10.3897/zookeys.1117.86984

#### Abstract

A new genus and species of Philopotamidae (Trichoptera), *Sumacodella elongata*, is described from the southern slope of Volcán Sumaco in Ecuador. This new genus differs from other philopotamid genera by having very elongate, narrow anterolateral apodemes on segment IX and the inferior appendages, a very elongate and narrow tergum X, and a very elongate, tubular phallus. In addition, two new species of *Wormaldia* are also described and illustrated from Sumaco as well as three new *Chimarra* (*Chimarra*), one new *Chimarra* (*Curgia*), and one new *Chimarra* (*Otarrha*) from the eastern and western slopes of the Ecuadorian Andes.

#### **Keywords**

Andes, biodiversity, caddisflies, Neotropics, South America

# Introduction

The caddisfly family Philopotamidae occurs around the world and currently contains approximately 1400 species, most of these in tropical regions. This is especially true for the Neotropics, where ~ 400 species occur across the region and where many new species have been described (Holzenthal and Calor 2017). Currently, the world fauna contains 24 genera in three subfamilies: Chimarrinae, Philopotaminae, and Rossodinae

(Blahnik 2005; Holzenthal et al. 2018), including the cosmopolitan genus Chimarra Stephens, 1829, which is the most species rich genus in the order Trichoptera (Kjer et al. 2014), but also regionally endemic genera such as Alterosa Blahnik, 2005, only found in Brazil (Dumas et al. 2013), Cryptobiosella Henderson, 1983, with less than five species only found in New Zealand (Holzenthal et al. 2007), and the recently described Aymaradella Holzenthal, Blahnik, & Ríos-Touma, 2018, with a single species known only from Bolivia (Holzenthal et al. 2018). In Ecuador, Chimarra is the most species rich genus of Philopotamidae, with 34 species, followed by Chimarrhodella Lestage, 1925, with five, Wormaldia MacLachlan, 1865, with four, and Hydrobiosella Tillyard, 1924, with H. andina Holzenthal, Blahnik, & Ríos-Touma, 2018, recently reported from Ecuador (Ríos-Touma et al. 2017; Holzenthal et al. 2018). Although more than 3500 species of Trichoptera have been described from the Neotropical region (Holzenthal and Calor 2017), the tropical Andes harbor several unexplored areas, and current species richness of the aquatic fauna, including Trichoptera, is underestimated (Ríos-Touma et al. 2017; Encalada et al. 2019). Here, we describe a new monotypic genus of Philopotamidae, Sumacodella, from the southern slope of Volcán Sumaco, a region known for its high endemicity (Valarezo et al. 2001). We also describe two new species of Wormaldia from Sumaco as well as three new species of Chimarra (Chimarra), one new species of Chimarra (Curgia) Walker, 1860, and one new species of Chimarra (Otarrha) Blahnik, 2002, all from mid-elevation localities on the eastern and western flanks of the Ecuadorian Andes (500-1500 m a.s.l.).

Volcán Sumaco is a 3830-m high, potentially active stratovolcano separated to the east from the principal volcanic belt of Ecuador. It is also geologically distinct from the main Ecuadorian volcanic belt in being composed largely of alkaline tephritic, basanitic, and phonolitic lavas (IGEPN 2022). The nearly symmetrical cone-shaped volcano is the dominant geological feature of Parque Nacional Sumaco Napo-Galeras and is generally surrounded by pristine, primary forest (Fig. 1).

## Materials and methods

Adult specimens of the new genus and new species were collected at UV fluorescent lights placed adjacent to streams. Lights were hung in front of a white bed sheet or placed over a white plastic pan containing 96% ethanol and powered by a small 12 V, sealed, lead-acid battery or a USB power pack (Fig. 2). Specimens were collected dry in cyanide or ammonium carbonate kill jars for later pinning or directly in ethanol. An additional specimen of *Chimarra (Chimarra) pacifica* sp. nov., was borrowed from the California Academy of Sciences (CAS). Association between males and females was done indirectly by overall similarity in body size and color with common occurrence. Adult specimens were prepared and examined following standard methods for pinned and al-cohol preserved material (Blahnik and Holzenthal 2004; Blahnik et al. 2007). Forewing length was measured from base to apex and is presented as a range when more than

![](_page_96_Picture_1.jpeg)

Figure I. Volcán Sumaco, Ecuador. Photograph credit: Xavier Amigo.

![](_page_96_Picture_3.jpeg)

Figure 2. Small stream on Coati Trail, Wildsumaco Lodge, UV-light pan trap. Photograph credit: Xavier Amigo.

one specimen was available. Philopotamid larvae are generally rarely collected in benthic samples from mid-elevation streams in Ecuador (BRT, pers. obs.) and no associated larval specimens were collected from ancillary Surber samples. Geocoordinates were taken in the field using Terra Map on a cellphone [https://www.globalterramaps.com/], except for the specimen from CAS, which was estimated using GeoLocate [https://www.geo-locate. org/]. EarthPoint [https://www.earthpoint.us/] was used to create a KML file of collection localities for import into Google Earth. This file is included as Suppl. material 1.

Male genitalia were soaked in 85% lactic acid heated to 125 °C for 20–40 min to dissolve internal soft tissues. An Olympus BX41 compound microscope outfitted with a drawing tube was used to examine specimens and to aid the rendering of detailed pencil drawings of genitalic structures. Pencil sketches were scanned and placed in Adobe Illustrator (Creative Cloud version) to serve as a template for vector illustrations. Morphological terminology follows that of Blahnik (1998, 2002) and Holzenthal et al. (2018). Each specimen or lot was affixed with a 2D data matrix barcode label bearing a unique alphanumeric sequence beginning with the prefix UMSP to serve as a specimen identifier for upload of collection, specimen, and taxonomic data to the University of Minnesota Insect Collection's Specify database [https://www.specifysoftware.org/], available via the SCAN portal [https://scan-bugs.org/portal/].

Types of the new species are deposited in the University of Minnesota Insect Collection, St. Paul, Minnesota, USA (**UMSP**), the Museo Ecuatoriano de Ciencias Naturales, Insituto Nacional de Biodiversidad, Quito, Ecuador (**MECN**), and the California Academy of Sciences, San Francisco, California, USA (**CAS**).

## **Systematics**

*Sumacodella* gen. nov. https://zoobank.org/0A53F62C-9EB6-40C6-96DF-EBCE65D18D3F

Type species. Sumacodella elongata sp. nov., original designation.

#### Sumacodella elongata sp. nov.

https://zoobank.org/BD6EAE2B-AFA1-471F-8F2B-F3C503219403 Figs 3, 4, 5

**Type material.** *Holotype.* Male (pinned). ECUADOR: Napo: Wildsumaco Lodge, small stream, Coati Trail @ wooden bridge, 0.67433°S, 77.60260°W, 1420 m a.s.l., 10.iii.2020, Ríos, Holzenthal, Frandsen, Pauls, Amigo, UMSP000500637 (UMSP). *Paratypes.* ECUADOR: same data as holotype, 2 males (pinned) (UMSP), 1 male, 1 female (pinned) (MECN).

**Diagnosis.** This new species is not easily placed in any established genus of Philopotaminae and consequently we are placing it in a new genus. Like other taxa that Ross (1956) assigned to *Sortosa* Navás, 1918 (subsequently reassigned to *Dolophilodes* 

Ulmer, 1909) it has the plesiomorphic trait of retaining all three anal veins in the hind wing. A character suggesting its possible relationship to the genus *Alterosa*, currently only known from eastern and southern Brazil, is the structure of the phallobase, which is uniformly tubular and lacks the basodorsal expansion typical of most genera of Philopotamidae. Also, like *Alterosa*, it lacks a ventral process on any of its abdominal segments, but, unlike *Alterosa*, it lacks a pair of intermediate appendages mesal to the preanal appendages, which was used as an apomorphic and defining character for that genus by Blahnik (2005). However, Dumas and Nessimian (2013) described two Brazilian species, *A. graciosa* and *A. inappendiculata*, that lack intermediate appendages, but otherwise these species conform morphologically to other species in the genus. *Sumacodella elongata*, in other features, is not similar to those two species and possesses several unique and unusual characters, which collectively serve as the basis for a generic diagnosis.

Characters of Sumacodella that can generally be regarded as plesiomorphic for Philopotaminae, as indicated by Ross (1956), include the venation of the forewing, which has a complete set of forks (I, II, III, IV, and V), a more or less linear and hyaline chord, composed of the s, r-m, and m crossveins, and looped anal veins, which converge basally and lack a crossvein, leaving a long common vein extending to the arculus (Fig. 4A). The hind wing has all three anal veins reaching the wing margin (Fig. 4B), a plesiomorphic character within Philopotaminae, also discussed by Ross (1956), and lacks fork IV, a character loss generally considered synapomorphic for the entire family Philopotamidae, exclusive of Rossodes tsaratananae (Ross, 1956). Sumacodella elongata has also lost fork III in the hind wing, probably convergently with several other taxa in the family, including some species of Wormaldia and some Chimarra. Also, plesiomorphic for Philopotaminae are the bi-segmented inferior appendages, each with an apicomesal pad of short spine-like setae (Fig. 3A), the elongate, digitate preanal appendages (Fig. 3A, B), and setation of the tergal segments anterior to segment IX, in which at least some segments have a pair of desclerotized patches near the posterior margin with several elongate setae, but the setation is otherwise confined to short and often sparse setae near the posterior margin. Distinctive characters for Sumacodella elongata, likely to be apomorphic because of their uniqueness within the family Philopotamidae, include an elongate and tapering segment IX (Fig. 3A), with an elongate ventral margin, but with the posterior margin nearly linearly narrowing dorsally, so that the posterior margin converges with the anterior margin dorsomesally, and from which the narrow, digitate preanal appendages emerge, as well as the base of tergum X. The very elongate, narrow anterolateral apodemes of segment IX are unique within Philopotamidae (Fig. 3A). Also unique within Philopotamidae is the very elongate and narrow tergum X, with sensilla confined to a narrow apicomesal projection, bordered by narrow lateral projections in the distal 3<sup>rd</sup> of the segment (Fig. 3A, B). Other characters unique to Sumacodella include the very elongate anteromesal apodeme of the inferior appendages (Fig. 3C, D) and the very elongate, tubular phallus, which is tubular anteriorly, rather than with a basodorsal projection, and has tracts of small, included spines (Fig. 3E, F). All these characters are diagnostic for the type species of the genus and any of them would serve as diagnostic characters for placement of additional species within the genus.

![](_page_99_Figure_1.jpeg)

**Figure 3.** *Sumacodella elongata* gen. nov., sp. nov. Male genitalia **A** segments VIII–X, lateral **B** segments VIII–X, dorsal **C** inferior appendage, dorsal **D** inferior appendage, ventral **E** phallus, dorsal **F** phallus, lateral.

**Description.** Adult. Forewing length male 5.0–5.7 mm (n = 4); female 5.9 mm (n = 1). Head short, rounded; postocular parietal sclerite less than half diameter of eye. Overall color dark brown, including palps and antennae; head and base of forewing with

longer, light brown setae, femora slightly paler, antennae with narrow annulations at intersection of segments, chord of forewing only indistinctly evident. Wings both relatively broad and rounded apically. Forewing with forks I, II, III, IV, and V; with chord nearly linear and hyaline (lacking pigmentation), anal loops of forewing with both 2A and 3A intersecting 1A in basal half of vein, 3A nearly convergent with 2A. Hind wing with forks I, II, and V, with all three anal veins reaching wing margin. Spur formula 2:4:4, spurs of foretibiae both short, outer spurs of mesotibiae slightly greater than half length of inner

![](_page_100_Figure_2.jpeg)

**Figures 4, 5.** *Sumacodella elongata* gen. nov., sp. nov. Male wings and female genitalia **4A** forewing **4B** hind wing **5** female genitalia, segment VII–X, lateral.

spurs, spurs of metatibia both elongate, outer spurs slightly shorter. Foretarsi of males unmodified, narrow.

Male. Segment VIII moderately elongate, sternum and tergum subequal in length, sternum densely covered with short, fine setae, tergum with setae confined to posterior region of segment, posterodorsally with pair of desclerotized patches with several more elongate setae (characteristic of most species in subfamily Philopotaminae). Segment IX, in lateral view, synscleritous, elongate, strongly tapering, with pair of very elongate apodemes on anterolateral margin at ca. mid-height, ventral margin strongly produced posteriorly, subtruncate as viewed dorsally or ventrally, posterior margin very obliquely narrowed dorsally, with lateral margin converging from ca. mid-height to anterior margin; as viewed dorsally, with posterior margin forming V-shaped convergence at anterior margin. Tergum X very elongate, narrow, and parallel-sided, weakly arched as viewed laterally, base distinctly narrowed at mesal juncture of anterior and posterior margins of segment IX, forming short tab-like projection; in apical 3rd or 4th forming elongate, narrow mesal lobe, bordered by pair of elongate, narrow lateral lobes, slightly shorter than mesal lobe; mesal lobe densely covered with sensilla, basally with pair of short, stalked projections at juncture with lateral lobes, each with one or two short terminal setae. Preanal appendage elongate, narrow, proximate basally, at juncture of tergum X and anterior and posterior convergence of dorsal margins of segment IX, appendage very narrow basally, gradually widening apically. Inferior appendage bi-segmented, segments subequal in length, nearly uniform in width; apical segment rounded, with dense pad of short, stiff apical spines, somewhat extended anteriorly on ventromesal surface. Phallus very elongate, narrow, tubular, without basodorsal projection; internally with several patches of fine, nail-like spines, varying in length, apical patch (in incompletely everted endotheca) preceded by two more elongate spines. Phallotremal sclerite very indistinct, weakly sclerotized, small, and ring-like.

**Female.** Genitalia very elongate, tapering from segment VII. Segment VII elongate, sternum covered with fine setae; tergum with setae confined to posterior half. Segment VIII with tergum and sternum not fused, shorter than segment VII, relatively undifferentiated in structure and shape, together forming narrow tube; sternum with very elongate, narrow apodemes from dorsolateral margins, at ca. mid-length, extending to ca. mid-length of segment VII. Segment IX shorter and somewhat narrower than segment VIII, sternum and tergum apparently divided, at least anteriorly, segment with very elongate, narrow apodemes, extending to ca. base of segment VIII. Segment X composed of pair of elongate, bulbous lobes, each lobe with short setae basally, apically with numerous sensilla and small, digitate cercus.

**Etymology.** The genus is named *Sumacodella*, feminine, for Volcán Sumaco, an isolated stratovolcano located in the Ecuadorian Amazon, which hosts an amazingly high diversity of endemic plants and animals. The termination *-della* is intended to make the name euphonious with *Chimarrhodella*, *Hydrobiosella*, and *Aymaradella*, other philopotamids known from the Neotropics. The specific epithet is from the Latin *elongatus*, meaning elongated and referring to the several elongate appendages and other structures of the male genitalia, which are very diagnostic for this new species.

https://zoobank.org/F1432B21-5E24-4894-93D6-6F4546BB04BB Figs 6, 7, 8

**Type material.** *Holotype.* Male (pinned). ECUADOR: Morona-Santiago: Macas, small gravel stream (Wallace/Real property), 2.20299°S, 78.08539°W, 1076 m a.s.l., 14.xi.2015, Ríos-Touma, Thomson, Amigo, Real-Wallace, UMSP000357522 (UMSP). *Paratypes.* ECUADOR: same data as holotype 28 males, 39 females (pinned) (UMSP); same locality as holotype, except 27.i.2015, Holzenthal, Huisman, Ríos-Touma, Amigo, 4 males, 11 females (pinned), 3 males (in alcohol) (MECN).

**Diagnosis.** *Chimarra asterae* is a member of the *Chimarra (Chimarra) bidens* group of Blahnik (1998), very similar to *C. duckworthi* Flint, 1967, particularly because of the general shape and length of the inferior appendages. The distinctly different sclerotization of the female genitalia provides the best evidence that the two forms are distinct species. The most distinctive differences in the male genitalia are in the structure of the inferior appendages, which have the basal part more broadly rounded, in lateral view, and the apex slightly more rounded, with a small notch or tooth-like projection pre-apically on the mesal surface (Fig. 6C–E), absent in *C. duckworthi* (Fig. 6G, H). *Chimarra asterae* could also be confused with *C. caribea* Flint, 1968, which also has a small tooth-like projection near the apex of the inferior appendage. However, the over-all length of the inferior appendage is longer in *C. caribea* and the tooth-like projection is somewhat more removed from the apex (Blahnik 1998: fig. 54C, D,F).

The female genitalia of *C. asterae* resemble *C. duckworthi* in having both a dorsal and paired ventral sclerites near the posterior opening of the vaginal apparatus, as well as distinct, membranous pocket-like lobes (probably receptacles for the inferior appendages of the male) associated with the ventral sclerites of segment IX (Fig. 8). The genitalia of *C. asterae* differs in that the sclerotized ventral furrows of the vaginal apparatus are short, and the lateral margins of the vaginal tract have distinct sclerites (Fig. 7). Also, it lacks the paired posteroventral sclerites, posterior to the sclerotized ventral furrows, which form an element of the vaginal apparatus in *C. duckworthi*.

**Description. Adult.** Forewing length male 4.8–5.4 mm (n = 5); female 5.4–6.0 mm (n = 5). Color nearly uniformly brownish black (fuscous), except femora slightly paler. Head relatively short and rounded, (postocular parietal sclerite ca. half diameter of eye). Third segment of maxillary much longer than second, subequal to 5<sup>th</sup>. Male protarsal claws enlarged, asymmetrical in size and shape, outer claw longer and twisted.

**Male.** Abdominal segment IX, in lateral view, with very pronounced sinuous extension of anteroventral margin and small apodemes from anterodorsal margin; Posteroventral process very narrow, length greater than width, subacute apically. Tergum X membranous mesally, with sclerotized lateral lobes, each bearing pair of sensilla on short, rounded protuberance near dorsal margin in basal half, apex of lobe somewhat mesally cupped and bluntly rounded. Preanal appendage short, rounded, knob-like. Inferior appendage, in lateral view, relatively elongate (similar in length to *C. duckworthi*, shorter than *C. caribea*), mesally curved, with apical rotation, apices

![](_page_103_Figure_1.jpeg)

**Figure 6.** *Chimarra* (*Chimarra*) *asterae* sp. nov. Male genitalia **A** segments IX, X, lateral **B** segments IX, X, dorsal **C** inferior appendages, oblique lateral **D** inferior appendage, ventral **E** inferior appendage, dorsal **F** phallus, lateral **G** inferior appendage, *C. duckworthi*, lateral (for comparison) **H** inferior appendage, *C. duckworthi*, ventral (for comparison).

![](_page_104_Figure_1.jpeg)

**Figures 7, 8.** *Chimarra (Chimarra) asterae* sp. nov. **7** female genitalia, ventral **8** *Chimarra (Chimarra) duckworthi*, female genitalia, ventral (for comparison). Abbreviations: vag. app. = vaginal apparatus.

apposed, chisel-like, basoventral margin of appendage more broadly rounded than in *C. duckworthi*, apex of inferior appendage somewhat enlarged, with small toothlike projection near apex on ventromesal margin. Phallobase with very distinct, acute apicoventral projection, two phallic spines, moderately elongate, differing slightly in length, endotheca tubular, more-or-less covered with small echinate spines, apicoventrally with curled sclerite and associated tract of very small spines. Phallotremal sclerite complex composed of elongate rod and ring structure and membranous structure with pair of associated wishbone-like sclerites apically.

**Female.** Ventral sclerites of sternum IX with prominent membranous lateral pouches (probably "clasper receptacles" of Blahnik, 1998). Vaginal apparatus moderately elongated with distinct rounded dorsal and paired ventral sclerites apically, ventrally with paired, narrow, furrow-like sclerites, beginning at mid-length, proximate posteriorly and diverging anteriorly; lateral margins of vaginal tract with elongate, narrow sclerites; vaginal tract narrowed anteriorly, with declivous, cup-like sclerite.

**Etymology.** This new species is named in honor of Aster Real-Wallace, a young nature enthusiast and member of the Real-Wallace family, owners and protectors of a beautiful patch of remnant Amazonian pre-montane riverine forest on a tributary of the Río Upano, where this species was discovered.

#### Chimarra (Chimarra) mashpi sp. nov.

https://zoobank.org/BCC116DA-64C2-42FF-93FB-909FB6361667 Fig. 9

**Type material.** *Holotype.* Male (pinned). ECUADOR: Pichincha: Quebrada Laguna, in Mashpi Lodge, 00.16693°N, 078.87122°W, 1111 m a.s.l., 23.vii.2015, Rázuri, Morabowen, Hernández, UMSP000380186 (UMSP). *Paratypes.* ECUADOR: Pichincha: Amagusa Reserve (private), Río Amagasu, 0.15508°N, 78.84330°W, 1160 m a.s.l., 17.i.2015, Holzenthal, Huisman, Ríos-Touma, 1 male (in alcohol) (MECN); Cotopaxi: Recinto Los Laureles (Jardín de los Suenos), stream, 0.84165°S, 79.20051°W, 473 m a.s.l., Holzenthal, Ríos, Amigo, Huisman, 1 male (in alcohol) (MECN).

**Diagnosis.** Chimarra mashpi is a distinctive species in the Chimarra (Chimarra) ortiziana group of Blahnik (1998), most closely resembling C. colmillo Blahnik & Holzenthal, 1992, especially in the spines of the endotheca, which has an array of short spines and two longer spines near the phallotremal opening (Fig. 9F). As compared to C. colmillo, the apical part of the inferior appendage is shorter, wider, and strongly, angularly mesally flexed (Fig. 9C–E). In the latter respect it somewhat resembles C. pollex Blahnik & Holzenthal, 1992, which also has the dorsal projection of the inferior appendage is shorter, narrower, and more dorsally directed, with the flexure forming a rounded notch. Tergum X of this new species is also diagnostic, with the lateral sensilla-bearing processes subtriangular and distinctly protruding (Fig. 9B).

**Description. Adult.** Forewing length 4.7 mm (n = 1). Color nearly uniformly brownish black (fuscous), except femora yellowish brown and head somewhat darker, with scattered whitish setae on vertex and anteromesal setal wart. Postocular parietal sclerite relatively short (less than half diameter of eye). Third segment of maxillary palp much longer than  $2^{nd}$ , subequal to  $5^{th}$ . Male protarsal claws enlarged, asymmetrical in size and shape, outer claw longer and twisted.

**Male.** Abdominal segment IX, in lateral view, with very pronounced sinuous extension of anteroventral margin and small apodemes from anterodorsal margin; posteroventral process moderately elongate, subacute apically. Tergum X membranous mesally, with sclerotized lateral lobes, each bearing pair of sensilla on subtriangular basolateral projection, apex of lobe with short, blunt projection. Preanal appendage short, rounded, knob-like. Inferior appendage, in lateral view, with relatively short and broad, apically rounded, dorsal process,  $-2 \times$  width of basal part of appendage, extending nearly straight on dorsal margin; as viewed ventrally or caudally, with dorsal process strongly and very angularly mesally flexed. Phallic apparatus with ventral margin of phallobase only weakly projecting; two phallic spines, subequal, moderately elongate; endotheca textured with small spines, also with sclerotic region with array of short spines and two more elongate spines. Phallotremal sclerite complex composed of elongate rod and ring structure and membranous structure with pair of associated wishbone-like sclerites apically.

Female. Unknown.

![](_page_106_Figure_1.jpeg)

**Figure 9.** *Chimarra (Chimarra) mashpi* sp. nov. Male genitalia **A** segments IX, X, lateral **B** segments IX, X, dorsal **C** inferior appendages, oblique lateral **D** inferior appendage, ventral **E** inferior appendage, dorsal **F** phallus, lateral.

**Etymology.** This new species is named for Mashpi Reserve, where this species was discovered, as a recognition of efforts to preserve the highly threatened Choco-Andean Tropical Forest.

**Habitat notes.** The streams of the Amagusa and Mashpi Reserves at this elevation (1100–1200 m a.s.l.) have higher flows between February and April. These highly forested streams are usually step-pool channels, with average flows ranging between 0.049–0.056 m<sup>3</sup>/s. Conductivity is low, ranging from 35–88  $\mu$ S/cm, oxygen is close to 100% saturation, and daytime water temperature ranges from 17–20 °C.

#### Chimarra (Chimarra) pacifica sp. nov.

https://zoobank.org/0DAB2295-FBF7-4976-B845-625DD139AF77 Fig. 10

**Type material.** *Holotype.* Male (pinned). ECUADOR: Pichincha: San José de Mashpi, Río Mashpi, 0.18954°N, 78.92117°W, 498 m a.s.l., 8.iii.2020, Ríos, Holzenthal, Frandsen, Amigo, UMSP000500813 (UMSP). *Paratype.* ECUADOR: El Oro: 9 mi. S Santa Rosa [3.581°S, 79.932°W, uncertainty 13,558 m], 23.i.1955, E.I. Schlinger & E.S. Ross, 1 male (in alcohol) (CAS).

**Diagnosis.** Chimarra pacifica is a new species in the Chimarra (Chimarra) beameri group of Blahnik (1998), very similar to both C. munozi Blahnik & Holzenthal, 1992 and C. dudosa Blahnik, 1998, resembling them in the general shape of the inferior appendages and spatulate lateral lobes of tergum X, and also in having an array of short spines associated with the phallotremal sclerite complex (Fig. 10E). It differs in that the apex of the inferior appendage has a short, but distinctive, protuberance from its ventral margin (Fig. 10A). Tergum X is also less strongly deflexed than in either of the compared species (Fig. 10A). The only other species of the beameri group currently reported from Ecuador is C. coheni Blahnik, 1998, which also has spatulate lateral lobes of tergum X, but differs in having a distinctly bifid apex of its inferior appendage and much longer phallic spines. The species also seems to lack the array of short spines associated with the phallotremal sclerite complex seen in the new species, but these are easily overlooked in specimens in which the endotheca is not everted.

**Description. Adult.** Forewing length male 4.0–4.3 mm (n = 2). Color nearly uniformly brownish black (fuscous), except femora yellowish brown and head somewhat darker, with scattered whitish setae on vertex and anteromesal setal wart. Head relatively short and rounded, postocular parietal sclerite short (less than half diameter of eye). Second segment of maxillary palp stout and elongate, subequal to  $3^{rd}$ , with stout apicomesal bristles,  $5^{th}$  segment shorter than  $3^{rd}$ . Male protarsal claws enlarged, asymmetrical in size and shape, outer claw longer and twisted.

**Male.** Abdominal segment IX, in lateral view, with well-developed anterodorsal apodemes, anteroventral margin distinctly extended, nearly linearly narrowing to dorsal apodemes, posterior margin weakly convex. Posteroventral process subtriangular, very large and prominent, length subequal to width at base. Tergum X membranous mesally, with sclerotized lateral lobes; lateral lobes, as viewed dorsally, spatulate in apical half, with two


**Figure 10.** *Chimarra (Chimarra) pacifica* sp. nov. Male genitalia **A** segments IX, X, lateral **B** segments IX, X, dorsal **C** inferior appendages, oblique lateral **D** inferior appendage, ventral **E** phallus, lateral.

widely spaced sensilla near dorsal margin; as viewed laterally, turned down, with lateral crease in apical half. Preanal appendage short, rounded, knob-like, somewhat flattened as viewed dorsally. Inferior appendage, in lateral view, with rounded basal part and relatively narrow, moderately elongate; basal expansion weakly rounded to subtruncate, dorsal

lobe with small tooth-like projection apicoventrally. Phallobase tubular, with basodorsal expansion, apicoventral margin only weakly projecting, two phallic spines, moderately elongate, subequal in length, endotheca apparently elongate, with small echinate spines. Phallotremal sclerite complex composed of rod and ring structure, rod short and curved and ring with prominent apicodorsal extension; apically with membranous structure subtending rod, anterior margin forming pair of weakly sclerotized, fishhook-like sclerites.

Female. Unknown.

**Etymology.** This new species is named "pacifica," referring to the localities where the species was collected, both on the Pacific slope of the Ecuadorian Andes.

**Habitat notes.** Río Mashpi is a clear water river with base flow ~ 4 m<sup>3</sup>/s and with peak flows between March to May. Conductivity is low, ranging from 46–58.5  $\mu$ S/ cm, oxygen is close to 100% saturation, and daytime water temperature ranges from 20–23 °C year-round.

#### Chimarra (Curgia) amigo sp. nov.

https://zoobank.org/85DE674F-A25E-455A-B5D4-694766D804A6 Fig. 11

**Type material.** *Holotype.* Male (pinned). ECUADOR: Carchi: small stream 1, road from Chilmá Bajo to Moldanado, 0.90574°N, 78.21870°W, 1669 m a.s.l., 15.ii.2017, Ríos-Touma, Holzenthal, Amigo, Huisman, UMSP000378196 (UMSP). *Paratypes.* ECUADOR: Carchi: Río Blanco between El Goaltal and Las Juntas, 0.80433°N, 78.16975°W, 1258 m a.s.l., Holzenthal and Huisman, 1 male (pinned) (UMSP); Pichincha: Quebrada Amagusa, 0.15561°N, 78.85356°W, 1254 m a.s.l., 21.vii.2015, Rázuri, Morabowen, Hernández, 2 males (pinned) (MECN).

**Diagnosis.** Chimarra amigo has a general similarity to other species of the Chimarra (Curgia) fernandezi group of Flint (1998). The group is characterized by the form of tergum X, with the dorsomesal part forming a projecting lobe, either entire or slightly notched apically, and with projecting ventrolateral lobes on either side, and particularly by having an enlarged and distinctly sclerotized phallotremal sclerite complex, varying in shape and complexity among the different species of the group. Chimarra amigo differs from other described species of the group by having the preanal appendages flattened and almost completely fused (Fig. 11A, B), much as in C. oztucoensis Flint & Reyes, 1991, which Flint (1998) placed in its own species group, largely because of having a tergum X that is deeply divided mesally. Chimarra amigo further differs from other species of the fernadezi group in the form of its inferior appendages, which are nearly subquadrate in lateral view, with the apicomesal projection very short and acute, not visible in lateral view (Fig. 11A). Also distinctive for this species is its elongate tubular endotheca, which is very sharply bent or elbowed (Fig. 11E).

**Description.** Adult. Forewing length male 5.8–6.5 mm (n = 4); female 6.8 mm (n = 1). Head setae brownish black, slightly darker than wings. Wings brownish black, chord hyaline, distinct. Appendages dark brown. Pretarsal claws of male foreleg unmodified.



**Figure 11.** *Chimarra (Curgia) amigo* sp. nov. Male genitalia **A** segments VIII–X, lateral **B** segments VIII–X, dorsal **C** inferior appendage, ventral **D** inferior appendage, dorsal **E** phallus, lateral.

Male. Tergum VIII longer than sternum; dorsomesal margin projecting, entire; sternum VIII short ventrally, widening dorsally. Segment IX short dorsally, long ventrally, subtriangular in lateral view; anterolateral margin slightly concave, ventral margin produced; posterolateral margin angularly produced at level of inferior appendage; ventral process long, narrow, projecting, subacute apically; anteroventral margin concave mesally; dorsomesal margin broadly concave. Preanal appendage completely fused to tergum X represented by elevated patch of setae. Tergum X moderately long; divided into dorsal and ventrolateral lobes, dorsal lobe strongly rounded apicodorsally, apex laterally compressed, crest-like, ventrolateral lobes ~ 1/2 length of dorsal lobe, rounded apically; in dorsal view with apex of dorsal lobe shallowly cleft, ventrolateral lobes very narrow; apicomesally with numerous sensilla. Inferior appendage moderately long in lateral view, subquadrate; apex in ventral view abruptly narrowed, strongly inturned, forming short subapicomesal tooth-like projection. Endotheca elongate, tubular, with dorsomesal membranous projection when everted and inflated; endotheca strongly bent ventrad at dorsomesal projection; phallotremal sclerite forming large, complex, lightly sclerotized structure with single ventral and paired lateral blade-like sclerites, but much shorter than in other *fernandezi* group species.

Female. Unknown.

**Etymology.** This species is named in honor of Xavier Amigo, one of the collectors of the new species. He has provided essential support as a member of our field expeditions in Ecuador and is the beloved husband of Blanca Ríos-Touma.

#### Chimarra (Otarrha) ramosa sp. nov.

https://zoobank.org/C65DFAD7-6D25-444B-9987-D8541C2B84A5 Figs 12, 13

**Type material.** *Holotype.* Male (pinned). ECUADOR: Orellana: river, road between Wawa Sumaco and Loreto [UV], 0.73632°S, 77.49507°W, 610 m a.s.l., 11.iii.2020, Ríos, Holzenthal, Frandsen, Pauls, Amigo, UMSP000501575 (UMSP). *Paratypes.* EC-UADOR: same data as holotype, 6 males (pinned) (UMSP), 5 males, 1 female (in alcohol) (MECN); Pastaza: small stream ca. 3.8 km (rd) SE Cuwitayo, 1.92251°S, 77.79459°W, 703 m a.s.l., Ríos, Holzenthal, Frandsen, Errigo, Amigo, 2 females (pinned) (UMSP).

**Diagnosis.** This is a species in the *Chimarra (Otarrha) patosa* group, as defined by Blahnik (2002). Other species belonging to this group include *C. amazonia* Blahnik, 2002, *C. parene* Blahnik, 2002, *C. parilis* Blahnik, 2002, *C. particeps* Blahnik, 2002, *C. patosa* Ross, 1956, and *C. peruana* Blahnik, 2002. Like other members of the group, it has tine-like projections from the mesal margin of the inferior appendages. It is the 1<sup>st</sup> member of the group known from Ecuador; all others in the group have known distributions confined to Peru. Like *C. patosa* and *C. peruana*, the new species has distinct, digitate projections from the posterior margin of tergum VIII; however, they are much shorter than in either of those species and the apical spines are very short and inconspicuous (Fig. 12A, B). Other distinctive aspects of the new species include additional spine-like projections from the inferior appendages, both basally and apically (Fig. 12C,

D), and a single pair of very short phallic spines (Fig. 12E), much shorter and less conspicuous than those of other species in the group. *Chimarra amazonia* also has spine-like basal projections on the inferior appendages and, on this basis, as well as the presence of very short dorsal projections on tergum VIII, is the likely sister taxon of *C. ramosa*.



**Figure 12.** *Chimarra (Otarrha) ramosa* sp. nov. Male genitalia **A** segments VIII–X, lateral **B** segments VIII–X, dorsal **C** inferior appendage, segment IX, ventral **D** inferior appendage, dorsal **E** phallus, lateral.



**Figure 13.** *Chimarra (Otarrha) ramosa* sp. nov. Female genitalia **A** segments VII–X, lateral **B** segments IX, X, dorsal **C** segment IX, X, vaginal apparatus, ventral.

The genitalia of the female of *Chimarra (Otarrha) ramosa* closely resemble those of *C. parilis* and *C. particeps*, particularly in that the ventral margin of segment VIII is somewhat produced and subtruncate, but has a distinct, shallow, U-shaped mesal invagination, bordered on either side by ventral setal warts composed of several elongate submarginal setae (Fig. 13A). It is most readily diagnosed by a very elongate V-shaped sclerite in the vaginal apparatus, most distinctly evident in ventral view (Fig. 13C).

**Description. Adult.** Forewing length male 3.9-4.3 mm (n = 5); female 4.7-5.0 mm (n = 2). Color nearly uniformly brownish black (fuscous). Chord of forewing hyaline, linear, indistinct. Palps short. Head relatively flat, moderately elongate (postocular parietal sclerite ca. half diameter of eye).

**Male.** Tergum VIII with pair of digitate projections from posterior margin, ca. as long as tergum, apices slightly dilated, with very short spines. Segment IX, in lateral view, with anterior margin nearly straight (slightly expanded in ventral half), segment longest ventrally, just above ventral process, posterior margin obliquely, nearly linearly, narrowed dorsally; posteroventral process relatively elongate (length more than 2 × width), nearly uniform in width, apex subtruncate, acute apicodorsally. Mesal lobe of tergum X (or posteromesal projection of tergum IX) very narrow and relatively short, less than half length of lateral lobes of tergum X, distinctly sclerotized, pre-apically with short, acute dorsal projection. Tergum X divided mesally, forming two sclerotized lateral lobes, narrowly separated to base; lateral lobes, as viewed laterally, each with apical invagination, extending nearly half length of segment and forming narrow, apically acute, dorsal and

ventral lobes; dorsal lobe slightly shorter than ventral lobe and more strongly sclerotized, with scattered sensilla, except apically, beginning from base of lateral invagination, dorsally with three or four short setae; dorsal lobe with apex narrowed, strongly sclerotized, and distinctly downturned, ventral lobe rather weakly sclerotized, except apically, apex slightly upturned. Preanal appendage relatively large, flattened, ear-like. Inferior appendage elongate, narrow, mesally curved, tapering apically, apex inturned and acute; mesal surface with several tine-like projections: basal tine short and acute, spine-like, median tine very elongate, narrow, distinctly evident in lateral view, somewhat flexed or bent basally, pre-apical tine short and somewhat irregular in shape. Phallic apparatus with phallobase relatively short and tubular, with basodorsal expansion, distinctly ventrally flexed on ventral margin; endotheca with membranous, sack-like basodorsal lobe, lacking spines, apex of endotheca dilated, with two very short, closely apposed, subequal dorsal spines or sclerites. Phallotremal sclerite complex composed of moderately elon-gate rod and ring structure, with pair of short, weakly sclerotized, apicoventral sclerites.

**Female.** Segment VII with short, rounded ventral process near posterior margin. Segment VIII short, synscleritous; anterolateral margin very obtusely angular, with a weakly developed apodeme at ca. mid-height; posterior margin, as viewed laterally, with dorsal setal wart absent, lateral setae on a broadly rounded protrusion in ventral half, composed of marginal array of elongate setae; ventral margin of segment distinctly produced, in ventral view forming subtruncate projection with shallow posteromesal invagination, bordered on either side by ventral setal warts, each composed of more or less linear array of several elongate submarginal setae; dorsal margin of segment very short, but continuously sclerotized. Tergum IX short and very wide, with short, ventrally projecting, anterolateral apodemes; posterior margin with rounded mesal projection bearing cluster of short setae; sternum IX absent or not evident. Tergum X forming pair of subdivided lobes, each with short apical cercus; basal part of lobe large, distinctly sclerotized, and setose; apical part of lobe more weakly sclerotized, with ventromesal tract of short setae. Vaginal apparatus short, with distinct anteromesal cup-like sclerite and longitudinal anterior sclerites, including very elongate, narrowly V-shaped, posteromesal sclerite.

**Etymology.** From the Latin *ramus*, meaning branches or antlers, and referring to the very branched inferior appendages of the male genitalia.

**Habitat notes.** The small stream in Pastaza where paratypes were collected had a flow of 0.05 m<sup>3</sup>/s, a specific conductivity of 60  $\mu$ S/cm, oxygen saturation was 90.7%, and daytime water temperature was 23.2 °C at the time of collection.

#### Wormaldia natalis sp. nov.

https://zoobank.org/84769896-75D5-4104-ABAF-24B17AF6CDAC Fig. 14

**Type material.** *Holotype.* Male (pinned). ECUADOR: Napo: Wildsumaco Lodge, small stream, Coati Trail @ wooden bridge, 0.67433°S, 77.60260°W, 1420 m a.s.l., 10.iii.2020, Ríos, Holzenthal, Frandsen, Pauls, Amigo, UMSP000500642 (UMSP). *Paratype.* ECUADOR: same data as holotype, 1 male (pinned) (MECN).



**Figure 14.** *Wormaldia natalis* sp. nov. Male genitalia **A** segments VIII–X, lateral **B** segments VIII–X, dorsal **C** segments VIII, IX, inferior appendage, ventral **D** phallus, lateral, detail: endothecal spines.

**Diagnosis.** *Wormaldia natalis* is undoubtedly most closely related to *W. aymara* Muñoz-Quesada & Holzenthal, 2015, described from Bolivia and resembling it in several respects, but particularly in the short, broad posteromesal projections from the

posterior margin of tergum VIII (Fig. 14A, B) and the strongly tapering apical segment of the inferior appendage (Fig. 14A). However, it differs in the shorter, more basally inflated preanal appendages (Fig. 14B), less angular projection of segment IX below the inferior appendages (Fig. 14A), details of the apex and shape of the lateral projections of tergum X (Fig. 14A, B), development of the apical segment of the inferior appendages, and in having a longer phallic spine (Fig. 14D). Additionally, the two short projections from the posterior margin of tergum VIII have a more V-shaped than U-shaped basal separation (Fig. 14B).

**Description.** Adult. Forewing length male 5.2 mm (n = 1). Head brown, with yellowish setae. Antenna with overall color yellowish, indistinctly annulated with light brown, somewhat flattened setae. Palps very short, with dark brown setae. Dorsum of thorax brown. Legs medium brown, spurs slightly darker; hind tibiae with sparse brush of long setae. Forewing covered with dark brown setae; apical forks I, II, III, IV, and V present. Hind wing translucent, with very fine, small, brown setae; apical forks I, II, III, and V present.

Male. Segment VIII slightly shorter than segment IX, tergum with pair of short, diverging, subtruncate posteromesal projections. Segment IX, as viewed laterally, with anterior margin weakly angularly produced at ca. mid-height, posterior margin nearly linear, angularly narrowing just below preanal appendage, dorsal margin, as viewed dorsally, very short, with short projecting anterolateral apodemes. Segment X, in lateral view, elongate narrow, somewhat down-curved, apex with short, angular apicodorsal projection, sensilla confined to apex; as viewed dorsally, elongate, tapering, subtriangular, apex subacute, with scattered sensilla, apex continuous laterally with short, diverging, rounded projections, each with several sensilla. Preanal appendage elongate, irregular in shape, with distinct basodorsal expansion, narrowing apically. Inferior appendage bi-segmented, both segments tapering from base to apex, basal segment very wide basally, ca. half height of segment IX, apical segment with apex subacute and slightly down-turned; as viewed ventrally, with basal segment bulbous,  $-2 \times$  width of apical segment, apical segment with dense apicomesal pad of short spines. Phallus, when viewed laterally, with phallobase very short, with basodorsal expansion, weakly sclerotized, tapering apically, endotheca with two spines, one short and strongly curved, the other elongate, narrow, more sinuously curved.

Female. Unknown.

**Etymology.** From the Latin *natalis*, meaning birthday in reference to the date when the species was collected, March 10<sup>th</sup>, the shared birthday of collectors Frandsen and Holzenthal.

## Wormaldia sumaco sp. nov.

https://zoobank.org/E76ACD87-7852-417B-BD24-719118C15619 Figs 15, 16

**Type material.** *Holotype.* Male (pinned). ECUADOR: Napo: Wildsumaco Lodge, small stream, Coati Trail @ wooden bridge, 0.67433°S, 77.60260°W, 1420 m a.s.l., 10.iii.2020, Ríos, Holzenthal, Frandsen, Pauls, Amigo, UMSP000500644 (UMSP).



**Figure 15.** *Wormaldia sumaco* sp. nov. Male genitalia **A** segments VII–X, lateral **B** segments VII–IX, inferior appendage, ventral **C** segments VIII–X, dorsal (segment VIII offset for clarity) **D** phallus, lateral.

**Diagnosis.** Among Neotropical species of *Wormaldia*, this species is unusual in several respects and more closely resembles several North American species (Muñoz-Quesada and Holzenthal 2008) than others from the Neotropical region (Muñoz-

Quesada and Holzenthal 2015). Particularly unusual is the well-developed ventral projection from sternum VII (Fig. 15A, B), along with the relatively simple tergum X (Fig. 15A, C), absence of dorsal modifications on tergum VIII, and the rather simple, digitate preanal appendages (Fig. 15A, B). The phallus lacks the pair of spines typical of Neotropical species and instead has a tract of granular short spines (Fig. 15D), more typical of some North American species. Additionally, the phallus is less membranous and tapered apically than most Neotropical *Wormaldia*, more resembling that of other philopotamid genera (e.g., *Chimarra*). Finally, the species lacks R2 in the hind wing and thus fork I (Fig. 16B). In this respect it resembles *W. gabriella* (Banks, 1930), *W. lacerna* Denning, 1958, *W. shawnee* (Ross, 1938), and *W. strota* (Ross, 1938) in the North American fauna, which also lack the fork I in the forewing.

**Description. Adult.** Forewing length male 5.2 mm (n = 1). Head brown, with yellowish setae. Antenna with underlying color yellowish, overlaid with dark brown, somewhat flattened setae, giving antennae an overall dark, but somewhat annulated appearance. Maxillary palps yellowish, with light brown setae. Labial palps yellowish, with light brown setae. Dorsum of thorax brown. Legs medium brown, spurs slightly darker; hind tibiae with rather dense brush of long setae. Forewing with medium brown setae for the most part, except base, apical third, and small spot at base of thyridial cell darker; apical forks I, II, III, IV, and V present. Hind wing translucent, with very few fine, small, brown setae; apical forks II, III, and V present.

Male. Sternum VII with flattened, digitate, setose, posteromesal process projecting beyond middle of segment VIII; as viewed ventrally, with process subtriangular, wide basally, subacute apically, set off from segment VII by sclerotized line. Segment VIII moderate in length, both sternum and tergum unmodified. Segment IX lightly sclerotized, with evident sclerotized lines anteriorly and posteriorly, lines connected midlaterally, converging ventrally; as viewed laterally, segment relatively short, with both anterior and posterior margins moderately, angularly projecting at ca. mid-height. Segment X, in dorsal view, simple in structure, subtriangular, wide basally, narrowed apically, apex rounded, slightly upturned, with numerous sensilla. Preanal appendage elongate, digitate; as viewed dorsally, widely separated, emerging at lateral margins of tergum X, not fused basally. Inferior appendage bi-segmented, segments subequal in length; when viewed laterally, basal segment stout, subrectangular, distinctly wider than apical segment, apical segment nearly uniform in width, slightly expanded and rounded apically; as viewed ventrally, with basal segment bulbous, apical segment much narrower and flatter, with dense patch of short spine-like setae apically, extending basally on ventral margin. Phallus, when viewed laterally, with basodorsal expansion, phallobase relatively short, uniform in width, endotheca with indistinct patch of short, granular spines.

Female. Unknown.

**Etymology.** Named for Volcán Sumaco, an isolated stratovolcano in the Ecuadorian Amazon, where this species was discovered.

Habitat notes. Sumacodella elongata, Wormaldia natalis, and Wormaldia sumaco share the same type locality. Small permanent forest streams, similar to the type locality



Figure 16. Wormaldia sumaco sp. nov. Male wings A forewing B hind wing.

of these species, are common on the mid-elevation slopes of Volcán Sumaco. Leaflitter is abundant and waters are clear with very low conductivity (20–55  $\mu$ S/cm), highly oxygen saturated (close to 100%), and warm (17–20 °C).

# Discussion

Ríos-Touma et al. (2017) predicted that ~ 50% of caddisflies species are yet to be discovered in Ecuador. In 2018, three new species of Philopotamidae were described and two new records, one of them a new continental record, were established for Ecuador (Holzenthal et al. 2018). Here, we describe eight new species of philopotamids, including one new genus, which indicates, on one hand, the amazing diversity of caddisflies of the Tropical Andes, and on the other, that there are still many species to be discovered in this area, probably more than previously thought. Moreover, the biogeographical and phylogenetic relationships of Philopotamidae in this highly diverse area are still unknown (Holzenthal et al. 2018).

# Acknowledgements

We are grateful to Mashpi Lodge and Reserve (Carlos Moroczh, Mateo Roldán), Mashpishungo Reserve (Agustina Arcos and Alejandro Solano), Wildsumaco Lodge (Jonas Nilsson), and Finca Upano (RhoAnn Wallace and Galo Real) for the facilities during our field trips. Ernesto Rázuri-Gonzales, Andres Morabowen, Paul Frandsen, Jolanda Huisman, Steffen Pauls, Olivier Amigo and Xavier Amigo (Nature Experience) provided essential assistance and warm companionship during the field work. Appreciation is extended to Allan Santos and Gleison Desidério, as well as Associate Editor Ana Previšić, who thoroughly reviewed the manuscript and offered many suggestions to improve it. This research was funded by the Dirección General de Investigación, Universidad de Las Américas (Ecuador): "Montane freshwater diversity, from taxonomy to functional genomics, an approximation from Trichoptera" (CODE AMB. BRT:19.02) and by the University of the Minnesota Agricultural Experiment Station projects MIN-017-029, -079, -094.

# References

- Blahnik RJ (1998) Revision of the Neotropical species of the genus *Chimarra*, subgenus *Chimarra* (Trichoptera: Philopotamidae). Memoirs of the American Entomological Institute 59: 1–318. https://doi.org/10.3897/zookeys.184.2911
- Blahnik RJ (2002) Systematics of *Otarrha*, a new Neotropical subgenus of *Chimarra* (Trichoptera: Philopotamidae). Systematic Entomology 27(1): 65–130. https://doi.org/10.1046/ j.0307-6970.2001.00166.x
- Blahnik RJ (2005) Alterosa, a new caddisfly genus from Brazil (Trichoptera: Philopotamidae). Zootaxa 991(1): 1–60. https://doi.org/10.11646/zootaxa.991.1.1
- Blahnik RJ, Holzenthal RW (2004) Collection and curation of Trichoptera, with an emphasis on pinned material. Nectopsyche, Neotropical Trichoptera Newsletter 1: 8–20 https://conservancy.umn.edu/handle/11299/190744
- Blahnik RJ, Holzenthal RW, Prather AL (2007) The lactic acid method for clearing Trichoptera genitalia. In: Bueno-Soria J, Barba-Álvarez R, Armitage BJ (Eds) Proceedings of the 12<sup>th</sup> International Symposium on Trichoptera. The Caddis Press Columbus, Ohio, 9–14.
- Dumas, LL, Nessimian JL (2013) New species of the caddisfly genus Alterosa Blahnik 2005 (Trichoptera: Philopotamidae: Philopotaminae) from Brazil. Zootaxa 3609 (1): 26–48. https://doi.org/10.11646/zootaxa.3609.1.2.
- Dumas LL, Calor AR, Nessimian JL (2013) The genus Alterosa Blahnik, 2005 (Trichoptera, Philopotamidae, Philopotaminae) in northeastern Brazil, including the description of three new species and an identification key for the genus. ZooKeys 317: 1–15. https://doi. org/10.3897/zookeys.317.5437
- Encalada AC, Flecker AS, Poff NL, Suárez E, Herrera-R GA, Ríos-Touma B, Jumani S, Larson EI, Anderson EP (2019) A global perspective on tropical montane rivers. Science 365(6458): 1124–1129. https://doi.org/10.1126/science.aax1682
- Flint Jr OS (1998) Studies of Neotropical caddisflies, LIII: a taxonomic revision of the subgenus *Curgia* of the genus *Chimarra* (Trichoptera: Philopotamidae). Smithsonian Contributions to Zoology 594(594): 1–131. https://doi.org/10.5479/si.00810282.594
- Holzenthal RW, Calor AR (2017) Catalog of the Neotropical Trichoptera (Caddisflies). ZooKeys 654: 1–566. https://doi.org/10.3897/zookeys.654.9516

- Holzenthal RW, Blahnik RJ, Prather AL, Kjer KM (2007) Order Trichoptera Kirby, 1813 (Insecta), caddisflies. Zootaxa 1668(1): 639–698. https://doi.org/10.11646/zootaxa.1668.1.29
- Holzenthal RW, Blahnik RJ, Ríos-Touma B (2018) New species and a new genus of Philopotamidae from the Andes of Bolivia and Ecuador (Insecta, Trichoptera). ZooKeys 780: 89–108. https://doi.org/10.3897/zookeys.780.26977
- IGEPN [Instituto Geofísico Escuela Politécnica Nacional] (2022) Instituto Geofísico Escuela Politécnica Nacional. https://www.igepn.edu.ec/sumaco [retrieved 19 May 2022]
- Kjer KM, Zhou X, Frandsen PB, Thomas JA, Blahnik RJ (2014) Moving toward species-level phylogeny using ribosomal DNA and COI barcodes: an example from the diverse caddisfly genus *Chimarra* (Trichoptera: Philopotamidae). Arthropod Systematics & Phylogeny 72: 345–354. https://www.senckenberg.de/wp-content/uploads/2019/08/07\_asp\_72\_3\_ kjer\_et\_al\_345-354.pdf
- Muñoz-Quesada FJ, Holzenthal RW (2008) Revision of the Nearctic species of the caddisfly genus *Wormaldia* McLachlan (Trichoptera: Philopotamidae). Zootaxa 1838: 1–75. https://www.biotaxa.org/Zootaxa/article/view/zootaxa.1838.1.1
- Muñoz-Quesada FJ, Holzenthal RW (2015) Revision of the Neotropical species of the caddisfly genus Wormaldia McLachlan (Trichoptera: Philopotamidae). Zootaxa 3998(1): 1–138. https://doi.org/10.11646/zootaxa.3998.1.1
- Ríos-Touma B, Holzenthal RW, Huisman J, Thomson RE, Rázuri-Gonzales E (2017) Diversity and distribution of the caddisflies (Insecta: Trichoptera) of Ecuador. PeerJ 5: e2851. https://doi.org/10.7717/peerj.2851
- Ross HH (1956) Evolution and classification of the mountain caddisflies. University of Illinois Press, Urbana, 213 pp.
- Valarezo V, Gómez J, Mejía L, Célleri Y (2001) Plan de manejo de la Reserva de Biosfera Sumaco. Tena, Ecuador. http://documentoskoha.s3.amazonaws.com/14594.pdf

# Supplementary material I

## Map S1

Authors: Ralph W. Holzenthal, Roger J. Blahnik, Blanca Ríos-Touma Data type: kml file.

- Explanation note: Collection locality data for new species of Ecuadorian Philopotamidae (Trichoptera) for import into Google Earth.
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Link: https://doi.org/10.3897/zookeys.1117.86984.suppl1

RESEARCH ARTICLE



# A new species of *Rhacophorus* (Anura, Rhacophoridae) from Guangxi, China

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| Academic editor: Anthony Herre   Received 26 April 2022   Accepted 25 July 2022   Published 12 August 2022       |  |  |  |  |  |  |  |  |
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| https://zoobank.org/40EAE999-C6B0-4AA8-A3B8-2C0867A27D30   |  |  |  |  |  |  |  |  |
| Citation: Li L Liu S. Vu C. Sun T. (2022) A new species of <i>Phasabharus</i> (Apura Rhasanharidae) from Guangyi |  |  |  |  |  |  |  |  |

Citation: Li J, Liu S, Yu G, Sun T (2022) A new species of *Rhacophorus* (Anura, Rhacophoridae) from Guangxi, China. ZooKeys 1117: 123–138. https://doi.org/10.3897/zookeys.1117.85787

## Abstract

Based on morphological and molecular evidence of five male adult specimens collected from Napo County, Baise City, Guangxi Zhuang Autonomous Region, China, we describe a new species of *Rhacophorus*, *Rhacophorus napoensis* **sp. nov.** This new species is similar to *Rhacophorus rhodopus* Liu & Hu, 1959 and *Rhacophorus bipunctatus* Ahl, 1927 in morphology, but it can be distinguished from the latter two by the following morphological characteristics: head width is greater than head length, snout pointed, loreal region oblique, tympanum distinct, maxillary teeth distinct, tongue cordiform, external single subgular vocal sac, tibiotarsal articulation reaches the snout, tibia length is greater than foot length and slightly greater than half of snout-vent length, and single outer metatarsal tubercle is flat. The phylogenetic tree constructed based on 16S rRNA sequence shows that all individuals of this species clustered into the same clade, and genetically this new species differs from *R. rhodopus* and *R. bipunctatus* by 7.71% and 7.98% in 16S rRNA sequences, respectively.

## Keywords

16S rRNA, morphology, Rhacophorus napoensis sp. nov., Rhacophorus rhodopus, taxonomy

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# Introduction

Currently, the genus Rhacophorus Kuhl & Van Hassalt, 1822 contains 43 species (Frost 2021), distributed widely across China, Japan, India, and from the Philippines to Sulawesi (O'Connell et al. 2018). The main common morphological characteristics of *Rhacophorus* are: relatively medium or large body size, intercalary cartilage between terminal and penultimate phalanges of digits present, terminal phalanges of finger and toes Y-shaped, end of the finger expands into a circular disks bearing circum-marginal grooves, webbing exists on all fingers, pupil horizontal, skin is not co-ossified with the skull, extensive dermal folds along the forearm or tarsus present, and dorsal color (usually) brown or green (Li et al. 2012; Pan et al. 2017; Jiang et al. 2019). Rhacophorus rhodopus Liu & Hu, 1959 can be distinguished from other members of the genus by its medium-sized and slim body size, well-developed dermal folds along the limbs and a square skin fold dorsal to the anus present, orange brown back with small black spots, a large black spot on the flank, scarlet webbing between the toes, and a golden iris (Fei et al. 2009; Nguyen et al. 2020). Rhacophorus rhodopus is mainly distributed in Myanmar (Kachin and Shan states), northeast India, northern Thailand, Laos, Vietnam and southern China (Yunnan, Guangxi and Hainan) (Zug and Kahn 2022).

The classification of *Rhacophorus* has been of concern to many scholars. With the completion of several phylogenetic studies in recent years, the classification of Rhacophorus has been clarified, but it has not been completely solved. There are still many disputes. Inger et al. (1999) once thought that R. rhodopus and R. bipunctatus Ahl, 1927 were the same species, but subsequent studies have shown that they can be distinguished according to the back color, the number of axillary black spots and the color of web. However, the above characteristics of the two species may be unstable with time, region and individual genetic differences, therefore, this conclusion is not completely reliable (Bordoloi et al. 2007; Fei et al. 2009). Although it is difficult to distinguish them completely in morphology, molecular research results show that they are indeed different species. Li et al. (2008, 2012) showed a distant genetic relationship between R. rhodopus from Mengyang, Yunnan (type locality) and R. bipunctatus from Myanmar (non-type locality), which provides strong evidence to support the view that *R. rhodopus* and *R. bipunctatus* are two different species. In addition, the above research also showed that there are morphological and genetic differences among R. rhodopus from different places, and there may be cryptic species within that species. Recent phylogenetic studies showed that R. rhodopus is not monophyletic, but belongs to complex (Yu et al. 2007; Tao et al. 2014; Chan et al. 2018). According to these authors and the re-division of Rhacophorus by Jiang et al. (2019), the genetic relationship between R. rhodopus and R. bipunctatus is the closest and they form a main clade with Rhacophorus norhayatii Chan & Grismer, 2010, Rhacophorus reinwardtii Schlegel, 1840, Rhacophorus borneensis Matsui, Shimada & Sudin, 2013, Rhacophorus helenae Rowley, Tran, Hoang & Le, 2012, and Rhacophorus kio Ohler & Delorme, 2006.

During a field survey in Napo County, Baise City, Guangxi Zhuang Autonomous Region, China (Fig. 1), we collected five specimens of *Rhacophorus* resembling *R. rhodopus* and *R. bipunctatus* in the presence of black spots at axillary region and



**Figure 1.** Map shows the collection site of the specimens used in morphological part of this study. The circle represents the type locality of *R. napoensis* sp. nov. at Napo County, Baise City, Guangxi Zhuang Autonomous Region and the star represents the collection site of *R. rhodopus* specimens in the Caiyanghe Nature Reserve, Simao District, Pu'er City, Yunnan Province (the type locality of *R. rhodopus*).

skin folds above the anus. However, they can be distinguished from *R. rhodopus* and *R. bipunctatus* in morphological and molecular characteristics. Therefore, we consider that these specimens represent a new species of *Rhacophorus*.

# Materials and methods

# Sampling

Specimens collected in Napo County, Baise City, Guangxi Zhuang Autonomous Region, China were euthanized and preserved in 75% ethanol. Liver tissue was preserved in 99% ethanol. The specimens are stored in Guangxi Normal University (**GXNU**).

# Morphology

Morphological data were measured with electronic vernier calipers to the accuracy of 0.1 mm. Morphological terms were referred to Fei (1999). The measured data included the following 16 measurements: snout-vent length (**SVL**, the length from the tip of snout to vent); head length (**HL**, the length from the tip of snout to the posterior edge of the

mandibular joint); head width (**HW**, the maximum distance between two sides of the head); snout length (**SL**, the length from the tip of snout to anterior border of eye); internasal space (**INS**, the distance between the inner edges of the left and right nostrils); interorbital space (**IOS**, the narrowest distance between the medial edges of the left and right upper eyelids); width of upper eyelid (**UEW**, the maximum width of upper eyelid); diameter of eye (**ED**, the diameter of the eye parallel to the body axis); diameter of tympanum (**TD**, the maximum diameter of tympanum); distance from nostril to eye (**DNE**, the length from the anterior border of the eye to the inner edge of the ipsilateral nostril); distance from snout to nostril (**SN**, the length from the tip of snout to the inner edge of the ipsilateral nostril); to the tip of the third finger); thigh length (**THL**, the length from vent to knee); tibia length (**TIL**, the length from knee to ankle); length of the foot and tarsus (**TFL**, the length from the tibial tarsal joint to the tip of the fourth toe); foot length (**FL**, the length from the endial tarsal process to the tip of the fourth toe).

SPSS statistical software was used to analyze 16 morphological measurements of five specimens collected from Napo County, Baise City, Guangxi Zhuang Autonomous Region and four specimens of *R. rhodopus* collected from type locality (Caiyanghe Nature Reserve, Simao District, Pu'er City, Yunnan Province). The snout-vent length data was used to correct the other 15 data measurements (the corrected data was the snout-vent length divided by the quotient of each data). The obtained data were imported into SPSS (statistical product and service solutions) ver. 17.0 for principal component analysis (PCA). The two principal components with the highest contribution rate were used to make a scatter diagram to compare the morphological characteristics between the new species and *R. rhodopus*.

#### Molecular analyses

Total genomic DNA was extracted from liver tissue. Tissue samples were digested with proteinase K, and then purified by standard phenol/chloroform separation and ethanol precipitation. The fragment encoding partial 16S ribosomal RNA (16S) was amplified and sequenced according to the protocol of Yu et al. (2019). All new sequences were deposited in GenBank under accession Nos. ON217794–ON217798.

Homologous sequences of *R. rhodopus, R. bipunctatus*, the related species mentioned above, and that for the outgroup, were downloaded from GenBank (Table 1). *Zhangixalus smaragdinus* Blyth, 1852 was selected as outgroup according to Jiang et al. (2019). All sequences were aligned using the default parameters of MUSCLE in MEGA 7 (Kumar et al. 2016). Uncorrected pairwise distances (P-distances) between species were calculated in MEGA 7. The best alternative model was selected as TIMef in MODELTEST ver. 3.7 (Posada and Crandall 1998). Bayesian phylogenetic analysis was conducted using MRBAYES ver. 3.2.6. Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 5,000,000 generations and sampled every 100 generations. When the average standard deviation of split frequencies was less than 0.01, the first 25% of sampled trees were discarded as burn-in and the remaining trees were used

| Species                      | Voucher         | Locality   | Accession No. |
|------------------------------|-----------------|--|---------------|
| Z. smaragdinus               |                 |  | MN613221      |
| R. bipunctatus               | PUCZM/IX/SL360  | Mizoram, India                                       | MH087073      |
| R. bipunctatus               | PUCZM/IX/SL612  | Mizoram, India                                       | MH087076      |
| R. helenae                   |                 | Nui Ong Nature Reserve, Binh Thuan Province, Vietnam | JQ288090      |
| R. helenae                   |                 | Nui Ong Nature Reserve, Binh Thuan Province, Vietnam | JQ288091      |
| R. kio                       | SCUM 37941C     | Xishuangbanna, Yunnan, China                         | EU215532      |
| R. kio                       | VN.2018.057     | Kon Tum, Vietnam                                     | LC548742      |
| R. rhodopus                  | KIZ589          | Longling, Yunnan, China                              | EF564578      |
| R. rhodopus                  | KIZ587          | Longling, Yunnan, China                              | EF564577      |
| R. rhodopus                  | clone 5         | Xishuangbanna, Yunnan, China                         | EF646366      |
| R. rhodopus                  | SCUM 060692L    | Mengyang, Jinghong, China                            | EU215531      |
| R. borneensis                | BORN:22410      | Sabah, Maliau Basin, Malaysia                        | AB781693      |
| R. borneensis                |                 | Sabah, Maliau Basin, Malaysia                        | AB781694      |
| R. norhayatii                |                 | Johor, Endau Rompin, Malaysia                        | AB728191      |
| R. reinwardtii               | ENS 16447 (UTA) | Sumatra, Bandung, Indonesia                          | KY886335      |
| R. reinwardtii               | ENS 16179 (UTA) | Java, Patuha, Indonesia                              | KY886328      |
| R. rhodopus                  | L062456         | Motuo, Xizang, China                                 | JX219442      |
| R. rhodopus                  | L06245          | Motuo, Xizang, China                                 | JX219441      |
| R. napoensis sp. nov.        | GXNU YU000169   | Napo, Guangxi, China                                 | ON217794      |
| R. napoensis sp. nov.        | GXNU YU000170   | Napo, Guangxi, China                                 | ON217795      |
| R. napoensis <b>sp. nov.</b> | GXNU YU000171   | Napo, Guangxi, China                                 | ON217796      |
| R. napoensis <b>sp. nov.</b> | GXNU YU000172   | Napo, Guangxi, China                                 | ON217797      |
| R. napoensis sp. nov.        | GXNU YU000173   | Napo, Guangxi, China                                 | ON217798      |

**Table 1.** Species of *Rhacophorus* (and the outgroup, *Zhangixalus smaragdinus*) used in phylogenetic analyses of this study, together with locality and voucher and GenBank accession numbers.

to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs). In addition, we performed maximum likelihood analysis using IQ-TREE2 (Minh et al. 2020) with 1000 bootstrap replicates.

# Results

# Morphological study

Morphological data are summarized in Table 2. We retained the first two principal components which accounted for 80.19% of the total variance (Table 3). Loadings for PC1, which accounted for 44.60% of the total variance, were mainly loaded on head width (HW), snout length (SL), interorbital space (IOS), length of forearm and hand (LAHL), thigh length (THL), tibia length (TIL), length of foot and tarsus (TFL) and foot length (FL). Loadings for PC2, which accounted for 35.59% of the total variance, were mainly loaded on head length (HL), width of upper eyelid (UEW), diameter of eye (ED), diameter of tympanum (TD) and distance from nostril to eye (DNE) (Table 3). The scatter plot based on PC1 and PC2 showed that the new species and *R. rhodopus* can be well distinguished in the X-axis direction, but there is no obvious separation in the Y-axis, indicating that PC1 can be used to distinguish new species

from *R. rhodopus* (Fig. 2). The results showed that the snout length of the new species is longer than that of *R. rhodopus*, but the head width, interorbital space, length of forearm and hand, thigh length, tibia length, length of foot and tarsus and foot length are shorter than that of *R. rhodopus*.

Additionally, the specimens from Napo can be morphologically distinguished from *R. bipunctatus* by a series of characters: i.e., head width greater than head length, tympanum distinct, and two or three black spots at axillary region.

**Table 2.** Measurements (mm) and the number of dark spots (left, right, total) at axillary region of *R. napoensis* sp. nov. from Napo County, Baise City, Guangxi Zhuang Autonomous Region, China and *R. rhodopus* from Caiyanghe Nature Reserve, Simao District, Pu'er City, Yunnan Province, China. Abbreviations defined in Materials and methods.

| Species                       | Vouchers      | Sex | SVL  | HL   | HW   | SL   | INS  | IOS  | UEW  | ED   | Т     | D     |
|-------------------------------|---------------|-----|------|------|------|------|------|------|------|------|-------|-------|
| R. napoensis sp. nov.         | GXNU YU000169 | М   | 39.6 | 11.7 | 14.6 | 7.3  | 4.7  | 5.9  | 3.7  | 4.6  | 2.4   |       |
| R. napoensis <b>sp. nov.</b>  | GXNU YU000170 | М   | 43.6 | 13.5 | 16.6 | 8.0  | 5.1  | 5.2  | 4.3  | 6.0  | 2.8   |       |
| R. napoensis sp. nov.         | GXNU YU000171 | М   | 39.8 | 16.6 | 15.5 | 7.1  | 5.2  | 5.0  | 4.7  | 6.7  | 3.7   |       |
| R. napoensis <b>sp. nov</b> . | GXNU YU000172 | М   | 38.6 | 14.7 | 15.0 | 6.9  | 4.9  | 4.2  | 4.3  | 5.8  | 3.    | .4    |
| R. napoensis <b>sp. nov.</b>  | GXNU YU000173 | М   | 41.1 | 15.8 | 16.3 | 7.2  | 5.0  | 5.3  | 4.8  | 6.3  | 3.6   |       |
| R. rhodopus                   | 090142        | М   | 35.1 | 12.7 | 13.8 | 5.7  | 4.4  | 4.6  | 3.3  | 5.1  | 2.8   |       |
| R. rhodopus                   | 090143        | М   | 31.4 | 10.8 | 12.7 | 5.1  | 4.1  | 4.4  | 3.1  | 4.7  | 2.4   |       |
| R. rhodopus                   | 090144        | М   | 35.8 | 11.7 | 13.9 | 5.6  | 4.7  | 4.8  | 3.3  | 5.3  | 2.6   |       |
| R. rhodopus                   | 090145        | М   | 31.2 | 11.7 | 12.5 | 5.1  | 3.7  | 4.7  | 2.9  | 4.2  | 2.4   |       |
| Species                       | Vouchers      | Sex | DNE  | SN   | LAHL | THL  | TIL  | TFL  | FL   | Left | Right | Total |
| R. napoensis <b>sp. nov.</b>  | GXNU YU000169 | М   | 3.1  | 4.0  | 20.6 | 19.5 | 19.9 | 28.4 | 18.7 | 3    | 2     | 5     |
| R. napoensis <b>sp. nov.</b>  | GXNU YU000170 | М   | 3.6  | 4.1  | 20.3 | 21.5 | 21.7 | 29.2 | 18.7 | 3    | 2     | 5     |
| R. napoensis <b>sp. nov.</b>  | GXNU YU000171 | М   | 2.7  | 4.2  | 19.4 | 19.8 | 20.1 | 29.2 | 18.6 | 2    | 2     | 4     |
| R. napoensis <b>sp. nov</b> . | GXNU YU000172 | М   | 2.6  | 3.8  | 18.2 | 18.8 | 19.6 | 27.4 | 17.6 | 2    | 3     | 5     |
| R. napoensis <b>sp. nov.</b>  | GXNU YU000173 | М   | 2.9  | 3.9  | 20.1 | 21.0 | 20.8 | 29.3 | 18.0 | 2    | 3     | 5     |
| R. rhodopus                   | 090142        | М   | 2.6  | 3.1  | 18.5 | 18.6 | 19.0 | 26.3 | 17.2 | 2    | 1     | 3     |
| R. rhodopus                   | 090143        | М   | 2.1  | 3.0  | 16.4 | 17.1 | 16.9 | 23.1 | 14.6 | 2    | 2     | 4     |
| R. rhodopus                   | 090144        | М   | 2.6  | 3.2  | 19.1 | 19.3 | 18.6 | 25.9 | 17.2 | 1    | 1     | 2     |
| R. rhodopus                   | 090145        | М   | 2.2  | 2.6  | 16.6 | 16.3 | 16.7 | 22.4 | 15.5 | 1    | 1     | 2     |

**Table 3.** Factor loadings of the first two principal components of 15 size-adjusted morphometric characters of *R. napoensis* sp. nov. from Napo County and *R. rhodopus* from Caiyanghe Nature Reserve. Absolute values of loading greater than 0.7 in boldface. Abbreviations defined in Materials and methods.

| Character              | PC1    | PC2    |
|------------------------|--------|--------|
| HL                     | 0.247  | 0.887  |
| HW                     | 0.703  | 0.458  |
| SL                     | -0.957 | 0.051  |
| INS                    | 0.574  | 0.591  |
| IOS                    | 0.830  | -0.152 |
| UEW                    | -0.399 | 0.887  |
| ED                     | 0.159  | 0.935  |
| TD                     | 0.158  | 0.930  |
| DNE                    | -0.530 | -0.771 |
| SN                     | -0.562 | 0.670  |
| LAHL                   | 0.772  | -0.471 |
| THL                    | 0.864  | -0.300 |
| TIL                    | 0.917  | -0.159 |
| TFL                    | 0.709  | 0.234  |
| FL                     | 0.842  | -0.091 |
| Percentage of variance | 44.604 | 35.588 |



**Figure 2.** Scatter plot of the principal component analysis of size-adjusted morphological data. The black circles represents *R. rhodopus* from the type locality and the white circles represents *R. napoensis* sp. nov..

### Molecular study

The specimens from Napo County form a clade with strong support (Clade A; Fig.3). *Rhacophorus rhodopus* from different localities were grouped into three clades, one (Clade B) consisting of samples from the type locality (Xishuangbanna), one (Clade D) consisting of samples from western Yunnan (Longlin), and one (Clade E) containing samples from Motuo, Tibet. The clade consisting of specimens from Napo (Clade A) is not directly related to any one of these clades, although the support values are not strong. The Clade B is sister to the Clade D with strong support. Genetically the clade containing specimens from Napo (Clade A) differs from *R. bipunctatus* (Clade C) and *R. rhodopus* from the type locality (Clade B) by 7.98% and 7.71%, respectively (Table 4). The genetic distance between the specimens from Napo County (Clade A) and *R. rhodopus* from Longling (Clade D) is 6.55%, and the distance between the Napo specimens and *R. rhodopus* from Motuo (Clade E) is 7.59%. All these estimations of genetic distances are greater than 3.0%, the conventional threshold of species-level divergence in 16S rRNA gene of Anura (Vences et al. 2005), meaning that the clade of specimens from Napo is not conspecific with other clades.



**Figure 3.** Bayesian phylogenetic tree of *R. rhodopus* and the related species mentioned above constructed with 965bp 16S rRNA gene. The values above the branch are Bayesian posterior probabilities (BPP) and maximum likelihood (ML) bootstrap value, respectively (only values greater than 50% are displayed).

| Species                           | 1     | 2     | 3     | 4     | 5     | 6     | 7    | 8    | 9    | 10   |
|-----------------------------------|-------|-------|-------|-------|-------|-------|------|------|------|------|
| 1 R. napoensis sp. nov. (Clade A) |       |       |       |       |       |       |      |      |      |      |
| 2 R. rhodopus (Clade D)           | 6.55  |       |       |       |       |       |      |      |      |      |
| 3 R. rhodopus (Clade B)           | 7.71  | 4.84  |       |       |       |       |      |      |      |      |
| 4 R. rhodopus (Clade E)           | 7.59  | 5.97  | 7.69  |       |       |       |      |      |      |      |
| 5 R. bipunctatus (Clade C)        | 7.98  | 7.26  | 8.73  | 9.14  |       |       |      |      |      |      |
| 6 Z. smaragdinus                  | 15.28 | 11.34 | 13.79 | 15.60 | 14.35 |       |      |      |      |      |
| 7 R. borneensis                   | 6.55  | 5.86  | 8.06  | 6.54  | 9.06  | 14.99 |      |      |      |      |
| 8 R. helenae                      | 6.67  | 7.20  | 9.47  | 6.65  | 7.78  | 9.68  | 5.30 |      |      |      |
| 9 R. kio                          | 8.50  | 7.96  | 9.67  | 7.78  | 8.82  | 15.22 | 7.60 | 4.60 |      |      |
| 10 R. norhayatii                  | 7.83  | 5.76  | 7.26  | 7.94  | 9.59  | 16.49 | 5.47 | 7.39 | 9.03 |      |
| 11 R. reinwardtii                 | 4.64  | 5.74  | 6.49  | 5.00  | 6.79  | 11.03 | 4.89 | 6.34 | 7.67 | 4.04 |

Table 4. Average uncorrected pairwise distance (%) between groups of 16S rRNA sequences used in this study.

Therefore, based on the above morphological and molecular evidence, we considered that the specimens from Napo County (Clade A) are different from *R. rhodopus* (Clades B, D and E) and *R. bipunctatus* (Clade C) and represent a new species of *Rhacophorus*, which is described in the Taxonomy section below. As for Clades D and E, we suppose that they likely represent two cryptic species confused with *R. rhodopus*, pending further morphological studies.

# Taxonomy

## Rhacophorus napoensis sp. nov.

https://zoobank.org/66C47824-DE9B-4EA7-AFF1-CFBA0F8D239E Figs 4–6

Material examined. *Holotype*. GXNU YU000172, adult male, collected on 25 March 2019 by Shuo Liu from Napo County, Baise City, Guangxi Zhuang Autonomous Region,

China (23°1'20"N, 105°50'58"E, ca 1032 m a.s.l.). *Paratypes.* GXNU YU000169, GXNU YU000170, GXNU YU000171 and GXNU YU000173, four adult males, collected at the same time as the holotype from the type locality by Shuo Liu.

**Etymology.** The specific epithet is named for the type locality. We suggest the English common name as "Napo tree frog" and the Chinese common name as "那坡树蛙".

**Diagnosis.** Morphologically, there are the following differences between Napo County specimens and other species belonging to *Rhacophorus*: (1) Medium body size (adult males SVL 38.6–43.6 mm); (2) snout pointed, projecting beyond margin of lower jaw in ventral view, and the tip has a distinct bulge; (3) tympanum distinct, rounded; (4) maxillary teeth distinct; (5) tongue cordiform, notably notched posteriorly; (6) external single subgular vocal sac; (7) the tibiotarsal articulation reaches the snout; (8) TIL longer than FL and slightly longer than half of SVL; (9) entire web between fingers and toes; (10) single inner metatarsal tubercle, flat; (11) banding exists in dorsal surface of limbs posterior part of dorsum; (12) two to three black spots at axillary region; (13) web is not black; and (14) dorsal color hoary with numerous black spots when the species is kept in preservative.

**Description of holotype.** Adult male, body size medium (SVL 38.6 mm); head width (HW 15.0 mm) longer than head length (HL 14.7 mm); snout pointed, longer than diameter of eye (ED 5.8 mm), protruding from the margin of the lower jaw; canthus rostralis distinct; loreal region oblique; nostril small, closer to eye than to tip of snout; internasal space (INS 4.9 mm) longer than interorbital space (IOS 4.2 mm) and width of upper eyelid (UEW 4.3 mm); interorbital space (IOS 4.2 mm) almost equal to width of upper eyelid (UEW 4.3 mm); pineal ocellus absent; pupil horizontal; tympanum distinct, rounded, diameter of tympanum (TD 3.4 mm) longer than half eye diameter (ED 5.8 mm), internasal space (INS 4.9 mm) and half interorbital space (IOS 4.2 mm); supratympanic fold distinct; vomerine teeth present; maxillary teeth distinct; tongue cordiform, attached anteriorly, notably notched posteriorly; choanae oval; external single subgular vocal sac, vocal sac opening at the bottom of the mouth on either side.

Forelimbs stubby, length of lower arm and hand (LAHL 18.2 mm) shorter than snout-vent length (SVL 38.6 mm); fingers short, relative length of fingers: I < II < IV < III; tips of all fingers expanded into discs; entire web between fingers; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; supernumerary tubercles below the base of finger absent; single thenar (inner metacarpal) tubercle large, oval, distinct (Fig. 5a).

Hindlimbs long and thin, tibia length (TIL 19.6 mm) longer than thigh length (THL 18.8 mm) and foot length (FL 17.6 mm), tibiotarsal articulation reaches the snout; when the legs are at right angles to the body, the heels overlap; relative length of toes is I < II < III < V < IV; tips of all toes expanded into discs; entire web between toes; subarticular tubercles prominent and rounded, formula 1, 1, 2, 3, 2; supernumerary tubercle below the base of toe absent; single inner metatarsal tubercle, flat (Fig. 5b).

The skin of throat, ventral part of tibia, foot and tarsus smooth; the skin of chest, venter, vent and thigh rough and granular; some warts are found around the vent and flanks; dermal fringe along joint, vent and the outer sides of limbs (Fig. 4b); three black spots at the right armpit (Fig. 6a), and two black spots at the left armpit (Fig. 6b).



**Figure 4.** Dorsal views (**a**) and ventral views (**b**) of the holotype of *R. napoensis* sp. nov. (GXNU YU000172) in preservative. Ventral view (**c**) of *R. rhodopus* from type locality (090142) in preservative.



**Figure 5.** Ventral view of hand (**a**) and foot (**b**) of the holotype of *R. napoensis* sp. nov. (GXNU YU000172) in preservative.



**Figure 6.** Right armpit and flank view (**a**) and the left armpit and flank view (**b**) of the holotype of *R. napoensis* sp. nov. (GXNU YU000172) in preservative.

*Color of holotype in preservative.* Dorsal color hoary with numerous black spots; horizontal banding on dorsum and dorsal surface of limbs (Fig. 4a).

*Male secondary sexual characteristics.* No nuptial pad and lineae masculinae were observed.

**Morphological variation.** The morphological measurement and the number of dark spots at axillary region of the holotype and paratypes are shown in Table 2. The total number of dark spots at axillary region varies between individuals, and the number of dark spots on the left and right axillary region also varies. Because all specimens are male, sexual dimorphism cannot be determined.

**Distribution and ecology.** The new species was found near several large rocks in the bushes, 306 m southeast of Nongyao, Napo County, Baise City, Guangxi Zhuang Autonomous Region, China (Fig. 7). Vocal recordings and tadpoles of this new species were not collected.

**Comparisons.** The new species is obviously distinguishable from most of the closely-related species including *R. norhayatii*, *R. reinwardtii*, *R. kio*, *R. borneensis*, and *R. helenae* by smaller body size (SVL of adult males 38.6–43.6 mm vs. 64.7 mm in *R. norhayatii*, 41.1–52.5 mm in *R. reinwardtii*, 70.5 mm in *R. kio*, 50.9 mm in *R. borneensis*, and 72.3–85.5 mm in *R. helenae*) and lack of black coloration on the webs (vs. webs between toes black).

The new species differs from *R. rhodopus* by head width greater than head length (vs. head length almost equal to head width), snout pointed and the tip has a distinct bulge (vs. snout oblique and pointed) (Fig. 4), tongue cordiform, notably notched posteriorly (vs. tongue narrow and long, deeply notched posteriorly), external single subgular vocal sac (vs. internal single subgular vocal sac), the tibiotarsal articulation reaches the snout (vs. the tibiotarsal articulation reaches the eye), tibia length is slightly greater than half of snout-vent length (vs. tibia length is about half of snout-vent length), two to three black spots at axillary region (vs. one black or dark round spot at axillary region); and from *R. bipunctatus* by head width greater than head length (vs. head length almost equal to head width), snout pointed, and the tip has a distinct bulge (vs. snout broad and pointed), loreal region oblique (vs. loreal region concave), tympanum distinct (vs. tympanum indistinct), maxillary teeth distinct (vs. tooth-like projection on maxilla absent), tongue cordiform, notably notched posteriorly (vs. tongue medium size, round, slight notched posteriorly, median lingual process absent), slender toes (vs. toes rather short, thin), tibia length is slightly greater than half of snout-vent length (vs. tibia length is slightly less than half of snout-vent length), two to three black spots at axillary region (vs. one big and one small black spot at axillary region).

# Discussion

The phenomenon of cryptic species was first discovered by Derham in 1718 (Winker 2005), which refers to species that are highly similar in morphology and are hidden as known species (Lincoln et al. 1983). Cryptic species and known species form a species complex (Liu 2012). The phylogenetic tree constructed in this study shows that



Figure 7. Habitat of *R. napoensis* sp. nov. at the type locality.

*R. rhodopus* is clustered into three clades (Clades B, D, and E) and the new species from Napo (Clade A) is not directly related to these clades or *R. bipunctatus* (Clade C) with strong supports. Furthermore, genetically the new species differs from Clades B–E by over 6.5%, which is greater than the conventional threshold of species-level divergence in 16S rRNA sequence of Anura (3%; Vences et al. 2005), and morphologically the new species can be distinguished from *R. rhodopus* and *R. bipunctatus* by a series of characters (i.e., longer snout, shorter tibia length, and head width greater than head

length). Therefore, we think that *Rhacophorus napoensis* sp. nov. should be diagnosed as an independent species.

Genetically, the clade containing specimens from the type locality of R. rhodopus (Clade B) differs from specimens from Longling, Yunnan, China (Clade D) and specimens from Motuo, Tibet, China (Clade E) by 4.84% and 7.69%, and the differences were greater than 3%, which means that probably the Clades D and E are not R. rhodopus, but two separate species (Vences et al. 2005; Vieites et al. 2009), which are in line with the concept of cryptic species. A morphological study is necessary to confirm the taxonomic status of the Clades D and E. This result supports that there may be cryptic species in R. rhodopus and that R. rhodopus belongs to complex. Species are the basic units of biological taxonomy. Only by making a clear and scientific distinction between species can we further study the interspecific relationship, biodiversity and evolution of species. With the maturity of research technology, especially the rapid development of molecular systematics, more and more species complexes have been deeply studied (Villalobos-Guerrero 2019; Amorim et al. 2022; Guimarães et al. 2022;), which shows that there may be some mistakes in the current species classification, and there are more cryptic species waiting to be discovered. The study of cryptic species and complexes will have a far-reaching impact on evolutionary theory, biogeography and conservation planning, which needs attention (Bickford et al. 2007).

Recently, Poyarkov et al. (2021) mentioned that the existing records of *R. bipunctatus* in Thailand, Laos, Cambodia and Vietnam seem to be related to *R. rhodopus*, and the classification of this complex needs to be further clarified. These countries are located in the Indochina Peninsula, bordering on the southwest of China. The terrain of this area is mainly mountainous and plateau (Qiu et al. 2021). The complex landform may lead to geographical isolation within the widely distributed species, thus forming new species (Wu et al. 2020). Napo County, Baise City, Guangxi Zhuang Autonomous Region, where the new species was collected, and the type locality of *R. rhodopus*, are located in this area. Therefore, we speculate that there may be more undiscovered new species in the Indochina Peninsula and its surrounding areas. In conclusion, the intraspecific classification of *R. rhodopus* and the morphological differences between this species and *R. bipunctatus* are still vague. There may be taxonomic disputes and errors among different geographical populations of *R. rhodopus*. The intraspecific morphological differences and phylogenetic relationship of *R. rhodopus* need to be further studied.

## Acknowledgements

Thanks go to Decai Ouyang for his assistances during the field survey. This work was supported by grants from the National Natural Science Foundation of China (32060114), Guangxi Natural Science Foundation Project (2022GXNSFAA035526), Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education (ERESEP2020Z22), and Guangxi Key Laboratory of Rare and Endangered Animal Ecology, Guangxi Normal University (19-A-01-06).

# References

- Amorim AM, Marinho LC, Francener A (2022) Deciphering the *Heteropterys pannosa* species complex (Malpighiaceae). PeerJ 10: e12937. https://doi.org/10.7717/peerj.12937
- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22(3): 148–155. https://doi.org/10.1016/j.tree.2006.11.004
- Bordoloi S, Bortamuli T, Ohler A (2007) Systematics of the genus *Rhacophorus* (Amphibia, Anura): Identity of red-webbed forms and description of a new species from Assam. Zootaxa 1653(1): 1–20. https://doi.org/10.11646/zootaxa.1653.1.1
- Chan KO, Grismer LL, Brown RM (2018) Comprehensive multi-locus phylogeny of Old World tree frogs (Anura: Rhacophoridae) reveals taxonomic uncertainties and potential cases of over- and underestimation of species diversity. Molecular Phylogenetics and Evolution 127: 1010–1019. https://doi.org/10.1016/j.ympev.2018.07.005
- Fei L (1999) Atlas of Amphibians of China. Henan Science and Technology Press, Zhengzhou City, Henan Province China. [in Chinese]
- Fei L, Hu SQ, Ye CY (2009) FAUNA SINICA Amphibia Vol. 2 Anura. Science Press, Beijing, China. [in Chinese]
- Frost DR (2021) Amphibian Species of the World: An Online Reference. Version 6.1. American Museum of Natural History, New York. https://amphibiansoftheworld. amnh.org/Amphibia/Anura/Rhacophoridae/Rhacophorinae/Rhacophorus [Accessed on: 20.12.2021]
- Guimarães KLA, Lima MP, Santana DJ, de Souza MFB, Barbosa RS, Rodrigues LRR (2022) DNA barcoding and phylogeography of the *Hoplias malabaricus* species complex. Scientific Reports 12(1): 1–12. https://doi.org/10.1038/s41598-022-09121-z
- Inger RF, Orlov N, Darevsky I (1999) Frogs of Vietnam: A report on new collections. Fieldiana Zoology New Series 92: 1–46.
- Jiang DC, Jiang K, Ren JL, Wu J, Li JT (2019) Resurrection of the Genus *Leptomantis*, with Description of a New Genus to the Family Rhacophoridae (Amphibia: Anura). Asian Herpetological Research 10(01): 1–12. https://doi.org/10.16373/j.cnki.ahr.180058
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054
- Li JT, Che J, Bain RH, Zhao EM, Zhang YP (2008) Molecular phylogeny of Rhacophoridae (Anura): A framework of taxonomic reassignment of species within the genera *Aquixalus*, *Chiromantis*, *Rhacophorus*, and *Philautus*. Molecular Phylogenetics and Evolution 48(1): 302–312. https://doi.org/10.1016/j.ympev.2008.03.023
- Li JT, Li Y, Murphy RW, Rao DQ, Zhang YP (2012) Phylogenetic resolution and systematics of the Asian tree frogs, *Rhacophorus* (Rhacophoridae, Amphibia). Zoologica Scripta 41(6): 557–570. https://doi.org/10.1111/j.1463-6409.2012.00557.x
- Lincoln RJ, Boxshall GA, Clark PF (1983) A Dictionary of Ecology, Evolution, and Systematics. Systematic Botany 8(3): 339. https://doi.org/10.2307/2418488
- Liu SS (2012) *Bemisia tabaci* is a species complex. Chinese Journal of Biological Control 28(04): 466. [in Chinese] https://doi.org/10.16409/j.cnki.2095-039x.2012.04.001

- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Teeling E (Ed.). Molecular Biology and Evolution 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015
- Nguyen TV, Brakels P, Maury N, Sudavanh S, Pawangkhanant P, Idiiatullina S, Lorphengsy S, Inkhavilay K, Suwannapoom C, Poyarkov NA (2020) New herpetofaunal observations from Laos based on photo records. Amphibian & Reptile Conservation 14(2): 33.
- O'Connell KA, Smart U, Smith EN, Hamidy A, Kurniawan N, Fujita MK (2018) Withinisland diversification underlies parachuting frog (*Rhacophorus*) species accumulation on the Sunda Shelf. Journal of Biogeography 45(4): 929–940. https://doi.org/10.1111/jbi.13162
- Pan T, Zhang YN, Wang H, Wu J, Kang X, Qian LF, Li K, Zhang Y, Chen JY, Rao DQ, Jiang JP, Zhang BW (2017) A New Species of the Genus *Rhacophorus* (Anura: Rhacophoridae) from Dabie Mountains in East China. Asian Herpetological Research 8(01): 1–13. https:// doi.org/10.16373/j.cnki.ahr.160064
- Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817
- Poyarkov NA, Nguyen TV, Popov ES, Geissler P, Pawangkhanant P, Neang T, Suwannapoom C, Orlov NL (2021) Recent Progress in Taxonomic Studies, Biogeographic Analysis, and Revised Checklist of Amphibians in Indochina. Russian Journal of Herpetology 28(3A): 1–110. https://doi.org/10.30906/1026-2296-2021-28-3A-1-110
- Qiu T, Zhang SF, Xian W (2021) Study on water resources zoning of five countries in Indo-China Peninsula. Geographical Research 40(05): 1421–1431. [in Chinese] https://doi. org/10.11821/dlyj020200402
- Tao NT, Matsui M, Eto K, Orlov NL (2014) A preliminary study of phylogenetic relationships and taxonomic problems of Vietnamese *Rhacophorus* (Anura: Rhacophoridae). Russian Journal of Herpetology 21(4): 274–280.
- Vences M, Thomas M, Meijden A, Chiari Y, Vieites DR (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. Frontiers in Zoology 2(1): 5. https://doi.org/10.1186/1742-9994-2-5
- Vieites DR, Wollenberg KC, Andreone F, Kohler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the United States of America 106(20): 8267–8272. https://doi.org/10.1073/pnas.0810821106
- Villalobos-Guerrero TF (2019) Redescription of two overlooked species of the *Perinereis nuntia* complex and morphological delimitation of *P. nuntia* (Savigny *in* Lamarck, 1818) from the Red Sea (Annelida, Nereididae). Zoosystema 41(24): 465–496. https://doi.org/10.5252/ zoosystema2019v41a24
- Winker K (2005) Sibling species were first recognized by William Derham (1718). The Auk 122(2): 706–707. https://doi.org/10.1093/auk/122.2.706
- Wu ZY, Li L, Zhang L, Qiu FG, Yang SL, E GX (2020) Genetic Diversity Analysis of *Fejervarya multistriata* Population in Fanjingshan Region Based on 12S rRNA Sequence. Journal of Agricultural Biotechnology 28(12): 2209–2220. [in Chinese]
- Yu GH, Rao DQ, Yang JX, Zhang MW (2007) Non-monophyly of *Rhacophorus rhodopus*, *Theloderma* and *Philautus albopunctatus* Inferred from Mitochondrial 16S rRNA Gene

Sequences. Zoological Research 04: 437–442. https://doi.org/10.3321/j.issn:0254-5853.2007.04.015

- Yu GH, Hui H, Hou M, Wu ZJ, Rao DQ, Yang JX (2019) A new species of *Zhangixalus* (Anura: Rhacophoridae), previously confused with *Zhangixalus smaragdinus* (Blyth, 1852). Zootaxa 4711(2): 275–292. https://doi.org/10.11646/zootaxa.4711.2.3
- Zug G, Kahn TR (2022) Amphibians and Reptiles of Myanmar: Checklists and Keys. I. Amphibians, Crocodilians, and Turtles. Smithsonian Scholarly Press, Washington, D.C.

RESEARCH ARTICLE



# Two new alkali-sink specialist species of *Paruroctonus* Werner 1934 (Scorpiones, Vaejovidae) from central California

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| Academic editor: José Antonio Ochoa   Received 25 October 2021   Accepted 10 May 2022   Published 15 August 20 | 22 |
|--|----|
| https://zoobank.org/ADF4CFE4-019A-4544-8AF9-E2183F255A52   |    |

Citation: Jain P, Forbes H, Esposito LA (2022) Two new alkali-sink specialist species of *Paruroctonus* Werner 1934 (Scorpiones, Vaejovidae) from central California. ZooKeys 1117: 139–188. https://doi.org/10.3897/zookeys.1117.76872

#### Abstract

Herein we describe two new species of *Paruroctonus* (Werner 1934) from California: *Paruroctonus soda* **sp. nov.** from the Soda Lake playa at the center of the Carrizo Plain in San Luis Obispo county and *Paruroctonus conclusus* **sp. nov.** from the Koehn Lake playa in the Mojave Desert of Kern County. They can be differentiated from other *Paruroctonus* by a combination of morphological features including deeply scalloped pedipalp fingers in males, specific patterns of fuscous pigmentation, unique setal counts, and unique morphometric ratios. They can also be separated from one another by the latter three characters. Photographs of a large selection of live scorpions are provided, including detailed images and figures of many morphological features. Their distributions, habitats, and ecologies are discussed; and important steps towards their conservation are described.

#### Keywords

Conservation, desert, playa, salt flat, scorpions

# Introduction

*Paruroctonus* Werner 1934 is the most speciose genus of scorpions in California. Including the two species described in this paper, it consists of 17 recorded species in the state. This includes wide-ranging generalists such as *Paruroctonus silvestrii* (Borelli, 1909), *P. becki* Gertsch & Allred, 1965, and *P. boreus* (Girard, 1854), as well as rangerestricted specialists such as *Paruroctonus bantai* Gertsch & Soleglad, 1966, *P. arenicola*  Haradon, 1984, and *P. shulovi* Williams, 1970. Most specialist desert species are restricted to habitats that retain more water than the surrounding environment. Examples found in California include sand dune specialists such as *Paruroctonus arenicola nudipes* Haradon, 1984, *P. xanthus* Gertsch & Soleglad, 1966, *P. hirsutipes* Haradon, 1984, and *P. baergi* Williams & Hadley, 1967 as well as playa/spring specialists such as *Paruroctonus bantai* Gertsch & Soleglad, 1966 (both ssp.).

The most recent large-scale taxonomic work on the genus *Paruroctonus* was conducted by Haradon in three publications in 1984 and 1985 (Haradon 1984a, b, 1985). He described several new species in *Paruroctonus*, including four from California (Haradon 1984a, b, 1985), and split the genus into three infragroups: *gracilior* group, *stahnkei* group, and *boreus* group. He then further split each of the latter two into an additional four microgroups (Haradon 1985). These groups have not been rigorously tested through empirical data, and morphological diagnoses for both infragroups and microgroups contain multiple exceptions (Haradon 1984a, b, 1985), making it impossible to accurately assign a new species to any of them. Phylogenetic evidence has also not yet supported any of these group distinctions, and in one case, has suggested that the microgroups in the *boreus* infragroup may be incorrectly constructed (Haradon 1985; Miller et al. 2014). Although some or many of these groups may be valid, we have not suggested an infragroup or microgroup placement for the two species described herein and advise against using these or other groups within *Paruroctonus* until more phylogenetic work is conducted on the genus.

The two species described herein are specialist species restricted to alkali-sink environments surrounding desert playas: *Paruroctonus soda* sp. nov. (Figs 1–14) is found around Soda Lake in the Carrizo Plain, part of the San Joaquin Desert in San Luis Obispo county, and *Paruroctonus conclusus* sp. nov. (Figs 15–27) is found around Koehn Lake in the Fremont Valley, part of the Mojave desert in Kern county. Both the San Joaquin Desert and the Mojave Desert have an abundance of ephemeral water bodies, in large part due to several substantial rivers that flow into them from the surrounding mountains. We summarize the paleogeographic history of these playas and hypothesize what historic trends could predict for the future of these species. We also propose steps towards the conservation of *Paruroctonus conclusus* sp. nov., which should be considered threatened due to a restricted distribution, and discuss a possible instance of hypomelanism in *Paruroctonus soda* sp. nov. Lastly, we discuss the significance of morphological adaptations playa-specialist *Paruroctonus* have in common.

## Materials and methods

Specimens were photographed using a Canon EOS 7D camera with the Canon 100 mm F/2.8 macro lens. Habitat photos were taken using a Canon EOS 7D camera with the Canon 24–70 mm F/2.8 wide-angle lens or the Laowa 15 mm F/4 wide-angle macro lens. Stacked photographs were taken using the StackShot macro rail and were combined using Helicon Focus 7. Minor touch-ups to clean up the background and maintain even lighting were done using Gnu Image Manipulation Program and Adobe Photo-

shop. Satellite imagery for the maps is sourced from Google Earth and elevation data is sourced from NASA Shuttle Radar Topography Mission. Maps were constructed using QGIS, Gnu Image Manipulation Program, and Adobe Photoshop. Scale bars on figures are constructed using the pixel measurements in the photograph or traced illustration of the largest completely sclerotized precisely-measurable morphological feature parallel to the plane of the image and the corresponding length measurement on the scorpion.

Nomenclature and measurements largely follow Stahnke (1970) with a few exceptions. Basitarsal and telotarsal spine and setal nomenclature follows McWest (2009). Metasomal carinal nomenclature follows González-Santillán and Prendini (2013) with "lateral median" replaced with "lateral supramedian," consistent with Sissom and Francke (1981) (Figs 9, 23). Metasomal setae follow the name of their associated carinae. Pedipalp and trichobothrial nomenclature follows González-Santillán and Prendini (2013) (Figs 6–8, 20–22). Setae on the manus follow the name of their associated carinae.

All elements in the diagnosis, unless otherwise noted, are not sexually dimorphic and apply to late instar juveniles as well. Counts and measurements separated by a "/" indicate a difference on the left/right sides of a single specimen, while those separated by a "-" indicate a range across multiple examined specimens. Setal counts used in the diagnosis are taken as the maximum number of macrosetae on either the right or left side of the individual scorpion (e.g., if a scorpion had two macrosetae on the left and three on the right, the setal count would be "3"). Total length includes telson and is not calculated additively. Measurements are made using digital calipers and are given in mm.

Specimens examined and photographed are either maintained alive in captivity or preserved in 95% ethanol. Preserved specimens examined are deposited at the California Academy of Sciences (**CAS**).

## Data resources

The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility, and are available at https://doi.org/10.15468/zwgv36.

## **Systematics**

Family Vaejovidae Thorell, 1876 Genus *Paruroctonus* Werner, 1934

#### Paruroctonus soda sp. nov.

https://zoobank.org/7BF88AF5-E85F-4627-ACE1-1ED7B1C5084C Figs 1–14, Table 1

**Type material.** *Holotype*: USA • 1 ♂; California, San Luis Obispo County, southern tip of North Basin of Soda Lake; 35.2038, -119.8553; 585 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light; CASENT 9101932.

**Paratypes.** USA • 1♂, 2♀; same data as for holotype; CASENT 9101933 • 1♂, 2♀; California, San Luis Obispo County, northeastern edge of North Soda Lake Plain; 35.2476, -119.8630; 587 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light; CASENT 9101934 • 1♂; California, San Luis Obispo County, western edge of North Basin of Soda Lake; 35.2186, -119.8958; 580 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light; CASENT 9101935.

Additional material examined. USA • 1  $\bigcirc$ ; California, San Luis Obispo County, eastern edge of North Basin of Soda Lake; 35.2263, -119.8548; 586 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 2  $\bigcirc$ , 4 $\bigcirc$ ; California, San Luis Obispo County, southern tip of North Basin of Soda Lake; 35.2038, -119.8553; 585 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 1 $\bigcirc$ ; California, San Luis Obispo County, northeastern edge of North Soda Lake Plain; 35.2476, -119.8630; 587 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 1 $\bigcirc$ ; California, San Luis Obispo County, western edge of North Soda Lake Plain; 35.2476, -119.8630; 587 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 7 $\bigcirc$ ; California, San Luis Obispo County, western edge of North Basin of Soda Lake; 35.2186, -119.8958; 580 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collector leg Harper Forbes, Prakrit Jain; collecter edge of North Basin of Soda Lake; 35.2186, -119.8958; 580 m a.s.l.; 30 May 2021; collector leg Harper Forbes, Prakrit Jain; collector leg Harper Forbes, Prakrit Jai

Diagnosis. Differs significantly from other Paruroctonus species found in the San Joaquin Valley and its surrounding mountains (the Inner Southern Coast Range, the Sierra Nevada, the Tehachapis, and the northern mountains of the Transverse Range) by a combination of the following characteristics: 1: Fuscous markings entirely absent from the metasoma and the posterior margin of the tergites (Figs 1, 2). 2: Chelal fingers deeply scalloped in adult males, leaving a large proximal gap when closed. 3: Metasomal macrosetae along dorsolateral, ventrolateral, and ventral submedian carinae of segments I-IV follow the patterns 0,0,0,1; 1-2,2,2,2-3; and 1-2,2,2,2, respectively (Fig. 9). 4: All macrosetae on the manus greatly reduced in size; dorsal median, retrolateral median, ventral prosubmedian, prolateral ventral, and prolateral median carinae lacking any macrosetae except those at the proximal extent of their respective carinae (Fig. 6). 5: No large medial or distal retrolateral macrosetae on the pedipalp patella. 6: Length / Width ratios of metasomal segment V in adult males 2.22-2.59 and in adult females 2.21-2.28. 7: Chela length / Manus width and Chela length / Manus thickness ratios 2.05-2.16 and 2.96-3.14 in adult males, respectively and 2.15-2.30 and 2.98–3.14 in adult females, respectively.

**Comparisons.** Comparisons are provided against other *Paruroctonus* sp. scorpions found in the San Joaquin Valley and its surrounding ranges, ordered ascendingly by the distance of the nearest record to the distribution of *P. soda* sp. nov. No *Paruroctonus* has been recorded within 13 kilometers of *P. soda* sp. nov., and while this distance could decrease significantly with more sampling, the habitat of *P. soda* sp. nov. is sufficiently distinct from that of any other nearby *Paruroctonus* that we consider sympatry to be unlikely.

Paruroctonus variabilis Hjelle, 1982 differs from *P. soda* sp. nov. in the following characters relating to the numeration in the above diagnosis: (2) Chelal fingers not scalloped (straight), leaving a negligible proximal gap when closed. (3) Metasomal macrosetae along dorsolateral, ventrolateral and ventral submedian carinae on segments I–IV follow the patterns 0,1,1,2; 3,3–5,4–5,5–6; and 3–4,4,4–5,4–8, respectively. (4) Many large macrosetae on the manus; macrosetae along chelal dorsal median, retrolateral median, ventral prosubmedian, prolateral ventral, and prolateral median carinae, excluding any near the proximal extent of their respective carinae, follow the pattern 1–3,2–4,3–4,1–2,1–2. (5) Pedipalp patella with 3–5 large medial and 2 large distal retrolateral macrosetae. (6) length/width ratios of metasomal segment V in adult males 2.85–3.02, in adult females 2.63–2.89. (7) Chela length/manus width and chela length/manus thickness ratios in adult males 2.49–3.10, 3.39–4.03, respectively, in adult females 2.90–3.37, 3.94–4.27, respectively.

*Paruroctonus silvestrii* differs from *P. soda* sp. nov. in the following characters relating to the numeration in the above diagnosis: (1) Extensive fuscousity present on the ventral surface of the metasoma, mesosomal fuscousity extending to the posterior edge of the tergites. (2) Chelal fingers not scalloped (straight), leaving a negligible gap when closed. (3) Metasomal macrosetae along dorsolateral, ventrolateral, and ventral submedian carinae on segments I–IV follow the patterns 0,1,1,2; 2,3,3,3–4; and 2–3,3,3–4,3–4, respectively. (4) Many large macrosetae on the manus; macrosetae along chelal dorsomedian, retrolateral median, ventral prosubmedian, prolateral ventral, and prolateral median carinae, excluding any near the proximal extent of their respective carinae, follow the pattern 0–1,1–2,1–2,1,1. (5) Pedipalp patella with 2–4 large medial and 2 large distal retrolateral macrosetae. (6) Length/width ratios of metasomal segment V in adult males 2.72–2.90, in adult females 2.46–2.63. (7) Chela length/manus width and chela length/manus thickness ratios in adult males 2.59–2.70 and 3.36–3.65, respectively; in adult females 2.75–3.06 and 3.73–4.15, respectively.

*Paruroctonus boreus* differs from *P. soda* sp. nov. in the following characters relating to the numeration in the above diagnosis: (1) Fuscousity present on the ventral surface of the metasoma, especially on segments II–IV. (3) Metasomal macrosetae along dorsolateral, ventrolateral and ventral submedian carinae on segments I–IV follow the patterns 0,0-1,1,1-2; 2,3,3,3-4, and 2,2,2-3,3, respectively. (4) Several large macrosetae on the manus; macrosetae along chelal dorsal median, retrolateral median, ventral prosubmedian, prolateral ventral, and prolateral median carinae, excluding any near the proximal extent of their respective carinae, follow the pattern 0,1-2,1,1,1. (5) Pedipalp patella with 1-2 large medial and 2 large distal retrolateral macrosetae. (6) Length/width ratios of metasomal segment V in adult males 2.72-3.12, in adult females 2.50-2.71.

*Paruroctonus conclusus* sp. nov. differs from *P. soda* sp. nov. in the following characters relating to the numeration in the above diagnosis: (3) Metasomal macrosetae along dorsolateral and ventral submedian carinae on segments I–IV follow the patterns 0,1,1,2 and 2,2,2,3, respectively. (4) Several large macrosetae on the manus; macrosetae along chelal dorsal median, retrolateral median, ventral prosubmedian, prolateral ventral, and prolateral median carinae, excluding any near the proximal extent of their respective carinae, follow the pattern 0,1-2,1,1,1. (5) Pedipalp patella with 1 large medial and 2 large distal retrolateral macrosetae. (6) Length/width ratios of metasomal segment V in adult males 2.86–3.05, in adult females 2.47–2.56.

**Description of male holotype.** *Coloration* (Figs 1–3). Carapace orange-brown with faint fuscous markings present directly posterior to the median eyes, at the posterior-lateral corners of the carapace, and along the posterior edges of the interocular triangle. Tergites I–VI with fuscousity occupying the majority of the segment with the exception of the posterior and lateral margins; fuscousity somewhat reduced on VII. Legs pale cream to slightly tan. Pedipalps tan to orange with slightly darker orange carinae and dark orange fingers. Metasoma tan with faintly orangish carinae. Telson pale yellow, base of aculeus dark reddish, and aculeus black. Sternites brown, with tan spiracles. Pectines, sternum, and genital operculum tan to cream.

*Carapace* (Figs 4, 5). Anterior margin roughly straight with three pairs of distinct macrosetae. Surface irregularly granular, with the largest granules near the center of the



Figure 1. Variation of *Paruroctonus soda* sp. nov. from across their range. Top two rows, males; bottom row females; holotype male top left.


Figure 2. Dorsal habitus photographs of *Paruroctonus soda* sp. nov. holotype male (left) and female (right). Scale bars: 10 mm, silhouettes to scale.

carapace. Very fine, evenly spaced granules present between the large granules. Lateral margins finely crenulate. Posterior median sulcus narrow and moderately deep, free of granulation. Anterior median, median ocular, lateral ocular, and posterior lateral sulci broad and shallow, entirely free of granules. Anterior and posterior marginal sulci shallow and sparsely granular. Median ocelli separated by a distance greater than the width of one ocellus. Three pairs of lateral ocelli present. Single pairs of macrosetae present posterior to the median ocelli, between the lateral ocelli and the margin of the carapace, and roughly halfway between the posterior median sulcus and the margin of the carapace, in line with the posterior edge of the ocular tubercle.

*Mesosoma* (Figs 2, 3). Tergites I–VI very finely granular to smooth, except on the posterior and lateral thirds, which are weakly granular to granular. These areas become increasingly granular on subsequent segments. Median longitudinal carina absent on



Figure 3. Ventral habitus photographs of *Paruroctonus soda* sp. nov. holotype male (left) and female (right). Scale bars: 10 mm, silhouettes to scale.

tergites I–II, indistinct and very weakly crenulate on III–VI. Submedian longitudinal sulci indistinct. One pair of small posterior sub-median setae on tergites I–VI. Tergite VII essentially smooth anteriorly and irregularly granular elsewhere. Posterior margin finely granular; lateral marginal, dorsolateral, and dorsal sub-median carinae crenulate. Median longitudinal carina indistinct. Sternites III–VI sparsely setose and smooth. Sternite VII smooth anteriorly, finely granular posteriorly, and granular laterally, with ventral submedian carinae indistinct and very weakly crenulate and lateral marginal carinae irregular and weakly crenulate.

*Genital operculum* (Fig. 5). Sclerites roughly triangular with rounded corners, wider than long. Overlapping medially and separated slightly only at the posterior edge, with protruding genital papillae. Several macrosetae present on each sclerite.



**Figure 4.** Carapace of *Paruroctonus soda* sp. nov. holotype male (left) and paratype female (right). Scale bars: 5 mm.

*Sternum* (Fig. 5). Type 2 with posterior emargination absent. Lateral lobes concave anteriorly, roughly straight laterally, convex posteriorly. Apex deep. Sclerite slightly wider than long, smooth to finely granular, especially along the slopes of the apex. Three pairs of macrosetae.

**Pectines** (Fig. 5). Long, thin, and densely hirsute, with 21/21 tightly packed teeth on each side. Middle lamellae roughly circular distally, highly irregular in size and shape proximally; roughly 16/15 distinct and separated sclerotized sections are visible under ultraviolet illumination.

*Legs. Carinae.* Retroventral carinae on Leg I femur unpigmented and finely crenulate; proventral carinae sparsely, finely and weakly crenulate on Leg I patella. Both decreasingly distinct on subsequent legs, proventral carinae on patella absent by leg IV. Other carinae indistinct to absent on all legs. On all legs, femur irregularly and very finely granular; other surfaces smooth.

**Telotarsi.** Telotarsal retroinferior terminal macrosetae on legs I–IV 1/2, 2/2, 2/2, 2/2; other telotarsal retroinferior macrosetae on the distal half of telotarsi I–IV 1/1, 1/1, 1/1, 2/2. Two telotarsal retromedial macrosetae on each leg, with one always at the retromedial terminal position. Two large telotarsal retrosuperior macrosetae on each leg with consistent positions, with an additional smaller retrosuperior seta on dextral leg III. Single proinferior terminal macroseta on each leg, single other proinferior macroseta on legs II–IV except none on sinistral leg III. Two telotarsal promedial macroseta on legs I–IV at terminal and distal positions; one on leg IV in the terminal position. Two large



**Figure 5.** Carapace (upper) and sternopectinal region (lower) of *Paruroctonus soda* sp. nov. holotype male (left) and female (right). Taken under ultraviolet illumination. Scale bars: 5 mm.

telotarsal prosuperior macrosetae on each leg in terminal and medial positions. Single telotarsal superioterminal and superior macroseta present on all legs.

**Basitarsi.** Three basitarsal spine rows present on legs I and II; proventral and retroventral spine rows equally dense and retrosuperior spine row less dense. The retroventral spine row extends ca. two-thirds the entire length of the segment, the proventral spine row extends through ca. half the segment, and the retrosuperior spine row extends through less than half. On leg III, proventral spine row absent and the retroventral and retrosuperior spine rows heavily reduced both in size and density. On leg IV, both the proventral and retroventral spine rows are absent and the retrosuperior spine row is heavily reduced in size and density, almost absent. Basitarsal retroventral

macrosetae on legs I–IV, excluding only the distal retroventral spinoid macroseta at the end of the retroventral spine row, follow the pattern 2/3, 5/5, 4/5, 5/4, with variable sizes. Spinoid basitarsal proventral macrosetae on legs I–IV follow the pattern 2, 2, 3, 3; an additional thinner distal ventral macroseta is present on legs II–IV. Superior basitarsal macrosetae on legs I–IV consist of two spinoid macrosetae at the distal and mid retrosuperior positions; one macroseta at the distal prosuperior position; one macroseta at the distal retrosuperior macroseta, except on sinistral leg IV and dextral legs II–IV; and large superiomedian macrosetae following the pattern 4/4, 5/5, 5/5, 4/4. Prolateral macrosetae on legs I–IV, excluding one on the margin, follow the pattern 3/3, 3/3, 2/3, 2/2.

**Pedipalps** (Figs 6–8). *Femur.* Dorsal prolateral carina crenulate with two macrosetae on the proximal half; dorsal retrolateral carina also crenulate with two macrosetae on the proximal three-fourths. Dorsal surface sparsely granular. Retrolateral dorsosubmedian carina weakly crenulate; retrolateral surface otherwise smooth aside from a few proximal granules. Two long median macrosetae on the retrolateral surface. One large inframedian macroseta on the distal fourth of the retrolateral surface. Ventral retrosubmedian carina vestigial, irregularly granular with granules decreasing in size distally. Prolateral surface granular with two prolateral ventral macrosetae on the proximal half, one prolateral ventrosubmedian macroseta at the midpoint, and a pair of macrosetae on the distal margin.

**Patella.** Dorsal retrolateral carina weakly crenulate with a large proximal macroseta; dorsal prolateral carinae crenulate with a small proximal macroseta. Dorsal surface essentially smooth. Retrolateral median carinae indistinct and very weakly crenulate, retrolateral surface otherwise smooth. Two very small and indistinct retrolateral distal marginal macrosetae present. Ventral retrolateral carina weakly crenulate; ventral prolateral and ventral median carinae crenulate. Ventral surface smooth. Prolateral median carina indistinct to absent, represented by a few large granules. Prolateral surface sparsely and weakly granular. Prolateral surface with large proximal supramedian, proximal inframedian, and distal inframedian macrosetae; heavily reduced distal supramedian macroseta. No large macrosetae present on the ventral and external surfaces.

**Chela.** Dorsal prolateral carina indistinct, non-linear, and crenulate with no macrosetae, smooth on the fixed finger. Dorsal median carina weakly crenulate proximally and smooth distally, stopping at the base of the fixed finger, with a single small macroseta at its proximal extent. Dorsal retrosubmedian carina vestigial, consisting of only a few weak granules, and extending through less than the proximal fifth of the manus. Dorsal retrosubmedian accessory carina weakly crenulate, extending through less than the proximal fifth of the manus, with a small proximal macroseta. Dorsal retrolateral carina very weakly crenulate proximally and smooth distally, entirely smooth on the fixed finger, with a small distal macroseta near the base of the fixed finger. Retrolateral median carina very weakly granular and unpigmented, lacking setation. Ventral retrolateral carina indistinct and weakly crenulate, with 0/1 small macroseta at its proximal extent. Intercarinal spaces on the dorsal and retrolateral surfaces smooth. Ventral prosubmedian carina indistinct and very



**Figure 6.** Pedipalp of *Paruroctonus soda* sp. nov., holotype male (above) and female (below). Macrosetae indicated with open circles (proximal) and closed circles (diagnosis character 4). Carinae abbreviations: retrolateral median (rm), dorsal retrolateral (drl), dorsal median (dm), dorsal prolateral (dpl), ventral retrolateral (rel (vrl), ventral prosubmedian (vps), prolateral ventral (plv), prolateral median (plm). Scale bars: 10 mm.

weakly crenulate, with a single small macroseta at its proximal extent. Ventral surface smooth to granular near the base of the movable finger. Prolateral ventral and median carinae both crenulate to weakly crenulate with a single small macroseta at their respective proximal extents. Two additional small carinae are present near the base



**Figure 7.** Illustrations of pedipalp chela of *Paruroctonus soda* sp. nov. **A–D** holotype male and **E–H** female **A, E** retrolateral **B, F** prolateral **C, G** dorsal **D, H** ventral. Trichobothria indicated with open circles. Scale bars: 5 mm.

of the fixed finger, both of which are evenly and finely crenulate. Prolateral surface of the manus otherwise mostly smooth with some weak and irregular granulation in the distal half. The fingers are heavily scalloped, leaving a large proximal gap when closed. The chela is uniformly finely granular at the base of this gap. Retrolaterally, the fingers are smooth except some fine proximal granulation. Prolaterally, the fingers are smooth aside from a few patches of granulation on the proximal half. 19/16 small macrosetae and numerous microsetae are present on the ventral surface of the movable finger. No movable finger ventral prolateral, fixed finger prolateral median, or fixed finger prolateral dorsolateral macrosetae are present. The movable finger has one proximal prolateral median macroseta. A single proximal retrolateral median macroseta is present on the movable finger and a single dorsal prolateral seta is present near the distal end of the fixed finger. Both the fixed and movable fingers



**Figure 8.** Illustrations of pedipalp patella and femur of Paruroctonus soda sp. nov. holotype male (**B**, **C**) paratype female (**A**, **D**). **A** dorsal patella **B** retrolateral patella **C** ventral patella **D** dorsal femur. Trichobothria indicated with open circles. Scale bars: 2 mm.

have five enlarged denticles dividing the primary denticles into six sub-rows, with an additional enlarged denticle at the distal extent of the movable finger, alongside the distal hook. On the fixed finger, rows I–VI contain 5/5, 7/6, 7/7, 7/8, 10/9, 12/10 primary denticles with a total row I–V count of 36/35. On the movable finger, rows I–VI contain 6/6, 8/8, 10/9, 9/10, 13/13, 9/10 primary denticles with a total row I–V count of 46/46. Each enlarged denticle as well as the distal finger-tip hook is accompanied by a single prolateral supernumerary denticle, for a total of six on the fixed finger and seven on the movable finger. There is a single macroseta posterior to each supernumerary denticle apart from the two most distal ones on each finger for a total of four on the fixed finger and five on the movable finger. Two further macroseta e are present near the proximal primary denticle row on the fixed finger.

Metasoma (Fig. 9). Dorsal surface I-V smooth with a few scattered granules. Dorsolateral carinae on segments I-IV strongly crenulate to serrate, weakly crenulate on V. Lateral supramedian surface smooth with a few scattered granules. Lateral supramedian carinae I-IV crenulate. Lateral surface smooth. Lateral inframedian carinae crenulate on I-III, extend through only the posterior fifth of segments II-III. Lateral median carinae weakly crenulate on V, extending ca. a third of the way up the segment. Ventrolateral carinae I-IV smooth to weakly crenulate, becoming weakly crenulate on the posterior fourth of each segment. Ventrolateral carinae on segment V strongly crenulate to serrate. Ventral surface of segment I-IV smooth; ventral surface granular on segment V. Ventral sub-median carinae on I-IV smooth, unpigmented, and indistinct. Ventromedian carinae on segment V are crenulate, irregular, and disconnected. Dorsolateral macrosetae I-V follow the pattern 0,0,0,1,2. Lateral supramedian macrosetae I-IV follow the pattern 0,1,1,1. One Lateral median macroseta on V. Lateral inframedian macrosetae I-III follow the pattern 1,0,0. Ventrolateral macrosetae I-V, excluding any on the posterior margin of the segment, follow the pattern 1,2,2,2,3/4. Ventral submedian macrosetae I-IV, excluding those on the posterior margin of the

segment, follow the pattern 1,2,2,2. Three pairs of macrosetae are present between the ventromedian and ventrolateral carinae on segment V. Two pairs of macrosetae on the ventral posterior margin of metasomal segment V; a single pair of macrosetae on the ventral posterior margins of other metasomal segment.

*Telson* (Fig. 9). Very weakly granular on the ventral anterior portion, otherwise smooth. Sparsely setose ventrally and laterally.



**Figure 9.** Metasoma of *Paruroctonus soda* sp. nov. holotype male (above) and female (below); ventral, lateral, and dorsal aspects (top to bottom). Ventral sub-median, ventrolateral, lateral submedian, and dorsolateral macrosetae on segments I-IV indicated with black circles (diagnosis character 3). Carinae abbreviations: Dorsolateral (dl), lateral median (lm), lateral supramedian (lsm), lateral inframedian (lim), ventrolateral (vm), ventral submedian (vs), and ventromedian (vm). Scale bars: 10 mm.

*Hemispermatophore* (Fig. 10). Hemispermatophore roughly equal in width from pedicel to stalk, three fold bauplan (Monod et al. 2017). Stalk wide and relatively straight and dorso-ventrally flattened. Distal carina and lamelar hook scletertized, lamelar hook bifurcate at terminus. Mating plug weakly scleretized, moderate in size with a wide bilobed base and relatively long stem terminating in a barb.

**Female.** Larger size. Relatively thinner chela with less curved fingers, weakly scalloped with a negligible gap when closed. Most proximal row on the chelal fixed finger with 16–21 primary denticles; most proximal row on the chelal movable finger with 10–13 primary denticles. Metasoma more robust. Pectines smaller overall with smaller teeth; teeth count 17–19 (17 n = 4.5, 18 n = 1.5, 19 n = 1) and middle lamella count 12–15 on a side. Sclerites separated narrowly through their entire length with the gap slowly increasing toward the posterior half.

Variation. Coloration (Figs 1–3). Fuscous markings posterior to the median eyes and in the posterior-lateral corners of the carapace range from typically prominent to sometimes indistinct to absent. Those along the edges of the interocular triangle are typically faint but range to absent. Fuscous markings on tergites I–VI also highly variable, ranging in extensiveness from covering the entire tergite except the posterior and lateral margins to covering only a small area around the anterior half of the submedian sulci. Fuscous markings on tergite VII also variable, ranging from covering approximately the anterior three-fourths of the segment to only being present in small areas along the dorsal submedian carinae. Other aspects of coloration in preserved specimens relatively consistent. In life, carapace, metasoma, and pedipalp coloration ranges from dark brown to tan, but is typically orange.

*Carapace* (Figs 4, 5). Level and density of granulation variable. The elevated area of the interocular crescent on either side of the anterior median sulcus is sometimes largely free of granulation.

*Tergites* (Fig. 2). Posterior granulation on tergites I–VI ranging from weakly granular through the posterior third of the segment to indistinct, very weakly granular, and restricted to the posterior margin of the segment. Lateral granulation sometimes absent.

*Pectines* (Fig. 5). Pectines in males 21–24 (21 n = 2.5, 22 n = 5, 23 n = 5.5, 24 n = 2) with 15–20 middle lamellae per side.

Legs. Retroventral carinae on the leg patella ranging from finely crenulate to very weakly crenulate, almost absent. Prosuperior carinae on the leg femur ranging from very finely crenulate to weakly finely crenulate, almost absent. Retroventral spine row on basitarsus III ranging from equal in length and density to retrosuperior spine row to indistinct, almost absent. Terminal retroinferior macrosetae on telotarsus II 1–2, other retroinferior macrosetae on telotarsus III 1–2, other retroinferior macrosetae on telotarsus III 1–2, retromedian macrosetae on telotarsus IV 2–3. Additional small retrosuperior macrosetae present occasionally on legs II–III. Other large telotarsal retrolateral macrosetae described in the holotype description consistent with the exception of occasional asymmetrical additions or deletions. Second promedian macroseta occasionally present on leg IV and third promedian macroseta occasionally present on leg I; other large telotarsal asymmetrical additions asymmetrical additions and the holotype description consistent with the exception of occasional asymmetrical macrosetae described in the holotype description consistent with the exception of occasional asymmetrical additions are described in the holotype description consistent with the exception of occasional asymmetrical additions are described in the holotype description consistent with the exception of occasional problem and the promedian macroseta occasionally present on leg IV and third promedian macroseta occasionally present on leg IV and the promedian macroseta occasional asymmetrical additions or deletions.



**Figure 10.** Right hemispermatophore of *Paruroctonus soda* sp. nov.: anterior aspect (left) and posterior aspect (right). Scale bar: 5 mm.

or deletions. Number of retroventral basitarsal setae on legs I–IV highly variable, within the following ranges for legs I–IV: 3–4, 5–6, 5–6, 5–6 with occasional asymmetrically added or missing setae. Proventral basitarsal macrosetae consistent. Large superior basitarsal macrosetae on legs I–IV, excluding the large spinoid distal and mid retrosuperior macrosetae; the large distal prosuperior and sometimes present small medial prolateral macrosetae; and the often absent macroseta at the distal superiomedian position adjacent to the distal retrosuperior macroseta, are highly variable, within the ranges 4–5, 5–6, 5–6, 4–5 with occasional asymmetrical deletions or additions of small macrosetae. Prolateral macrosetae on legs I–IV, excluding one on the margin, highly variable and often non-linear, within the ranges 3, 2–4, 2–4 with occasional asymmetrical deletions. The smaller distal superiomedian macroseta is often missing on any leg.

Pedipalps (Figs 6-8). Macrosetae on femur variable: prolateral ventrosubmedian sometimes missing; retrolateral dorsosubmedian excluding those on distal margin 2-4; other occasional asymmetrical deletions. Proximal macroseta on the pedipalp patella dorsal prolateral carina small or large, other large macrosetae on patella consistent. Patella retrolateral median carina weakly crenulate to very weakly crenulate with an inconsistent pattern. Two very small and indistinct distal median macrosetae sometimes present on the external surface of the pedipalp patella; no large medial or distal macrosetae ever present on the external surface of the pedipalp patella. On the chela, a small median macroseta on the dorsal prolateral carina and the ventral retrolateral carina sometimes present. A small proximal macroseta on the ventral retrolateral carina and a small macroseta along the dorsal retrolateral carina near the base of the fixed finger sometimes absent. On the fixed finger, prolateral median macroseta rarely present. Ventral macrosetae on the movable finger 15–19. Number of primary denticles in rows I–V on the fixed finger within the ranges 3–6, 5–7, 6–8, 7–9, 9–12. Number of primary denticle in row VI on the fixed finger of males 9-14. Number of primary denticles in rows I-VI on the movable finger within the ranges 4-7, 6-9, 8-10, 10-12, 11-17. Number of primary denticle in row VI on the movable finger of males 8-10. Primary denticles on the fixed finger excluding those on the proximal row 30-38 and primary denticles on movable finger excluding those on the proximal row 42-50 with no obvious sexual dimorphism in either.

*Metasoma* (Fig. 9). Crenulation on metasomal ventrolateral carinae variable, ranging from very weakly crenulate on the posterior third to weakly crenulate on the posterior half. Lateral inframedian carina on II–III from ca. one fifth to one third the length of the segment. Dorsolateral medial macroseta on V ranging from indistinct to absent to small but distinct for a total of 2–3 macrosetae. Ventrolateral macrosetae on I 1–2, ventral submedian macrosetae on I 1–2, ventrolateral macrosetae on IV 2–3, ventrolateral macrosetae on V 3–6, and lateral supramedian macrosetae on IV 1–2. Other metasomal setae are consistent with the exception of occasional asymmetrical deletions.

Remarks. The most valuable taxonomic characters for *P. soda* sp. nov. are:

1. The macrosetal patterns on the pedipalps and metasoma are very consistent and unique, provide excellent diagnostics against almost all other *Paruroctonus*.

2. The morphometric ratios of different aspects of the metasomal segments and chela are fairly consistent and do not overlap with those of several other *Paruroctonus*.

3. The lack of fuscous markings on the metasoma and chelae is very consistent and provides a helpful diagnostic for comparison with several other *Paruroctonus*.

4. The overall color pattern and the fuscous patterning on the carapace and tergites is somewhat variable but is still a reliable diagnostic character.

Other taxonomic characters which may be taxonomically valuable in some cases, but are typically not useful, include:

1. The telotarsal macrosetae are somewhat variable but have different counts than those of certain other *Paruroctonus*.

2. The extent of granulation on the carapace and tergites is fairly variable but is notably different from certain other *Paruroctonus*. This character, however, can be difficult to quantify.

3. The basitarsal macrosetae are generally extremely variable and are only helpful for differentiating *P. soda* sp. nov. from psammophilous *Paruroctonus*. The basitarsal spinoid distal and mid retrosuperior macrosetae are not variable but are still only helpful for differentiating *P. soda* sp. nov. from these psammophiles.

4. The granulation on the pedipalps, legs, and metasoma is somewhat variable and difficult to quantify. It is fairly similar to that of most other *Paruroctonus* species, although in isolated examples may be used for diagnosis.

5. The pectinal tooth counts are somewhat variable and are only useful as a diagnostic against some other *Paruroctonus*. Middle lamellae counts are also not taxonomically valuable, as they are typically ambiguous.

6. The chelal primary denticle counts are somewhat variable and overlap with those of most other *Paruroctonus*.

**Habitat, distribution, and ecological notes.** *Paruroctonus soda* sp. nov. is known only from the area immediately surrounding Soda Lake in the Carrizo Plain, an area of the San Joaquin Valley in San Luis Obispo county, California (Fig. 11). Soda Lake is a complex of a single large and dozens of smaller typically dry lake beds draining the Carrizo Plain and its surrounding ranges, an endorheic watershed ca. 1230 km<sup>2</sup> in size (Stephenson 2013). The lake complex began to form during the Pliocene epoch when tectonic activity severed a connection to the ocean (Cooper 1990; Stephenson 2013). A permanent, deep, brack-ish lake persisted from ca. 75 kya to around 16–17 kya, at which point a drying climate and hotter temperatures led to the lake shrinking and increasing in alkalinity (Stephenson 2013). The habitat surrounding the lake is now an alkali sink largely dominated by *Atriplex spinifera, Atriplex vallicola, Allenrolfea occidentalis*, and various small wildflower and grass species (Munz and Keck 1949; Buck-Diaz et al. 2013 Stout et al. 2013).

The Carrizo Plain receives approximately 230 mm of sporadic winter rain in an average year resulting in an arid climate. Water drainage from the surrounding Temblor, La Panza, and Caliente ranges, which receive a slightly greater amount of rainfall, keeps the Soda Lake complex and the immediately surrounding area comparatively moist (Stephenson 2013). The summer climate in the region is hot and arid, with little to no rainfall and temperatures typically in excess of 35 °C during the hottest months.

Along the western, southwestern, and eastern edges of the largest basin (North Basin), we found *Paruroctonus soda* sp. nov. to be present only in a thin band of soft clay soil dominated by *Allenrolfea occidentalis* immediately adjacent to the edge of the dry lakebed (Figs 11, 12). To the northeast of the North Basin is a relatively large area of soft clay soils including a multitude of smaller basins (North Soda Lake Plain). Specimens of *Paruroctonus soda* sp. nov. were collected at the point in the North Soda Lake Plain region furthest from the edge of the North Basin, suggesting that it is likely found



**Figure 11.** Soda Lake and the surrounding Carrizo Plain, the type locality of *Paruroctonus soda* sp. nov. Above, satellite imagery of the central Carrizo Plain taken in June 2019; below, a regional overview taken from the eastern side of the North Basin and facing southwest across the Soda Lake taken in May 2021.



Figure 12. Habitat of *Paruroctonus soda* sp. nov. in May 2021. Note the dominance of *Allenrolfea occidentalis* and the soft, exposed clay soil along the lakebed.

throughout a significant portion of the North Soda Lake Plain. *Paruroctonus soda* sp. nov. was not found along the edge of the second-largest basin (South Basin) and the smaller basin immediately to its north despite significant sampling. We hypothesize that *Paruroctonus soda* sp. nov. is absent from these basins because the band of soft clay soil surrounding these basins is too narrow to support a population of this species. It is, how-ever, impossible to make high-confidence conclusions of absence from a single night of sampling. *Paruroctonus soda* sp. nov. was also not found in any of the areas of relatively

tougher soil dominated by *Atriplex polycarpa* or *A. spinifera*. We hypothesize that this species' reliance on soft clay soils may be due to the high summer surface temperatures in the area, as the softer soils form deep cracks and are relatively easier to burrow into.

No scorpions were found in sympatry with *Paruroctonus soda* sp. nov. However, the presence of *Hadrurus obscurus* Williams, 1970 and *Paravaejovis* sp. is possible, as both



Figure 13. Photographs of a typical juvenile *Paruroctonus soda* sp. nov. (left), and atypical coloration observed in one late instar juvenile female. Not to scale.



Figure 14. Adult female Paruroctonus soda sp. nov. with 51 newly born juveniles.

|                        | Holotype<br>male | Paratype<br>male | Paratype<br>male | Paratype<br>male | Paratype<br>female | Paratype<br>female | Paratype<br>female | Paratype<br>female |
|------------------------|------------------|------------------|------------------|------------------|--------------------|--------------------|--------------------|--------------------|
| CASENT#                | 9101932          | 9101934          | 9101933          | 9101935          | 9101934            | 9101934            | 9101933            | 9101933            |
| Total L                | 49.54            | 45.23            | 50.20            | 41.95            | 60.99              | 61.28              | 59.71              | 56.08              |
| Carapace L             | 5.95             | 5.89             | 6.59             | 5.33             | 7.47               | 7.27               | 7.41               | 7.55               |
| Prosoma posterior W    | 5.41             | 5.89             | 6.16             | 5.15             | 7.16               | 7.04               | 7.28               | 8.18               |
| Prosoma median W       | 4.40             | 4.33             | 5.02             | 4.29             | 5.92               | 5.77               | 5.65               | 5.77               |
| Mesosoma L             | 13.12            | 10.62            | 12.80            | 11.85            | 18.47              | 21.75              | 18.89              | 13.21              |
| Metasoma L             | 30.10            | 28.29            | 30.65            | 25.80            | 33.67              | 33.01              | 34.25              | 34.32              |
| Metasoma I L           | 3.32             | 3.21             | 3.73             | 3.34             | 3.85               | 4.08               | 4.06               | 3.63               |
| Metasoma I W           | 3.23             | 3.15             | 3.56             | 2.83             | 4.32               | 4.31               | 4.43               | 4.42               |
| Metasoma I H           | 2.52             | 2.47             | 2.71             | 2.41             | 3.27               | 3.10               | 3.31               | 3.28               |
| Metasoma II L          | 3.78             | 3.63             | 4.38             | 3.68             | 4.38               | 4.55               | 4.27               | 4.77               |
| Metasoma II W          | 3.19             | 3.10             | 3.53             | 3.14             | 4.21               | 4.48               | 4.08               | 4.16               |
| Metasoma II H          | 2.56             | 2.50             | 2.74             | 2.27             | 3.23               | 3.09               | 3.25               | 3.39               |
| Metasoma III L         | 3.90             | 3.93             | 4.44             | 3.50             | 4.97               | 4.75               | 4.69               | 4.97               |
| Metasoma III W         | 3.11             | 3.05             | 3.54             | 2.70             | 4.14               | 3.88               | 4.23               | 4.04               |
| Metasoma III H         | 2.56             | 2.51             | 2.76             | 2.33             | 3.18               | 3.14               | 3.21               | 3.39               |
| Metasoma IV L          | 4.82             | 4.43             | 5.29             | 4.58             | 5.49               | 5.72               | 5.35               | 5.53               |
| Metasoma IV W          | 3.03             | 2.84             | 3.35             | 2.70             | 3.84               | 3.52               | 3.77               | 3.94               |
| Metasoma IV H          | 2.59             | 2.51             | 2.96             | 2.32             | 3.21               | 3.49               | 3.34               | 3.39               |
| Metasoma V L           | 6.69             | 6.33             | 7.27             | 6.32             | 7.95               | 7.98               | 7.90               | 8.34               |
| Metasoma V W           | 2.87             | 2.76             | 3.28             | 2.44             | 3.60               | 3.32               | 3.54               | 3.66               |
| Metasoma V H           | 2.40             | 2.46             | 2.64             | 2.00             | 3.16               | 2.82               | 3.23               | 3.08               |
| Telson L               | 6.85             | 6.50             | 7.25             | 6.74             | 8.65               | 8.02               | 8.34               | 8.43               |
| Vesicle L              | 4.63             | 4.57             | 4.62             | 4.15             | 5.99               | 5.72               | 5.50               | 6.04               |
| Vesicle W              | 2.87             | 2.73             | 3.06             | 2.76             | 3.86               | 3.51               | 3.77               | 4.17               |
| Vesicle H              | 2.16             | 2.09             | 2.47             | 2.04             | 2.90               | 2.77               | 2.98               | 2.83               |
| Aculeus L              | 2.53             | 2.72             | 2.45             | 2.38             | 2.71               | 3.02               | 2.63               | 3.32               |
| Pedipalp L             | 20.48            | 20.06            | 20.50            | 18.48            | 24.96              | 24.22              | 24.30              | 25.63              |
| Pedipalp femur L       | 4.80             | 4.75             | 5.30             | 4.16             | 5.75               | 5.79               | 5.73               | 6.14               |
| Pedipalp femur W       | 1.81             | 1.69             | 1.81             | 1.59             | 2.19               | 2.06               | 2.37               | 2.43               |
| Pedipalp femur H       | 1.26             | 1.21             | 1.41             | 1.16             | 1.65               | 1.59               | 1.67               | 1.81               |
| Pedipalp patella L     | 4.72             | 4.68             | 5.05             | 4.51             | 6.18               | 5.92               | 6.11               | 6.07               |
| Pedipalp patella W     | 2.23             | 2.14             | 2.27             | 1.88             | 2.71               | 2.60               | 2.73               | 2.87               |
| Pedipalp patella H     | 2.10             | 2.02             | 2.08             | 1.83             | 2.37               | 2.22               | 2.73               | 2.59               |
| Pedipalp Chela L       | 9.53             | 9.45             | 9.91             | 8.88             | 11.47              | 11.21              | 11.50              | 12.08              |
| Pedipalp Manus W       | 4.66             | 4.48             | 4.59             | 4.15             | 5.33               | 5.05               | 4.99               | 5.60               |
| Pedipalp Manus T       | 3.18             | 3.01             | 3.35             | 2.93             | 3.85               | 3.65               | 3.69               | 3.85               |
| Chela Finger fixed L   | 3.45             | 3.96             | 3.81             | 3.39             | 4.40               | 4.80               | 4.91               | 4.73               |
| Chela finger movable L | 6.27             | 6.35             | 6.21             | 6.01             | 7.88               | 7.46               | 7.52               | 7.90               |

Table I. Table of measurements of 4 adult male and 4 adult female Paruroctonus soda sp. nov., in mm.

species have records from the Panorama Hills a few kilometers from Soda Lake and the latter has records a short distance to the north of the North Basin. The geographically closest *Paruroctonus* is *P. variabilis*, found in the nearby Panorama Hills and Temblor range. However, we consider it unlikely that *P. variabilis* is found in the flat portion of the Carrizo Plain near Soda Lake as we have been unable to locate any after significant sampling.

5.13

1.55

5.09

1.01

5.38

1.18

5.39

1.24

4.75

1.06

Pectine L

Pectine W

5.48

1.42

5.67

1.43

6.16

1.91

All specimens included in the description of this species were found by blacklight on 30 May 2021 and additional specimens were found by users of iNaturalist.org on 19 May 2021 and 1 March 2022. *Paruroctonus soda* sp. nov. was abundant at all localities where it was found. We found a higher density of surface-active adult males than surface-active adult females, and a higher density of surface-active adults than surfaceactive juveniles. A single late-instar juvenile female *Paruroctonus soda* sp. nov. was found to be whitish in color and completely lacked fuscousity (Fig. 13), possibly indicating hypomelanism. It is unclear whether this was a low-probability chance event or if there is a significant portion of the population of *Paruroctonus soda* sp. nov. with this trait. A gravid adult female collected and maintained alive in captivity gave birth in mid-August to 51 offspring, of which all except one survived until the first molt (Fig. 14).

**Conservation.** Fortunately, the entirety of the range of *Paruroctonus soda* sp. nov. is encompassed within the Carrizo Plain National Monument, rendering the species safe from the primary anthropogenic threats to scorpions: land alteration and habitat destruction due to human development.

**Etymology.** *Paruroctonus soda* sp. nov. is named after Soda Lake, which is the only locality this species is known from. The name also reflects the highly alkaline soils this species inhabits.

## Paruroctonus conclusus sp. nov.

https://zoobank.org/3C830C30-4F3E-400B-9661-046EB7726D1F Figs 15–27, Table 2

**Type material.** *Holotype*: USA • 1 ♂; California, Kern County, southeastern edge of Koehn Lake; 35.3123, -117.8614; 581 m a.s.l.; 3 July 2021; collector leg Prakrit Jain; collected at night using handheld UV light; CASENT 9101936.

*Paratypes.* USA • 4 ♂, 1 ♀; California, Kern County, southeastern edge of Koehn Lake; 35.3123, -117.8614; 581 m a.s.l.; 3 July 2021; collector leg Prakrit Jain; collected at night using handheld UV light; CASENT 9101937.

USA• 1<sup>Q</sup>; California, Kern County, southeastern edge of Koehn Lake; 35.3123, -117.8614; 581 m a.s.l.; 2 August 2021; collector leg Harper Forbes; collected at night using handheld UV light; CASENT 9101938.

**Diagnosis.** Differs from other *Paruroctonus* species found in the Northwestern Mojave Desert and its surrounding mountains (The Tehachapis, the southern Sierra Nevada, and the northeastern Transverse Range) by a combination of the following characteristics: 1: Fuscous markings entirely absent from the metasoma and pedipalps and heavily reduced to absent from the carapace and tergites (Figs 15, 16, 18, 20). 2: Chelal fingers deeply scalloped in adult males, leaving a large proximal gap when closed. 3: Macrosetae on the metasomal lateral supramedian and ventral submedian carinae of metasomal segments I–IV follow the patterns 0,1,1,2 and 2,2,2,3, respectively (Fig. 23). 4: Presence of only a single large retrolateral median macroseta on the pedipalp patella (between the  $em_1$  and esttrichobothria). 5: The number of primary denticles on the fixed and movable fingers, excluding the proximal row, 31–36 and 42–51, respectively. 6: Chela length / Manus width ratio 2.20–2.4 in adult males and 2.46–2.52 in adult females. 7: Mid-retrosuperior macroseta always present on basitarsus II. 8: Chelal dorsomedian carina strong and smooth on its distal half, curving prolaterally between the *db* and *dsb* trichobothria (Fig. 20). 9: Prolateral ventral macroseta absent on movable finger of the chela (Fig. 20).

Comparisons are provided for the four other *Paruroctonus* found in the Northwestern Mojave desert and its surrounding mountains (The Tehachapis, the southern Sierra Nevada, and the northeastern Transverse Range), *P. becki, P. marksi, P. boreus*, and *P. silvestrii*. Of these, only *P. becki* is found in sympatry with *P. conclusus* sp. nov. The other three are found at a considerable distance away in very different habitats: sand dunes for *P. marksi*; grassland or chaparral for *P. silvestrii*; and high desert, scrubland, or conifer woodland, typically well above 800 m elevation, for *P. boreus* (especially in the Mojave Desert and surrounding regions). *P. becki* can be easily differentiated by its significantly more slender chela. Morphological comparisons are also provided for the other species described in this paper, *Paruroctonus soda* sp. nov., but the two species can be easily separated by range. Other *Paruroctonus* which may have certain morphological similarities to *P. conlcusus* sp. nov. are entirely allopatric.

*P. becki* differs from *P. conclusus* sp. nov. in the following characters relating to the numeration in the above diagnosis: (2) Chelal fingers not scalloped (straight), leaving no proximal gap when closed. (3) Metasomal macrosetae on the lateral supramedian and ventral submedian carinae of segments I–IV follow the patterns 0,2,3,2–3 and 3,4,4–5,4–5, respectively. (4) Presence of 4–6 retrolateral median macrosetae on the pedipalp patella. (5) Primary denticles 46–50 on the fixed finger and 61–66 on the movable finger. (6) Chela length/manus width ratio 3.40–3.51 in males, 3.44–3.56 in females.

*P. marksi* differs from *P. conclusus* sp. nov. in the following characters relating to the numeration in the above diagnosis: (2) Chelal fingers in males moderately scalloped, leaving a small gap when closed. (3) Metasomal macrosetae on the lateral supramedian and ventral submedian carinae of segments I–IV follow the patterns 0,1,1,2 and 2–3,3–5,3–4,4–5, respectively (Haradon 1984a). (7) Mid-retrosuperior macroseta always absent on basitarsus II (Haradon 1984a). (8) Chelal dorsomedian carina weakly crenulate and irregular on its distal half and terminates near the *db* trichobothria without curving (Haradon 1984a).

*P. boreus* differs from *P. conclusus* sp. nov. in the following characters relating to the numeration in the above diagnosis: (1) Heavy fuscous markings present on the tergites, carapace, and the ventral surface of the metasoma, especially on segments II–IV. (3) Macrosetae on the ventral submedian carinae of metasomal segments I–IV follow the pattern 2,2–3,3,3 (rarely 2,2,2,3). (5) The number of primary denticles on the fixed finger, excluding the proximal row, 35–46 (37–52 according to Haradon 1985). (8) Chelal dorsal median carina continues to be weakly crenulate and irregular on its distal half and ends near the *db* trichobothria without curving. (9) A single prolateral ventral macroseta is typically present on the proximal half of the movable finger of the chela. Of these characters, (3), (5) and (9) may overlap with *P. conclusus* sp. nov., however, overlap is rare and ambiguity in all 3 characters is highly unlikely.



**Figure 15.** Photographs of four adult *Paruroctonus conclusus* sp. nov. illustrating intraspecific variation, holotype male is at top left.

*P. silvestrii* differs from *P. conclusus* sp. nov. in the following characters relating to the numeration in the above diagnosis: (1) Heavy fuscous markings present on the tergites, carapace, pedipalps, and the ventral surface of the metasoma. (2) Chelal fingers not scalloped (straight), leaving no proximal gap when closed. (3) Macrosetae on the ventral submedian carinae of metasomal segments I–IV follow the pattern 2–3,3,3–

4,3–4. (4) Presence of 2–4 retrolateral median macrosetae on the pedipalp patella. (5) Primary denticles 41–52 on the fixed finger and 54–68 on the movable finger. (6) Chela length/manus width ratio 2.59–2.70 in males, 2.75–3.06 in females. (8) Chelal dorsal median carina weakly crenulate to smooth and weak on its distal half and ends near the db trichobothria without curving. (9) Two prolateral ventral macrosetae present on the movable finger of the chela.

*P. soda* sp. nov. differs from *P. conclusus* sp. nov. in the following characters relating to the numeration in the above diagnosis: (1) Significant fuscous markings present on the carapace and tergites. (3) Macrosetae on the ventral submedian carinae of metasomal segments I–IV follow the pattern 1–2,2,2,2. (4) No large retromedian macrosetae present on the pedipalp patella.

**Description of male holotype.** *Coloration* (Figs 15–17). Carapace pale yellow anteriorly to tan posteriorly. Very faint fuscous markings restricted to the posterior extent of the interocular triangle. Tergites mostly brown with lighter, yellowish posterior and lateral margins. Fuscosity on the tergites extremely indistinct, almost absent. Legs whitish to pale cream. Pedipalps light tan with darker carinae and orangish fingers. Metasoma tan, with slightly darker carinae. Telson pale yellow, base of aculeus dark reddish-brown, and aculeus black. Sternites dark brown with brown spiracles. Pectines, sternum, and genital operculum tan to pale yellow.

*Carapace* (Figs 18, 19). Anterior margin roughly straight to very slightly concave with three pairs of distinct macrosetae. Large granules present sparsely and irregularly; very fine and evenly spaced granules present between the large granules. The large st granules are clustered in the posterior median portion of the carapace, and large granules decrease in size anteriorly and laterally. Posterior, lateral, and anterior margins finely crenulate. Posterior median sulcus narrow and moderately deep, with some posterior granulation. Anterior median and median ocular sulci shallow, free of large granules. Lateral ocular and posterior lateral sulci broad and shallow, free of large granules. Central lateral sulcus broad and shallow, with sparse granules. Interocular region of the carapace smooth with sparse granules anteriorly. Median ocelli separated by a distance greater than the width of one ocellus. 2/3 lateral ocelli present on each side. Single pairs of macrosetae present posterior to the median ocelli, situated between the lateral ocelli and the margin of the carapace, and roughly halfway between the posterior median sulcus and the posterior margin of the carapace, in line with the posterior edge of the ocular tubercle.

*Mesosoma* (Figs 16–18). Tergites I–VI smooth to very finely granular, except on the posterior-lateral half of each side, which is smooth (tergite I) to granular (tergite VI), and the posterior margin, which is ranges from smooth (tergite I) to weakly and finely granular (tergite VI). Median longitudinal carina weak, smooth on I and weakly irregularly crenulate to irregularly crenulate on II–VI. Submedian longitudinal sulci indistinct. One pair of small posterior sub-median setae on tergites I–V, vestigial on VI. Tergite VII essentially smooth anteriorly and posteriorly, lateral-median areas sparsely granular. Lateral marginal carina finely crenulate; dorsolateral and dorsal sub-median carinae strongly crenulate. Median longitudinal carina weakly crenulate. Sternites III–VI sparsely setose and smooth. Sternite VII smooth anteriorly and finely granular laterally, with ventral submedian carinae indistinct and very weakly crenulate and lateral marginal carinae finely crenulate.

*Genital operculum* (Fig. 19). Sclerites roughly triangular with rounded corners, ca. as wide as long. Overlapping medially and separated slightly only at the posterior edge, with protruding genital papillae. Several macrosetae present on each sclerite.

*Sternum* (Fig. 19). Type 2 with posterior emargination absent, apex deep, slightly wider than long, smooth except very finely granular along the slopes of the apex. Three pairs of macrosetae.

**Pectines** (Fig. 19). Long, thin, and densely hirsute, with 25/26 tightly packed teeth on each side. Middle lamellae roughly circular distally, highly irregular in size and shape proximally; roughly 21/22 distinct and separated sclerotized sections are visible under ultraviolet illumination.

*Legs. Carinae.* Retroventral carina on leg I femur finely crenulate and nonlinear; linear on subsequent legs. Superior carina on leg I femur weakly and finely crenulate, decreasingly distinct on subsequent legs. Proventral carina sparsely, finely, and weakly crenulate on leg I patella, decreasingly distinct on subsequent legs and nearly absent by leg IV. Intercarinal spaces on legs smooth with occasional sparse, fine granules on the femur.

**Telotarsi.** Telotarsal retroinferior terminal macrosetae on legs I–IV 1/1, 1/2, 2/2, 2/2; other telotarsal retroinferior macrosetae on the distal half of telotarsi I–IV 1/1, 1/1, 2/2, 2/2. Two telotarsal retromedial macrosetae on each leg, with one always at the retromedial terminal position. Two large telotarsal retrosuperior macrosetae on each leg with consistent positions. Single proinferior terminal macroseta on each leg, single other proinferior macroseta on legs II–IV. Two telotarsal promedial macrosetae on legs I–II at terminal and distal positions; one on legs III–IV in terminal position. Two large telotarsal prosuperior macrosetae on each leg in terminal and medial positions. Telotarsal superior macrosetae on legs I–IV 1/1, 1/1, 1/0, 0/0. Single telotarsal superioterminal macroseta present on all legs.

**Basitarsi.** Three basitarsal spine rows present on legs I and II; proventral and retroventral spine rows equally dense and retrosuperior spine row less dense. The retroventral spine row extends ca. three-fourths the entire length of the segment, the proventral spine row extends through ca. half the segment, and the retrosuperior spine row extends irregularly through around half. On leg III, the proventral spine row is absent and the retrosuperior and retroventral spine rows are heavily reduced in density. On leg IV, both the proventral and retroventral spine rows are absent and the retrosuperior spine row is heavily reduced in density, nearly absent. Basitarsal retroventral macrosetae on legs I–IV follow the pattern 2/3, 4/5, 6/6, 4/4 (excluding the distal retroventral spinoid macroseta at the terminus of the retroventral spine row), with variably sized setae. Spinoid basitarsal proventral macroseta is present on legs II–IV. Superior basitarsal macrosetae on legs I–IV consist of two spinoid macrosetae at the distal and



Figure 16. Dorsal habitus of *Paruroctonus conclusus* sp. nov. holotype male (left) and paratype female (right). Scale bars: 10 mm, silhouettes to scale.

mid retrosuperior positions; two macrosetae at the distal and mid prosuperior positions, except leg IV which has only the distal prolateral macroseta; one macroseta at the distal superiomedian position adjacent to the distal retrosuperior macroseta on legs I–III; and large superiomedian macrosetae following the pattern 5/5, 5/5, 5/5, 4/5. Prolateral macrosetae on legs I–IV, excluding one on the margin, follow the pattern 3/3, 3/3, 3/3, 2/2.

**Pedipalps** (Figs 20–22). *Femur.* Dorsal prolateral carina crenulate with two macrosetae on the proximal half; dorsal retrolateral carina also crenulate with two macrosetae on the proximal three-fourths. Dorsal surface sparsely granular. Retrolateral dorsosubmedian carina weakly crenulate with 3/4 median macrosetae and an additional one on the distal margin; retrolateral surface otherwise smooth aside from a few proximal granules. One additional large macroseta in a distal inframedian position on the retrolateral surface. Three small ventral retrolateral macrosetae present. Ven-



**Figure 17.** Ventral habitus of *Paruroctonus conclusus* sp. nov. holotype male (left) and paratype female (right). Scale bars: 10 mm, silhouettes to scale.

tral retrosubmedian carinae vestigial, irregularly granular with granules decreasing in size distally. Ventral prolateral carina irregularly crenulate. Prolateral surface irregularly granular with three prolateral ventral macrosetae on the proximal two-thirds including one on the proximal marginal carina, one prolateral ventrosubmedian macroseta near the midpoint, and a pair of macrosetae on the distal margin.

**Patella.** Dorsal retrolateral carina weakly crenulate with a proximal macroseta; dorsal prolateral carina crenulate, also with a proximal macroseta. Dorsal surface smooth. Retrolateral median carinae indistinct and very weakly crenulate, retrolateral surface otherwise smooth. A single median and two distal macrosetae are present on the retrolateral surface. Ventral retrosubmedian carina weakly crenulate with a distal macroseta; ventral prolateral carina crenulate, also with a distal macroseta. A proximal macroseta



**Figure 18.** Dorsal trunk of *Paruroctonus conclusus* sp. nov. holotype male (above), paratype female (below). Scale bars: 5 mm.

is present at the junction of the ventral retrosubmedian carina and the finely crenulate ventral median carina. Ventral surface smooth. Prolateral median carina indistinct, represented by a few large granules. Prolateral surface otherwise smooth. Prolateral surface with large proximal supramedian, proximal inframedian, distal inframedian, and distal supramedian macrosetae.

**Chela.** Dorsal prolateral carina indistinct, non-linear, and crenulate on the manus with a medial macroseta. Dorsal median carina weakly crenulate proximally and smooth distally, curving prolaterally between the *db* and *dsb* trichobothria and terminating at the dorsal prolateral carina. A single macroseta is present at the proximal terminus of the dorsal median carina. Dorsal retrosubmedian carina vestigial, consisting of only a few weak granules, and extending through less than the proximal fifth of the manus. Dorsal retrosubmedian accessory carina also vestigial, extending through less than the proximal terrolateral carina very weakly crenulate proximally and smooth distally with a medial and distal macroseta on the manus. Retrolateral median carina very weakly granular and unpigmented, with a single medial macroseta. Ventral retrolateral carina irregular and weakly crenulate, with 1/0 proximal and three non-linear medial macrosetae. Inter-

carinal spaces on the dorsal and retrolateral surfaces smooth aside from occasional sparse granules. Ventral prosubmedian carina irregular and weakly crenulate, with a one proximal and one medial macroseta. Ventral surface mostly smooth with some distal granulation. Prolateral ventral carina crenulate to weakly crenulate with a proximal and distal macroseta. Prolateral median carina crenulate to weakly crenulate with a proximal and medial macroseta. Two further small carinae are present near the base of the fixed finger, both of which are evenly and finely crenulate. Prolateral surface of



**Figure 19.** Carapace and sternopectinal region of *Paruroctonus conclusus* sp. nov. holotype male (left) and paratype female (right). Shown under ultraviolet illumination. Scale bars: 5 mm.



**Figure 20.** Pedipalp of *Paruroctonus conclusus* sp. nov., holotype male (above) and female (below). Trichobothria db and dsb (diagnosis character 8) indicated with closed circles. Carinae abbreviations: retrolateral median (rm), dorsal retrolateral (drl), dorsal median (dm), dorsal prolateral (dpl), ventral retrolateral (vrl), ventral prosubmedian (vps), prolateral ventral (plv), prolateral median (plm). Scale bars: 10 mm.

the manus otherwise mostly smooth with some weak and irregular granulation in the distal half. The fingers are heavily scalloped, leaving a large proximal gap when closed. The chela is uniformly finely granular at the base of this gap. Retrolaterally and prolaterally, the fingers are smooth except some fine proximal granulation. 18/21 small



**Figure 21.** Illustrations of pedipalp chelae of *Paruroctonus conclusus* sp. nov. **A–D** holotype male **E–H** paratype female **A, E** retrolateral **B, F** prolateral **C, G** dorsal **D, H** ventral. Trichobothria indicated with open circles. Scale bars: 5 mm.

macrosetae and numerous microsetae are present on the ventral surface of the movable finger. No prolateral ventrolateral macrosetae are present on the movable finger. The movable finger has one proximal and one medial prolateral median macroseta and one proximal retrolateral median macroseta. No proximal prolateral ventral macroseta is present on the movable finger. The fixed finger has one prolateral medial macroseta and one proximal prolateral dorsolateral macroseta. The fixed finger has one retrolateral medial and one distal dorsal retrolateral macroseta. Both the fixed and movable fingers have five retrolateral enlarged denticles dividing the primary denticles into six sub-rows, with an additional retrolateral enlarged denticle at the distal extent of the movable finger, alongside the distal hook. On the fixed finger, rows I–VI contain 5/3, 6/4, 6/7, 7/8, 10/10, 10/11 primary denticles with a total row I–V count of 34/33.



**Figure 22.** Illustrations of pedipalp patella and femur of *Paruroctonus conclusus* sp. nov. Holotype male, dorsal patella **A** retrolateral patella **B** ventral patella **C** dorsal femur **D** Trichobothria indicated with open circles. Scale bars: 2 mm.

On the movable finger, rows I–VI contain 6/5, 7/8, 10/9, 10/10, 11/13, 8/8 primary denticles with a total row I–V count of 44/45. Each retrolateral enlarged denticle as well as the distal finger-tip hook is accompanied by a single prolateral supernumerary denticle, for a total of six on the fixed finger and seven on the movable finger. There is a single macroseta posterior to each supernumerary denticle with the exception of the two most distal ones on each finger. Two further macrosetae are present near the proximal to the most proximal primary denticle on the fixed finger.

Metasoma (Fig. 23). Dorsal surface I-V smooth. Dorsolateral carinae on segments I-IV strongly crenulate to serrate, weakly crenulate on V. Lateral supramedian surface essentially smooth with a few scattered granules. Lateral supramedian carinae I-IV strongly crenulate to serrate. Lateral surface smooth. Lateral inframedian carinae crenulate on I-III, extending through only the posterior fifth of segments II-III. Lateral median carinae indistinct and weakly crenulate on V, extending ca. a third of the way up the segment. Ventrolateral carinae I-IV smooth, becoming weakly crenulate on the posterior fifth of segment III and the posterior half of segment IV. Ventrolateral carinae on segment V strongly crenulate to serrate. Ventral surface of segment I-IV smooth; ventral surface sparsely granular on segment V. Ventral sub-median carinae on I-IV smooth and unpigmented, indistinct on I. Ventromedian carinae on segment V are crenulate and irregular. Dorsolateral macrosetae I–IV follow the pattern 0,1,1,2. Four dorsolateral macrosetae on V. Lateral supramedian macrosetae I-IV follow the pattern 0,1,1,2. Two Lateral median macroseta on V. Lateral inframedian macrosetae I-III follow the pattern 1,0,0. Ventrolateral macrosetae I-V, excluding any on the posterior margin of the segment, follow the pattern 2,3,3,3. Six ventrolateral macrosetae on V excluding any on the posterior margin. Ventral submedian macrosetae I-IV, excluding those on the posterior margin of the segment, follow the pattern 2,2,2,3. Four

pairs of macrosetae are present between the ventromedian and ventrolateral carinae on segment V. Two pairs of macrosetae on the ventral posterior margin of metasomal segments IV and V; a single pair of macrosetae on the ventral posterior margins of other metasomal segments.

*Telson* (Fig. 23). Very weakly granular on the ventral anterior portion, otherwise smooth. Sparsely setose ventrally and laterally.

*Hemispermatophore* (Fig. 24). Hemispermatophore decreasing in width from pedicel to stalk, three-fold bauplan (Monod et al. 2017). Stalk wide, relatively straight, and dorso-ventrally flattened. Distal carina and lamellar hook sclerotized, lamellar hook prominent and weakly bifurcate at terminus. Mating plug weakly sclerotized, moderate in size with a single lobed base and long stem terminating in a prominent barb.

**Female.** Larger carapace in comparison to the male. Carapace smoother, with essentially smooth interocular triangle and very weak granulation in the posterior-lateral areas. Tergites also smooth, with granulation largely restricted to the posterior and lateral margins and lateral fifth or less on each side. Chela less incrassate, with fingers not scalloped, leaving a negligible gap when closed. Most proximal row on the chelal fixed finger with 16–19 primary denticles; most proximal row on the chelal movable finger with 8–10 primary denticles. Metasoma more robust than in males. Pectines smaller overall with fewer teeth, 16–19; middle lamellae slightly more regular than in males; 13–15 separated and sclerotized sections visible under ultraviolet illumination. Genital operculum sclerites do not overlap, and are slightly separated through their entire length.

**Variation.** *Coloration* (Figs 15–17). Coloration largely constant, with pale yellow-brown carapace, pedipalps, and metasoma; lighter legs; and a darker mesosoma. Fuscousity very faint, restricted to the edges of the interocular triangle, the extreme posterior-lateral corners of the carapace, and the anterior portion of the tergites.

*Carapace* (Figs 18, 19). Density and distribution of granulation variable. Highest density of large granulation is found in the posterior median section. Posterior-lateral margins with moderate to very weak granulation; interocular triangle with weak to essentially absent granulation. Lateral eyes typically three on each side.

*Mesosoma* (Figs 16–18). Tergites with variable amounts of granulation. Margins granular to weakly granular, posterior and lateral portions granular to smooth. Small posterior submedian setae sometimes absent on VI.

**Pectines** (Fig. 19). Males with 23–26 teeth. Roughly 18/23 distinct and separated sclerotized middle lamellae are visible under ultraviolet illumination

Legs. Telotarsal setation somewhat variable. Retroinferior terminal and other retroinferior macrosetae on the distal half of telotarsi I–IV both within the ranges 1,1–2,2,2. Typically two retrosuperior and two retromedial macrosetae, with an additional large macroseta rarely present on legs III–IV. One or multiple extra small retrosuperior or retromedian macrosetae occasionally present on any leg. Telotarsal proinferior and prosuperior macrosetae consistent with occasional asymmetric additions or deletions. Promedian macrosetae on legs I–IV within the ranges 2–3,2,1–2,1–2. Superior median macroseta on legs I–IV within the ranges 1,1,0–1,0–1, with variation in size. Basitarsal setation highly variable. Retroventral macrosetae on legs I–IV, excluding



**Figure 23.** Metasoma of *Paruroctonus conclusus* sp. nov. male holotype (above) and female (below); ventral, lateral, and dorsal aspects (top to bottom). Ventral sub-median and lateral submedian macrosetae on segments I–IV indicated with black circles (diagnosis character 3). Carinae abbreviations: Dorsolateral (dl), lateral median (lm), lateral supramedian (lsm), lateral inframedian (lim), ventrolateral (vm), ventral submedian (vs), and ventromedian (vm). Scale bars: 10 mm.

only the one on the distal margin, within the ranges 2–4,4–7,6–7,4–6. Proventral macrosetae on legs I–IV, excluding the thinner proventral terminal macroseta on legs II–IV, within the ranges 2,2–3,2–3,3. Spinoid retrosuperior macrosetae always present in the mid and distal positions. Prosuperior macrosetae typically present at the mid and distal positions on legs I–III and at the distal position on leg IV but one or both



**Figure 24.** Right hemispermatophore of *Paruroctonus conclusus* sp. nov.: anterior aspect (left) and posterior aspect (right). Scale bar: 5 mm.

may be present, absent, or accompanied by an additional prosuperior macroseta on any leg. Distal superiomedian macroseta typically adjacent to the distal retrosuperior macroseta but variable in position and occasionally absent on any leg. Large retrosuperior setae excluding the aforementioned retrosuperior, prosuperior, and distal superiomedian seta typically consist of three distal and two proximal ones for a total of five on legs I–III and two or three distal and two proximal ones for a total of four or five on leg IV; however, an additional large macroseta may be present on legs I–III and additional small macrosetae may be present on all legs. Larger prolateral macrosetae on legs I–IV variable and non-linear, within the ranges 3,3–4,3–4,2–3; typically three on each.

**Pedipalps** (Figs 20–22). Femur with 3–5 large retrolateral dorsosubmedian macrosetae and an occasionally present small distal dorsal retrolateral macroseta. Other macrosetae on the pedipalp femur and patella consistent apart from occasional asymmetrical deletions, which are most frequent on the patella retrolateral median macroseta. On the chela, most macrosetae consistent. An additional ventral retrolateral for a total of four and an additional retrolateral median macroseta for a total of two occasionally present. Asymmetrical deletions sometimes occur on most macrosetae on the manus. Fixed finger retrolateral median macroseta sometimes missing. Movable finger with 17–21 ventral macrosetae. Number of primary denticles in rows I–V on the fixed finger within the ranges 2–5, 4–7, 6–8, 7–10, 9–12. Number of primary denticle in row VI on the fixed finger of males 5–10, 9–10, 11–16. Number of primary denticle in rows I–V on the fixed and movable finger of males 5–10. Total number of primary denticles on rows I–V on the fixed and movable fingers 31–36 and 42–51, respectively with no obvious sexual dimorphism.

*Metasoma* (Fig. 23). Dorsolateral macrosetae on I–IV, lateral superiomedian macrosetae on I–IV, lateral inframedian macrosetae on I–IV, ventral submedian macrosetae on I–IV, posterior marginal macrosetae on I–IV, and macrosetae on V consistent. Ventrolateral macrosetae on I–IV follow the pattern 2–3,2–3,3,3. Occasional asymmetrical macrosetal deletions on dorsal and lateral surfaces; frequent asymmetrical macrosetal deletions on ventral surface.

Remarks. The most valuable taxonomic characters for P. conclusus sp. nov. are:

1. The lack of fuscousity on the carapace and tergites is very consistent and is reliably different than in certain other *Paruroctonus*.

2. The macrosetal patterns on the pedipalps and metasoma are mostly consistent and provide an excellent diagnostic against many other *Paruroctonus*.

Other taxonomic characters which may be valuable in some cases, but are typically not useful, include:

1. The telotarsal macrosetae are somewhat variable and have different counts than certain other *Paruroctonus*.

2. The granulation on the carapace and tergites is fairly variable but is notably different from certain other *Paruroctonus*. This character, however, can be difficult to quantify.

3. The basitarsal macrosetae are generally extremely variable and are only helpful for differentiating *P. conclusus* sp. nov. from certain psammophilous *Paruroctonus*. The basitarsal spinoid distal and mid retrosuperior macrosetae are not variable but are still only helpful for differentiating *P. conclusus* sp. nov. from these psammophiles.

4. The granulation on the pedipalps, legs, and metasoma is somewhat variable and difficult to quantify. It is fairly similar to that of most other *Paruroctonus* species, although in isolated examples may be used for diagnosis.

5. The morphometric ratios of different aspects of the metasomal segments and chela are typically fairly consistent but overlap with those of many other *Paruroctonus*.

6. The pectinal tooth counts are somewhat variable and overlap with those of most other *Paruroctonus*. Middle lamellae counts are also not taxonomically valuable, as they are typically ambiguous.

7. The chelal primary denticle counts are somewhat variable and overlap with those of most other *Paruroctonus*.

**Habitat, distribution, and ecological notes.** *Paruroctonus conclusus* sp. nov. is known from only a single locality along the edge of Koehn Lake, which is located within Kern County, California (Fig. 25). Koehn lake is an ephemeral, alkaline desert lakebed at the center of the Fremont Valley in the northwestern Mojave Desert. This valley is bounded on the north by the El Paso mountains and on the south by the Rand mountains, resulting in it being an endorheic basin draining primarily into its lowest point, Koehn Lake, which lies at ca. 570 m a.s.l. (RWMG 2019).

Over the past million years, water levels in Mojave Desert lakes have varied significantly, with several periods of increased moisture where Koehn lake, with other lakes in the Mojave, expanded in size and filled with perennial water and other periods where these lakes shrunk and dried up (Stoffer 2004). Since the end of the most recent Pleistocene ice age, these lakes have generally decreased in size with some minor fluctuation (Stoffer 2004). Currently, playas throughout the Mojave Desert only occasionally hold water (Enzel et al. 1992). Marsh-specialist flora and fauna species distributions typically increase and decrease in area with the surface area of their associated lakes (Stoffer 2004), so we hypothesize that the distribution of *P. conclusus* sp. nov. was historically more extensive than it is currently and that it has shrunk with a drying climate.

The Fremont Valley region is typical of the Mojave desert with characteristic low levels of precipitation concentrated in the winter months, around 15 cm annually, and high summer temperatures, typically in excess of 35 °C (RWMG 2019). This results in an overall arid desert climate with relatively more moisture concentrated at and around Koehn Lake.

The type locality of *Paruroctonus conclusus* sp. nov. is on the southeast edge of this lakebed in an area of increased moisture (Fig. 25). *P. conclusus* sp. nov. was found at the type locality on two moonless summer nights in 2021, with a moderate level of surface activity on July 3 and a low level of surface activity on August 2. Both dominant plant species found at the type locality, *Allenrolfea occidentalis* and *Suaeda nigra*, are alkali sink specialists (Munz and Keck 1949). Correspondingly, the soil at the type locality is



**Figure 25.** Koehn Lake and the surrounding Fremont Valley. Above, satellite imagery of Koehn Lake with the type locality of *Paruroctonus conclusus* sp. nov. indicated with a star, taken in September 2015. Below, a habitat overview looking north-northeast across Koehn Lake towards the El Paso mountains, taken in July 2021.

mostly clay, although in a few spots, it is covered with a thin layer of sand. *P. conclusus* sp. nov. does not appear to prefer either the open clay or the sand-covered clay over the other. Several *P. conclusus* sp. nov. were seen partially or fully concealed within burrows or cracks in the clay soil, indicating that they are a largely fossorial species. We hypothesize that the increased moisture and softer clay-rich soil facilitate burrowing and are the primary factors restricting *P. conclusus* sp. nov. to the lakeside alkali-sink habitat (Fig. 26).

*P. conclusus* sp. nov. is sympatric with three other scorpion species: *Hadrurus arizonensis* Ewing 1928, *Paravaejovis confusus* (Stahnke, 1940), and *Paruroctonus becki*. The former two can be found throughout the desert flats habitat surrounding the



Figure 26. Habitat of Paruroctonus conclusus sp. nov. at the type locality, taken in July 2021.

alkali-sink area adjacent to the Koehn lakebed; however, *P. becki* was only observed immediately adjacent to the lakebed, in sympatry with *P. conclusus* sp. nov. We conducted significant additional sampling at three other localities around Koehn Lake: the southernmost point, the northernmost point, and the northwestern corner. Suitable


Figure 27. Early instar juvenile (left) and late instar juvenile (right) *Paruroctonus conclusus* sp. nov. Not to scale.

habitat, which is dominated by *A. occidentalis* and *S. nigra*, was not found at the former two locations. The northwestern corner of the lakebed had a small area of seemingly suitable habitat, and while *P. becki* was found to be surface-active in high density, no *P. conclusus* sp. nov. were observed despite significant sampling effort. While more sampling is necessary to make a high-confidence determination of absence, we currently believe that it is unlikely *P. conclusus* sp. nov. is found at the northwestern edge of Koehn Lake. Another locality where the habitat appears to be potentially suitable for *P. conclusus* sp. nov. based on satellite imagery exists along the western edge of the lakebed; however, we were unable to sample it due to it being on privately-owned land.

Predation by *P. conclusus* sp. nov. was recorded once, by an adult male on an adult *Paruroctonus becki*. This indicates that these two species exist at least partially in microsympatry.

**Conservation.** *Paruroctonus conclusus* sp. nov. has one of the smallest known distributions of any species of *Paruroctonus*, existing in a stretch of suitable habitat only a couple kilometers in length and no more than a few hundred meters wide. This limited range makes it especially susceptible to extinction. Both primary threats to this scorpion are anthropogenic in origin or extent: destruction of habitat and alterations in climate. The Fremont Valley region of California was home to ca. 20,800 residents in 2020, a number that is projected to grow to 29,400 over the next 20 years (RWMG 2019). This will cause further degradation of the land, not only in the form of housing development but also due to water extraction, electricity production, and other economic activity. The small community of Cantil abuts Koehn Lake on its western shore and contains some agricultural activity. The formerly inhabited town of Saltdale is at the northern extent of Koehn Lake, from where it historically mined valuable salts from the lakebed. While the mine is currently not operational, the entirety of Koehn Lake remains open to the potential of mining (BLM 2005). Another major industry in the Fremont Valley is solar electricity production (RWMG 2019). Two operational farms, owned by Beacon Solar and Springbok, are located in close proximity to Koehn Lake (RWMG 2019). These factors significantly threaten the habitat of *Paruroctonus conclusus* sp. nov., not only by direct habitat alteration but also by indirect downstream effects such as production of waste products, usage of groundwater, and possible

|  | Holotype<br>male                                     | Paratype<br>male                                     | Paratype<br>male                                     | Paratype<br>male                                     | Paratype<br>male                                     | Paratype<br>female                                   | Paratype<br>female                                   |
|--|--|--|--|--|--|--|--|
| CASENT #   | 9101936  | 9101937  | 9101937  | 9101937  | 9101937  | 9101938  | 9101937  |
| Total L  | 43.29  | 38.11  | 40.96  | 36.87  | 38.91  | 41.09  | 43.68  |
| Carapace L   | 5 32   | 4 56   | 5.04   | 4 35   | 4 94   | 5.82   | 5.88   |
| Prosoma posterior W  | 4 69   | 4.12   | 4 47   | 4 10   | 4 45   | 5.52   | 5.47   |
| Prosoma median W   | 4 13   | 3.28   | 3.67   | 3 23   | 3 50   | 4 20   | 4 70   |
| Mesosoma L   | 10.98  | 8.65   | 9.07   | 9 33   | 10.02  | 11.28  | 10.74  |
| Metasoma L   | 28.13  | 24 38  | 26.74  | 23.56  | 25.03  | 26.35  | 27 35  |
| Metasoma LL  | 3.09   | 2.88   | 3.04   | 2.53   | 2.94   | 2.73   | 2.86   |
| Metasoma I W   | 2 73   | 2.30   | 2 49   | 2.33   | 2.66   | 2.89   | 2.80   |
| Metasoma I H   | 2.08   | 1.95   | 2.19   | 1.71   | 2.00   | 2.09   | 2.61   |
| Metasoma II I  | 3 53   | 3.18   | 3.47   | 3.00   | 3.72   | 3.61   | 3 41   |
| Metasoma II W  | 2.81   | 2 24   | 2 50   | 2.46   | 2.60   | 2.76   | 2 72   |
| Metasoma II H  | 2.01   | 1.97   | 2.00   | 1.76   | 1.97   | 2.70   | 2.72   |
| Metasoma III I   | 3.90   | 3.42   | 3.66   | 3.28   | 3.65   | 3.45   | 3 74   |
| Metasoma III W   | 2 74   | 2.06   | 2.21   | 2.25   | 2.38   | 2.75   | 2.60   |
| Metasoma III H   | 2.7 1  | 1.98   | 1.97   | 1.77   | 1.95   | 2.16   | 2.00   |
| Metasoma IV I  | 4 65   | 4 19   | 4 53   | 3.94   | 4 4 4  | 4 29   | 4 42   |
| Metasoma IV W  | 2 40   | 2.08   | 2.17   | 2.05   | 2 19   | 2.70   | 2 43   |
| Metasoma IV H  | 2.10   | 1.94   | 2.08   | 1.71   | 1.98   | 2.70   | 2.15   |
| Metasoma V I   | 6.75   | 5.83   | 6.46   | 5.70   | 6.23   | 6.09   | 6.57   |
| Metasoma V W   | 2.36   | 1.92   | 2.12   | 1.87   | 2.07   | 2 47   | 2.57   |
| Metasoma V H   | 2.50   | 1.92   | 1.92   | 1.70   | 1.77   | 2.47   | 2.37   |
| Telson I   | 5 79   | 5.37   | 5.87   | 5 34   | 5.38   | 6.60   | 6.63   |
| Telson vesicle I   | 4.32   | 3.60   | 3.96   | 3.68   | 3.54   | 4.12   | 4.26   |
| Telson vesicle W   | 2 32   | 1.95   | 2.16   | 1.80   | 2.03   | 2 44   | 2 49   |
| Telson vesicle H   | 1.87   | 1.55   | 1.63   | 1.60   | 1.56   | 2.14   | 2.4)   |
| Telson aculeus I   | 1.86   | 1.73   | 1.89   | 1.44   | 1.75   | 2.00   | 2.15   |
| Pedipalp I   | 18.35  | 16.28  | 17.87  | 15.80  | 16.88  | 19.48  | 19.51  |
| Pedipalp femur I   | 4.56   | 4.08   | 4 25   | 3.80   | 4.08   | 4 54   | 1 38   |
| Pedipalp femur W   | 4.50   | 1.03   | 1.31   | 1.23   | 1.39   | 1.51   | 4.58   |
| Pedipalp femur H   | 0.95   | 0.90   | 0.94   | 0.83   | 0.90   | 1.01   | 1.05   |
| Pedipalp patella I   | 5.14   | 4 20   | 4 35   | 3.69   | 4 64   | 4.92   | 4.92   |
| Pedipalp patella W   | 1.71   | 4.20   | 1.50   | 1.37   | 1.54   | 1.75   | 1.91   |
| Pedipalp patella H   | 1.71   | 1.42   | 1.56   | 1.33   | 1.54   | 1.79   | 1.91   |
| Pedipalp chela I   | 8.52   | 7.51   | 8.25   | 7.14   | 7.53   | 8.95   | 0.21   |
| Pedipalp manus W   | 3.83   | 3.11   | 3.50   | 3.12   | 3.43   | 3.64   | 3.65   |
| Padipalp manus W   | 2.85   | 2.05   | 2.42   | 2.16   | 2.42   | 2.51   | 2.61   |
| Chelp finger fixed I   | 2.00   | 2.0)   | 2.42   | 2.10   | 2.42   | 2.51   | 2.41   |
| Chela finger movable I   | 5.45   | 4.79   | 5.41   | 2./0<br>4.61   | 4 56   | 5.55   | 5.80   |
| Dectine I  | 5.20   | 5.08   | 5.51   | 4.00   | 5.08   | <i>4</i> 37  | 1.00   |
| Pectine W  | 2.14   | 1.80   | 1.88   | 1.68   | 1.88   | 1.37   | 1.23   |
| Pedipalp patena II<br>Pedipalp chela L<br>Pedipalp manus W<br>Pedipalp manus T<br>Chela finger fixed L<br>Chela finger movable L<br>Pectine L<br>Pectine W | 8.52<br>3.83<br>2.80<br>3.45<br>5.26<br>5.35<br>2.14 | 7.51<br>3.11<br>2.05<br>3.13<br>4.79<br>5.08<br>1.80 | 8.25<br>3.50<br>2.42<br>3.22<br>5.41<br>5.51<br>1.88 | 7.14<br>3.12<br>2.16<br>2.78<br>4.61<br>4.90<br>1.68 | 7.53<br>3.43<br>2.42<br>2.84<br>4.56<br>5.08<br>1.88 | 8.95<br>3.64<br>2.51<br>3.53<br>5.56<br>4.37<br>1.27 | 9.21<br>3.65<br>2.41<br>3.96<br>5.80<br>4.25<br>1.23 |

Table 2. Table of measurements of 5 adult male and 2 adult female Paruroctonus conclusus sp. nov., in mm.

alterations to the region's hydrology. Agriculture and mining use large amounts of water, and solar farms can have large-scale destructive effects on desert ecosystems.

These negative changes to the habitat of *Paruroctonus conclusus* sp. nov. will likely be further compounded due to climate change in the Mojave Desert. Typical summer daytime high temperatures in Fremont Valley are projected to increase by ca. 6 °C by 2100; furthermore, the frequency of extreme heat days is projected to increase by 8–15 times compared to pre-1990 levels (RWMG 2019). We hypothesize that *P. conclusus* sp. nov. is restricted to this small lakeside area due to the soft soils and increased moisture providing shelter from the daytime heat. Historically, decreases in water levels and increases in temperature have coincided with range reductions and die-offs in desert flora and fauna species associated with playa habitats (Stoffer 2004). This trend is likely to apply to *P. conclusus* sp. nov. as well. Fortunately, the known range of *Paruroctonus conclusus* sp. nov. is entirely on lands managed by the Bureau of Land Management (BLM), meaning that these lands may be eligible for protection. We urge the BLM to consider creating a conservation area for *P. conclusus* sp. nov. and work towards reducing external threats to its habitat.

**Etymology.** The specific epithet *conclusus* translates to restricted or confined, in reference to the high degree of habitat specialization and severely limited range of *Paruroctonus conclusus* sp. nov.

## Discussion

Most low-elevation desert *Paruroctonus*, including all California species with the exception of *P. variabilis* and *P. becki*, specialize in habitats of increased moisture such as sand dunes, springs, or alkali-sinks (Gertsch and Soleglad 1966; Haradon 1985; Fet et. al 1998). *Paruroctonus soda* sp. nov. and *Paruroctonus conclusus* sp. nov. both inhabit alkali-sink/playa environments, making them the second and third species in the state to specialize in this habitat. A morphological comparison of all three reveals some important shared characteristics:

1. A reduction in setation: In comparison to other *Paruroctonus*, the number of macrosetae on the pedipalp chela, pedipalp patella, and metasoma is moderately to heavily reduced on alkali-sink species. This is most prominent in *P. bantai* and *P. soda* sp. nov., both of which entirely lack large macrosetae on the manus and have high degrees of macroseta reduction on the pedipalp patella and metasoma. *P. conclusus* sp. nov., also has a moderate degree of macrosetal reduction in these areas (Haradon 1985). Superior macrosetae on the basitarsi and retrosuperior macrosetae on the telotarsi are also slightly reduced. The difference is most prominent when these species are compared to dune-dwelling *Paruroctonus*, which have especially hirsute legs as an adaptation to a psammophilous lifestyle (Haradon 1984a, 1984b).

2. A moderate reduction in pigmentation: In comparison to several other *Paruroctonus*, especially those that occur outside of desert regions such as *P. silvestrii*, *P. boreus*, and *P. maritimus*, these alkali-sink *Paruroctonus* have significant reduction in

pigmentation. This is most prominent on the legs, pedipalps, and metasoma, where fuscous markings are entirely absent, but is also the case to a lower degree on the carapace and tergites. The lowest degree of pigment reduction is present in *P. soda* sp. nov. while the highest degree of pigment reduction is present on *P. conclusus* sp. nov. The level of pigment reduction, however, is not as significant as it is in desert dune-dwelling *Paruroctonus* such as *P. xanthus*, *P. luteolus*, or *P. baergi* (Haradon 1984a, 1984b).

3. An enlargement of the chela: the chelae of all three alkali-sink species are very incrassate with heavy scalloping in males, leaving a large proximal gap between the fingers when closed. This is most prominent in *P. soda* sp. nov. and *P. conclusus* sp. nov. While some other species, such as *P. boreus*, *P. arenicola*, and *P. baergi* also possess a similar level of chelal enlargement, especially in males, they are in the minority (Haradon 1983, 1984a, 1984b, 1985).

Most estimates place the formation of the Tehachapi mountains and Sierra Nevada separating the San Joaquin Desert from the Mojave Desert in the Eocene (Buwalda 1954; Mix et al. 2016), far before the formation of the Soda Lake endorheic basin in the Pliocene. Furthermore, alkali sinks typically form in endorheic basins, which in most cases require surrounding mountain ranges to prevent outflow to the ocean. It is unlikely that these alkali-sink specialist Paruroctonus species would have been able to traverse a mountain range, suggesting they likely speciated as the present day geographies of California emerged (Fig. 28). The Paruroctonus with the greatest degree of morphological similarity to these three alkali sink species is *P. boreus*, a species which can also be found in higher-elevation alkali sink areas such as the Deep Springs Valley in California or the Great Salt Lake region in Utah. The three alkali sink Paruroctonus may have shared a common ancestor with *P. boreus* that specialized in mountainous habitats. In times of cooler temperatures or higher moisture, such a species' range could extend into lowland areas, subsequently contracting as temperatures warmed and conditions dried. This range contraction would have resulted isolated populations in alkali sink areas which would eventually diverge into distinct species while still exhibiting either plesiomorphic morphology or convergent evolution from similar ecological constraints. Such a process may be ongoing in populations of *P. boreus* in areas such as Deep Springs Valley.

*P. soda* sp. nov. and *P. bantai* share a much higher degree of morphological similarity than either does with *P. conclusus* sp. nov. This includes very similarly reduced pedipalp and metasomal setation and more similar patterns of fuscousity. However, *P. soda* sp. nov. and *P. bantai* are separated by many more geographic barriers (in the form of mountain ranges) from each other than either is from *P. conclusus* sp. nov. Future phylogenetic work on the group will be important to determine not only the relationships between these three species, but also the relative influence of past geologic events on the speciation of the genus *Paruroctonus*. The existence of these species in remnant alkali sinks mean that their distributions are extremely restricted, and their conservation should be prioritized alongside the preservation of their habitat.



**Figure 28.** Distribution overview of the 3 Alkali sink *Paruroctonus* species: *P. soda* sp. nov. (red), *P. conclusus* sp. nov. (green), *P. bantai bantai* (purple), and *P. bantai saratoga* (blue). Enlarged map represents the rectangle on the inset map at the top left, shading represents elevation with dark being low and light being high elevations, and lines represent county borders.

# Additional material examined

## Paruroctonus variabilis

USA • 1  $\bigcirc$  1  $\bigcirc$ ; California, Kern County, Bitter Creek NWR, Klipstein Cyn Rd; 34.9512, -119.4064; 929 m a.s.l.; 29 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 1  $\bigcirc$ ; California, Fresno County, along W Whitesbridge Rd near Mendota; 36.7285, -120.2964; 50 m a.s.l.; 15 Jul. 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 3  $\bigcirc$ ; California, Fresno County, Silver Creek near Panoche; 36.5717, -120.7024; 239 m a.s.l.; 5 Oct 2019; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 1  $\bigcirc$ ; California, Contra Costa county, Empire Mine Road near Antioch; 37.9396, -121.8026; 113 m a.s.l.; 24 Sep 2021; collector leg Prakrit Jain; collected at night using handheld UV light. • 1  $\bigcirc$ ; California, Kings county, Jackson avenue near Lemoore; 36.2519, -119.8059; 66 m a.s.l.; 3 Sep 2021; collector leg Prakrit Jain; collected at night using handheld UV light.

# Paruroctonus becki

USA • 2 🖑; California, Kern County, southeastern edge of Koehn Lake; 35.3123, -117.8614; 581 m a.s.l.; 3 July 2021; collector leg Prakrit Jain; collected at night using

handheld UV light • 2  $\bigcirc$ ; California, Los Angeles County, Wilsona Gardens; 34.6804, -117.8278; 798 m a.s.l.; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light.

## Paruroctonus marksi

USA • 1  $\bigcirc$  1  $\bigcirc$ ; California, Los Angeles County, Wilsona Gardens; 34.6804, -117.8278; 798 m a.s.l.; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light.

# Paruroctonus boreus

USA •  $3 \circ 1 \circ$ ; California, Alpine County, W side Monitor Pass, Hwy 89 × 4 intersection; 38.6605, -119.7264; 1738 m a.s.l; 15 Sep. 1980; collector leg Stan C. Williams. •  $1 \circ 3 \circ 2$ ; California, Alpine County, W. side along Hwy 89; 18 Jun. 1980; collector leg Stan C. Williams. •  $1 \circ 3 \circ 2$ ; California, Alpine County, W. side along Hwy 89; 18 Jun. 1980; collector leg Stan C. Williams. •  $1 \circ 3 \circ 2$ ; California, Inyo County, White Mountains, Sierra Vista; 37.3563, -118.1868; 2836 m a.s.l.; 12 Jun. 2020; collector leg Harper Forbes, Prakrit Jain; flipped under rocks. •  $2 \circ 2$ ; California, Inyo County, Big Pine Creek Campground; 37.1254, -118.437; 2377 m a.s.l.; 11 Jun. 2020; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. •  $2 \circ 2$ ; California, Inyo County, Deep Springs Lake; 37.2866, -118.0395; 1501 m a.s.l.; 13–15 Jun. 2021; collector leg Harper Forbes, Prakrit Jain; flipped under debris. •  $1 \circ 3$ ; Nevada, Lander County, Pete's Summit; 39.1848, -116.7914; 2420 m a.s.l.; 4 July 2021; collector leg Corey Lange; collected at night using handheld UV light.

# Paruroctonus silvestrii

USA • 1  $\bigcirc$ ; California, Colusa County, Bear Creek; 38.9772, -122.3391; 302 m a.s.l.; 15 May 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 1  $\bigcirc$ ; California, Orange County, Santa Ana Mountains, Lost Woman Cyn; 33.7525, -117.5513; 767 m a.s.l.; 4 August 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • 1  $\bigcirc$ ; California, Stanislaus County, Del Puerto Canyon Road; 37.4750, -121.2388; 107 m a.s.l.; 1 March 2020; collector leg Harper Forbes; flipped under rocks. • 1  $\bigcirc$ ; California, Alameda County, Patterson Pass Road; 37.6961, -121.5894; 235 m a.s.l.; 26 June 2021; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light. • Mexico • 2  $\bigcirc$ , 2  $\bigcirc$ ; Baja California Norte, Puerto Santo Tomas; 8 m a.s.l.; 11 Jul. 1969; collector leg Stan C. Williams, V. F. Lee.

# Paruroctonus bantai

USA • 1  $\bigcirc$ ; California, Inyo County, Saline Valley, west of dunes; 36.7500, -117.8617; 350 m a.s.l.; 14 June 2020; collector leg Harper Forbes, Prakrit Jain; collected at night using handheld UV light.

## Acknowledgements

The authors would like to thank Edmundo González Santillán and an anonymous reviewer for their careful consideration of this manuscript. We would also like to thank the three users on iNaturalist.org who uploaded observations of these species, they helped to bring these scorpions to our attention for the first time. The first and second authors would also like to thank our families, who accompanied us in doing fieldwork.

## References

- Buck-Diaz J, Ratchford J, Evens J (2013) Report to the Natural Resources Conservation Service: California Rangeland Monitoring and Mapping: Focusing upon Great Valley and Carrizo Plain Grassland Habitats. California Native Plant Society, Sacramento, USA, 74 pp. https://www.cnps.org/wp-content/uploads/2018/04/grassland\_nrcs\_report.pdf
- Bureau of Land Management (2005) Final environmental impact report and statement for the west Mojave plan: A habitat conservation plan and California desert conservation area plan amendment. US Department of the Interior, USA. https://eplanning.blm.gov/public\_projects/lup/72544/97517/117675/wemo\_plan\_vol-1\_2004.pdf
- Buwalda JP (1954) Geology of the Tehachapi Mountains, California. In: Jahns, RH (Ed) Geology of Southern California. State of California Division of Mines Bulletin 170(1): 131–142. https:// ia800703.us.archive.org/12/items/boxsouthgeology00calirich/boxsouthgeology00calirich.pdf
- Cooper JW (1990) A geophysical study of the hydrogeology of the Carrizo Plain area San Luis Obispo County, California. PhD Thesis, San Jose State University, San Jose, USA. https:// scholarworks.sjsu.edu/cgi/viewcontent.cgi?article=4248&context=etd\_theses
- Enzel Y, Brown WJ, Anderson RY, McFadden LD, Wells SG (1992) Short-duration Holocene lakes in the Mojave River drainage basin, southern California. Quaternary Research 38(1): 60–73. https://doi.org/10.1016/0033-5894(92)90030-M
- Fet V, Polis GA, Sissom WD (1998) Life in sandy deserts: The scorpion model. Journal of Arid Environments 39(4): 609–622. https://doi.org/10.1006/jare.1997.0386
- Gertsch WJ, Soleglad ME (1966) The scorpions of the Vejovis boreus group (subgenus Paruroctonus) in North America (Scorpionida, Vejovidae). American Museum Novitates no. 2278. http://hdl.handle.net/2246/3294
- González-Santillán E, Prendini L (2013) Redefinition and generic revision of the North American vaejovid scorpion subfamily Syntropinae Kraepelin, 1905, with descriptions of six new genera. Bulletin of the American Museum of Natural History 2013(382): 1–71. https:// doi.org/10.1206/830.1
- Haradon RM (1983) Smeringurus, a new subgenus of Paruroctonus Werner (Scorpiones, Vaejovidae). The Journal of Arachnology 11(2): 251–270. https://www.americanarachnology. org/journal-joa/joa-all-volumes/detail/volume/108/
- Haradon RM (1984a) New and redefined species belonging to the *Paruroctonus baergi* group (Scorpiones, Vaejovidae). The Journal of Arachnology 12(2): 205–221. https://www.americanarachnology.org/journal-joa/joa-all-volumes/detail/volume/105/

- Haradon RM (1984b) New and redefined species belonging to the *Paruroctonus borregoen*sis group (Scorpiones, Vaejovidae). The Journal of Arachnology 12(3): 317–339. https:// www.americanarachnology.org/journal-joa/joa-all-volumes/detail/volume/106/
- Haradon RM (1985) New groups and species belonging to the nominate subgenus *Paruroc-tonus* (Scorpiones, Vaejovidae). The Journal of Arachnology 13(1): 19–42. https://www.americanarachnology.org/journal-joa/joa-all-volumes/detail/volume/101/
- McWest KJ (2009) Tarsal spinules and setae of vaejovid scorpions (Scorpiones: Vaejovidae). Zootaxa 2001(1): 1–126. https://doi.org/10.11646/zootaxa.2001.1.1
- Miller AL, Makowsky RA, Formanowicz DR, Prendini L, Cox CL (2014) Cryptic genetic diversity and complex phylogeography of the boreal North American scorpion, *Paruroctonus boreus* (Vaejovidae). Molecular Phylogenetics and Evolution 71: 298–307. https://doi. org/10.1016/j.ympev.2013.11.005
- Mix HT, Ibarra DE, Mulch A, Graham SA, Chamberlain CP (2016) A hot and high Eocene Sierra Nevada. Geological Society of America Bulletin 128(3–4): 531–542. https://doi. org/10.1130/B31294.1
- Monod L, Cauwet L, González-Santillán E, Huber S (2017) The male sexual apparatus in the order Scorpiones (Arachnida): a comparative study of functional morphology as a tool to define hypotheses of homology. Frontiers in Zoology 14: e51. [48 pp] https://doi. org/10.1186/s12983-017-0231-z
- Munz PA, Keck DD (1949) California plant communities. Aliso: A Journal of Systematic and Evolutionary Botany 2(1): 87–105. https://scholarship.claremont.edu/aliso/vol2/iss1/
- RMWG [Regional Water Management Group of the Fremont Basin Integrated Regional Water Management Region] (2019) Fremont Basin integrated regional water management plan, Fremont, USA. https://www.californiacity-ca.gov/CC/images/\_Fremont-Basin-IRWMP\_ FINAL\_2019-02-22.pdf
- Sissom WD, Francke OF (1981) Scorpions of the genus *Paruroctonus* from New Mexico and Texas (Scorpiones, Vaejovidae). The Journal of Arachnology 9(1): 93–108. https://www. jstor.org/stable/pdf/3705219.pdf
- Stahnke HL (1970) Scorpion nomenclature and mensuration. Entomological News 81(12): 297–316. https://archive.org/details/biostor-77637/
- Stephenson RK (2013) Lithostratigraphic record of North Soda Lake, Carrizo Plain, San Luis Obispo County, California: Implications for late Pleistocene paleoclimate in a closed hypersaline lake basin. Master's Thesis, California State University of Bakersfield, Bakersfield, USA. https://scholarworks.calstate.edu/concern/theses/x920g203d
- Stoffer PW (2004) Desert landforms and surface processes in the Mojave National Preserve and vicinity. USGS Open-File Report 2004–1007. https://doi.org/10.3133/ofr20041007
- Stout D, Buck-Diaz J, Taylor S, Evens JM (2013) Vegetation mapping and accuracy assessment report for Carrizo Plain National Monument. California Native Plant Society, Sacramento USA. https://www.cnps.org/wp-content/uploads/2018/04/carrizo-mapping-report-2013.pdf

RESEARCH ARTICLE



# A new species of the hitherto monospecific genus *Pleonoporus* Attems, 1938 (Diplopoda, Spirostreptida, Odontopygidae)

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| Academic editor: Pavel                                   | Stoev | Received | 14 June 2022 | Accepted | 8 July 2022 | Publishee | d 15 August 2 | 2022 |
|--|-------|----------|--------------|----------|-------------|-----------|---------------|------|
| https://zoobank.org/2D882D31-2E76-4F9D-AB6B-62B183FFD554 |       |          |              |          |             |           |               |      |

**Citation:** Enghoff H, Akkari N (2022) A new species of the hitherto monospecific genus *Pleonoporus* Attems, 1938 (Diplopoda, Spirostreptida, Odontopygidae). ZooKeys 1117: 189–202. https://doi.org/10.3897/zookeys.1117.87765

#### Abstract

The hitherto monospecific genus *Pleonoporus* is revised based on the syntypes of *P. robustus* Attems, 1938, housed in Naturhistorisches Museum Wien (NHMW), as well as on specimens of what we interpret as a new species of the genus, *Pleonoporus tanzanicus* sp. nov., collected in Tanzania and housed in the Museum of Nature – Zoology, Leibnitz Institute for the Analysis of Biodiversity Change (ZMH) for more than a century. Both species are described based on habitus and gonopod structures and illustrated with micrographs, whereas scanning electron microscope images are also provided for the new species. This paper further highlights the importance of natural history collections for taxonomic research and studies on species diversity in general.

#### Keywords

Africa, gonopods, natural history collections, new species, syntypes, taxonomy

# Introduction

In his work on "Diplopoden des Belgischen Congo", Attems (1938) continued with the colossal task of studying the myriapod diversity of the Congo, which he started almost a decade earlier (see also Attems 1929, 1934, 1935), aiming to document and describe the species of the area based on material sent to him for study by the Museum of the Belgian Congo (now The Royal Museum for Central Africa). In the same publication, Attems (1938) recorded 70 species and subspecies for the region, including 25 taxa new to science. Among these, 13 belonged to the family Odontopygidae (Diplopoda, Spirostreptida), classified in the genera *Odontopyge* Brandt, 1841; *Haplothysanus* Attems, 1909; *Chaleponcus* Attems, 1928; *Spinotarus* Attems, 1909; *Plethocrossus* Attems, 1909; *Rhamphidarpe* Attems, 1914; *Solenozophyllum* Attems, 1914 and *Pleonoporus* Attems, 1938. While some of these genera have recently been the subject of taxonomic revisions (e.g., Enghoff 2014, 2022), the genus *Pleonoporus* has remained known only from its original description and its only species *Pleonoporus robustus* Attems, 1938, described from Elisabethville (now Lubumbashi) in the south-western part of the Democratic Republic of the Congo (Attems 1938). The species *Pleonoporus robustus* has never been collected again, nor had its type series been re-studied. At the same time, no other representatives of the genus had ever been discovered.

In this work, we describe a new species of the genus *Pleonoporus*, *P. tanzanicus* sp. nov., based on material collected from Tanzania and housed in ZMH, and document the type species of the genus, *P. robustus*, based on the syntypes housed in NHMW. Both species are described, illustrated and compared, and the genus *Pleonoporus* is redefined.

## Material and methods

The specimens on which the new species is based were collected by "Ostafrika-Expedition der Hamburger Geographischen Gesellschaft in 1911."

The syntypes of *Pleonoporus robustus* were examined using a Nikon SMZ25 stereomicroscope and images were obtained with a Nikon DS-Ri2 camera mounted on the same stereomicroscope, using NIS-Elements Microscope Imaging Software (version 5.02) with an Extended Depth of Focus (EDF) patch (Nikon Corporation, Tokyo, Japan). A male of the new species was studied by scanning electron microscopy (SEM): body parts were cleaned with ultrasound, transferred to 96% ethanol, then to acetone, air dried, mounted on aluminium stubs or on triangles of flexible aluminium tape in turn mounted on a stub, coated with platinum/palladium and studied in a JEOL JSM-6335F scanning electron microscope. Images were processed in Adobe PhotoShop CS6 and assembled into figure plates with InDesign CS6 or Microsoft Publisher.

Morphological terminology mostly follows Enghoff (2022), with additional terms taken from Enghoff (2014).

Specimens are kept in the following collections:

| NHMD | Natural History Museum of Denmark   |
|------|---|
| NHMW | Naturhistorisches Museum Wien   |
| ZMH  | Museum of Nature – Zoology, Leibnitz Institute for the Analysis of Biodi- |
|      | versity Change, Bonn  |

## Taxonomy

Class Diplopoda De Blainville in Gervais, 1844 Order Spirostreptida Brandt, 1833 Family Odontopygidae Attems, 1909 Subfamily Archepyginae Manfredi, 1939 Tribe Prionopetalini Hoffman, 1991

#### Genus Pleonoporus Attems, 1938

**Diagnosis.** Differs from all other genera of Archepyginae by having ozopores on body ring 5, a condition paralleled in the subfamily Peridontopyginae (see Enghoff 2022: 128). In other characters, especially the structure of the gonopod telomere, *Pleonoporus* resembles the genus *Spinotarsus* Attems, 1909.

**Type species.** *Pleonoporus robustus* Attems, 1938, by monotypy **Other included species.** *Pleonoporus tanzanicus* sp. nov.

#### Pleonoporus tanzanicus sp. nov.

https://zoobank.org/D825A23A-B618-41E9-B98B-0871CF67545B Figs 1–4

**Diagnosis.** Differs from *P. robustus*, its only congener, by having a long, slender, distolaterad hooked spine on the gonopod coxa; in *P. robustus*, there is no trace of such spine.

**Material examined.** (total 5  $\Im$   $\Im$ , 1 (juvenile)  $\bigcirc$ ). *Holotype.* TANZANIA •  $\Im$ ; Singida Region, Manyoni District, Kilimatinde; 05°50'S, 34°58'E; 26 Jan. – 08 Feb. 1911; E. Obst leg.; *verbatim* label text: "Ostafr.-Exp. d. Hamb. Geogr. Ges. Kilimatinde, Landschaft Ugogo Dr, E. Obst leg., 26.I –8.II. 1911. Geogr-. Ges. ded. 3.x.1912"; (ZMH) *Paratypes.* TANZANIA •  $\Im$ ; Singida Region, Manyoni District, Saranda-Sawa [here understood as the village of Saranda ca. 14 km N of Kilimatinde]; 05°43'S, 34°59'E; 15–16 Feb. 1911; E. Obst leg. ZMH-A0016675; NHMD 621850; *verbatim* label text: "Ostafr.-Exp. d. Hamb. Geogr. Ges. Ugogo und Turu, Saranda-Sawa, Dr. E. Obst leg., 15.–16.II. 1911, Geogr-. Ges. ded. 3.x.1912"; additional labels: "*Spinotarsus*?, R.L. Hoffman det. 1966", "Odontopygidae: ?n.gen. oder viell. *Spinotarsus*?" [label by Krabbe?] •  $\Im$ ; Singida Region, Manyoni District, Mahalala [here understood as the village of Muhalala ca. 11 km WNW of Kilimatinde]; 05°47'S, 34°53'E; 12–14 Feb. 1911; E. Obst leg.; ZMH-A0016676; *verbatim* label text: "Ostafr.-Exp. d. Hamb. Geogr. Ges. Mahalala, Landschaft Ugogo leg. Obst, 12/14.II. 1911" • 2  $\Im$ , 1 (juvenile)  $\bigcirc$ ; same collection data as holotype, NHMW MY 10277 ( $\Im$ ).

**Description.** (males). SIZE. Length ca. 8 cm. Diameter 5.5–6.0 mm. 60–62 podous rings, no apodous rings in front of telson.

**Colour** (Fig. 1). Quite faded after 111 years in alcohol. Head below antennal sockets, antennae and legs yellowish brown. Head above antennal sockets, collum, rings 2



**Figure 1.** *Pleonoporus tanzanicus* sp. nov. **A** paratype ♂, habitus (ZMH ZMH-0016676) **B** holotype ♂ (ZMH-0016675) Last four body rings and telson **C** paratype ♂, head and first nine body rings (NHMW MY10277). Scale bars: 5 mm (**A**); 1 mm (**B**, **C**).

and 6 almost uniformly dark brown. Other body rings greyish or whitish, with some irregular darker blotches; posterior part of metazonites amber. Traces of a pattern can be seen in some specimens, in which the prozonites from midway between the ozopore level and the midline are yellowish, white in the anterior half and a contrasting blackish



**Figure 2.** *Pleonoporus tanzanicus* sp. nov., paratype,  $3^{\circ}$  (NHMD 621850) **A, B** sternum 9 **A** anterior view **B** ventral view **C–E** first pair of legs **C** anterior view **D** sublateral view **E** ventral view **F** limbus. Abbreviations: *APS* = mesapical prefemoral setae; *CXS* = coxosternal setae; *LPS* = lateral prefemoral setae. Scale bars: 0.2 mm (**A–E**); 0.02 mm (**F**).

in the posterior half; anterior part of body with traces of a narrower dorsal longitudinal light band flanked by a darker coloured zone.

*Head.* Area below supralabral setae vertically wrinkled, otherwise smooth; parietal furrow distinct, interocular furrow very faint; supralabral setae abraded and uncountable in most specimens, but six setae/sockets can be seen in some; eyes extending to medial tangent to antennal sockets. Mandibular stipes with a not very pronounced disto-ventral lobe; distal margin very shallowly emarginated.

**Collum**. With rectangular lateral lobes; a marginal furrow and one further furrow extending almost halfway to dorsal midline, two short furrows between them.



**Figure 3.** *Pleonoporus tanzanicus* sp. nov., paratype,  $\delta$  (NHMD 621850), left gonopod coxa **A** anterior view **B** posterior view **C** mesal view. Abbreviations: CU = cucullus; hu = lateral hump; LCS = lateral coxal spine; MF = metaplical flange; mk = metaplical knob; MP = metaplica; ms = metaplical shelf; msp = metaplical spine; mst = metaplical shelf tooth; mts = metaplical transverse spine; PP = proplica. Scale bars: 0.2 mm.

**Body rings**. Unvaulted; prozonites with fine, finely punctate ring furrows; suture straight, simple; metazonites with rather dense, deep longitudinal furrows in ventral part; on anterior body rings the furrows reach almost to ozopores level, further back they stop well below the pores and are not so deep. Ozopores starting on ring 5, visible as black dots (Fig. 1C). Limbus (Fig. 2F) with pointed, triangular lobes, lobes slightly longer than broad at base.

*Telson* (Fig. 1B). Preanal ring not keeled. Anal valves smooth, with well-developed dorsal spine and raised margins; no ventral spine or "corner"; setae not visible, no rave-lins. Subanal scale simple.

*Legs*. Length ca. 0.8 × body diameter. Prefemoral and tibial pads present on all legs from pair 6 backwards.

*First pair of legs* (Fig. 2C–E). With short, broad, regularly rounded lobes, almost semicircular in ventral view. Ca. 6 long coxosternal setae (*CXS*) in a group lateral to, and well separated from, prefemoral lobe. Prefemora with two to three mesapical setae (*APS*) and ca. five lateral setae (*LPS*).

**Sternum 9** (Fig. 2B, C). Massive; in anterior view triangular with a basal nonsclerotized incision resulting in an inverted V-shaped appearance; on posterior side strongly swollen.



**Figure 4.** *Pleonoporus tanzanicus* sp. n., paratype,  $\mathcal{O}$  (NHMD 621850), left gonopod telopodite **A** anterior view **B** posterior view (basomere partly covered by mounting tape (orange)) **C** ventral (apical) view **D** basal (dorsal) view **E** mesal view **F** tip of solenomere. Abbreviations: *al* = anterior lobe of telomere; *ARC* = arculus; *fl* = basal flap of solenomere; *pl* = posterior lobe of telomere; *SLM* = solenomere; *TL I* = torsotope lobe; *TM* = telomere; *TT* = torsotope. Scale bars: 0.2 mm (**A–E**); 0.02 mm (**F**).

**Gonopod coxa** (Fig. 3). In anterior view with a large hump (hu) on lateral margin; a long, slender, disto-laterad hooked spine (*LCS*) originating from distal part of hump. Mesal margin of proplica (*PP*) slightly sinuous, proplical lobe hid-

den from view by process (msp) from metaplica. Metaplica (MP) with large flange (MF) extending almost until half-height of coxa, ending abruptly but without a distal process. Metaplica distal to flange with a large horizontal shelf (ms) across mesal surface, ending posteriorly in triangular tooth (mst); a rounded knob (mk) projecting from mesal margin of metaplica, facing *mst*. Further distally, a lateral extension of the metaplica gives rise to a long, stout, transverse spine (mts) which curves posteriad across mesal surface of coxa, ending between *mst* and *mk*. Distal part of coxa, cucullus (CU) sensu Enghoff (2022), in anterior view resembling a bird's head with the curved "beak" pointing meso-basad; a long, slender, slightly curved spine (msp) originating from base of cucullus on anterior side, extending basad along mesal margin of proplica.

**Gonopod telopodite** (Fig. 4). Arculus (ARC) 90°; torsotope (TT) compact, with a rounded torsotope lobe (TL); no post-torsal spine. Solenomere (SLM) much longer than telomere, slender, whip-like, without a basal solenomeral spine, but with a small tongue-like flap (fl) at base; without any other process or modification. Telomere (TM) complex; basal shape a broad sheet curved in a semicircle and with the lateral margins partly folded in. Telomere distally separating into two lamellae, anterior lamella (al) with largely smooth margins, posterior lamella (pl) coarsely and irregularly serrate.

# Pleonoporus robustus Attems, 1938

Figs 5-7

**Diagnosis.** Very similar to *Pleonoporus tanzanicus* sp. nov., from which it differs by the absence of the lateral coxal spine (*LCS*) on the gonopods.

**Material examined.** *Syntypes:*  $6 \ \circled{S} \ \circ$ 

**Comments.** Attems (1938) mentioned a small projection on the ventral side of the anal valves "ein winziges Höckerchen" in his original description of the species. This structure was visible only in two of the male syntypes (Fig. 5E, arrow), whereas the rest of the specimens showed a regular ventral margin or just a slightly angular one. We dissected one of the males having anal valves with a regular ventral margin, and it showed gonopods identical to those described by Attems.

It was not possible to find the specimen from which Attems dissected and extracted the gonopods. However, the gonopod illustrations (Attems 1938: figs 86–89) perfectly match our illustrations (Figs 6–7) of the loose gonopods found in the jar, which we put in a separate vial (NHMW MY 9062). These may very well be the gonopods illustrated by Attems in the original description of the species.



**Figure 5.** *Pleonoporus robustus* Attems, 1938. Syntype & (NHMW MY 2666) **A** male, in toto lateral view **B** head and anteriormost rings, lateral view **C** close-up of gonopod in situ, lateral view **D** posteriormost rings and telson, lateral view **E** close-up of the telson, arrow indicating ventral projections. Scale bars: 5 mm (**A**); 1 mm (**B–D**).



**Figure 6.** *Pleonoporus robustus* Attems, 1938. Syntypes  $\mathcal{S}$  gonopods **A** gonopod block (NHMW MY9061), anterior view **B** left gonopod, posterior view (NHMW MY 9062). Abbreviations: CU = cucullus; hu = lateral hump; MP = metaplica; msp = metaplical spine; mts = metaplical transverse spine; PP = proplica; Scale bars: 1 mm.



**Figure 7.** *Pleonoporus robustus* Attems, 1938. Syntype  $\mathcal{J}$  gonopods (NHMW MY 9062) **A** right gonopod, anterior view **B** antero-mesal view. Abbreviations: al = anterior lobe of telomere, CU = cucullus; *hu* = lateral hump; *MF* = metaplical flange, *MP* = metaplica; *msp* = metaplical spine; *mts* = metaplical transverse spine; *PP* = proplica; pl = posterior lobe of telomere, *SLM* = solenomere; *TM* = telomere; *TT* = torsotope. Scale bars: 1 mm.

## Discussion

The new species from Tanzania described here is very similar to *P. robustus* from the Democratic Republic of the Congo, but although there is really only one distinguishing character, i.e., the lateral coxal spine present in *P. tanzanicus* sp. nov. and absent in *P. robustus*, this spine is so conspicuous that we consider the Tanzanian specimens different from the Congolese ones at the species level. The geographical distance between the type localities of the two species is 1000 km, which in itself suggests (but does not prove) that different species are involved.

Another long-distance disjunction involving odontopygids was recorded by Hoffman and Howell (2012), although in this case the authors found no differences between type specimens of *Calyptomastix kakandae* (Kraus, 1958) from Upemba National Park in D.R. Congo and specimens referred to the same species but collected in SW Tanzania, some 600 km from the type locality. A similar distance separates the type locality of a third odontopygid, *Helicochetus mutaba* Kraus, 1960 (Kirungu, D.R. Congo), from two localities in the Iringa Region, Tanzania, from where *H. mutaba* was recorded by Enghoff (2018). The family Odontopygidae, in addition to containing a very large number of undescribed species, obviously also offers interesting distributional patterns.

Natural history collections continue to play a pivotal role as a source of undescribed species. A high number of unknown taxa are housed in museum collections, awaiting to be studied and described. An average shelf life of 20.7 years (range 0–206 years) was estimated for all species described in 2007 (Fontaine et al. 2012), and was explained by what these authors called the "taxonomic impediment", referring to the shortage of taxonomists capable of identifying, describing and documenting species (Fontaine et al. 2012). The type specimens of *P. tanzanicus* sp. nov. were collected in 1911, and although at least one of them had already been studied by two specialists, they had a "shelf life" of 111 years before finally being described herein. Although this is less than the species *Ommatoiulus schubarti* Akkari & Enghoff, 2012 (149 years, see Akkari and Enghoff 2012), *P. tanzanicus* sp. nov. has largely exceeded the average estimated shelf life of undescribed taxa in museum collections.

In other instances, species have randomly been discovered when studying related taxa and recovered among the type material of another previously described species. The relatively recently described species *Annamina attemsi* Golovatch, Geoffroy & Akkari 2017 is an example of such a species, which was described based on specimens mixed with the syntypes of *Annamina xanthoptera* Attems, 1937 housed in NHMW and discovered by chance by one of us (NA) when studying that type series. The species *Haasea gruberi* Antić & Akkari, 2020 is another example of a species discovered when revising the genus based on the NHMW collection. The species had been misidentified as *Haasea flavescens* (Latzel, 1884) by Attems in 1954, remained cryptic for some 70 years and was eventually described at the same time as a cavernicolous congener from Serbia (Antić and Akkari 2020). Misidenfications often happened in the past and in some cases discoveries of new taxa happened by comparing original species

descriptions with subsequent literature related to these taxa. For example, Read (2005) noticed differences between the gonopods of *Cylindroiulus distinctus* (Lucas, 1846) as illustrated in the original description (Lucas 1846) and those presented in the redescription of the species by Attems (1927) based on specimens collected in 1893 and housed in NHMW, the latter actually representing a completely new species, *Cylindroiulus attemsi* Read, 2005. Read (2005) based her description also on material from other natural history collections, with the oldest specimens housed in NHMD (then ZMUC) and dating back to 1869.

### Acknowledgements

Thanks to Danilo Harms (ZMH) for kindly loaning the specimens of the new species, and to Sree Gayathree Selvantharan (NHMD) and Oliver Macek (NHMW) for help with photographing the specimens. Our thanks are extended to Sergei Golovatch (Russian Academy of Sciences, Moscow) and Tarumbera Mwabvu (University of Mpumalanga, South Africa) for commenting on an earlier version of the manuscript.

### References

- Akkari N, Enghoff H (2012) Review of the genus *Ommatoiulus* in Andalusia, Spain (Diplopoda: Julida) with description of ten new species and notes on a remarkable gonopod structure, the fovea. Zootaxa 2528(1): 1–53. https://doi.org/10.11646/zootaxa.3538.1.1
- Antić D, Akkari N (2020) *Haasea* Verhoeff, 1895 a genus of tumultuous history and chaotic records – redefinition, revision of taxonomy and geographic distributions, with descriptions of two new species from Austria and Serbia (Diplopoda, Chordeumatida, Haaseidae). Zootaxa 4798 (1): 001–077. https://doi.org/10.11646/zootaxa.4798.1.1
- Attems C (1927) Über palaearktische Diplopoden. Archiv für Naturgeschichte 92(1–2): 1–256.
- Attems C (1929) Diplopoden des Belgischen Congo. I. Polydesmoidea. Revue de Zoologie et de Botanique Africaines 17(3): 253–378.
- Attems C (1934) Diplopoden des Belgischen Congo. II. Spirostreptoidea und Spiroboloidea. Revue de Zoologie et de Botanique Africaines 26(1): 98–111.
- Attems C (1935) Diplopoden des Belgischen Congo II. Spirostreptoidea und Spiroboloidea. Revue de Zoologie et de Botanique Africaines 26: 98–11 + 158–201 + 327–396.
- Attems CG (1938) Diplopoden des Belgischen Congo. Polydesmoidea, 2. Nachtrag und Spirostreptoidea, 1. Nachtrag. Revue de Zoologie et de Botanique Africaines 31(2): 225–313.
- Enghoff H (2014) A mountain of millipedes I: An endemic species-group of the genus Chaleponcus Attems, 1914, from the Udzungwa Mountains, Tanzania (Diplopoda, Spirostreptida, Odontopygidae). European Journal of Taxonomy 100(100): 1–75. https://doi. org/10.5852/ejt.2014.100
- Enghoff H (2018) A mountain of millipedes VI. New records, new species, a new genus, and a general discussion of Odontopygidae from the Udzungwa Mts, Tanzania (Diplopoda, Spi-

rostreptida, Odontopygidae). –. European Journal of Taxonomy 394(394): 1–29. https://doi.org/10.5852/ejt.2018.394

- Enghoff H (2022) Mountains of millipedes: The family Odontopygidae in the Eastern Arc Mountains of Tanzania (Diplopoda, Spirostreptida). European Journal of Taxonomy 803: 1–136. https://doi.org/10.5852/ejt.2022.803.1691
- Fontaine B, Perrard A, Bouchet P (2012) 21 years of shelf life between discovery and description of new species. Current Biology 22(22): R943–R944. https://doi.org/10.1016/j.cub.2012.10.029
- Golovatch S, Geoffroy J-J, Akkari N (2017) Revision of the Vietnamese millipede genus Annamina Attems, 1937, with descriptions of three new species (Diplopoda, Polydesmida, Paradoxosomatidae). ZooKeys 669: 1–18. https://doi.org/10.3897/zookeys.669.12561
- Hoffman RL, Howell KM (2012) A new genus of odontopygid millipeds from Tanzania (Diplopoda: Spirostreptida: Odontopygidae). Journal of East African Natural History 101(1): 67–72. https://doi.org/10.2982/028.101.0104
- Lucas M. H.1846. Notes sur quelques nouvelles espèces d'insectes qui habitent les possessions francaises du nord de l'Afrique. Revue zoologique, par la Société Cuvierienne: 283–289
- Read H (2005) A revision of the *Cylindroiulus distinctus* Lucas group from north Africa, with descriptions of six new species (Diplopoda, Julida, Julidae). Journal of Natural History 39(18): 1491–1532. https://doi.org/10.1080/0022293042000193689

RESEARCH ARTICLE



# A new species of the genus Prosopistoma Latreille, 1833 (Ephemeroptera, Prosopistomatidae) from Morocco

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| Academic editor: L. Pereira-da-Conceicoa   Received 10 March 2022   Accepted 25 July 2022   Published 15 August | 2022 |
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**Citation:** El Alami M, Benlasri M, Sartori M, Vuataz L, Ghamizi M (2022) A new species of the genus *Prosopistoma* Latreille, 1833 (Ephemeroptera, Prosopistomatidae) from Morocco. ZooKeys 1117: 203–218. https://doi.org/10.3897/ zookeys.1117.83539

#### Abstract

We describe a new species of *Prosopistoma* collected in the High Atlas Mountains of Morocco. *Prosopistoma maroccanum* **sp. nov.** appears to be morphologically more similar to the European highly endangered *P. pennigerum* (Müller, 1785) than to the other Maghrebian species, *P. alaini* Bojkova & Soldán, 2015. A gene tree including the few available barcode sequences of Palearctic *Prosopistoma* specimens is provided. Possible affinities with West African species are also discussed.

### Keywords

High Atlas Mountains, mayfly, North Africa, Western Palearctic

# Introduction

Prosopistomatidae is an Old-World family of Ephemeroptera with all 29 species belonging to the genus *Prosopistoma* Latreille, 1833. *Prosopistoma* is represented by 15 species in the Oriental region, six in the Afrotropical region, six species in the Palearctic

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region, and two in the Australasian region (Barber-James et al. 2008, 2013; Barber-James 2009, 2010a; Shi and Tong 2013; Bojkova and Soldán 2015; Balachandran et al. 2016; Schletterer et al. 2016, 2021; Roopa et al. 2017; Kazanci and Türkmen 2018; Boonsoong and Sartori 2019). The six Palearctic species occur in its western region: *Prosopistoma pennigerum* (Müller, 1785) (Europe), *Prosopistoma oronti* Alouf, 1977 (Levant), *Prosopistoma orhanelicum* Dalkiran, 2009 (Turkey), *Prosopistoma turcica* Kazanci & Turkmen, 2018 (Turkey), *Prosopistoma helenae* Bojkova & Soldán, 2015 (Middle East), and *Prosopistoma alaini* Bojkova & Soldán, 2015 (Algeria). Thus far, only this last species has been identified from the Maghreb.

*Prosopistoma pennigerum* is the most widespread species, with a range from France and Portugal to Russia (Schletterer et al. 2021); currently only three isolated populations in Spain, Albania, and Russia are documented.

The first record of a North African *Prosopistoma* population was made by Gagneur and Thomas (1988) who listed specimens from the Tafna watershed in western Algeria under the name *Prosopistoma ?pennigerum*. This population was described later by Bojkova and Soldán (2015) as the new species *Prosopistoma alaini*. This species is probably extinct in the wild, as documented by Benhadji et al. (2019). Touabay et al. (2002) made the second report of *Prosopistoma* nymphs in Maghrebian rivers, as *Prosopistoma* sp.; they collected their material at Tizguit Wadi in the Moroccan Middle Atlas (El Alami et al. 2022).

In the present study, we describe a new species of *Prosopistoma* based on nymphs from the High Atlas Mountains of Morocco. In addition, distinctive characters of Western Palearctic *Prosopistoma* species are provided.

#### Materials and methods

The nymphs of *Prosopistoma maroccanum* sp. nov. were collected at two sites on the Oued Laabid, a tributary of the Oum Errbia River which originates in the High Atlas Mountains (Fig. 1). The sampling was performed by Ghamizi team (director of the Museum of Natural Sciences of Marrakech) in 2016 and by Benlasri in 2021. They were subsequently preserved in 95% ethanol for description and DNA extraction. Nymphal dissection was performed in Cellosolve or in 10% KOH, and specimens were mounted on slides with Euparal medium, or the dissected parts of the nymphs were mounted directly in Hoyer's liquid (Alba-Tercedor 1988).

Several specimens belonging to our new species were processed for DNA extraction, targeting a fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI). Despite numerous attempts, the COI of only one nymph was successfully sequenced. Total genomic DNA was extracted using the BioSprint 96 extraction robot (Qiagen Inc., Hilden, Germany), following the supplier's instructions. The non-destructive protocol described by Vuataz et al. (2011), which enables postextraction morphological study of specimens, was implemented. We then amplified a 658-bp fragment at the 5' end of the COI, corresponding to the standard animal barcode region, using the HCO2198 and LCO1490 primers (Folmer et al. 1994). Polymerase chain reaction (PCR) was conducted in a volume of 25  $\mu$ l, consisting of



Figure 1. The sampling site localization of Prosopistoma maroccanum sp. nov. in Laabid River, Morocco.

5  $\mu$ l (unknown concentration) of template DNA, 1.3  $\mu$ l (10  $\mu$ M) of each primer, 0.2 µl (25 mM) of dNTP solution (Promega), 5 µl of 5× buffer (Promega) containing 7.5 mM of MgCl<sub>2</sub>, 2.5 µl (25 mM) of MgCl<sub>2</sub>, 1 U of Taq polymerase (Promega), and 9.7 µl of sterile ddH<sub>2</sub>O. Optimized PCR conditions included initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, with final extension at 72 °C for 7 min. Purification and automated sequencing was carried out in Microsynth (Balgach, Switzerland). We also included all Palearctic Prosopistoma COI sequences available in GenBank and BOLD, resulting in four additional sequences from two species (Table 1). We explored the evolutionary divergence between our new species and the others using the COI genetic distances. The number of parsimony-informative sites and the mean distances between species were calculated in MegaX (Kumar et al. 2018; Stecher et al. 2020) under the Kimura 2-parameter (K80) substitution model (Kimura 1980). We then applied the recently developed species delimitation method ASAP (Assemble Species by Automatic Partitioning; Puillandre et al. 2021) to our COI dataset using the graphical web-interface at https://bioinfo.mnhn.fr/ abi/public/asap/asapweb.html. This distance-based method is similar to the popular ABGD (Automatic Barcode Gap Discovery; Puillandre et al. 2012) approach but has the advantage of providing a score that specifies the most likely species delimitation. Pairwise genetic distances were computed under the K80 model, and all other settings were set to default. Finally, we conducted a Bayesian inference gene tree reconstruction in MrBayes v. 3.2.7a (Ronquist et al. 2012), using the best evolutionary model (HKY +  $\Gamma$ ) selected in JModelTest 2.1.10 (Darriba et al. 2012) following the second-order Akaike information criterion (AICc; Hurvich and Tsai 1989). We used 11 substitution scheme and six gamma categories, with all other parameters set to default. To accommodate different substitution rates among COI codon positions, we analyzed our data set in two partitions, one with first and second codon positions, and one with third positions (1 + 2, 3). Two independent analyses of four MCMC chains run for one million generations with trees sampled every 1000 generations were implemented, and 200,000 generations were discarded as a burn-in after visually verifying run stationarity and convergence in Tracer v. 1.7.2 (Rambaut et al. 2018). The consensus tree was visualized and edited in iTOL v. 6 (Letunic and Bork 2021). Two *Baetisca* sequences were chosen as the outgroup.

The material is deposited in the collections of the Museum of Natural History in Marrakech (**MHNM**), the Laboratoire Ecologie, Systématique, Conservation de la Biodiversité (**LESCB**) in Tétouan, and the Museum of Zoology in Lausanne (**MZL**).

| Species  | Specimen catalogue | Stage | Locality                | GPS<br>coordinates                 | Date        | GenBank ID | GenSeq<br>Nomenclature |
|--|--------------------|-------|-------------------------|------------------------------------|-------------|------------|------------------------|
| Prosopistoma   | GBIFCH 00970951    | Nymph | Morocco, Oued           | 32°8.252 N,                        | 6.iii.2016  | ON920528   | genseq-2 COI           |
| maroccanum <b>sp. nov.</b><br>Prosopistoma<br>pennigerum |                    | Nymph | Albania, Vjosa          | 7 1.764 W<br>40.316°N,<br>20.030°E | 2018        | MZ707155   | genseq-4 COI           |
| Prosopistoma<br>pennigerum                               |                    | Nymph | Russia, Volga,<br>Rzhev | 56.260°N,<br>34.321°E              | 2018        | MZ707154   | genseq-4 COI           |
| Prosopistoma oronti                                      |                    | Nymph | Israel, En Tina         | 33.078°N,<br>35.644°E              | 27.iii.2019 | MN958840   | genseq-4 COI           |
| Prosopistoma oronti                                      |                    | Nymph | Israel, En Tina         | 33.078°N,<br>35.644°E              | 27.iii.2019 | MN958841   | genseq-4 COI           |

Table 1. Codes, origin, and nomenclature of sequences used in molecular study.

# Results

#### Molecular analysis

The COI ingroup data set were 95% complete and included 14% of parsimony informative sites. The missing data resulted from the two *P. pennigerum* sequences from GenBank that lacked 5' and 3' ends. All COI gene tree relationships were highly supported, with the *Prosopistoma maroccanum* sp. nov. COI sequence recovered as sister to the other *Prosopistoma* sequences, which were split into two well-supported monophyletic clades according to their species affiliation (Fig. 2). The three *Prosopistoma* species, including *P. maroccanum*, were supported as distinct species in the ASAP analysis. The K80 mean genetic distance between *P. maroccanum* and the other two species ranged from 34.3% (mean distance to *P. pennigerum*) to 35.6% (mean distance to *P. oronti*).



**Figure 2.** Bayesian majority-rule consensus tree reconstructed from the COI data set of the available Palearctic *Prosopistoma* species. The branch labelled with the GBIF code represents the newly sequenced specimen (species name in bold), other codes correspond to previously published GenBank sequences. Vertical colored boxes indicate species delimitation hypothesis according to the ASAP analysis. The outgroups are represented in grey. Circles on branches indicate Bayesian posterior probabilities of 1.

## Morphological analysis

# Prosopistoma maroccanum El Alami, Benlasri & Sartori, sp. nov.

https://zoobank.org/9C03C91D-8E32-47C7-9FD1-DE01A7093072

**Material examined.** *Holotype:* MOROCCO • 1 nymph in ethanol; Béni-Mellal Province, Oued Laabid, in Bzou village, 32°6.076'N, 7°2.644'W, 372 m alt., 14 December 2021, coll. M. Benlasri (MZL GBIFCH01119080). *Paratypes:* MOROCCO • 1 nymph in ethanol, same data as holotype (MZL GBIFCH01119081); 1 nymph on slide, same data as holotype (MZL GBIFCH00608997; 7 nymphs in ethanol, same data as holotype (LESCB); 1 nymph on slide, same data (LESCB); 3 nymphs in ethanol, same sampling site, 08 May 2021; 4 nymphs in ethanol, same sampling site, 14 October 2021, Coll. M. Benlasri (MHNM) • 4 nymphs in ethanol, Béni-Mellal Province, Oued Laabid, in Imdahen village, 32°8.252'N, 7°1.764'W, 364 m alt., 06 March 2016, coll. H. Hajjani (MZL GBIFCH00980869); 1 nymph on slide, same data (MZL GBIFCH00970951); 5 nymphs in ethanol, same site, 14 October 2021, Coll. M. Benlasri (MHNM).

**Description.** Nymph (in alcohol). Body length 3–4 mm excluding caudal filaments.

Notal shield (carapace) length along median suture 2 mm and total length of body 4.0 mm. Carapace (Fig. 3), wider than long, width/length ratio: 1.15–1.25; head width/length ratio:2.5, head width/carapace width ratio: 0.58–0.60; distance between eyes/head width ratio: ca 0.53 for male nymphs and 0.58 for female nymphs. Carapace flange relatively wide (Fig. 3).

*Head.* Yellowish-brown, with a brownish spot above the median ocellus and between antennae (Fig. 3). Compound eyes roughly oval, blackish, slightly larger than oval lateral ocelli in male nymphs (Fig. 4A). Epicranial suture hardly discernible crossing the middle part of lateral ocelli, and between compound eyes and antennal bases (Fig. 4A) and progressing to the head's lateral margin. Antennae 7-segmented (including scape and pedicel) in mature nymphs (Fig. 4B), 6-segmented in younger specimens (Fig. 4C); segment III shorter than the total length of segment IV–VI or VII (respectively 0.64× and 0.95×), length of segment III/segments IV–V ratio: 1.18–1.21; antennae not extending beyond the head's anterior edge.

Labrum (Fig. 5A) narrow, 3.25× broader than long, anterior margin convex in medial section and straight laterally, anterior margin fringed with fine setae.

Left and right mandibles similar (Fig. 5B). Outer canine distinctly longer and wider than inner canine with three apical teeth, inner one slightly longer than the two others. Outer margin of outer canine with 4–6 micro teeth, inner margin of outer canine with 4 or 5 micro teeth; first distal micro tooth large, conspicuous, more than twice as long and wider than others. Mandible inner canine, one-third shorter than outer canine, rod-shaped, apically bifurcate and forming a pair of elongate and thin pointed teeth with three subapical sharply pointed teeth. 6 or 7 long serrated bristles arising from base of inner canine (Fig. 5C); two first anterior bristles shorter than remaining, and one long simple bristle in the middle of outer margin of mandible.

Maxillae (Fig. 5D, E) crowned by a rigid canine and three moveable, strong dentisetae of subequal length (Fig. 5E). A row of 2 or 3 strong serrated bristles appear below the dentisetae and a single finer bristle on proximal part of sclerotized galea (Fig. 5E). Maxillary palp 3-segmented; segment II long and clearly longer than segment I (1.3–1.5 times) (Fig. 5D).



Figure 3. Nymphal dorsal view of Prosopistoma maroccanum sp. nov.



Figure 4. *Prosopistoma maroccanum* sp. nov. A head of larva B antennae with 7 segments C antennae with 6 segments.

Labial palps 3-segmented, reaching front margin of labium (Fig. 5F). Labial palp segment II 0.9× length of segment I, segment III 0.5× length of segment II (Fig. 5G).

**Thorax.** Carapace yellowish brown, with distinct ornamentation (Fig. 6A) and four irregular brownish markings, two on each side of midline of central region of carapace at approximately 0.20× length of carapace from posterior margin of head (Fig. 3); also, there are markings from the anterior end of the carapace at approximately 60° angle to the



**Figure 5.** *Prosopistoma maroccanum* sp. nov. **A** labrum (dorsal view) **B** right mandible (ventral view) **C** bristles arising from base of inner canine **D** left maxilla (ventral view) **E** canine (c) and moveable strong dentisetae (d1, d2, d3) **F** labium (ventral view) **G** labial-palp.



**Figure 6.** *Prosopistoma maroccanum* sp. nov. **A** posteromedial part of carapace **B** scale-like pattern on femur (ventral view) **C** ventral margin of fore tibia **D** gill I **E** gill II **F** gill III **G** abdominal segments VII–IX.

midline; carapace flange and distal part of carapace transparent but not apparently translucent; relatively wide. Posteromedial part of carapace distinctly concave (Figs 3, 6A).

*Legs.* Fore femora with reticulate pattern on dorsal surface consisting of scalelike structures more accentuated at the anterior and posterior border (Fig. 6B); dorsal margin of femora with simple and fine setae; ventral margin of fore tibia with 6–8 pectinate setae (Fig. 6C). All tarsal claws sharp, slender, smooth, and without denticles.

**Abdomen.** Abdominal gills (Fig. 6D–F). Gill I upper portion lamellate with serrated margin, apically slightly asymmetric and rounded; lower section cleaved in numerous filaments with about 6–8 major branches divided into 17–22 filaments (Fig. 6D). Gill II with rectangular lamella (ratio width/length ca 1.10); posterior margin and outer lateral margin concave with short, pointed spines; inner lateral margin convex without spines (Fig. 6E), covering gills III–V appearing with multiple branching filaments, decreasing in number towards gill V (Fig. 6F); gill III with 6 main stems; gill VI conical in shape, very small and unbranched. Abdominal segments VII–IX apparently angular with straight posterior margins; posterolateral projections nearly symmetric, with straight inner margins and relatively pointed apex (Fig. 6G). Segment X rectangular, relatively longer than wider. Caudal filaments, retractile, short, plume-like.

#### Imago. Not known.

**Morphological remark.** As already mentioned by Gillies (1954) and Peters (1967), and well documented by Dalkiran (2009) and Schletterer et al. (2016), the number of segments forming the antenna is subject to some variation. In *P. maroccanum*, mature nymphs possess 7 segments, while younger ones have only 6 segments. Therefore, the ratio length of segment III vs length of the remaining segments is to be use cautiously and may have a taxonomic value only when applying to mature nymphs with 7 segments, since this ratio is around 0.95 (subequal) for 6-segmented antennae vs 0.65 (much shorter) for 7-segmented antennae.

**Diagnosis.** The nymph of *P. maroccanum* sp. nov. appears to be more closely similar to *P. pennigerum* than to *P. alaini* from Algeria (Table 2).

Indeed, it differs from the latter in several aspects, mainly the distinct ornamentation of the carapace, the lower number of setae on the right mandible (6 vs 8–9), the more numerous subapical teeth on outer margin of the outer canine (6–7 vs 5), the number of maxillary dentisetae (3 vs 4), the distinctly concave distal medial margin of carapace (almost straight in *P. alaini*), and above all by the fewer pectinate setae on the inner margin of fore tibia (7–8 vs 10–14). This last character also separates *P. maroccanum* from *P. pennigerum* (10–11), as well as the number of antennal segments in mature nymphs (7 vs 6), the antenna not reaching the anterior margin of the head (reaching in *P. pennigerum*), and the ratio width/length of the carapace higher in *P. maroccanum* (1.25 vs 0.9). Furthermore, gill I has the apical tip of the dorsal lamina shorter in *P. maroccanum* than in *P. pennigerum*. In addition, the ventral filamentous part possesses a number of main stems which overlap (7–8 vs 8–10) but with a greater number of filaments in *P. pennigerum* (24–28

| Characters  | P. maroccanum      | P. pennigerum        | P. alaini         | P. oronti          | P. orhanelicum | P. turcica         | P. helenae         |
|---|--------------------|----------------------|-------------------|--------------------|----------------|--------------------|--------------------|
| Antennal segments in mature nymphs (N)                | 7                  | 6                    | 7                 | 5                  | 7              | 6                  | 6                  |
| Antenna reaching/not reaching anterior margin of head | not reaching       | reaching             | not reach-<br>ing | not reach-<br>ing  | not reaching   | not reach-<br>ing  | reaching           |
| Setae on the right mandible $(N)$                     | 6–7                | 7-8                  | 8-9               | 7–9                | 7              | 5                  | 5                  |
| Subapical teeth on outer margin of outer canine $(N)$ | 6–7                | 6–8                  | 5                 | 6–9                | 7–8            | 4–5                | 46                 |
| Pectinate setae on inner margin of fore tibia (N)     | 6–8                | 10-11                | 10-14             | 6–7                | 9–10           | 7                  | 6–7                |
| Filaments on gill I (N)                               | 17-22              | 24-28                | 21-23             | 12-14              | >40            | 20-22              | 15-17              |
| Lateral outer margin of gill II                       | concave            | concave              | concave           | concave            | concave        | straight           | straight           |
| Shape of distal medial margin of carapace             | distinctly concave | shallowly<br>concave | straight          | distinctly concave | convex         | distinctly concave | distinctly concave |
| Ornamentation of the carapace                         | distinct           | distinct             | indistinct        | distinct           | indistinct     | distinct           | indistinct         |
| Ratio carapace width / length                         | 1.15-1.25          | 0.8-0.9              | 1.1               | 1.1 - 1.2          | 1.1 - 1.4      | 1.13               | 1.2-1.3            |
| Distribution  | Morocco            | Europe               | Algeria           | Levant             | Turkey         | Turkey             | Iraq               |

**Table 2.** Morphological discriminant characters between the seven *Prosopistoma* species from the Western Palearctic.

vs 17–22). Prosopistoma maroccanum differs from *P. oronti* mainly by the number of antennal segments (5 in *P. oronti*) and the length of segment II of the antenna shorter compared to the following segments (longer in *P. oronti*). It differs from *P. turcica* mainly by the number of antennal segments (6) and the setation of the right mandible, from *P. orhanelicum* by the fewer subapical teeth on inner margin of outer canine (4–5 vs 6–7), the shape of the distal medial margin of the carapace (convex in *P. orhanelicum*), and the fewer pectinate setae on the inner margin of fore tibiae (9–10 in *P. orhanelicum*). From *P. helenae*, *P. maroccanum* differs in having abdominal segments VII–IX angular (rounded in *P. helenae*) and in the number of antennal segments and length (antenna 6-segmented and reaching the anterior margin of the head in *P. helenae*).

**Ecology.** *Prosopistoma maroccanum* sp. nov. was collected at two sites in the Laabid River, 117 km from Marrakech. The greatest density, 10 specimens, was recorded in December 2021 at site 2 (Imdahen locality; Fig. 7B). At this site, the bottom structure was composed of 70% pebbles, 20% gravel, and 10% silt. During the sampling campaign, water temperatures were 22.4–23.7 °C, dissolved oxygen 6.57–8.26 mg/l, pH 8.05–8.17, and conductivity 977–999  $\mu$ S/cm. The channel, about 6 m wide and about 20 cm deep, had a moderate current velocity and turbid water. There was almost no riparian vegetation along the stream banks and no submerged macrophyte cover. *Prosopistoma* species are very sensitive to organic pollution and habitat degradation (Barber-James 2010a). However, there are small villages near the two sampled locations (Fig. 7A, B) which probably increased the turbidity and organic pollution in the water. These impacts probably explain the low density, or the absence of this species, at other sampled sites. The ecological aspects such as microhabitat, nutrition, life history, and phenology of *P. maroccanum* sp. nov. should be further investigated.



Figure 7. Sampling sites of *Prosopistoma maroccanum* sp. nov. A site 1 (Bzou locality) B site 2 (Imdahen locality).

## Discussion

The discovery of a new population and species of *Prosopistoma* in the High Atlas of Morocco is surprising and shows that the biodiversity of Maghrebian mayfly fauna is far from well known. *Prosopistoma* nymphs are so characteristic that we cannot consider their presence has been overlooked by previous studies. The occurrence of *P. maroccanum* sp. nov. is currently limited to a single stream, which may bring arguments that the species should be considered as Critically Endangered based on IUCN criteria. The true identity of the population studied by Touabay et al. (2002) from the Middle Atlas needs also to be explored.

We also investigated the possibility that *P. maroccanum* may represent a relict population of an Afrotropical species. During the African Humid Period, in the late Pleistocene and early Holocene, between roughly 120,000 and 11,500 years before present (de Menocal et al. 2000; Quade et al. 2018), the Sahara was covered by grass

savannah, shrubs, and trees, with lakes and running water abundant. In particular, the Tamanrasset paleoriver in western Africa was an important link between the Senegal and Niger watersheds in the south and the streams and rivers of the Atlas Mountains in the north, acting as a pathway for the spread of animals and humans (Skonieczny et al. 2015).

In her extensive and complete review of the Prosopistomatidae worldwide, Barber-James (2010b) studied all described species and several undescribed ones. Among the material, some specimens from West Africa were analysed (J.-M. Elouard collection housed in MZL) and sampled in Guinea, Ivory Coast, Togo, and Sierra Leone (Barber-James 2010b: 298); these she referred to as "African sp. 7". She recovered all investigated species as belonging to two clades, one called the "*P. variegatum* clade" and the other the "African clade". Based on the character matrix of Barber-James (2010b: 342–343), *P. maroccanum* would cluster within the "*P. variegatum* clade", together with *P. pennigerum* and other Palearctic species analysed; African sp. 7 is nested within the "African clade". We can therefore conclude that *P. maroccanum* is probably Palearctic in origin, separate from the African species, as shown by several characters, among which is the shorter inner incisor of the mandibles, which is subequal to the outer in all species of the "African clade".

## Acknowledgements

We express our gratitude to Céline Stoffel (MZL) for her dedicated work with the molecular lab. The sampling was supported by the Critical Ecosystem Partnership Fund (project 110217).

# References

- Alba-Tercedor J (1988) Ephemeroptera. In: Barrientos JA (Ed.) Bases para un curso práctico de Entomología, Asociación Española de Entomología 6(2): 359–372.
- Alouf NJ (1977) Sur la presence du genre Prosopistoma au Liban. Description de P. oronti n.sp. et de P. phoenicium n.sp. (Ephemeroptera). Annles de Limnologie. 13(2): 133–139. https://doi.org/10.1051/limn/1977006
- Balachandran C, Anbalagan S, Kannan M, Dinakaran S, Krishnan M (2016) A new species of *Prosopistoma* Latreille, 1833 (Ephemeroptera: Prosopistomatidae) from South India. Zootaxa 4178(2): 289–294. https://doi.org/10.11646/zootaxa.4178.2.7
- Barber-James HM (2009) A preliminary phylogeny of Prosopistomatidae (Ephemeroptera) based on morphological characters of the larvae, and an assessment of their distribution. Aquatic Insects 31(sup1): 149–166. https://doi.org/10.1080/01650420903020502
- Barber-James HM (2010a) Two new species of Prosopistomatidae (Ephemeroptera) from South Africa and Swaziland. African Entomology 18(1): 147–165. https://doi. org/10.4001/003.018.0112

- Barber-James HM (2010b) Systematics, morphology, phylogeny and historical biogeography of the mayfly family Prosopistomatidae (Ephemeroptera: Insecta) of the world. PhD thesis, Rhodes University, Grahamstown, South Africa, 395 pp.
- Barber-James HM, Gattolliat JL, Sartori M, Hubbard MD (2008) Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. Hydrobiologia 595(1): 339–350. https://doi. org/10.1007/s10750-007-9028-y
- Barber-James HM, Gattolliat J-L, Sartori M, Webb J (2013) World checklist of freshwater Ephemeroptera species. http://fada.biodiversity.be/group/show/35. [Accessed on: 2022-7-26]
- Benhadji N, Abdellaoui Hassaine K, Gattolliat J-L, Sartori M (2019) Thirty Years after: An update to the mayflies composition in the Tafna Basin (Algeria). Zoosymposia 16: 22–35. https://doi.org/10.11646/zoosymposia.16.1.6
- Bojkova J, Soldán T (2015) Two new species of the genus *Prosopistoma* (Ephemeroptera: Prosopistomatidae) from Iraq and Algeria. Zootaxa 4018(1): 109–123. https://doi.org/10.11646/ zootaxa.4018.1.6
- Boonsoong B, Sartori M (2019) Review and integrative taxonomy of the genus *Prosopistoma* Latreille, 1833 (Ephemeroptera, Prosopistomatidae) in Thailand, with description of a new species. ZooKeys 825: 123–144. https://doi.org/10.3897/zookeys.825.32443
- Dalkiran N (2009) A new species of *Prosopistoma* Latreille, 1833 (Ephemeroptera: Prosopistomatidae) from northwestern Turkey. Aquatic Insects 31(2): 119–131. https://doi.org/10.1080/01650420802642414
- Darriba D, Taboada G, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772–772. https://doi.org/10.1038/ nmeth.2109
- de Menocal P, Ortiz J, Guilderson T, Adkins J, Sarnthein M, Baker L, Yarusinsky M (2000) Abrupt onset and termination of the African Humid Period. Quaternary Science Reviews 19(1–5): 347–361. https://doi.org/10.1016/S0277-3791(99)00081-5
- El Alami M, El Yaagoubi S, Gattolliat JL, Sartori M, Dakki M (2022) Diversity and Distribution of Mayflies from Morocco (Ephemeroptera, Insecta). Diversity 14(6): 498. https:// doi.org/ 10.3390/d14060498
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
- Gagneur J, Thomas AGB (1988) Contribution à la connaissance des Ephéméroptères d'Algérie I. Répartition et écologie (1<sup>ere</sup> partie) (Insecta, Ephemeroptera). Bulletin de la Société d'Histoire naturelle de Toulouse 124: 213–223.
- Gillies MT (1954) The adult stages of *Prosopistoma* Latreille (Ephemeroptera) with descriptions of two new species from Africa. Transactions of the Royal Entomological Society of London 105(15): 355–372. https://doi.org/10.1111/j.1365-2311.1954.tb00768.x
- Hurvich CM, Tsai C-L (1989) Regression and time series model selection in small samples. Biometrika 76(2): 297–307. https://doi.org/10.1093/biomet/76.2.297
- Kazanci N, Türkmen G (2018) A new *Prosopistoma* (Ephemeroptera, Prosopistomatidae) species from Turkey, with ecological notes. Review of Hydrobiology 11: 1–22.
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
- Latreille PA (1833). Description d'un nouveau genre de Crustacés. Annales du Museum d'Histoire naturelle de Paris 3(2): 23–34.
- Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. Nucleic Acids Research 49(W1): W293–W296. https://doi.org/10.1093/nar/gkab301
- Müller OF (1785) Entomostraca seu testacea, quae in aquis Daniae et Norvegiae reperit, descripsit et inconibus illustravit. Lipsiae et Havniae. https://doi.org/10.5962/bhl.title.14414
- Peters WL (1967) New species of *Prosopistoma* from the Oriental Region (Prosopistomatidae: Ephemeroptera). Tijdschrift voor Entomologie 110: 207–222.
- Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21(8): 1864–1877. https://doi. org/10.1111/j.1365-294X.2011.05239.x
- Puillandre N, Brouillet S, Achaz G (2021) ASAP: Assemble species by automatic partitioning. Molecular Ecology Resources 21(2): 609–620. https://doi.org/10.1111/1755-0998.13281
- Quade J, Dente E, Armon M, Ben Dor Y, Morin E, Adam O, Enzel Y (2018) Megalakes in the Sahara? A Review. Quaternary Research 90(2): 253–275. https://doi.org/10.1017/qua.2018.46
- Rambaut A, Drummond A, Xie D, Baele G, Suchard M (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi. org/10.1093/sysbio/syy032
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Hohna S, Larget B, Liu L, Suchard M, Huelsenbeck J (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https:// doi.org/10.1093/sysbio/sys029
- Roopa SR, Selvakumar C, Subramanian KA, Sivaramakrishnan KG (2017) A new species of *Prosopistoma* Latreille, 1833 and redescription of *P. indicum* Peters, 1967 (Ephemeroptera: Prosopistomatidae) from the Western Ghats, India. Zootaxa 4242(3): 591–599. https:// doi.org/10.11646/zootaxa.4242.3.10
- Schletterer M, Bauernfeind E, Lechthaler W (2016) Larval redescription of *Prosopistoma* pennigerum (Müller, 1785) from the River Volga near Rzhev, Tver Region, Russia (Insecta: Ephemeroptera). Zoosymposia 11: 15–27. https://doi.org/10.11646/zoosymposia.11.1.6
- Schletterer M, Weiss SJ, Kuzovlev VV, Vitecek S, Borgwardt F, Graf W (2021) The rare and enigmatic mayfly *Prosopistoma pennigerum* (Müller, 1785): Habitat characteristics, recent records from the Volga (Russia) and Vjosa (Albania) rivers. Aquatic Conservation 31(12): 3636–3643. https://doi.org/10.1002/aqc.3748
- Shi W, Tong X (2013) A new species of *Prosopistoma* (Ephemeroptera: Prosopistomatidae) from China with a key to Oriental species. Zootaxa 3718(1): 89–96. https://doi.org/10.11646/ zootaxa.3718.1.8

- Skonieczny C, Paillou P, Bory A, Bayon G, Biscara L, Crosta X, Eynaud F, Malaizé B, Revel M, Aleman N, Barusseau JP, Vernet R, Lopez S, Grousset F (2015) African humid periods triggered the reactivation of a large river system in Western Sahara. Nature Communications 6(1): 8751. https://doi.org/10.1038/ncomms9751
- Stecher G, Tamura K, Kumar S (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Molecular Biology and Evolution 37(4): 1237–1239. https://doi.org/10.1093/ molbev/msz312
- Touabay M, Aouad N, Mathieu J (2002) Etude hydrobiologique d'un cours d'eau du Moyen-Atlas: L'oued Tizguit (Maroc). Annales de Limnologie 38(1): 65–80. https://doi. org/10.1051/limn/2002007
- Vuataz L, Sartori M, Wagner A, Monaghan MT (2011) Toward a DNA taxonomy of Alpine *Rhithrogena* (Ephemeroptera, Heptageniidae) using a mixed Yule-coalescent analysis of mitochondrial and nuclear DNA. PLoS ONE 6(5): e19728. https://doi.org/10.1371/journal. pone.0019728