

# ***Mayaweckelia troglomorpha*, a new subterranean amphipod species from Yucatán state, México (Amphipoda, Hadziidae)**

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## **Abstract**

A detailed description of a new stygobiont species of the amphipod family Hadziidae, *Mayaweckelia troglomorpha* Angyal, **sp. n.** is given, based on material collected in four cenotes of Yucatán federal state, México. Morphology was studied under light microscopy and with scanning electron microscopy. Morphological description is complemented with mitochondrial cytochrome c oxidase subunit I (COI) sequences as barcodes, with affinities to the related taxa and with notes on the species' ecology. Using COI Bayesian inference and genetic distance analyses, we show that the closest relative of the new species is *M. cenoticola*, forming a monophyletic group referring to the genus *Mayaweckelia*. Based on the available sequences, we also revealed that *Mayaweckelia* and *Tuluweckelia* are sister genera, standing close to the third Yucatán subterranean genus, *Bahadzia*. The data gathered on the habitat, distribution, abundance, and ecology will contribute to the conservation planning for *M. troglomorpha* Angyal, **sp. n.**

## Keywords

cenote, description, endemic, Hadziidae, mitochondrial marker, morphology, SEM, sinkhole, subterranean

## Introduction

To date, eleven species of amphipods have been recorded from subterranean habitats of the Yucatán Peninsula, belonging to five families (Ampithoidae, Hadziidae, Hyaliidae, Hyaellidae, and Melitidae) (e.g., Holsinger 1977, 1990, 1992, Alvarez and Iliffe 2008, Marrón-Becerra et al. 2014, Ortiz and Winifield 2015, Trujillo-Pisanty et al. 2010). Among them, six species are classified as stygobionts; they bind solely to aquatic subterranean habitats, exhibiting various degrees of morphological, physiological and behavioural adaptations to life in the hypogean environment (Notenboom 1991). These six species are endemic to the Yucatán Peninsula and inhabit mostly 'cenotes' (also referred as sinkholes), well-like water-filled karst features, which are formed by the collapse of limestone bedrock and are usually connected with extended submerged cave passages (e.g., Reddell 1981, Alvarez et al. 2015). Like other karst aquifers, the Yucatán cenotes are particularly vulnerable to contamination especially from tourist activities and infrastructure, pollution from growing human settlements, industrial and agricultural activities (Escolero et al. 2002, Bauer-Gottwein et al. 2011).

Cenotes of the Yucatán Peninsula are considered anchialine environments; they are filled with fresh and saltwater, separated by a halocline layer (Bauer-Gottwein et al. 2011). Contrary to the cenotes found on the Caribbean coast of the peninsula, those in Yucatán federal state (which is located on the north part of the peninsula, bordered by Campeche federal state to the southwest and Quintana Roo federal state to the east, with the Gulf of México on its north coast) are mainly inland, far from the coastline and therefore are filled with freshwater only. The saline intrusion can only be detected in a few rather deep cenotes, like Sabak-Ha (20.579974°N, 89.588353°W, halocline at 62 m, own data) and Ultimo Suspiro (21.403485°N, 88.568434°W halocline at 51 m, own data), or in few cenotes, which are located near the northern coast of the peninsula, like Cervera, in which the halocline occurs at about 25 m depth (Alvarez et al. 2005).

The hadziid *Tuluweckelia cernua* Holsinger, 1990, *Bahadzia bozanici* Holsinger, 1992 and *Bahadzia setodactylus* Holsinger, 1992 and the hyaellid amphipod *Hyaella cenotensis* Marrón-Becerra, Hermoso-Salazar & Solís-Weiss, 2014 have been described from caves and cenotes of Quintana Roo state near the Caribbean (eastern) coast of the peninsula (Holsinger 1990, 1992, Marrón-Becerra et al. 2014), while *Mayaweckelia yucatanensis* Holsinger, 1977 (Hadziidae) is reported from a cave pool in Campeche state (Holsinger 1977). The only stygobiont amphipod that has been described from Yucatán state is *Mayaweckelia cenoticola* Holsinger, 1977.

In his genus description where Holsinger (1977) described the type species *M. yucatanensis* and *M. cenoticola*, he highlighted the differences from the two known related subterranean hadziid genera, *Mexiweckelia* Holsinger & Minckley, 1971 and *Hadzia* S. Karaman, 1932. The new genus differs in some important characters, such as the three-

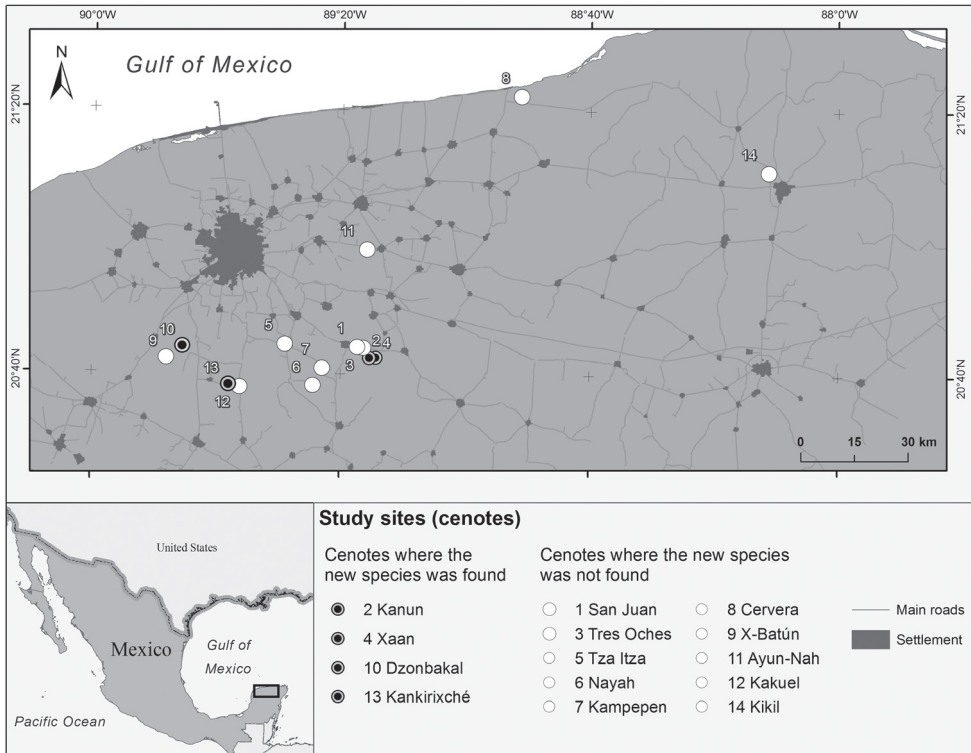
articulated accessory flagellum of the first antenna, the absence of robust setae on the inner margin of the maxilliped outer plate, the presence of ventrally produced lobe of gnathopod I merus and the absence of dorsal robust setae on urosomites I and II. Other diagnostic characters of *Mayaweckelia* are the absence of the mandibular palp, the completely separated telson halves, and the outer ramus of the third uropod with one article (Holsinger 1977). In his paper written about the description of the genus *Tuluweckelia* and the type species *T. cernua*, Holsinger (1990) also gave a second, complementary description of *M. cenoticola*, where he presented some characters that were found since the original description as: the presence of aesthetascs on flagellar articles ten-twelve on the first antenna, pereopod VI up to 15% longer than pereopod VII and the presence of a row of fine setae on the distal half of upper margin of pereopods V-VII dactyli.

This study results from a long-term research project using cave diving techniques, initiated in May 2016 to contribute to the understanding of Crustacea diversity and distribution in the cenote ecosystems of Yucatán federal state (Angyal et al., in preparation). As part of the project findings, the description of a new species of *Mayaweckelia* is presented herein, using several sources of data that increase the robustness of taxonomic conclusions (Padial et al. 2010). Cytochrome c oxidase subunit I (COI) sequences are provided as barcodes, as well as the first comparative scanning electron micrographs (SEM) of *Mayaweckelia*. Moreover, phylogenetic relationships based on mitochondrial sequences of the collected *Mayaweckelia* and *Tuluweckelia* samples are presented, including publicly available hadziid sequences. Field observations that may contribute to the species' conservation and ecological comprehension are summarised.

## Materials and methods

### Sampling sites and sampling

Fourteen cenotes were studied between May and July 2016 in seven municipalities of Yucatán federal state (Yucatán Peninsula, México) in order to characterize their Crustacea fauna (Angyal et al., in preparation). Most of these cenotes are situated near Mérida city and are part of the 'Ring of cenotes', which is a fracture zone that marks the outline of the Chicxulub asteroid impact crater with a high density of sinkholes (Gonzales-Herrera et al. 2002, Bauer-Gottwein et al. 2011). The new amphipod species was found in four of the sampled cenotes and their respective submerged cave passages (Figure 1). These were Cenote Dzonbakal (Umán, 20.669819°N, 89.778869°W), Cenote Kanún (Homún, 20.745599°N, 89.244638°W), Cenote Xaan (Homún, 20.727571°N, 89.256834°W) and Cenote Kankirixché (Abalá, 20.37225°N, 89.632892°W). Amphipods were collected individually in 50 ml sample tubes during cave dives. Habitat data of each sample (depth, temperature, found in cavern or cave zone, caught in fresh water or saltwater) were recorded. Photos and videos of the observed crustaceans and their habitats were also taken. After the dives the collected individuals were placed into 96% ethanol. All specimens were collected under the permission of the Secretary of Environment and Natural Resources of United Mexican States (SEMARNAT/SPGA/DGVS/05263/14; SEMAR-



**Figure 1.** Location of the studied area, showing the four cenotes where the new species was collected (Yucatán federal state, México).

NAT/SPGA/DGVS/02068/17). Type material is deposited in the National Crustacean Collection in the Institute of Biology of the National Autonomous University of México (UNAM), in the Yucatán Crustacea Collection at the Academic Multidisciplinary Unit of Teaching and Research of UNAM, and in the Collection of Crustaceans of the Hungarian Natural History Museum, as detailed in Table 1.

## Morphological studies

Selected specimens of the presumably new species and other Yucatán hadziids (*M. cenoticola* and *T. cernua*) were dissected on slides and were studied under compound (light) microscope. At first, they were heated in 10% KOH solution, rinsed with HCl and washed in distilled water. Cleared exoskeletons were stained with chlorazol black, partly dissected in glycerol, and mounted on slides in glycerol gelatine medium under a stereomicroscope (Fišer et al. 2009, Angyal et al. 2015). Slides were examined using a Leica DM 1000 light microscope. Drawings were made using a drawing tube mounted on the light microscope and were digitally edited afterwards. Scanning electron micrographs were made with a HITACHI S-2600 N scanning electron microscope. The



**Table 1.** Data of hadziid and hyallelid samples used for COI molecular studies. *Bahadzia jaraguensis* and *Hyalella azteca* sequences were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank>).

Sample codes and collection codes	Species	Date of collection	Cenote, municipality, state	Collected by	Cited in	GenBank IDs
Nr: 00046 HOLOTYPE CNR 34392	<i>Mayaweckelia troglomorpha</i> sp. n.	14.05.2016	Dzonbakal, Umán, Yucatán	D. Angyal, R. Acosta, J. Baduy & S. Reyes	present study	MF589977
Nr: 00113 ALLOTYPE HNHM Amphipoda -4094	<i>Mayaweckelia troglomorpha</i> sp. n.	11.06.2016	Cenote Kankirixché, Abalá, Yucatán	D. Angyal & E.M. Chávez Solís	present study	MF589981
Nr: 00043 PARATYPE CNR 34393	<i>Mayaweckelia troglomorpha</i> sp. n.	04.06.2016	Cenote Kanún, Homún, Yucatán	D. Angyal, R. Acosta, J. Baduy, B. Magaña & S. Reyes	present study	MF589976
Nr: 00056 PARATYPE HNHM Amphipoda -4095	<i>Mayaweckelia troglomorpha</i> sp. n.	14.05.2016	Dzonbakal, Umán, Yucatán	D. Angyal, R. Acosta, J. Baduy & S. Reyes	present study	MF589978
Nr: 00095 PARATYPE YUC-CC-255-11-003922	<i>Mayaweckelia troglomorpha</i> sp. n.	09.06.2016	Cenote Xaan, Homún, Yucatán	D. Angyal & E.M. Chávez Solís	present study	MF589979
Nr: 00110 PARATYPE HNHM Amphipoda -4096	<i>Mayaweckelia troglomorpha</i> sp. n.	11.06.2016	Cenote Kankirixché, Abalá, Yucatán	D. Angyal & E.M. Chávez Solís	present study	MF589980
Nr: 00042 YUC-CC-255-11-003923	<i>Mayaweckelia cenoticola</i> Holsinger, 1977	22.05.2016	Cenote Ayun-Nah, Cacalchén, Yucatán	D. Angyal & B. Magaña & E. Sosa Rodríguez	present study	MF589975
Nr: 00108 YUC-CC-255-11-003924	<i>Tuluweckelia cernua</i> Holsinger, 1990	11.06.2016	Cenote Kankirixché, Abalá, Yucatán	D. Angyal & E.M. Chávez Solís	present study	MF589983
Nr: 00101 YUC-CC-255-11-003925	<i>Tuluweckelia cernua</i> Holsinger, 1990	09.06.2016	Cenote Xaan, Homún, Yucatán	D. Angyal & E.M. Chávez Solís	present study	MF589982
MX16.82 YUC-CC-255-11-003926	<i>Tuluweckelia cernua</i> Holsinger, 1990	11.12.2016	Cenote Concha (Sistema Sac Actun), Tulum, Quintana Roo	G. Balázs, B. Lerner, R. Mier & N. Kamarás	present study	MF589984
NC_019661.1	<i>Bahadzia jaraguensis</i> Jaume & Wagner, 1998	no data	Ovideo, S. Hispaniola (Dominican Republic), cave	no data	Bauzá-Ribot et al. 2012	NC019661.1
HM_138032.1	<i>Hyalella azteca</i> (Saussure, 1858)	no data	Canada, within the frame of Canadian Aquatic Biomonitoring Network program	no data	Baird et al. 2011	HM138032.1

studied specimen was placed in absolute alcohol for one day, then was dissected and dried out in air. Dry samples were stuck onto holders and were sputter-coated with gold-palladium. Micrographs were digitally edited. The terminologies 'slender seta' and 'robust seta' were based on Watling's (1989) classification system for crustacean setae. The terms 'notched spine teeth' and 'unnotched spine teeth' are based on the descriptions of Yucatán subterranean hadziids (Holsinger 1977, 1990), and refer to strong, thick spine-like features, typically on the palm of gnathopod I and II propodus.

## Molecular studies

DNA extraction of six individuals of *Mayaweckelia troglomorpha* sp. n., one *M. cenoticola* specimen and three *T. cernua* individuals (two from Yucatán state and one from Quintana Roo state, see Table 1 for sample data) was performed using QIAamp DNA Microkit (Qiagen), following the manufacturer's instructions. Only a few pereopods were used for DNA isolation of each animal. For PCR amplification of mitochondrial cytochrome c oxidase subunit I (COI) we used the primer pair LCO 1490 and HCO 2198 (Folmer et al. 1994). PCR reactions (25 µl) were obtained by mixing 13.85 µl mQ water, 2.5 µl 10X PCR buffer, 2.5 µl dNTP mix (2mM), 1.5 µl of each primers (5µM), 0.15 µl Fermentas Dream Taq (5U/ µl) and 3 µl DNA extract. PCR temperature conditions were as follows: initial denaturation for 3 min at 94 °C, denaturation for 45 sec at 94 °C, hybridization for 45 sec at 48 °C, and polymerization for 1 min at 72 °C. After thirty cycles a final extension for 3 min at 72 °C was added. PCR products were cleaned using Exo SAP-IT Express PCR Product Cleanup (Affymetrix) according to manufacturer's instructions. The fragments were sequenced in both directions using PCR amplification primers with an ABI 3130 sequencer. 638 bp COI barcode sequences have been uploaded to NCBI GenBank database. Accession numbers are MF589975–MF589984 (see Table 1).

## Sequence analyses

In order to evaluate phylogenetic relationships and genetic distances of the newly collected hadziids (*Mayaweckelia* spp. and *T. cernua*) with other hadziid and hyalellid species with publicly available sequences, a dataset of COI sequences was compiled (Table 1). The widespread and abundant north and central American *Hyalella azteca* (Hyalellidae) was included in the dataset as outgroup taxon.

DNA sequences were edited using BioEdit 7.1.11 (Hall 1999) and aligned with ClustalW multiple sequence alignment program (Thompson et al. 1994). Nucleotide substitution model selection carried out with MEGA V 6.0 (Tamura et al. 2013) using the Akaike information criterion (AIC) (Akaike 1973) revealed that the best fitting model for COI is GTR+G+I. Bayesian inference was carried out on phylogeny.fr (Dereeper et al. 2008) using Metropolis coupled Markov chain Monte Carlo simulations

for 100.000 generations, sampling a tree in every 10 generations. The first 1000 trees were discarded as burn-in. FigTree 1.4.0 (Rambaut 2012) was used for visualisation. Pairwise genetic distances were calculated in MEGA V 6.0 using p-distance (Nei and Kumar 2000).

## Results

### Taxonomy

**Order Amphipoda Latreille, 1816**

**Suborder Senticaudata Lowry & Myers, 2013**

**Family Hadziidae S. Karaman, 1943**

**Genus *Mayaweckelia* Holsinger, 1977**

***Mayaweckelia troglomorpha* Angyal, sp. n.**

<http://zoobank.org/32D988B9-58D3-4224-9A21-53B9C2BFB8F5>

Figs 2–9

**Material examined.** *Holotype* ♂, 10 mm, Nr. 00046, 14 May 2016, Dzonbakal, 20.669819°N, 89.778869°W, San Antonio Mulix, Umán, Yucatán state, México, collected by D. Angyal, R. Acosta, J. Baduy & S. Reyes in cave part, 26.7 m depth in fresh water; dissected and mounted on slide. Collection ID: CNR 34392 (UNAM, Institute of Biology, National Crustacean Collection, México City.)

*Allotype* ♀, 10 mm, Nr. 00113, 11 June 2016, Cenote Kankirixché, 20.37225°N, 89.632892°W, Mucuyché, Abalá, Yucatán state, México, collected by D. Angyal & E.M. Chávez Solís in cavern part, 20.4 m depth in fresh water; dissected and mounted on slide. Collection ID: HNHM Amphipoda -4094 (Hungarian Natural History Museum, Collection of Crustaceans, Budapest).

*Paratypes* ♀, 7 mm, Nr. 00056, 14 May 2016, Dzonbakal, 20.669819°N, 89.778869°W, San Antonio Mulix, Umán, Yucatán state, México, collected by D. Angyal, R. Acosta, J. Baduy & S. Reyes in cave part, 26.3 m depth in fresh water; sputter-coated by gold-palladium. Collection ID: HNHM Amphipoda -4095 (Hungarian Natural History Museum, Collection of Crustaceans, Budapest).

♂, 8 mm, Nr. 00043, 4 June 2016, Cenote Kanún, 20.745599°N, 89.244638°W, Homún, Homún, Yucatán state, México, collected by D. Angyal, R. Acosta, J. Baduy, B. Magaña & S. Reyes in cave part, 24.3 m depth in fresh water; not dissected. Collection ID: CNR 34393 (UNAM, Institute of Biology, National Crustacean Collection, México City).

Juvenile, 3 mm, Nr. 00095, 9 June 2016, Cenote Xaan, 20.727571°N, 89.256834°W, Homún, Homún, Yucatán state, México, collected by D. Angyal & E.M. Chávez Solís in cave part, 25.4 m depth in fresh water; not dissected. Collection

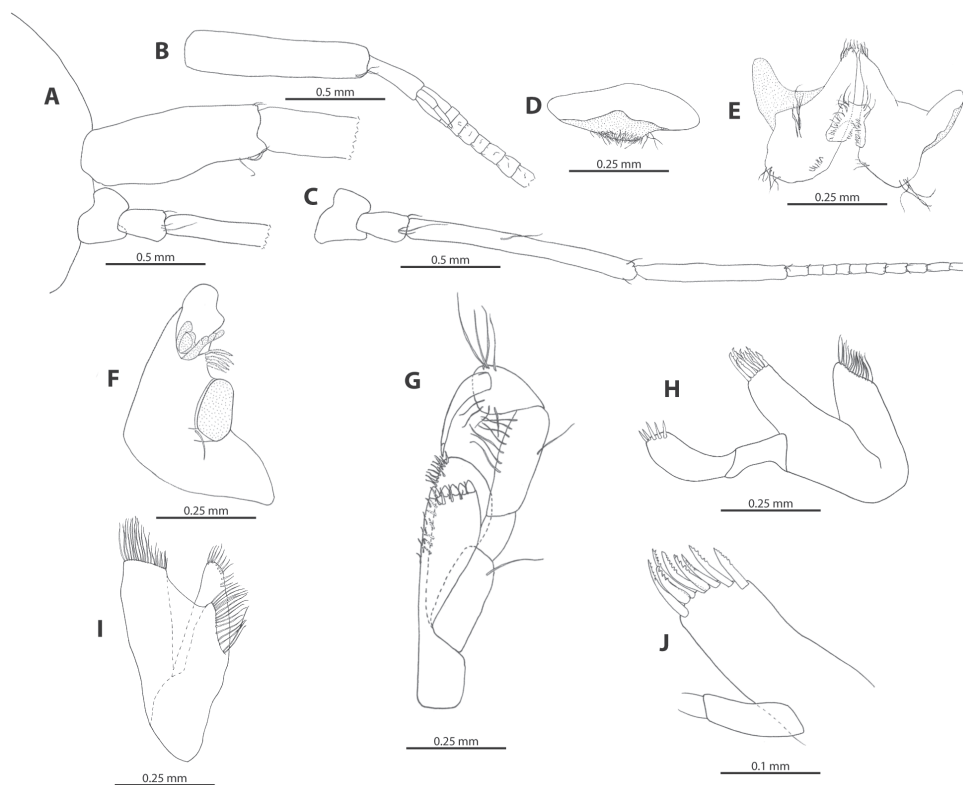


**Figure 2.** *M. troglomorpha* sp. n., living specimens. Above: allotype ♀ collected in Cenote Kankirixché; below: individual photographed in its natural habitat during research dive in Cenote Kanún (not collected).

ID: YUC-CC-255-11-003922 (UNAM, Academic Multidisciplinary Unit of Teaching and Research, Yucatán Crustacea Collection, Sisal).

Juvenile, 5 mm, Nr. 00110, 11 June 2016, Cenote Kankirixché, 20.37225°N, 89.632892°W, Mucuyché, Abalá, Yucatán state, México, collected by D. Angyal & E.M. Chávez Solís in cave part, 33.3 m depth in fresh water; dissected and mounted on slide. Collection ID: HNHM Amphipoda -4096 (Hungarian Natural History Museum, Collection of Crustaceans, Budapest).

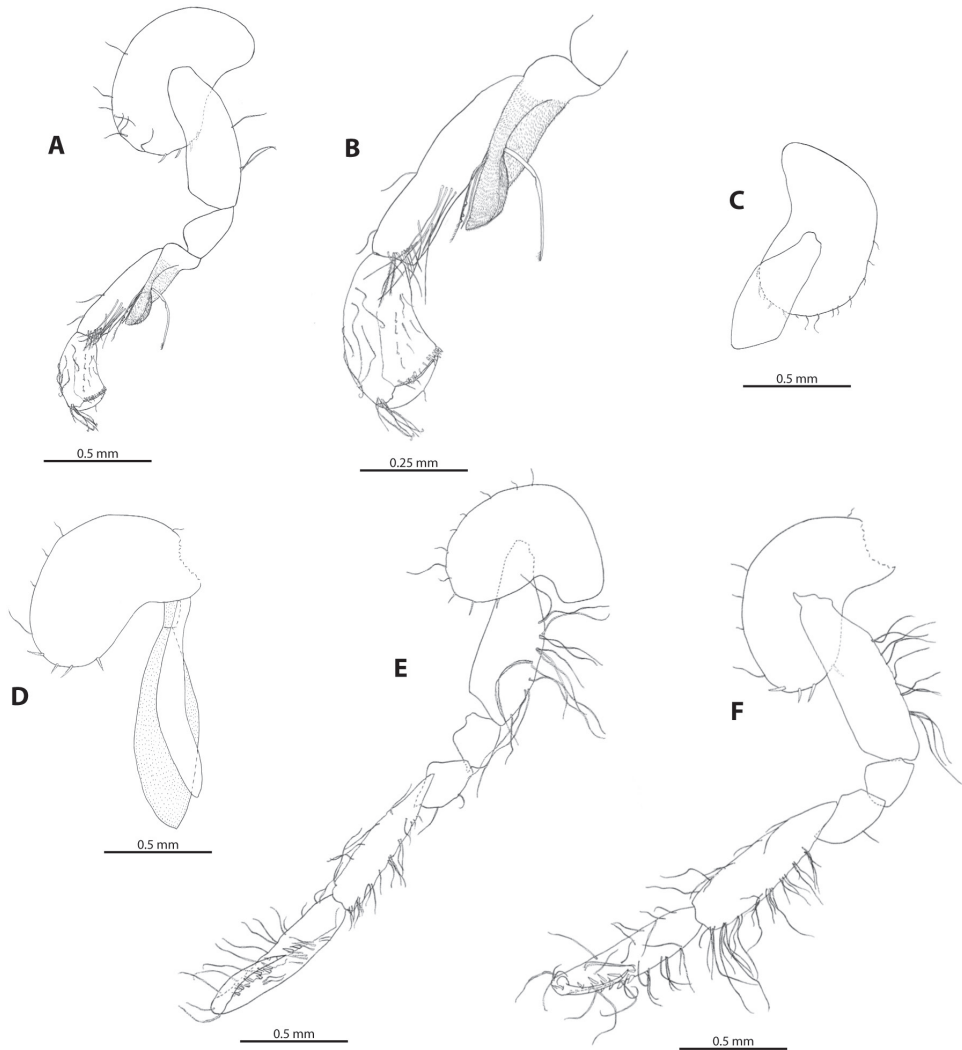
**Diagnosis.** Medium-sized, eyeless hadziid with conspicuous troglomorphic traits. The first antenna almost twice as long as body and three times as long as the second antenna; gnathopod I propodus palm armed with distally notched spine teeth, carpus more than 1.5 times as long as corresponding propodus, merus as broad as but shorter than carpus, ventrally produced lobe with three long sensory setae; gnathopod II propodus twice as long as propodus I, palm armed with unnotched spine teeth, carpus



**Figure 3.** *M. troglomorpha* sp. n. **A** detail of head and antenna I and II (♀ allotype) **B** detail of antenna I with accessory flagellum (♀ allotype) **C** antenna II peduncle articles and proximal part of flagellum (♀ allotype) **D** upper lip (♂ holotype) **E** lower lip (♂ holotype) **F** left mandible (♀ allotype) **G** maxilliped (8 mm ♂) **H** maxilla 1 (♀ allotype) **I** maxilla II (♀ allotype) **J** maxilla I outer plate (♂ holotype).

slightly shorter than propodus on males. Dactylus, propodus, and carpus of pereopods VI-VII extremely long; therefore, pereopods VI and VII are 1.3 times as long as body length; epimeral plates I-III ventro-posterior corner tiny but distinct, ventral margin without robust setae, posterior margins concave; surfaces of uropods I-III pubescent; telson lobes each possess five-six robust setae and one-three slender setae on outer margin and six-seven robust setae on inner margin. Largest males and females both measured 10 mm.

**Description.** (10 mm ♂, 8 mm ♂, 8 mm ♀, 7 mm ♀, 5 mm juvenile, 3 mm juvenile.) Antenna 1 (Figures 2, 3) 1.75 times as long as body; three times long as antenna II; primary flagellum with more than 60 articles; aesthetacs were not visible as distal half of the flagellum was missing from each animal (examination of the antennae was possible only using the photos of the living specimens); accessory flagellum with three articles. Antenna II (Figures 2, 3): flagellum with more than 20 articles. Mandibles (Figure 3) subequal; both molar with seta; setal row with four or five serrated setae; palp lacking. Maxilla I (Figure 3): inner plate with approximately 15 apical setae; outer

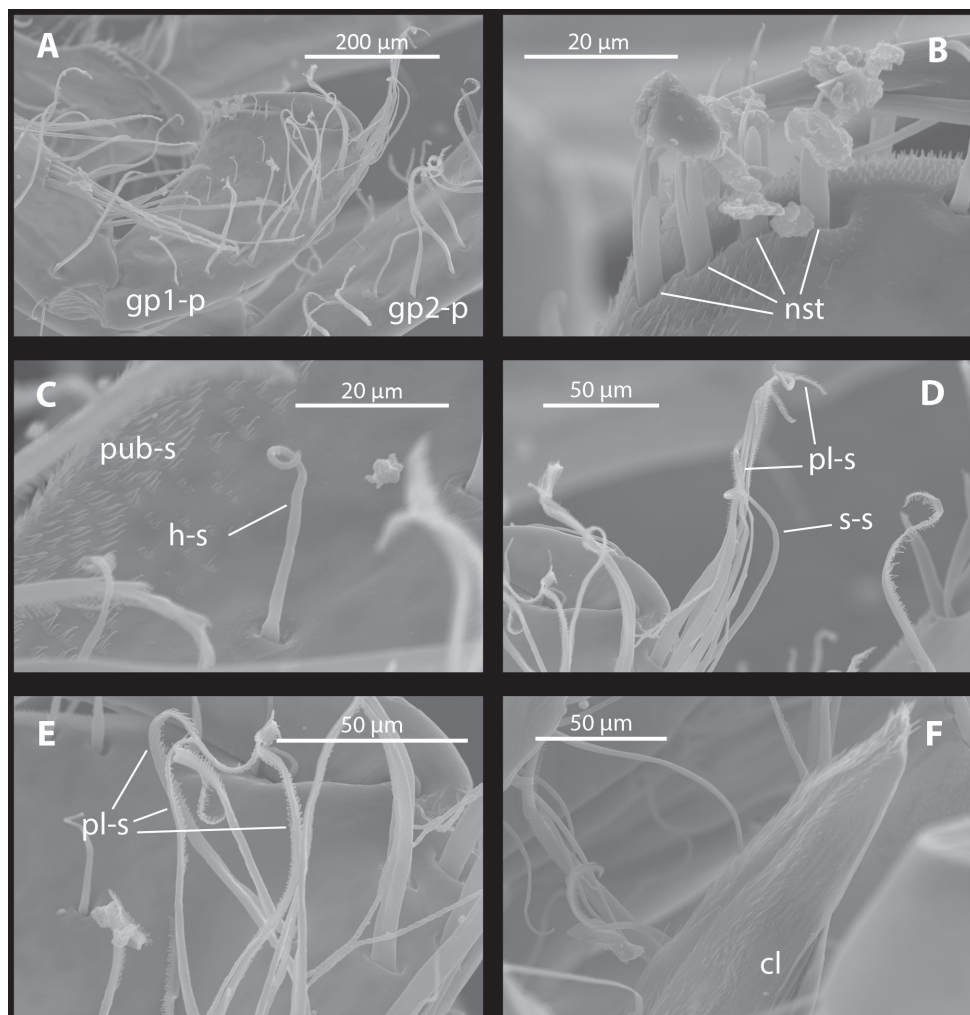


**Figure 4.** *M. troglomorpha* sp. n. **A** gnathopod I (♀ allotype) **B** gnathopod I propodus, carpus and merus (♀ allotype) **C** gnathopod I coxa (♂ holotype) **D** oostegite and gill on gnathopod II (♀ allotype) **E** gnathopod II (♂ holotype) **F** gnathopod II (♀ allotype).

plate with eight apical, pluri-toothed robust setae; second palpal article with five apical robust setae. Maxilla II (Figure 3): inner plate with 15–23 obliquely placed setae on inner margin. Maxilliped (Figure 3): inner plate with four or five cone shaped, thick robust setae and several coarse setae apically; outer plate with stiff setae apically. Lower lip (Figure 3): outer lobes narrowly rounded; lateral process prominent; inner lobes rather small.

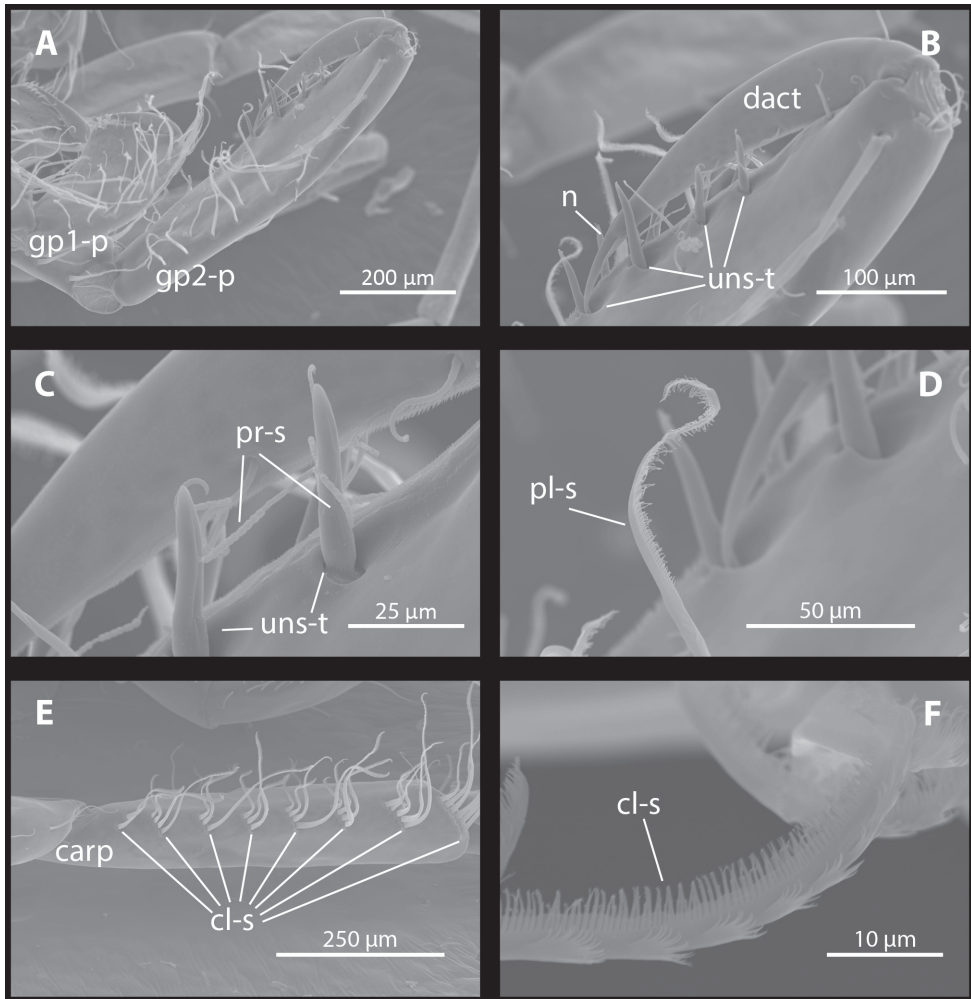
Gnathopod I (Figure 4, 5): dactyl thick, single seta present on anterior margin, inner margin without seta, unguis (nail) length 35% of total dactylus length. Propodus





**Figure 5.** *M. troglomorpha* sp. n., (7 mm ♀), scanning electron micrographs. **A** gnathopod I propodus; **B**, gnathopod I palmar corner **C** gnathopod I propodus posteromedial part **D** gnathopod I propodus anterodistal seta group **E** gnathopod I propodus anterior margin seta group **F** ventrally produced conspicuous lobe on gnathopod I merus. Abbreviations: gp1-p = gnathopod I propodus, gp2-p = gnathopod II propodus (**A**); nst = notched spine teeth (**B**); pub-s = pubescent setae, h-s = helical medial seta (**C**); pl-s = plumose seta, s-s = simple seta (**D**); pl-s = plumose seta (**E**); cl = conspicuous lobe (**F**).

small, longer than broad; palm short, slightly convex, in palmar corner double row of four-five distally notched spine teeth always present, additional notched spine teeth and spine-like setae sometimes present on palm, close to the base of dactylus; posterior margin of propodus slightly concave, surface near margin covered with pubescent setae; anterior margin with five-seven rows of long, plumose setae (sometimes singly inserted); antero-distal group with six-eight long plumose and simple setae; four singly



**Figure 6.** *M. troglomorpha* sp. n., (7 mm ♀), scanning electron micrographs. **A** gnathopod I and II propodus **B** gnathopod II propodus dactylus and palm **C** gnathopod II propodus palm **D** gnathopod II propodus posterior margin **E** gnathopod II carpus **F** comb-like plumose seta on gnathopod II carpus. Abbreviations: gp1-p = gnathopod I propodus, gp2-p = gnathopod II propodus (**A**); dact = dactylus, n = nail, unst-t = unnotched spine teeth (**B**); pr-s = pearl row-like seta, unst-t = unnotched spine teeth (**C**); pl-s = plumose seta (**D**); carp = carpus, cl-s = comb-like plumose setae (**E**); cl-s = comb-like plumose seta (**F**).

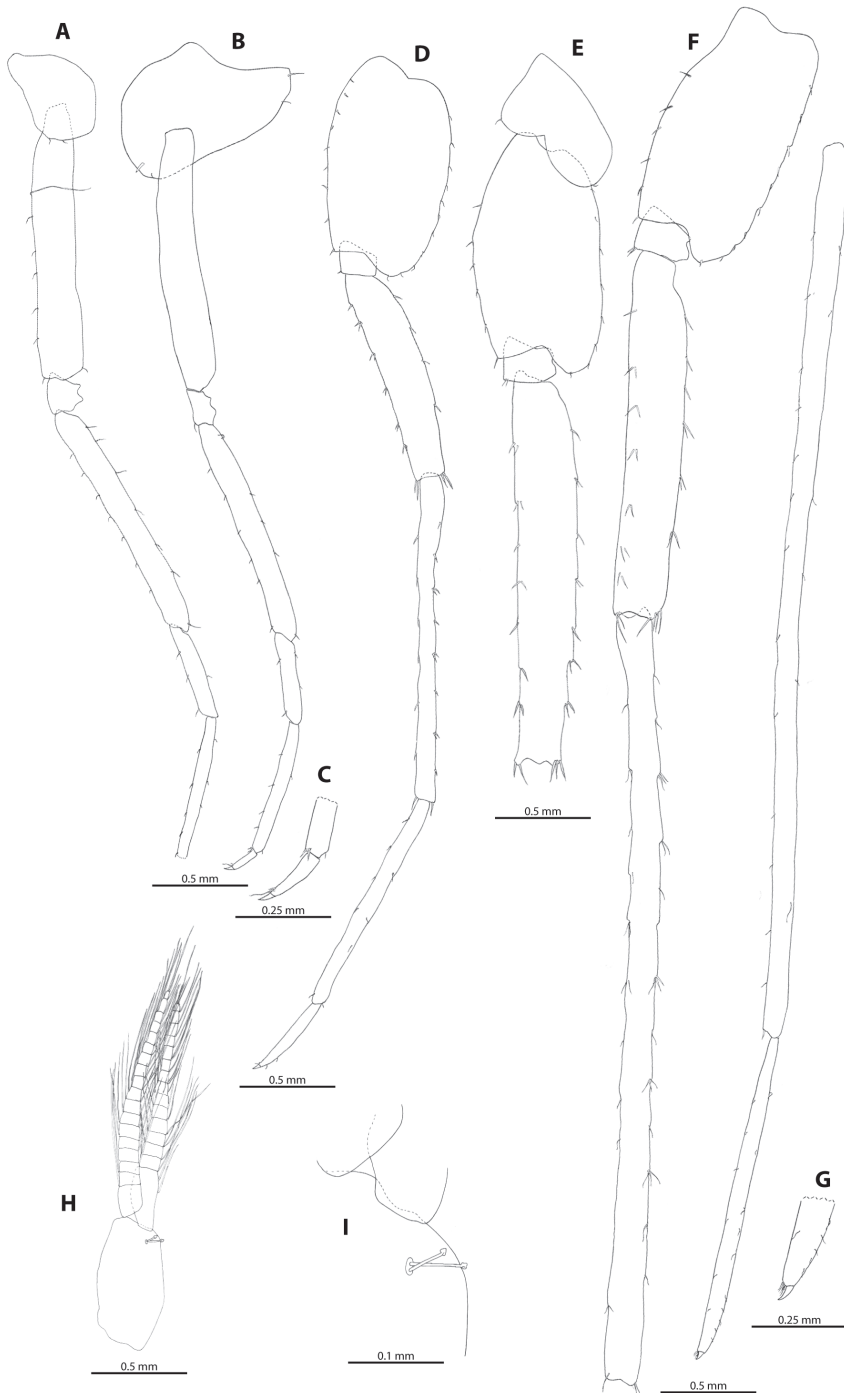
inserted helical medial setae always present, sometimes additional singly or doubly inserted medial setae present. Carpus narrow, 1.5–1.7 times longer than propodus. Merus: as broad as but shorter than carpus, ventrally produced into pubescence, conspicuous lobe with three long sensory setae. Sensory papillae visible on one of the setae. Coxal plate I large, deep, longer than broad, broadly rounded ventrally, margin with three-four robust setae and seven-eight slender setae on females and six robust setae and three-eight slender setae on males.

Gnathopod II (Figure 4, 6): dactylus thick, along anterior margin (close to antero-distal corner) a single seta present, inner margin with three-five setae. Propodus twice as long as gnathopod I propodus, narrow, subrectangular; palm length is more than 50% of propodus length on males and less than 50% of propodus length on females; palm armed with double row of five-six unnotched spine teeth which are sometimes accompanied by long, pearl row-like setae; surface near margin covered with pubescent setae; helical seta sometimes present below (proximal to) spine teeth; posterior margin with four-five, anterior margin with six-nine sets of plumose setae; three-four medial plumose setae. Carpus slightly shorter than propodus on males and slightly longer than propodus on females, armed with seven-eight rows of comb-like plumose setae on posterior margin. Merus as broad as but more than two times shorter than carpus; not produced ventrally into conspicuous lobe. Coxal plate II kidney-shaped, margin with three-four robust setae and eight slender setae.

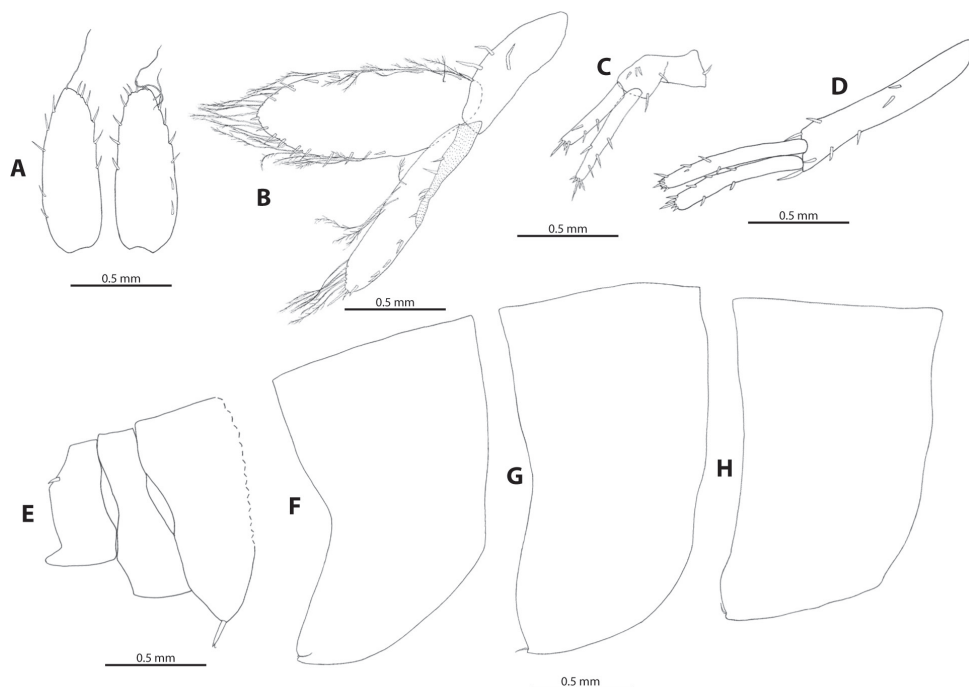
Coxal plate III (Figure 7) rather small and shallow, margin with two fine setae. Coxal plate IV (Figure 7) more than twice as broad as coxal plate III; posterior margin concave, ventral margin with four short stiff setae; dactylus length 28% of propodus; single robust seta and one long slender seta at the base of the unguis. Pereopod V (Figure 7) basis 1.7 times longer than broad, margins convex; pereopods VI (Figure 7) and VII (Figure 7) bases twice as long as broad, margins of pereopod VI slightly convex, posterior margin of pereopod VII straight; dactylus of pereopod V 40% length of corresponding propodus; outer margin with two slender setae; pereopod VI and VII extremely long (especially dactylus, propodus and carpus), 1.3 times as long as body; pereopod VI slightly longer than pereopod VII; pereopod VII dactylus with some short, slender setae at the base of the unguis. Coxal gills (Figure 4) large, almond shaped, pedicellate, present on pereon segments II-VI; oostegites (Figure 4) long, slender. Pleopods (Figs 7, 9) I-III with two-hooked retinaculae.

Epimeral plates I-III (Figure 8) ventro-posterior corner tiny but distinct with one slender seta at the corner; ventral margin without robust setae; posterior margin slightly concave on epimeral plate I, concave on epimeral plate II and strongly concave on epimeral plate III. Urosomites (Figure 8): urosomite I with one strong robust seta at the base of uropod I; urosomite II without robust and slender setae; urosomite III with one robust seta mid-dorsally.

Uropod I (Figures 8, 9) surface of rami and peduncle covered with pubescent setae; outer ramus 10–15% longer than inner ramus, outer ramus 20–33% shorter than peduncle; peduncle with five-eight spine-like robust setae; outer ramus with four-six robust setae (plus five apical robust setae), inner ramus with five-six robust setae (plus five apical robust setae). Uropod II (Figures 8, 9) surface of rami and peduncle covered with pubescent setae; outer ramus 13–22% longer than inner ramus on the studied males and 25–35% longer than inner ramus on females; outer ramus 5–15% longer than peduncle; peduncle with six spine-like robust setae; inner ramus with three-four robust setae (plus five apical robust setae); outer ramus with four-five robust setae (plus five apical robust setae). Uropod III (Figures 8, 9) surface of rami and distal end of peduncle covered with pubescent setae; uropod III 20%



**Figure 7.** *M. troglomorpha* sp. n., ♂ holotype. **A** pereopod III (dactylus was missing) **B** pereopod IV **C** pereopod IV dactylus **D** pereopod V **E** pereopod VI coxa, basis, ischium and merus **F** pereopod VII **G** distal part of pereopod VII with detail of unguis **H** pleopod III **I** retinacle on pereopod II.



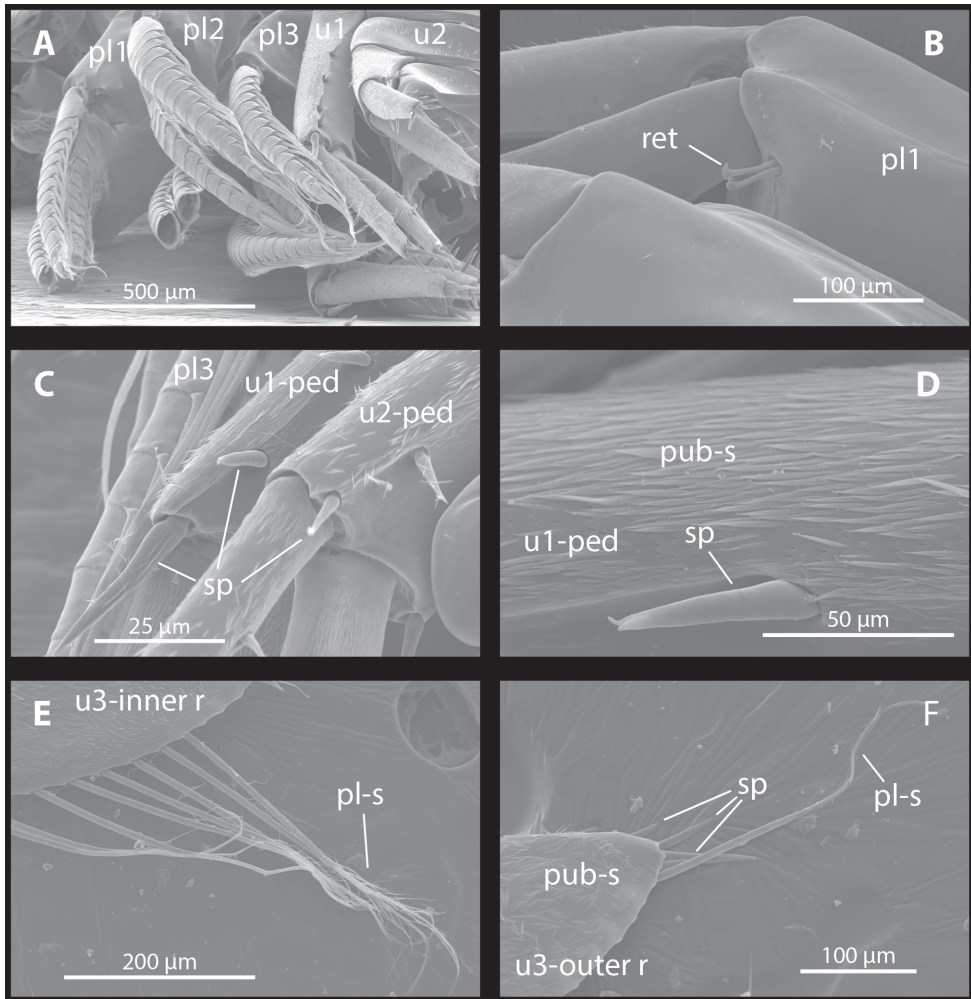
**Figure 8.** *M. troglomorpha* sp. n. **A** telson (♀ allotype) **B** uropod III (♀ allotype) **C** uropod II (♂ holotype) **D** uropod I (♂ holotype) **E** urosomites (♀ paratype, 7 mm) **F** pleonite III (♀ allotype) **G** pleonite II (♀ allotype) **H** pleonite I (♀ allotype).

as long as body; inner ramus slightly longer than outer ramus, margins with long, singly-inserted plumose and pappose setae and some short robust setae, apex with two short spine-like robust setae; outer ramus with long, plumose setae on inner margin and short robust setae on outer margin toward distal end, apex with three robust setae and sometimes additional with long spine-like seta; peduncle with two-four robust setae.

Telson (Figure 8) cleft to base, each half 2.65 times longer than broad; lobes each with five-six robust setae and one-three slender setae on outer margin and six-seven robust setae on inner margin.

**Variability.** Sexes very similar in size and appearance, except a few traits. Propodus I more oblong in males than in females. Coxal plate I margin with six robust setae and three-eight slender setae in males, and with three or four robust setae and seven-eight slender setae in females. Palm length of gnathopod II is more than 50 % of propodus length on males and less than 50 % of propodus length on females. Carpus of gnathopod II slightly shorter than propodus on males and slightly longer than propodus on females. Sexually mature females have long and narrow oostegites. Left and right side gnathopod I and II are unequal in length in both sexes. Outer ramus of uropod II 13–22 % longer than inner ramus on males and 25–35 % longer than inner ramus





**Figure 9.** *M. troglomorpha* sp. n., (7 mm ♀), scanning electron micrographs. **A** pleopods I-III and uropods I and II **B** retinacle on pleopod I; **C**, spine-like robust setae on uropod I and II **D** detail of uropod I peduncle article **E** setae on uropod III inner ramus **F** apical region of uropod III outer ramus. Abbreviations: pl1 = pleopod I, pl2 = pleopod II, pl3 = pleopod III, u1 = uropod I, u2 = uropod II (**A**); ret = retinacle, pl1 = pleopod I (**B**); pl3 = pleopod III, u1-ped = peduncle article of uropod I, u2-ped = peduncle article of uropod II, sp = spine-like robust setae (**C**); pub-s = pubescence setae, u1-ped = uropod I peduncle article, sp = spine-like robust seta (**D**); pa-s = pappose seta, u3-inner r = uropod III inner ramus (**E**); pl-s = plumose seta, sp = spine-like robust seta, pub-s = pubescent setae, u3-outer r = uropod III outer ramus (**F**).

on females, however this character should be further analysed on an elevated number of individuals of both sexes.

**Etymology.** The name *troglomorpha* refers to the highly adaptive troglomorphic features of the new species, particularly the elongation of appendages, the increased number of sensory setae and papillae, and general appearance of fragility. Gender feminine.



**Distribution and remarks on ecology.** The new species is known from four cenotes in the state of Yucatán, covering a distribution distance of 52 km (distance between the farthest cenotes Dzonbakal and Xaan). All the individuals were found in fresh water habitat, in most cases far from the cenote entrances, deeper in the associated cave passages, where sunlight does not penetrate. Water temperature was between 26 and 27 °C. Specimens were collected between 20 and 33 meters depth; in cenote Kankirixché some individuals were observed below 45 meters depth. The new species was represented in all four localities with low abundance, though it proved to be more common and more abundant than *M. cenoticola*, of which a single specimen was found in only one (Ayun-Nah) of the 14 visited cenotes, during an underwater waste collecting activity, hidden in a plastic soft drink bottle. In the type locality and in cenotes Xaan and Kankirixché the new species co-occurred with the hadziid amphipod *Tuluweckelia cernua*. Other co-occurring stygobiont macro-crustaceans (in the four cenotes) were the mysid *Antrommysis cenotensis* Creaser, 1936, the stygiomysid *Stygiomysis* cf. *holthuisi* (Gordon, 1958), the isopods *Creaseriella anops* (Creaser, 1936) and *Yucatalana robustispina* Botosaneanu & Illiffe, 1999, and the decapods *Typhlatya mitchelli* Hobbs & Hobbs, 1976, *Typhlatya pearsei* Creaser, 1936, and *Creaseria morleyi* (Creaser, 1936).

**Remarks and affinities to related species and genera.** Holsinger (1990) noted that his original description of *M. yucatanensis* (Holsinger 1977) 'was based on what appear to be submature specimens, therefore raising the strong possibility that the differences noted between the two species of *Mayaweckelia* are due primarily to age', and the two species probably should be synonymized. However, the synonymisation has not been published until now. *Mayaweckelia troglomorpha* sp. n. differs from *M. yucatanensis* by i) three times larger body size; ii) significantly increased number of flagellum articles in both antennae (three times more articles on primary flagellum); iii) gnathopod I carpus 1.5–1.7 times longer than propodus (vs. same length); iv) proportionally longer and differently ornamented propodus of gnathopod II (palm armed with unnotched spine teeth and pearl row-like setae); v) pereopods VI and VII 130 % of body length (vs. 60 % of body length) vi) more distinct ventro-posterior corner of epimeral plates. *M. troglomorpha* sp. n. differs from *M. cenoticola* by i) its two times larger body size; ii) elevated number of flagellum articles in both antennae; iii) less narrow and differently ornamented propodus of gnathopod I (palm armed with distally notched spine teeth); iv) gnathopod I carpus 1.5–1.7 times longer than propodus (vs. 0.7 times longer); v) longer and differently ornamented propodus of gnathopod II; vi) pereopods VI and VII 130 % of body length (vs. approximately 60 % of body length); and vii) more distinct ventro-posterior corner of epimeral plates. Scanning electron microscopy has revealed that uropods I–III are covered with pubescent setae (not mentioned in the description of *M. yucatanensis* and *M. cenoticola*), this character should also be checked on these species using SEM studies, as this trait is not visible using light microscopy.

The new species corresponds with the diagnostic characters of the genus *Mayaweckelia*. It differs from the related *Tuluweckelia* in the following traits: i) anterior body region does not bend markedly downward; ii) maxilla I outer lobe with seven-nine setae; iii); gnathopod II sexually dimorphic; iv) epimeral plates ventro-posterior corners less

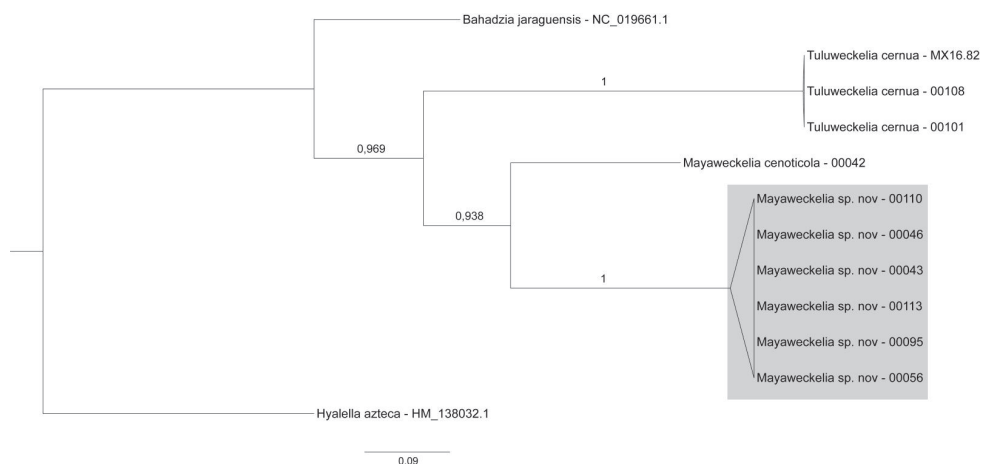
produced. *Mayaweckelia* differs from *Bahadzia* by i) the absense of palp from both mandibles; and ii) outer ramus of uropod III with one article (Holsinger and Yager 1985, Holsinger 1992). *Mexiweckelia* Holsinger & Minckley, 1971 and *Paramexiweckelia* Holsinger, 1982 are subterranean genera of the ‘weckeliid’ group known from north of México (e.g., Holsinger and Minckley 1971, Holsinger 1982). *Mayaweckelia* differs from them in some important ways: i) accessory flagellum of first antenna three-articulated (vs. single or vestigial); ii) presence of robust setae on inner margin of maxilliped outer lobe; iii) presence of large, ventrally produced lobe on gnathopod I merus; iii) sexually dimorphic gnathopod II (of *Paramexiweckelia* is not dimorphic); iv) pereopod VI little longer than pereopod VII; and v) completely separated telson halves (vs. deeply incised but fused in the other two genera).

Mitochondrial gene sequences

In accordance with the morphological data, the Bayesian analysis of COI sequences showed that the closest relative of the herein described new species is *M. cenoticola*, forming a monophyletic group referring to the genus *Mayaweckelia* (Figure 10). Comparing uncorrected p-distances (Table 2), the distance between *M. cenoticola* and the new species is 22 % (p = 0.221–0.224). Five individuals of *M. troglomorpha*, sp. n. show rather low intraspecific variance (p = 0.002–0.009). Among these, all substitutions proved to be synonymous (same sense), occurring in the third codon positions. However, individual ‘00110’ of the new species differs in 2 % (p = 0.016–0.022) from the other five specimens, and contains a nonsynonymous substitution. COI sequences of the three *Tuluweckelia cernua* individuals, including the one which was collected in Quintana Roo state, belonged to the same haplotype. Though, mitochondrial gene sequence of only a single species of the genus *Bahadzia* was available, the constructed phylogenetic tree is in accordance with the taxonomical ranks, *Tuluweckelia* being the sister group of *Mayaweckelia*. It is worth mentioning that the uncorrected p-distance

Table 2. Uncorrected p-distances between the studied hadziid species.

		1	2	3	4	5	6	7	8	9	10
1	<i>Mayaweckelia cenoticola</i> -00042										
2	<i>M. troglomorpha</i> sp. n. -00110	0.221									
3	<i>M. troglomorpha</i> sp. n. -00046	0.224	0.022								
4	<i>M. troglomorpha</i> sp. n. -00113	0.224	0.016	0.008							
5	<i>M. troglomorpha</i> sp. n. -00095	0.223	0.017	0.009	0.002						
6	<i>M. troglomorpha</i> sp. n. -00043	0.224	0.022	0.003	0.008	0.009					
7	<i>M. troglomorpha</i> sp. n. -00056	0.224	0.017	0.009	0.002	0.003	0.009				
8	<i>Tuluweckelia cernua</i> -MX16.820	0.265	0.288	0.296	0.296	0.298	0.296	0.295			
9	<i>Tuluweckelia cernua</i> -00108	0.265	0.288	0.296	0.296	0.298	0.296	0.295	0.000		
10	<i>Tuluweckelia cernua</i> -00101	0.265	0.288	0.296	0.296	0.298	0.296	0.295	0.000	0.000	
11	<i>Bahadzia jaraguensis</i> -NC 019661.1	0.251	0.248	0.243	0.246	0.245	0.241	0.246	0.277	0.277	0.277



**Figure 10.** Bayesian phylogenetic tree of COI sequences based on the collected *Mayaweckelia* and *Tuluweckelia* samples and publicly available hadziid and hyalellid sequences. *Hyalella azteca* was included as outgroup taxon. Posterior probability values are indicated. *Bahadzia jaraguensis* and *H. azteca* sequences are after Bauza-Ribot et al. (2012) and Baird et al. (2011), respectively.

value between the two *Mayaweckelia* species is almost as high (22 %) as the distance between the three genera (24–30 %).

## Discussion

The ‘weckeliid’ group of Hadziidae is composed of mostly monotypic, predominantly subterranean freshwater genera with a significant concentration of species in the old Tethyan remnants of the greater Caribbean and Gulf of México regions (e.g., Holsinger and Longley 1980, Barnard and Barnard 1983, Holsinger 1986, Holsinger and Ruffo 2002). Their evolution into freshwater stygobionts is explained by ‘stranding’ in newly developing hypogean freshwater habitats following marine regressions (e.g., Holsinger 1977, Stock 1980, Holsinger and Longley 1980, Holsinger 1986, 1992, 1994, Holsinger and Ruffo 2002). Holsinger (1986) stated that this group is primarily distinguished morphologically from other members of the family Hadziidae by the apomorphic character state of the third uropod. In the weckeliids, the rami are typically subequal in length and the outer ramus lacks a second article. A further important weckelioid character is the lack of mandibular palp (Stock 1985). Primarily because of the former two characters, *Mayaweckelia* and *Tuluweckelia* were previously considered to be members of the weckeliid group. However, as Holsinger (1990) pointed out, unlike all other genera previously classified to the weckeliids, the two Yucatán genera lack basofacial robust setae on the first uropod. Later on, Holsinger and Ruffo (2002) recommended the two genera to be assigned to separate groups, as they apparently belong to other lineages within the family.

Cladistic analysis performed including *Bahadzia*, the third stygobiont hadziid genus known from the peninsula and another 13 further hadziid genera suggested that *Mayaweckelia* and *Tuluweckelia* are sister genera to *Bahadzia* and may even be derived from a *Bahadzia*-like ancestor (Holsinger 1992, Sawicki and Holsinger 2004). Our mitochondrial sequence analysis supports this idea, though, it would be necessary to collect individuals of both Yucatán *Bahadzia* species (*B. bozanici* and *B. setodactylus*) to further solve this question.

*Mayaweckelia cenoticola* was previously recorded in 13 caves and cenotes and, except for two or three, they were all taken from fresh water habitats (Holsinger 1977, Reddell 1981, Holsinger 1990, Rocha et al. 1998, Alvarez and Iliffe 2008, Alvarez et al. 2015). Individuals of the new *Mayaweckelia* species were also found in freshwater in all cases, as well as the single specimen of newly collected *M. cenoticola*.

Intergeneric sympatry of subterranean Hadziidae, which is quite rare, can be explained by secondary contact (Bouin and Messouli 1988). This study revealed that the monotypic genus *Tuluweckelia*, which was previously known mostly from salt-water habitats of anchialine cenotes near the northeastern coastline of the peninsula (Holsinger 1990, Rocha et al. 1998, Alvarez and Iliffe 2008, Alvarez et al. 2015), exists in freshwater cenotes and submerged cave passages far from the coastline as well. *Tuluweckelia cernua* proved to be a relatively common species of the visited localities, as we found small populations in almost half of the sampled localities. Referring to the species' geographic distribution and ecology, Holsinger (1990) considered that the origin of *Tuluweckelia* from hypothetical marine ancestors is more recent than that of *Mayaweckelia* and 'may be related to the recession of a high sea stand during the Pleistocene'. Interestingly, despite the approximately 200 km distance between the most distant localities, only one COI haplotype occurred within the individuals collected in Yucatán state and Quintana Roo state (see sample data in Table 1). Botello and Alvarez (2010) pointed out that in case of the Yucatán cave shrimp *Creaseria morleyi*, genetic variation is a relict of an ancient marked genetic structure reduced by changes in sea level that resulted in a series of bottlenecks. A support to *Tuluweckelia*'s more recent marine originated subterranean colonisation hypothesis (Holsinger 1990) can be that unlike *Mayaweckelia*, during our thorough samplings in 14 cenotes, we have not discovered additional species of the genus *Tuluweckelia*, other than *T. cernua*. To study the origin of the peninsula's stygobiont hadziid fauna and to calibrate divergence times, an extended phylogenetic study would be needed, involving a series of species from different habitat types, using both mitochondrial and nuclear markers.

It is remarkable that in spite of the low intraspecific variability recorded of *M. troglomorpha* sp. n., COI sequence of one individual differed significantly from all the rest. This individual was found in Cenote Kankirixché, which is characteristically holds the most diverse subterranean crustacean fauna among the studied cenotes. In the same site, another individual of the new species was also collected, which shared the same haplogroup with the rest of the specimens from other cenotes. These two individuals were found in two distinct parts of the system: the former far from the entrance, below 30 meters depth in a descending cave passage, while the latter closer to the entrance, in

the cavern part. To study the possibility of cryptic speciation, further molecular studies of additional samples from Kankirixché could lead to interesting results.

Obtaining individuals for morphological and molecular genetic analyses from the type locality of *M. yucatanensis* (Grutas de Xtacumbilxunam, Campeche state) could aid in a comparison and validation of the species. To gain a better knowledge on the distribution range of the previously known and the newly described *Mayaweckelia* species and to contribute to their conservation planning, it would be important to explore additional cenotes and other subterranean ecosystems in Yucatán state and in the rest of the peninsula. Local regulations that target the protection of the species' habitats are necessary.

## Conclusions

To date, only a small proportion of the cenotes and other aquatic hypogean ecosystems have been studied in Yucatán state in zoological aspect. Our expedition has led to the discovery of a new species of subterranean hadziids, which confirms that exploration and further studies of the region's groundwater Crustacea diversity is necessary. Description of the new species was completed with comparative scanning electron microscopy, which was used for first time on *Mayaweckelia*. It proved to be a rather useful method for discovering, analysing, and illustrating barely visible diagnostic characters. As contributions to the future molecular genetic studies on Yucatán subterranean hadziids, COI sequences as barcodes of *M. troglomorpha* sp. n., *M. cenoticola*, and *T. cernua* are now publicly available in GenBank. The phylogenetic studies have shown that based on the available sequences, the closest relative of the new species is *M. cenoticola*. In accordance with the previous cladistic studies, *Mayaweckelia* and *Tuluweckelia* prove to be sister genera, closely related to *Bahadzia*, the third Yucatán subterranean Hadziidae genus. This knowledge may contribute to the species' future conservation planning.

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## References

- Akaike H (1973) Information theory and an extension of the maximum likelihood principle. Proceedings of the Second International Symposium on Information Theory, Budapest, 267–281.
- Alvarez F, Iliffe TM, Villalobos JL (2005) New species of the genus *Typhlatya* (Decapoda: Atyidae) from anchialine caves in México, the Bahamas and Honduras. Journal of Crustacean Biology 25(1): 81–94. <https://doi.org/10.1651/C-2516>
- Alvarez F, Iliffe TM (2008) Fauna anquihalina de Yucatán. In: Alvarez R, Rodríguez-Almaraz (Eds) Crustáceos de México: Estado Actual de su Conocimiento. Universidad Autónoma de Nuevo León-PROMEP, 379–418.
- Alvarez F, Iliffe TM, Benítez S, Brankovits D, Villalobos JL (2015) New records of anchialine fauna from the Yucatán Peninsula, México. Check List 11(1): 1505. doi: <http://dx.doi.org/10.15560/11.1.1505>
- Angyal D, Balázs G, Zakšek V, Krízsik V, Fišer C (2015) Redescription of two subterranean amphipods *Niphargus molnari* Méhely, 1927 and *Niphargus gebhardti* Schellenberg, 1934 (Amphipoda, Niphargidae) and their phylogenetic position. ZooKeys 509: 53–85. <https://doi.org/10.3897/zookeys.509.9820>
- Angyal D, Chávez Solís E, Magaña B, Simoes N (in prep.) New records of stygobiont crustaceans from cenotes of Yucatán state (México) with an illustrated identification guide.
- Baird DJ, Pascoe TJ, Zhou X, Hajibabaei M (2011) Building freshwater macroinvertebrate DNA-barcode libraries from reference collection material: formalin preservation vs specimen age. Journal of North American Benthological Society 30(1): 125–130. <https://doi.org/10.1899/10-013.1>
- Barnard JL, Barnard CM (1983) Freshwater Amphipoda of the World (Parts I & II), Hayfield Associates, Mt. Vernon, VA., 830 pp.
- Bauer-Gottwein P, Gondwe BRN, Chauvert G, Marín LE, Rebolledo-Vieyra M, Merediz-Alonso G (2011) The Yucatán Peninsula karst aquifer, México. Hydrogeology Journal 19(3): 507–524. <https://doi.org/10.1007/s10040-010-0699-5>



- Bauzá-Ribot MM, Nardi F, Oromí P, Pons J, Jaume D (2012) Mitogenomic phylogenetic analysis supports continental-scale vicariance in subterranean thalassoid crustaceans. *Current Biology* 22(21): 2069–2074. <https://doi.org/10.1016/j.cub.2012.09.012>
- Bouin CL, Messouli M (1988) *Longipodacrangonyx marrocanus*, n. gen., n. sp., nouveau représentant du groupe *Metacrangonyx* dans les eaux souterraines du Maroc. *Crustaceana* 13: 256–271.
- Botello A, Alvarez F (2010) Genetic variation in the stygobitic shrimp *Creaseria morleyi* (Decapoda: Palaemonidae), evidence of bottlenecks and re-invasions in the Yucatán Peninsula. *Biological Journal of the Linnean Society* 99(2): 315–325. <https://doi.org/10.1111/j.1095-8312.2009.01355.x>
- Dereeper A, Guignon V, Blanc G, Audic S, Buffet S et al. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 2008 Jul 1: 36. [Web Server issue: W465–9].
- Escolero OA, Marin LE, Steinich B, Pacheco AJ, Cabrera SA, Alcocer J (2002) Development of a protection strategy of karst limestone aquifers: The Merida Yucatan, México Case Study. *Water Resources Management* 16: 351–367. <https://doi.org/10.1023/A:1021967909293>
- Fišer C, Trontelj P, Luštrik R, Sket B (2009) Towards a unified taxonomy of *Niphargus* (Crustacea: Amphipoda): a review of morphological variability. *Zootaxa* 2061: 1–22.
- 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.
- Gonzalez-Herrera R, Sanchez-Pinto I, Gamboa-Vargas J (2002) Groundwater-flow modeling in the Yucatan karstic aquifer, México. *Hydrogeology Journal* 10(5): 539–552. <https://doi.org/10.1007/s10040-002-0216-6>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Holsinger JR, Minckley WL (1971) A new genus and two new species of subterranean amphipod crustaceans (Gammaridae) from Northern México. *Proceedings of the Biological Society of Washington* 83(37): 425–444.
- Holsinger JR (1977) A new genus and two new species of subterranean amphipod crustaceans (Gammaridae s. lat.) from the Yucatán Peninsula in México. *Association for Mexican Cave Studies, Bulletin* 6: 15–25.
- Holsinger JR, Longley G (1980) The subterranean amphipod crustaceana fauna of an artesian well in Texas. *Smithsonian Contributions to Zoology* 308: 1–62. <https://doi.org/10.5479/si.00810282.308>
- Holsinger JR (1982) *Paramexiweckelia*, a new genus of subterranean amphipod crustacean (Hadziidae) from Northern México. *International Journal of Speleology* 12: 37–44. <https://doi.org/10.5038/1827-806X.12.1.4>
- Holsinger JR, Yager J (1985) A new genus and two new species of subterranean amphipod crustaceans (Hadziidae) from the Bahamas and Turks and Caicos Islands. *Bijdragen tot de Dierkunde* 55(2): 283–294.
- Holsinger JR (1986) Zoogeographic patterns of North American subterranean amphipod crustaceans, in: Gore RH et al. (Eds) *Crustacean biogeography*. *Crustacean Issues* 4: 85–106.

- Holsinger JR (1990) *Tuluweckelia cernua*, a new genus and species of stygobiont amphipod crustacean (Hadziidae) from anchialine caves on the Yucatán Peninsula in México. *Beaufortia* 41: 97–107.
- Holsinger JR (1992) Two new species of the subterranean Amphipod genus *Bahadzia* (Hadziidae) from the Yucatan Peninsula region of southern México, with an analysis of phylogeny and biogeography of the genus. *Stygologia* 7(2): 85–105.
- Holsinger JR (1994) Pattern and process in the biogeography of subterranean amphipods. *Hydrobiologia* 287: 131–145. <https://doi.org/10.1007/BF00006902>
- Holsinger JR, Ruffo S (2002) *Indoweckelia superstes* n. gen. n. sp. from the subterranean waters of Socotra Island: the first weckeliid amphipod crustacean (Hadziidae) found in the Indo-West Pacific Region. *Bollettino del Museo Civico di Storia Naturale di Verona* 26: 27–36.
- IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. IUCN, Gland, Switzerland and Cambridge, UK, 32 pp. <https://doi.org/10.1111/cla.12055>
- Karaman S (1932) 5. Beitrag zur Kenntnis der Süßwasser-Amphipoden (Amphipoden unterirdischer Gewässer). *Prirodoslovne razprave, Ljubljana* 2: 179–232. [In German]
- Karaman S (1943) Die unterirdischen Amphipoden Südserbiens. *Srpska Akad. Nauk., Posebna Izd.* 135 (Prirod. Matem. Spisi) 34(4): 161–312. [In German]
- Latreille PA (1816) Amphipoda. *Nouveau Dictionnaire d'histoire naturelle, appliquée aux Arts, à l'Agriculture, à l'Économie rurale et domestique, à la Médecine, etc. Par une société de Naturalistes et d'Agriculteurs* 2nd edition. Volume 1. Deterville, Paris, 467–469. [In French]
- Lowry JK, Myers AA (2013) A phylogeny and classification of the Senticaudata subord. nov. (Crustacea: Amphipoda). *Zootaxa* 3610(1): 1–80. <https://doi.org/10.11646/zootaxa.3610.1.1>
- Marrón-Becerra A, Hermoso-Salazar M, Solís-Weiss W (2014) *Hyaella cenotensis*, a new species of Hyaellidae (Crustacea: Amphipoda) from the Yucatán Peninsula, México. *Zootaxa* (3811): 262–270. <https://doi.org/10.11646/zootaxa.3811.2.7>
- Nei M, Kumar S (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 333 pp.
- Notenboom J (1991) Marine regressions and the evolution of groundwater dwelling amphipods (Crustacea). *Journal of Biogeography* 18: 437–454. <https://doi.org/10.2307/2845485>
- Ortiz M, Winifield I (2015) new amphipod species (Peracarida: Amphipoda: Ampithoidae) collected from Cenote Aerolito, Cozumel Island, Quintana Roo. *Revista Mexicana de Biodiversidad* 86: 332–336. <https://doi.org/10.1016/j.rmb.2015.04.008>
- Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 16. <https://doi.org/10.1186/1742-9994-7-16>
- Rambaut A (2012) Figtree version 1.4.0. <http://tree.bio.ed.ac.uk/software/figtree/>
- Reddell JR (1981) A review of the cavernicole fauna of México, Guatemala, and Belize. *Texas Memorial Museum, The University of Texas at Austin, Bulletin* 27, 327 pp.
- Rocha CEF, Iliffe TM, Reid JW, Suárez-Morales E (1998) A new species of *Halicyclops* (Copepoda, Cyclopoida, Cíclopoidae) from cenotes of the Yucatan Peninsula, México, with an identification key for the species of the genus from the Caribbean region and adjacent areas. *Sarsia* 83: 387–399. <https://doi.org/10.1080/00364827.1998.10413698>

- Sawicki TR, Holsinger JR (2004) Systematics of the subterranean amphipod genus *Bahadzia* (Hadziidae), with description of a new species, redescription of *B. yagerae*, and analysis of phylogeny and biogeography. *Journal of Natural History* 38: 1397–1414. <https://doi.org/10.1080/0022293031000155386>
- Stock JH (1980) Regression model evolution as exemplified by the genus *Pseudoniphargus* (Amphipoda). *Bijdragen tot de Dierkunde* 50: 104–144.
- Stock JH (1985) Stygobiont amphipod crustaceans of the hadzioid group from Haiti. *Bijdragen tot de Dierkunde* 55(2): 331–426.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>
- Trujillo Pisanty D, Chávez Solís EM, Solís Weiss V, Hermoso Salazar M (2010) Peracarids (Crustacea: Malacostraca) from cenote Aerolito, Cozumel, Mexican Caribbean. *Cahiers de Biologie Marine* 51: 177–180.
- Watling L (1989) A classification system for crustacean setae based on the homology concept. In: Felgenhauer BWL, Thistle AB (Eds) *Functional morphology of feeding and grooming in Crustacea*. AA Balkema, Rotterdam, 15–26.



# A new species of the genus *Pseudocrangonyx* (Crustacea, Amphipoda, Pseudocrangonyctidae) from Korea

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## Abstract

A new subterranean species of pseudocrangonyctid amphipod, *Pseudocrangonyx daejeonensis* **sp. n.** is described from the interstitial waters in Daejeon, Korea. *Pseudocrangonyx daejeonensis* **sp. n.** is distinguished from three morphologically similar congeners, *P. coreanus* Uéno, 1966, *P. februs* Sidorov, 2009, and *P. gudariensis* Tomikawa & Sato, 2016, by the characteristics of antenna 1, antenna 2, mandible, gnathopod 2, pleopods, uropods 1–2, and telson. Molecular phylogenetic analyses based on nuclear 28S rRNA and histone H3, and mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA genes revealed that *P. daejeonensis* is a sister species of the unnamed *Pseudocrangonyx* sp. 3 inhabiting central Japan.

## Keywords

Crangonyctoidea, Korean Peninsula, interstitial water, molecular phylogeny

## Introduction

Amphipod species of the genus *Pseudocrangonyx* Akatsuka & Komai, 1922 have been known from subterranean waters and springs in Japan, the Korean Peninsula, Eastern China, and the Far East of Russia (Sidorov and Holsinger 2007; Tomikawa et al. 2016; Zhao and Hou 2017). Among the 22 known species of *Pseudocrangonyx*, only two

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species were recorded in Korean waters (Uéno 1966): *P. asiaticus* Uéno, 1934 and *P. coreanus* Uéno, 1966.

When Uéno (1966) described *P. coreanus* based on specimens collected from the Korean Peninsula, he clearly stated that morphological variations in the antennae, maxilla 1, uropod 3, and telson were observed among six populations of this species. Because recent systematic studies of *Pseudocrangonyx* in other regions have shown high species diversity within this genus (e.g., Tomikawa et al. 2016), it is highly possible that the true species diversity of *Pseudocrangonyx* amphipods inhabiting the Korean Peninsula remains under-estimated.

Recently, unidentified specimens of *Pseudocrangonyx* were collected during field surveys of interstitial invertebrates in Korea by the first author. In this paper, we describe and illustrate this amphipod as a new species. In addition, the phylogenetic position of the new species was estimated using nuclear 28S rRNA and histone H3, and mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rRNA sequence data.

## Materials and methods

### Sampling

*Pseudocrangonyx* specimens were collected from interstitial water in Heukseok-dong, Seo-gu, Daejeon, South Korea (Fig. 1) using a 50 µm fine-mesh net. Specimens were pumped up with 80–100 L of interstitial water at 1–1.5 m beneath hyporheic zones using a core (Lee and Park 2016). All specimens were immediately preserved in 95% ethanol. The specimens are deposited in the collection of the Nakdonggang National Institute of Biological Resources, Korea (NNIBR) and in the Zoological Collection of Kyoto University (KUZ).

### Morphological observation

The specimens were dissected in 70 % ethanol and mounted in gum-chloral medium on glass slides under a stereomicroscope (Model SZX-7; Olympus, Tokyo, Japan). Specimens were examined using a Nikon Eclipse Ni light microscope (Nikon, Tokyo, Japan) and illustrated with the aid of a drawing tube. The body length from the tip of the rostrum to the base of the telson was measured along the dorsal curvature to the nearest 0.1 mm. The nomenclature of the setal patterns on the mandibular palp follows that of Stock (1974).

### Molecular phylogenetic analyses

Methods of the genomic DNA extraction, PCR and DNA sequencing were performed following Tomikawa et al. (2016). Accordingly, nine DNA sequences of nuclear 28S





**Figure 1.** Map showing the collection locality of the specimens examined in this study.

rRNA, histone H3, COI and 16S rRNA from three Korean *Pseudocrangonyx* specimens were newly obtained in this study, and deposited into the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (Table 1).

The OTU set for phylogenetic analyses was almost identical to that used in the previous phylogenetic analyses in Tomikawa et al. (2016) with the DNA sequences of

**Table 1.** Samples used for the phylogenetic analyses. The information on the vouchers is accompanied by the collection locality and the INSDC accession numbers. Sequences marked with an asterisk were obtained for the first time in the present study. Acronyms: IZCAS, Institute of Zoology, Chinese Academy of Sciences; NNIBR, Nakdonggang National Institute of Biological Resources; NSMT, National Museum of Nature and Science, Tokyo.

Species	Voucher or isolate #	Locality or country	INSDC #			
			28S	Histone H3	COI	16S
<i>Pseudocrangonyx</i>						
<i>P. daejeonensis</i> sp. n.	NNIBRIV1 (Holotype)	Daejeon, Korea	LC322136*	LC322138*	LC322137*	LC322135*
<i>P. daejeonensis</i> sp. n.	NNIBRIV2 (Paratype)	Daejeon, Korea		LC322143*		
<i>P. daejeonensis</i> sp. n.	NNIBRIV3 (Paratype)	Daejeon, Korea	LC322140*	LC322142*	LC322141*	LC322139*
<i>P. gudariensis</i>	NSMT-Cr 24605	Aomori, Japan	LC171498	LC171500	LC171499	LC171497
<i>P. yezonis</i>	G1280	Hokkaido, Japan	LC171518	LC171520	LC171519	LC171517
<i>P. yezonis</i>	G1279	Akita, Japan	LC171514	LC171516	LC171515	LC171513
<i>Pseudocrangonyx</i> sp. 1	G400	Iwate, Japan				LC171479
<i>Pseudocrangonyx</i> sp. 1	G1281	Iwate, Japan				LC171521
<i>Pseudocrangonyx</i> sp. 2	G1283	Okayama, Japan	LC171525	LC171527	LC171526	LC171524
<i>Pseudocrangonyx</i> sp. 2	G1277	Yamaguchi, Japan	LC171506	LC171508	LC171507	LC171505
<i>Pseudocrangonyx</i> sp. 2	G1278	Yamaguchi, Japan	LC171510	LC171512	LC171511	LC171509
<i>Pseudocrangonyx</i> sp. 3	G404	Shiga, Japan	LC171488	LC171489		
<i>Pseudocrangonyx</i> sp. 3	G405	Shiga, Japan	LC171491	LC171493	LC171492	LC171490
<i>Pseudocrangonyx</i> sp. 3	G406	Shiga, Japan	LC171495	LC171496		LC171494
<i>Pseudocrangonyx</i> sp. 4	G1282	Shiga, Japan		LC171523		LC171522
<i>Pseudocrangonyx</i> sp. 5	G402	Shimane, Japan	LC171485	LC171487	LC171486	LC171484
<i>Pseudocrangonyx</i> sp. 5	G401	Shimane, Japan	LC171481	LC171483	LC171482	LC171480
<i>Pseudocrangonyx</i> sp. 5	G1271	Kagawa, Japan	LC171502	LC171504	LC171503	LC171501
<i>Pseudocrangonyx</i> sp. 5	G1295	Kochi, Japan	LC171533	LC171535	LC171534	LC171532
<i>Pseudocrangonyx</i> sp. 5	G1296	Kochi, Japan	LC171537	LC171539	LC171538	LC171536
<i>Pseudocrangonyx</i> sp. 5	G1294	Ehime, Japan	LC171529	LC171531	LC171530	LC171528
<i>Pseudocrangonyx</i> sp. 6	G1297	Gifu, Japan	LC171541	LC171543	LC171542	LC171540
<i>P. holsingeri</i>		Russian Far East	KJ871679		KF153111	
<i>P. korkishkorum</i>	B1	Russian Far East	KJ871678		KF153107	
<i>P. korkishkorum</i>	B2	Russian Far East			KF153108	
<i>P. korkishkorum</i>	B3	Russian Far East			KF153109	
<i>P. korkishkorum</i>	N1	Russian Far East	KJ871676		KF153105	
<i>P. korkishkorum</i>	N2	Russian Far East	KJ871677		KF153106	
<i>P. kseniae</i>		Russian Far East	KJ871675		KF153115	
<i>P. susanaensis</i>		Russian Far East			KF153113	
<i>P. sympatricus</i>		Russian Far East			KF153112	
<i>P. tiunovi</i>		Russian Far East	KJ871674		KF153110	
<i>P. elegantulus</i>	IZCAS I-A1602-2	China	KY436646		KY436647	
Outgroup						
<i>Crymostygius thingvallensis</i>						HQ286009
<i>Eocrangonyx primoryensis</i>						HQ286011
<i>Crangonyx floridanus</i>	G1322	Chiba, Japan	LC171549		LC171550	LC171548

*P. elegantulus* Hou in Zhao and Hou (2017) (Table 1). The alignments of H3 and COI were trivial, as no indels were observed. 28S and 16S sequences were aligned using MAFFT v. 7.310 (Katoh and Standley 2013). The lengths of the 28S, H3, COI and 16S were 1357, 328, 658, and 430 bp, respectively. The concatenated sequences thus yielded 2773 bp of alignment positions. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The ML phylogeny was constructed using RAxML v. 8.2.8 (Stamatakis 2014) with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (BS) conducted with 1000 replicates. The best-fit partition scheme was identified with Akaike information criterion using PartitionFinder v. 2.1.1 (Lanfear et al. 2017) with the “greedy” algorithm (Lanfear et al. 2012): 28S/1st and 2nd positions of H3/H3 3rd position/COI 1st position/COI 2nd position/COI 3rd position/16S. BI and Bayesian posterior probabilities (PPs) were estimated using MrBayes v. 3.2.6 (Ronquist et al. 2012). The best-fit partition scheme and models for each partition were selected based on the Bayesian information criterion using PartitionFinder with the “greedy” algorithm: for 28S, GTR+G; for H3 1st and 2nd position, JC+I; for H3 3rd position, K80+G; for COI 1st position, SYM+I+G; for COI 2nd position, F81+I; for COI 3rd position, GTR+I+G; and GTR+I+G for 16S. Two independent runs of four Markov chains were conducted for 20 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut and Drummond 2013), and the first 50001 trees were discarded based on the results.

## Taxonomy

### Family Pseudocrangonyctidae Holsinger, 1989

#### Genus *Pseudocrangonyx* Akatsuka & Komai, 1922

#### *Pseudocrangonyx daejeonensis* sp. n.

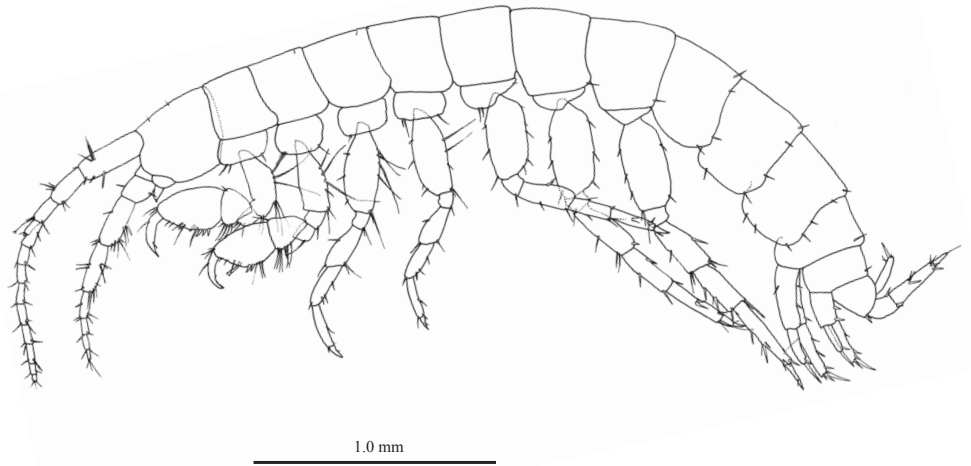
<http://zoobank.org/ECC7F708-DD43-4A48-9458-B6DA59265796>

Figs 2–9

**Material examined.** Holotype: Female (NNIBRIV1, 3.8 mm), Heukseok-dong (36°15.65'N, 127°20.43'E), Daejeon, Korea, collected by Lee CW on 31 May 2017. Paratypes: 1 male (NNIBRIV2, 2.7 mm), 1 female (NNIBRIV3, 2.3 mm), 3 females (KUZ Z1924), data same as for holotype.

**Etymology.** The specific name is an adjective derived from the type locality name of the new species.

**Description.** *Female* [NNIBRIV1, 3.8mm]. Head (Fig. 2) with short dorsal setae; rostrum reduced; lateral cephalic lobe rounded; antennal sinus shallow with rounded angle; eyes absent. Dorsal margin of pleonites 1–3 and urosomites 1–2 with setae (Fig. 2). Ventral margin of urosomite 1 without setae (Fig. 2). Ventral margin of epimeral plate 1 with seta, posteroventral corner rounded with seta (Fig. 2); ventral



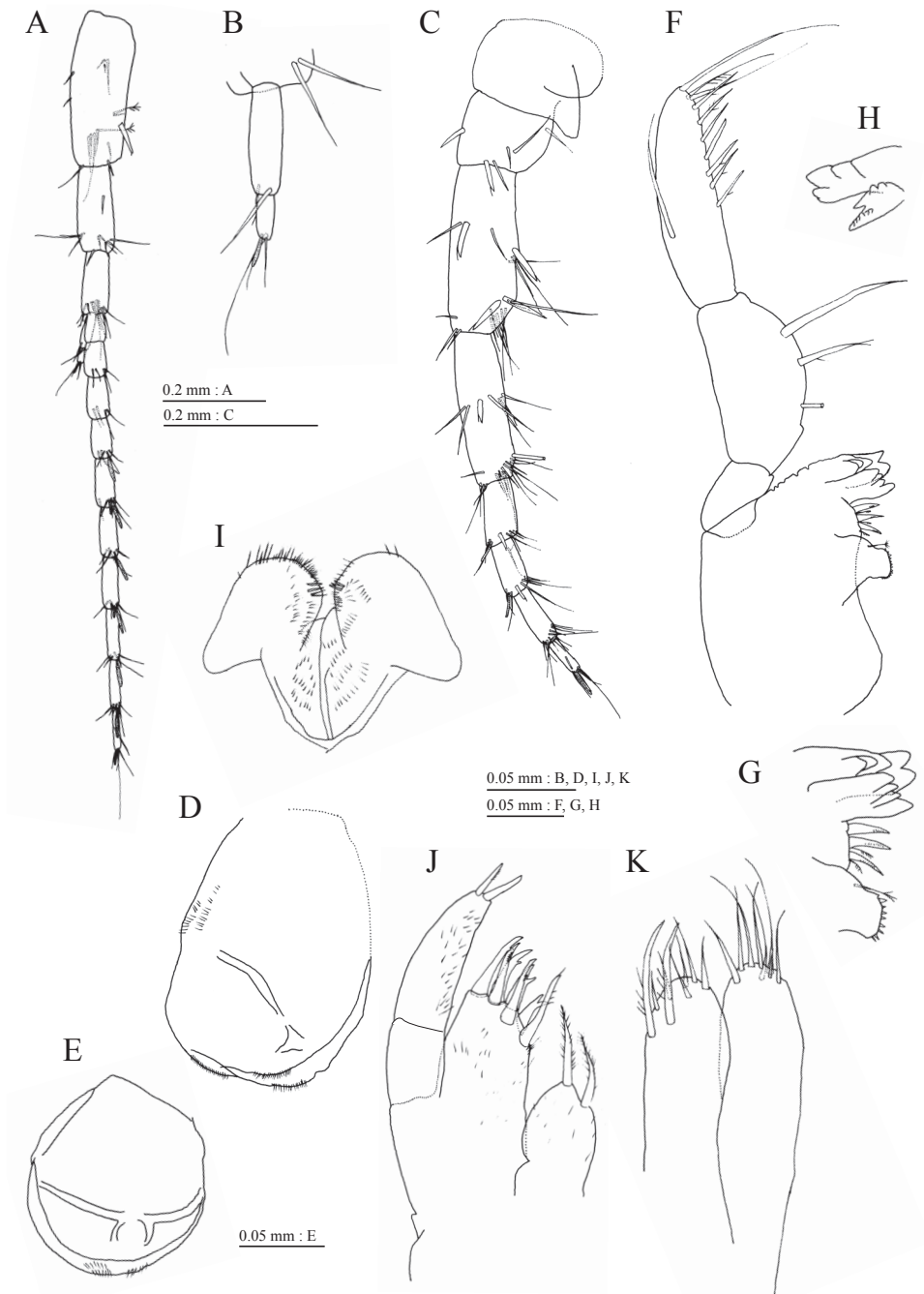
**Figure 2.** *Pseudocrangonyx daejeonensis* sp. n., holotype, female (3.8 mm), NNIBRIV1. Habitus, lateral view.

and posterior margins of plate 2 each with seta, posteroventral corner rounded with seta (Fig. 2); ventral and posterior margins of plate 3 each with 2 setae, posteroventral corner rounded with seta (Fig. 2).

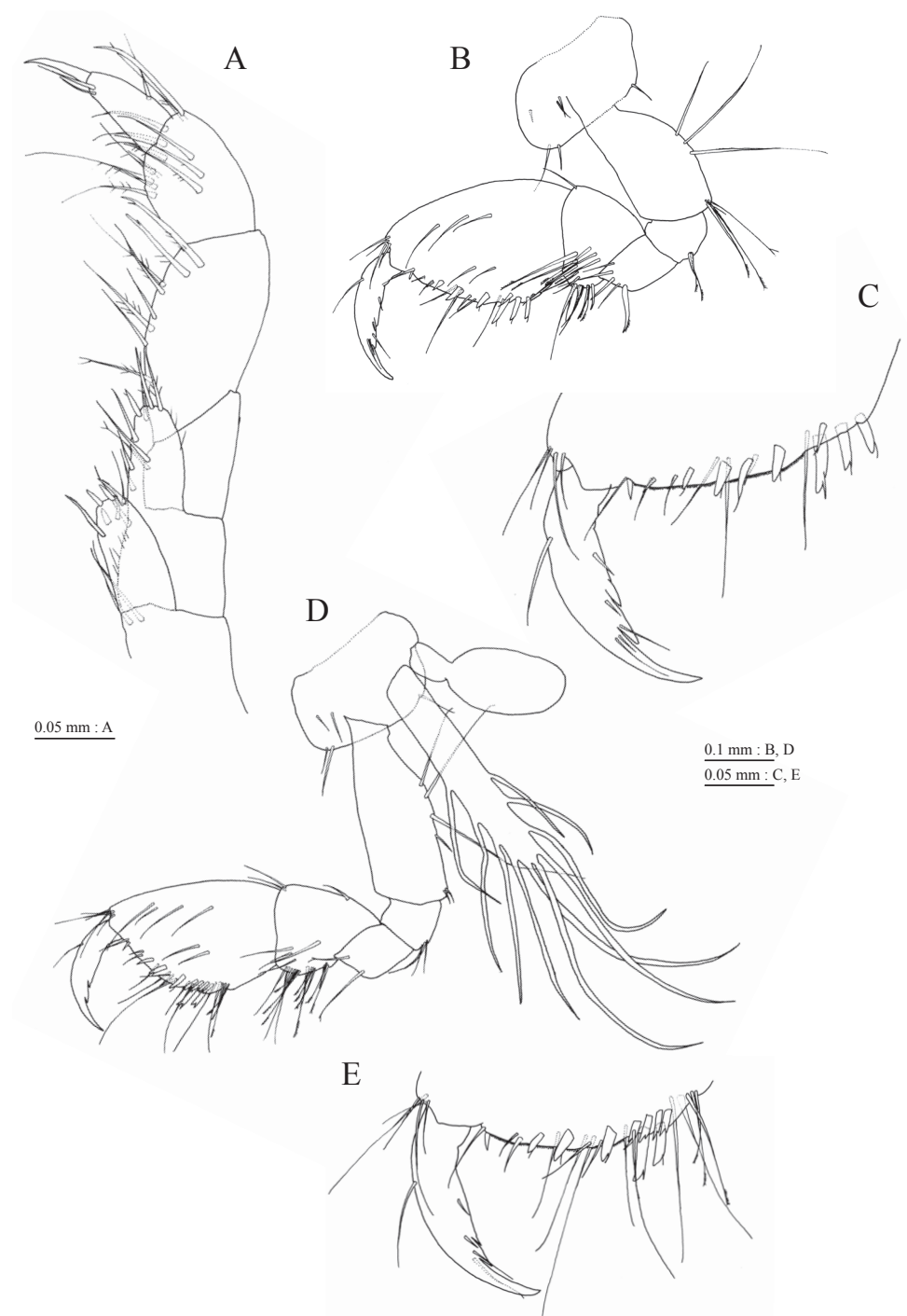
Antenna 1 (Fig. 3A) 0.38 times as long as body length, peduncular articles 1 to 3 in length ratio of 1.0 : 0.5 : 0.4; accessory flagellum (Fig. 3B) 2-articulate, terminal article with 3 setae and 1 aesthetasc; primary flagellum 10-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 3C) 0.58 times as long as antenna 1; flagellum 0.65 times as long as peduncular articles 4 and 5 combined, consisting of 4 articles; calceoli absent.

Upper lip (Fig. 3D) with rounded anterior margin, bearing fine setae. Mandibles (Fig. 3F, G, H) with left and right incisors with 5- and 4-dentate, respectively; left lacinia mobilis 4-dentate, right lacinia bifid, bearing many teeth; molar process tritritative; accessory setal rows of left and right mandibles with 3- and 2- pectinate setae, respectively; palp 3-articulate, article 3 with 1 A-, 7 D-, and 3 E-setae. Lower lip (Fig. 3I) with broad outer lobes with fine setae, mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 3J) with inner and outer plates, and palp; inner plate subovate with 2 plumose setae; outer plate subrectangular with 7 serrate teeth apically; palp 2-articulate, longer than outer plate, article 2 with 2 apical robust setae. Maxilla 2 (Fig. 3K) with oblique inner row of 2 setae on inner plate. Maxilliped (Fig. 4A) with inner and outer plates, and palp; inner plate reaching base of palp article 1, with 2 apical robust setae; outer plate not exceeding end of palp article 1, with 2 plumose setae and some medial setae; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

Gnathopod 1 (Fig. 4B) with subquadrate coxa, bearing setae on its anterodistal and posteroventral corners, width 1.9 times as long as depth; posterior margin of basis with 3 setae; posterodistal corner of carpus with slender setae, some weakly pectinate; propodus stout, subchelate, palmar margin with 3 medial and 3 lateral robust setae; posterior



**Figure 3.** *Pseudocrangonyx daejeonensis* sp. n., holotype, female (3.8 mm): **A–D, F–K** paratype female (2.3 mm): **E**. **A** antenna 1, lateral view **B** accessory flagellum of antenna 1, lateral view **C** antenna 2, medial view **D** upper lip, anterior view **E** upper lip, anterior view **F** left mandible, medial view **G** incisor, lacinia mobilis, and molar process of left mandible, medial view **H** incisor and lacinia mobilis of right mandible, medial view **I** lower lip, ventral view **J** maxilla 1, dorsal view **K** maxilla 2, ventral view.



**Figure 4.** *Pseudocrangonyx daejeonensis* sp. n., holotype, female (3.8 mm). **A** maxilliped, dorsal view **B** gnathopod 1, medial view **C** palmar margin of propodus and dactylus of gnathopod 1, medial view **D** gnathopod 2, medial view **E** palmar margin of propodus and dactylus of gnathopod 2, medial view.

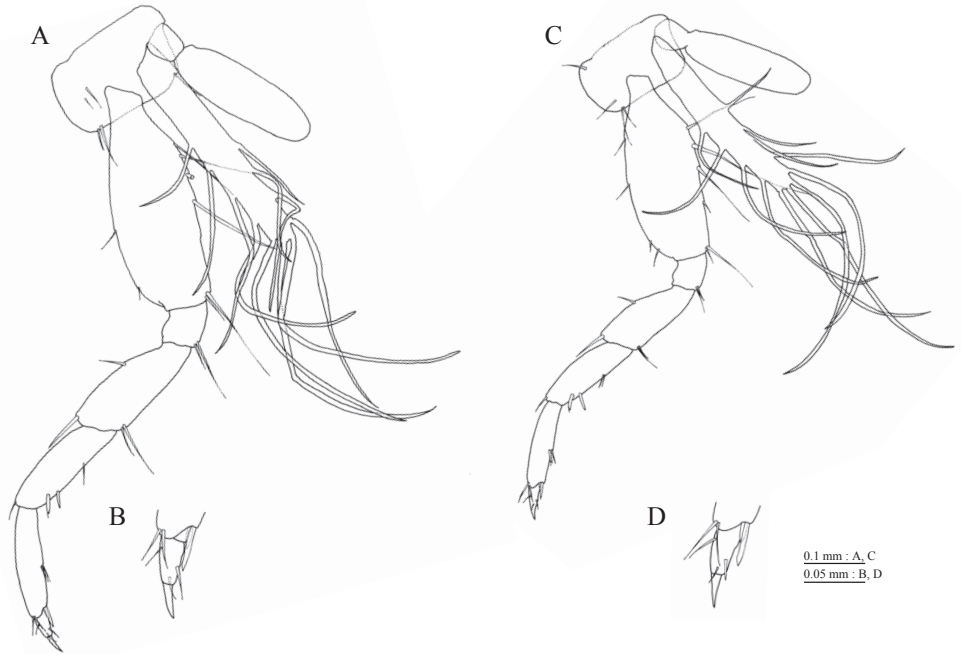
margin of dactylus dentate (Fig. 4C). Gnathopod 2 (Fig. 4D) with subquadrate coxa, bearing setae on its anterodistal and posteroventral corners, width 1.6 times as long as depth; posterior margin of basis with 4 setae; posterodistal corner of carpus with slender setae, some weakly pectinate; propodus stout, subchelate, palmar margin with 7 medial and 2 lateral robust setae; posterior margin of dactylus dentate (Fig. 4E). Pereopod 3 (Fig. 5A) with subquadrate coxa bearing setae on its anterodistal and posteroventral corners, width 1.6 times as long as depth; anterior and posterior margins of basis with 2 and 4 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 0.8; posterior margin and submargin of dactylus each with seta (Fig. 5B). Pereopod 4 (Fig. 5C) with subquadrate coxa bearing setae on its anterodistal corner, width 1.7 times as long as depth; anterior and posterior margins of basis each with 4 setae; merus, carpus, and propodus in length ratio of 1.0 : 0.7 : 0.8; posterior margin and submargin of dactylus each with seta (Fig. 5D). Pereopod 5 (Fig. 6A) with weakly bilobed coxa bearing setae on anterior and posterior lobes, width 1.7 times as long as depth; anterior and posterior margins of basis with 4 and 6 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 0.7 : 0.8; anterior margin of dactylus with 2 setae (Fig. 6B). Pereopod 6 (Fig. 6C) with coxa bearing concave lower margin, marginally bare; anterior and posterior margins of basis with 5 and 3 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 0.9; anterior margin of dactylus with 2 setae (Fig. 6D). Pereopod 7 (Fig. 6E) with subtriangular coxa, bearing seta on posteroproximal corner; anterior and posterior margins of basis with 3 and 4 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 1.1; anterior margin of dactylus with 2 setae (Fig. 6F).

Coxal gills (Figs 4D, 5A, C, 6A, C) on gnathopod 2 and pereopods 3–6; sternal gills absent. Brood plates (Figs 4D, 5A, C, 6A) slender, with numerous setae, on gnathopod 2 and pereopods 3–5.

Peduncle of pleopod 1 (Fig. 7A) with 1 outer marginal and 1 outerdistal seta; peduncle of pleopod 2 (Fig. 7C) with outerdistal seta; peduncle of pleopod 3 (Fig. 7D) lacking marginal and distal setae. Pleopods 1–3 with paired retinacula (Fig. 7B), and lacking bifid setae (clothes-pin setae) on inner basal margin of inner ramus; inner ramus of pleopods 1–3 3-, 3-, and 2-articulate (Fig. 7A, C, D); outer ramus of pleopods 1–3 4-, 3-, and 2-articulate (Fig. 7A, C, D).

Uropod 1 (Fig. 7E) with basofacial seta on peduncle; inner ramus 0.87 times as long as peduncle, inner margin of former with 2 robust setae, outer margin bare, basal part with slender seta; outer ramus 0.63 times as long as inner, marginally bare. Uropod 2 (Fig. 7F) with inner and outer rami; inner ramus 1.10 times as long as peduncle, its inner margin with robust seta, outer margin without setae; outer ramus 0.68 times as long as inner ramus, marginally bare. Uropod 3 (Fig. 7G) with peduncle 0.34 times as long as outer ramus, with 1 robust and 1 slender setae; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.32 times as long as proximal article, with 3 distal setae. Telson (Fig. 7H) length 1.3 times as long as wide, cleft for 0.08 times of length, each telson lobe with 2 lateral penicillate setae, 1 apical robust and 1 apical short setae.





**Figure 5.** *Pseudocrangonyx daejeonensis* sp. n., holotype, female (3.8 mm). **A** pereopod 3, medial view **B** dactylus of pereopod 3, medial view **C** pereopod 4, medial view **D** dactylus of pereopod 4, medial view.

*Male* [NNIBRIV2, 2.7 mm]. Antenna 1 (Fig. 8A, B) 0.46 times as long as body length, primary flagellum 7-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 8C) 0.57 times as long as antenna 1; flagellum 0.72 times as long as peduncular articles 4 and 5 combined, consisting of 4 articles, first 2 of which with calceoli (Fig. 8D).

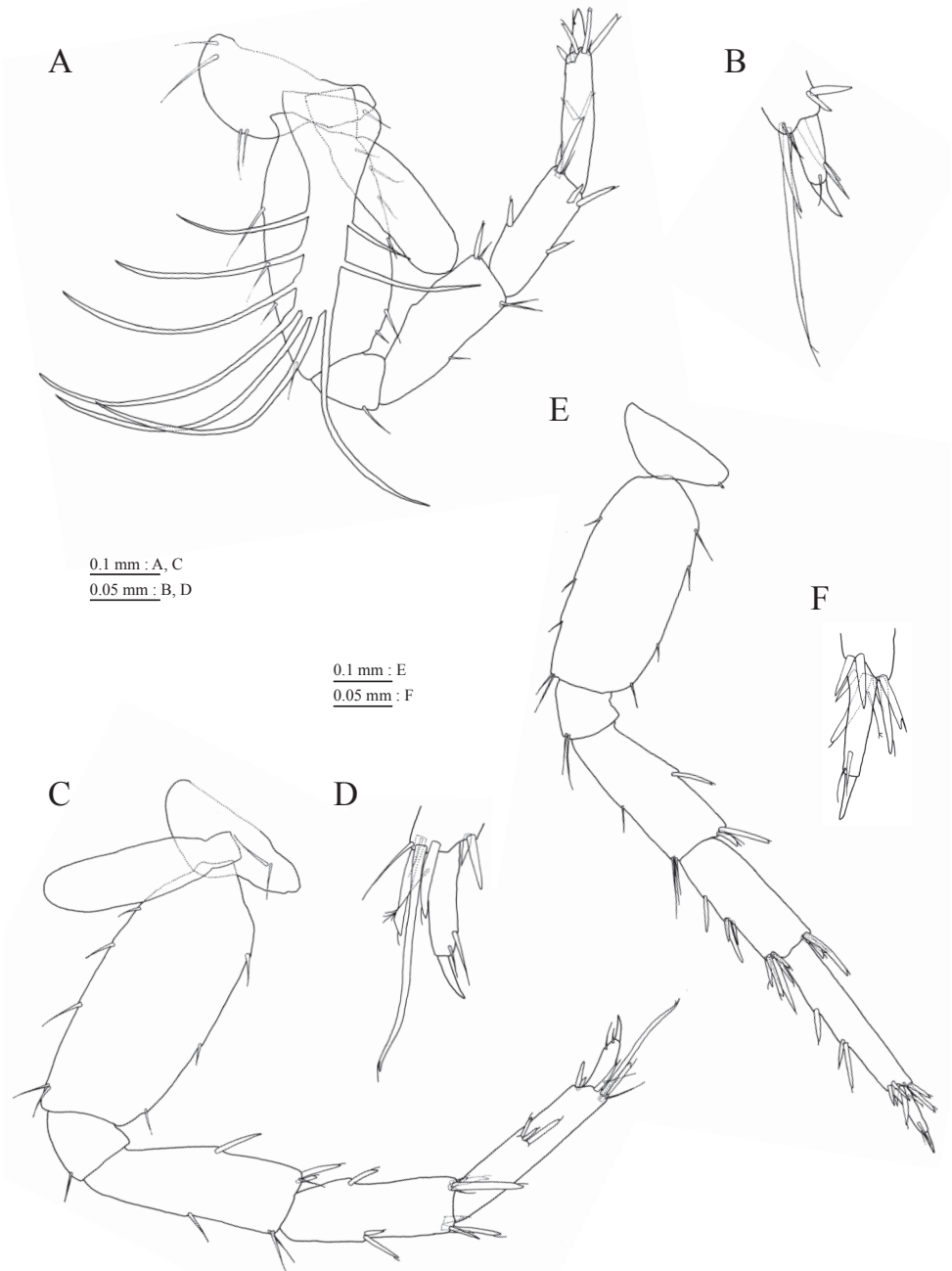
Gnathopod 1 (Fig. 8E) with coxa width 1.84 times as long as depth; palmar margin with 3 medial and 3 lateral robust setae (Fig. 8F). Gnathopod 2 (Fig. 8G) with coxa width 1.66 times as long as depth; palmar margin with 3 medial and 4 lateral robust setae (Fig. 8H).

Uropod 1 (Fig. 9A) with robust seta on inner margin of inner ramus; outer ramus 0.62 times as long as inner. Uropod 2 (Fig. 9B) with 2 serrate and 4 simple robust setae and slender seta at distal part. Uropod 3 (Fig. 9C) with peduncle 0.32 times as long as outer ramus; terminal article of outer ramus 0.5 times as long as proximal article.

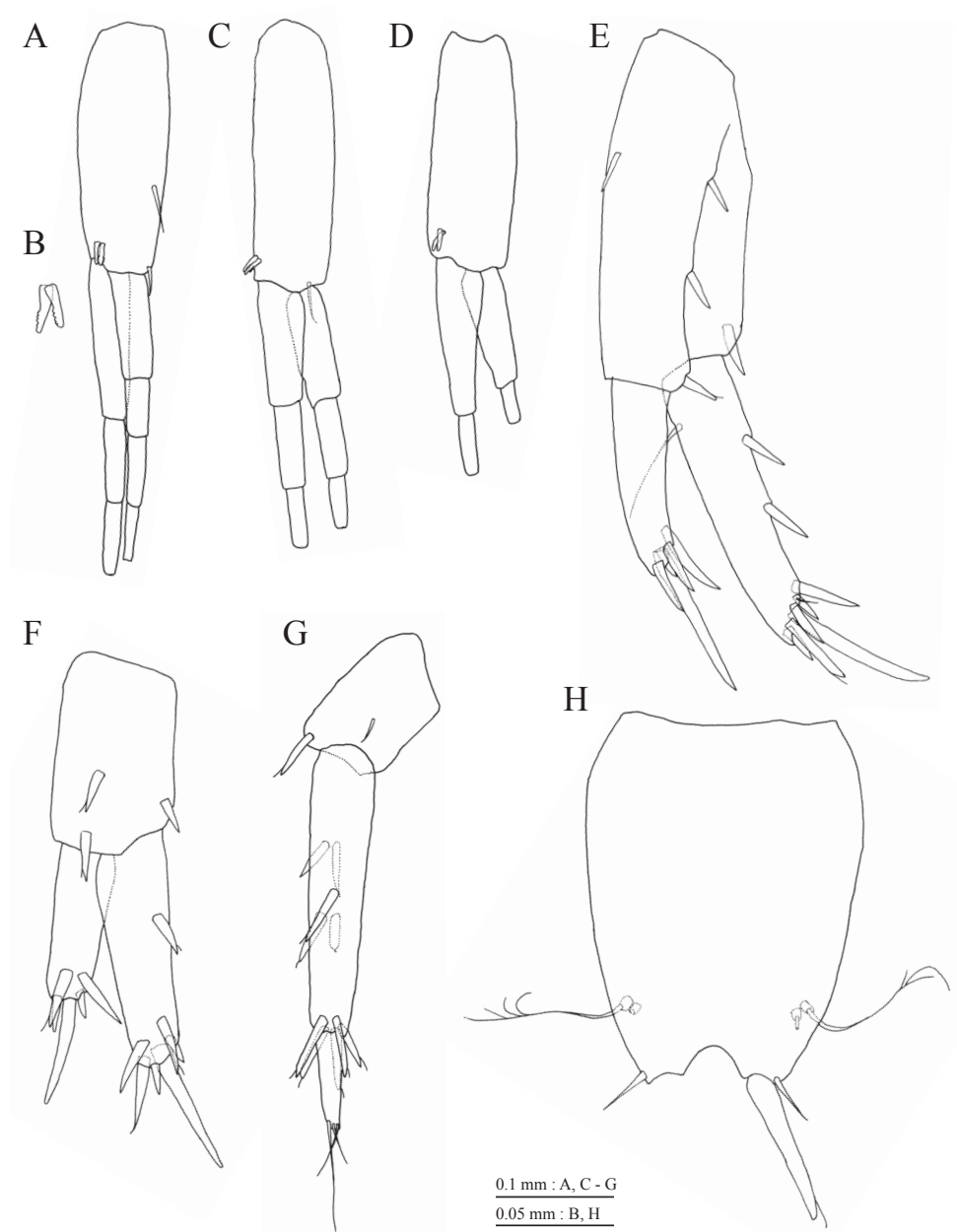
**Variation.** Peduncle of pleopod 1 with or without seta on outer margin.

**Distribution.** This species is known only from the type locality.

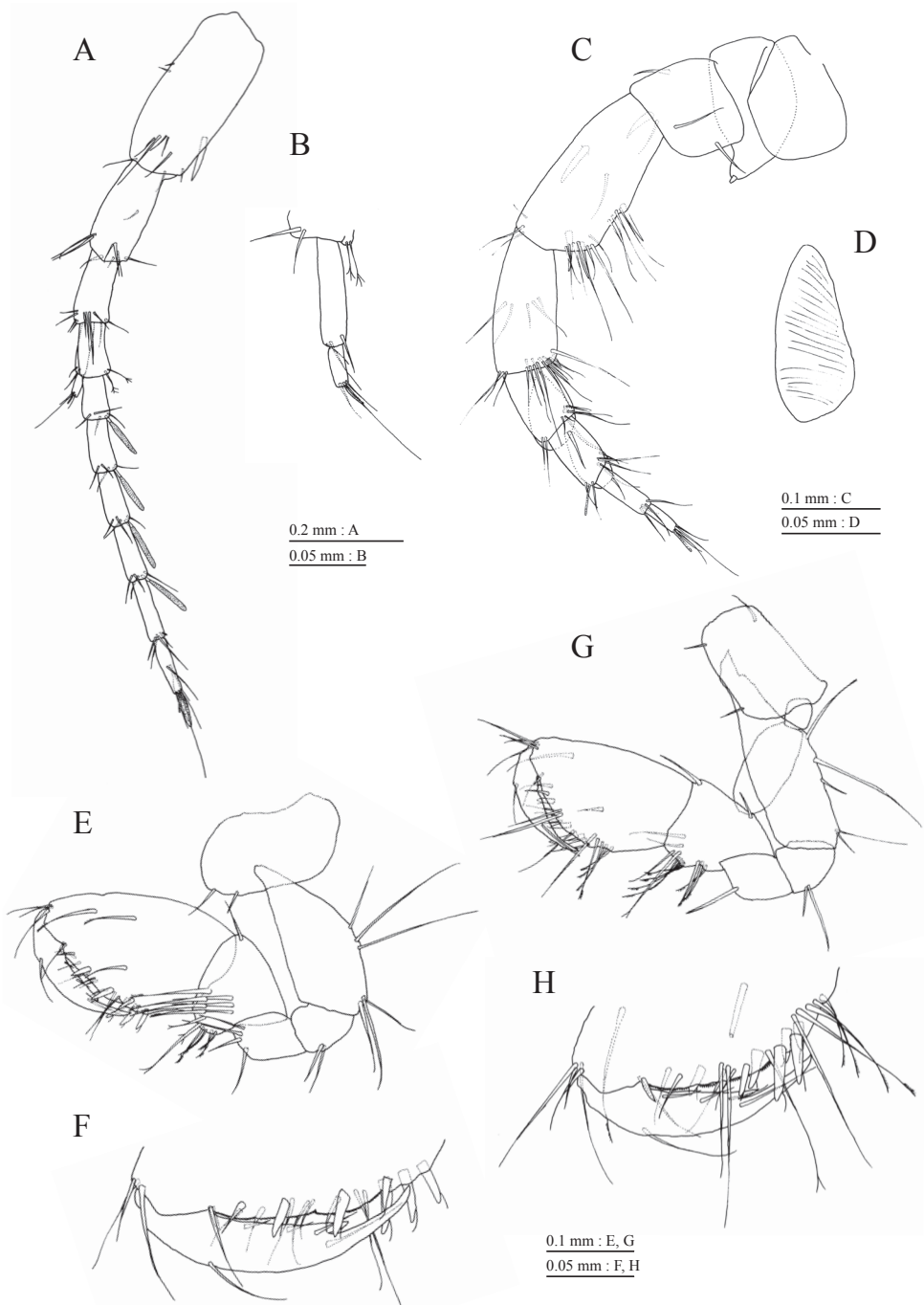
**Molecular phylogenetic position.** The BI tree (mean  $\ln L = -14039.10$ ; Fig. 10) for estimating the phylogenetic position of the new species had an identical topology to that of the ML tree ( $\ln L = -14504.12$ ; not shown). *Pseudocrangonyx daejeonensis* belonged to a well-supported clade (BS = 98 %, PP = 0.99) containing the three phylogroups known from the western parts of Honshu and Shikoku, i.e., *Pseudocrangonyx* spp. 3–5. The new



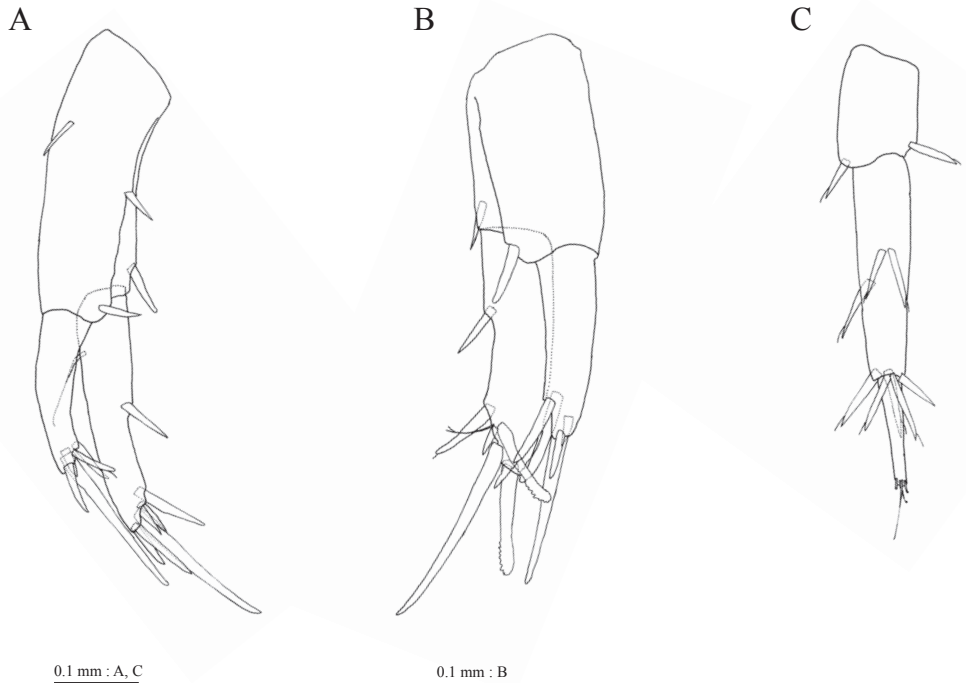
**Figure 6.** *Pseudocrangonyx daejeonensis* sp. n., holotype, female (3.8 mm). **A** pereopod 5, medial view **B** dactylus of pereopod 5, medial view **C** pereopod 6, medial view **D** dactylus of pereopod 6, medial view **E** pereopod 7, lateral view **F** dactylus of pereopod 7, lateral view.



**Figure 7.** *Pseudocrangonyx daejeonensis* sp. n., holotype, female (3.8 mm). **A** pleopod 1, anterior view **B** retinacula on peduncle of pleopod 1, anterior view **C** pleopod 2, anterior view **D** pleopod 3, anterior view **E** uropod 1, dorsal view **F** uropod 2, ventral view **G** uropod 3, dorsal view **H** telson, ventral view. Plumose setae on pleopodous rami omitted.



**Figure 8.** *Pseudocrangonyx daejeonensis* sp. n., paratype, male (2.7 mm). **A** antenna 1, lateral view **B** accessory flagellum of antenna 1, medial view **C** antenna 2, lateral view **D** calceolus of antenna 2, medial view **E** gnathopod 1, medial view **F** palmar margin of propodus and dactylus of gnathopod 1, medial view **G** gnathopod 2, lateral view **H** palmar margin of propodus and dactylus of gnathopod 2, lateral view.

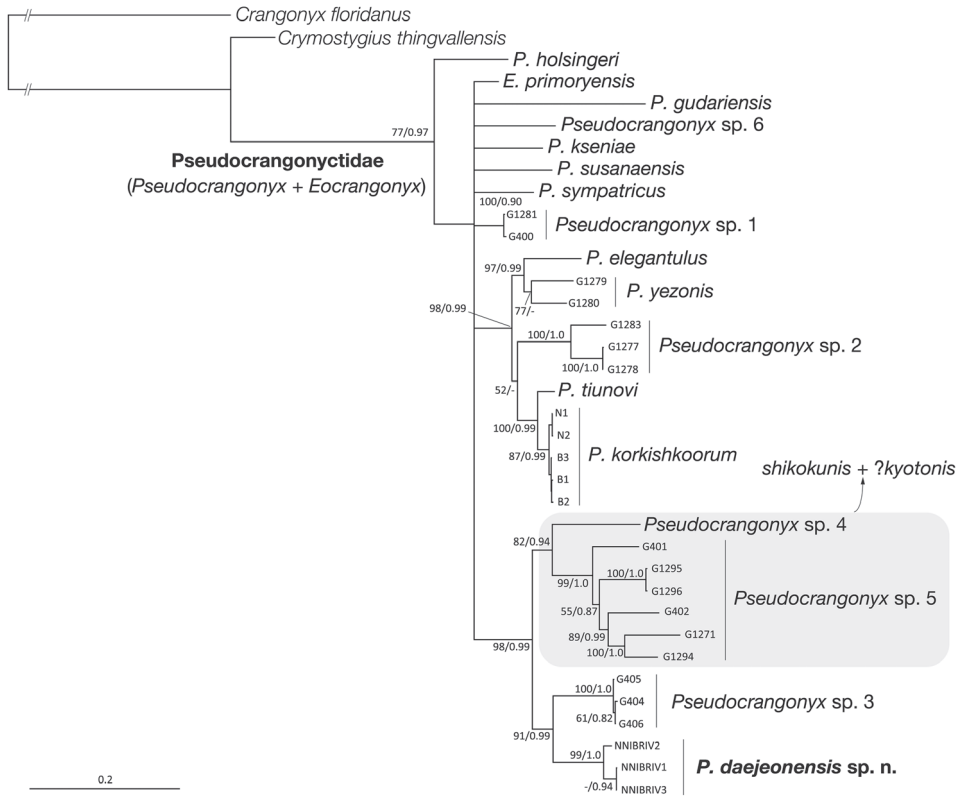


**Figure 9.** *Pseudocrangonyx daejeonensis* sp. n., paratype, male (2.7 mm). **A–C** uropods 1–3, respectively, dorsal views.

species formed a clade (BS = 91 %, PP = 0.99) with *Pseudocrangonyx* sp. 3 inhabiting the eastern part of Shiga Prefecture, Japan. Monophyly of the present specimens of *P. daejeonensis* was fully-supported (BS = 99 %, PP = 1.0).

**Remarks.** *Pseudocrangonyx daejeonensis* is morphologically similar to *P. coreanus* in having 1) relatively small body size (smaller than 6 mm), 2) eyes completely absent, 3) carpus of gnathopod 2 without serrate robust setae on posterodistal corner, 4) outer margin or outer distal corner of pleopods 1 and 2 with setae, 5) inner basal margin of inner ramus of pleopods without bifid setae, and 6) small number of articles (less than 5) of rami of pleopods. However, the former is distinguished from the latter by the following features (features of *P. coreanus* in parentheses): 1) antenna 1 shorter (longer) than 0.4 times as long as body length, 2) antenna 2 of female without calceoli (with calceoli), 3) uropod 1 not exceeding (slightly exceeding) tip of uropod 2, and 4) outer ramus of uropod 2 without robust seta (with robust seta).

*Pseudocrangonyx daejeonensis* is also similar to *P. febras* Sidorov, 2009 and *P. gudariensis* Tomikawa and Sato in Tomikawa et al. (2016) in having 1) relatively smaller body size, 2) eyes completely absent, and 3) urosomite 1 without basal setae. However, *P. daejeonensis* is distinguished from these two species by the following features: from *P. febras* (features of *P. febras* in parentheses), 1) antenna 1 shorter than 0.4 times as long as body length (longer than 0.7 times), 2) peduncular article 2 of antenna 1 0.5 (0.7)



**Figure 10.** Bayesian inference tree for 2773 bp of nuclear 28S rRNA, plus histone H3 and mitochondrial COI and 16S rRNA markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities. Specimen numbers are shown in Table 1.

times as long as article 1, 3) palp article 2 of mandible with 3 (7) setae, 4) carpus of male gnathopod 2 without serrate robust setae on posterodistal corner (with serrate robust setae), 5) fewer articles of pleopodal rami, up to 4 (more, up to 6), 6) inner ramus of uropod 1 with 2 inner marginal robust setae (5 inner and 3 outer marginal robust setae), 7) outer ramus of uropod 1 without setae (with 2 robust setae), and 8) inner ramus of uropod 2 with inner robust seta (3–4 inner and 2–3 outer marginal robust setae); from *P. gudariensis* (features of *P. gudariensis* in parentheses), 1) basal part of inner ramus of uropod 1 with 1 slender setae (with 3 slender setae), 2) outer ramus of uropod 1 without setae (with 2 setae), 3) inner margin of inner ramus uropod 2 with 1 robust setae (with 4 robust setae), and 4) telson lobe with 1 robust seta apically (with 2 robust setae apically).

Although the phylogenetic position of *P. coreanus* remains uncertain, the results of the previous molecular phylogenetic studies (Tomikawa et al. 2016; Zhao and Hou 2017) and our phylogenetic analyses showed that *P. daejeonensis* and the two morphologically similar species, *P. febras* and *P. gudariensis*, did not form a monophyletic

lineage with large genetic divergences. Because these three species inhabit interstitial waters, not subterranean habitats, morphological similarities observed among them may reflect their similar habitat preferences.

The phylogenetic position of *P. daejeonensis* also sheds light onto the complex faunistic relationships between the *Pseudocrangonyx* species inhabiting the Japanese Archipelago and those inhabiting the Far Eastern continental area. Common ancestors of the Japanese *Pseudocrangonyx* species were considered to have migrated from the continental part to the Japanese Archipelago (Sidorov and Holsinger 2007). Previous systematic studies revealed that the *Pseudocrangonyx* amphipods distributed in northern Japan and the western tip of Honshu, Japan, i.e., *P. yezonis* and *Pseudocrangonyx* sp. 2, are phylogenetically close to the continental species (Sidorov and Holsinger 2007; Tomikawa et al. 2016; Zhao and Hou 2017). As *P. daejeonensis* formed a well-supported clade with *Pseudocrangonyx* sp. 3, which is indigenous to the central part of Honshu, their phylogenetic relationship suggested that the species diversity of the Japanese *Pseudocrangonyx* has been increased as a result of multiple continental-origins. It is also feasible that *P. daejeonensis* diverged from a common ancestor indigenous to the Japanese Archipelago. To clarify the biogeographical history of *Pseudocrangonyx* amphipods, further faunistic surveys along with molecular phylogenetic analyses are essentially needed.

The uncorrected *p*-distance of 15.0 % for COI, calculated using MEGA7.0.16 (Kumar et al. 2016) between *P. daejeonensis* and *Pseudocrangonyx* sp. 3 is equivalent to sequence divergence thresholds for discriminating amphipod species (Witt et al. 2006; Rock et al. 2007; Hou et al. 2009). The former is distinguished from the latter in having the following features (features of *Pseudocrangonyx* sp. 3 in parentheses): 1) outerdistal corner of peduncle of pleopod 3 without seta (with seta), 2) each of inner and outer ramus of pleopod 3 2-articulate (3-articulate), 3) outer rami of uropods 1 and 2 without marginal robust setae (with marginal seta), and 4) robust setae on distal part of proximal article of uropod 3 short, not reaching tip of terminal article (long, exceeding tip of terminal article) (Tomikawa pers. observation).

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## References

- Akatsuka K, Komai T (1922) *Pseudocrangonyx*, a new genus of subterranean amphipods from Japan. *Annotationes Zoologicae Japonenses* 10: 119–126.
- Holsinger JR (1989) Allocrangonyctidae and Pseudocrangonyctidae, two new families of Holarctic subterranean amphipod crustaceans (Gammaridea), with comments on their phylogenetic and zoogeographic relationships. *Proceedings of the Biological Society of Washington* 102: 947–959.
- Hou Z, Li Z, Li S (2009) Identifying Chinese species of *Gammarus* (Crustacea: Amphipoda) using DNA barcoding. *Current Zoology* 55: 158–164.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lanfear R, Calcott B, Ho SYM, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773. <https://doi.org/10.1093/molbev/msw260>
- Lee CW, Park JG (2016) Distribution of meiobenthic arthropod communities in the hyporheic zone of Nakdonggang. *Journal of Species Research* 5: 157–165. <https://doi.org/10.12651/JSR.2016.5.1.157>
- Rambaut A, Drummond AJ (2013) Tracer v. 1.6. <http://tree.bio.ed.ac.uk/software/tracer/>
- Rock J, Ironside J, Potter T, Whiteley NM, Lunt DH (2007) Phylogeography and environmental diversification of a highly adaptable marine amphipod *Gammarus duebeni*. *Heredity* 99: 102–111. <https://doi.org/10.1038/sj.hdy.6800971>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sidorov DA (2009) New species of stygobiont amphipod (Crustacea: Amphipoda: Pseudocrangonyctidae) from Primorye, with description of female of *Pseudocrangonyx levanidovi* Birstein from the Khor River springs. *Amurian Zoological Journal* 1: 92–105.
- Sidorov D, Holsinger JR (2007) *Procrangonyx stygoedincus*, a new species of subterranean amphipod (Pseudocrangonyctidae) from the Far East of Russia, with remarks on biogeographic relationships. *Crustaceana* 80: 417–430. <https://doi.org/10.1163/156854007780440984>
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

- Stock JH (1974) The systematics of certain Ponto-Caspian Gammaridae (Crustacea, Amphipoda). Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 70: 75–95.
- Tomikawa K, Nakano T, Sato A, Onodera Y, Ohtaka A (2016) A molecular phylogeny of *Pseudocrangonyx* from Japan, including a new subterranean species (Crustacea, Amphipoda, Pseudocrangonyctidae). Zoosystematics and Evolution 92: 187–202. <https://doi.org/10.3897/zse.92.10176>
- Uéno M (1934) Subterranean Crustacea from Kwantung. Annotationes Zoologicae Japonenses 14: 445–450.
- 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: 501–535.
- Witt JDS, Threlloff DL, Hebert PDN (2006) DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. Molecular Ecology 15: 3037–3082. <https://doi.org/10.1111/j.1365-294X.2006.02999.x>
- Zhao S, Hou Z (2017) A new subterranean species of *Pseudocrangonyx* from China with an identification key to all species of the genus (Crustacea, Amphipoda, Pseudocrangonyctidae). ZooKeys 647: 1–22. <https://doi.org/10.3897/zookeys.647.11192>

# A new genus and species of Sphyrapodidae (Crustacea, Peracarida, Tanaidacea) from the southern coast of the South Korea

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## Abstract

*Wandogarida canalicula* **gen. n et sp. n.** (Tanaidacea, Sphyrapodidae) is described from the southern coast of Korea (NW Pacific). The genus is included in the subfamily Sphyrapodinae *Gufu*, 1980 by having a mandible lacking a palp. It has a close affinity with the genus *Poligarida* Bamber & Marshall, 2013 in the third pereonite having lateral pointed apophyses, the antennule of males with fringes of aesthetascs on articles 1 and 2 of the outer flagellum and the antenna with an elongate article 2. However, it is distinguished from *Poligarida* by having a maxillular palp. *Wandogarida* **gen. n.** can be differentiated from other genera within the subfamily Sphyrapodinae by the unique shape characterised by the male's antennule article 1 with its rough denticles and a deep groove, pleonites each with a ventral hyposphenium, the maxillule with uniarticulate palp and the presence of prominent sexual dimorphism in the mouthparts. An identification key to the five genera of the subfamily Sphyrapodinae is presented.

## Keywords

Tanaidacea, Apseudomorpha, Sphyrapodinae, *Wandogarida*, sexual dimorphism

## Introduction

The suborder Apseudomorpha Sieg, 1980 includes about 460 species and is widely distributed in various shallow marine benthic habitats, being quite abundant in coral reefs, estuaries and mangrove swamps, from the tropics to temperate regions (Błażewicz-Paszkowycz and Bamber 2012). Although many species of this suborder have been described from diverse regions, the current state of the systematics is unstable and unresolved (Larsen 2012). Guțu (1980) established the family Sphyrapidae, with two subfamilies Pseudosphyrapinae Guțu, 1980 and Sphyrapinae Guțu, 1980, to encompass a group of tanaidaceans characterised by the enlarged pereopod 1, the lack of spiniform apophyses on the carapace, the biramous antennule, the mandible with or without palp and the maxillule with palp. Later their familial and subfamilial names were emended to Sphyrapodidae Guțu, 1980 and Sphyrapodinae Guțu, 1980 and Pseudosphyrapodinae Guțu, 1980 by Larsen (2005). The family has undergone several systematic reviews to establish its current taxonomic status (see Anderson 2013). Initially, the family Sphyrapodidae had been placed in the superfamily Metapseudoidea Guțu, 1981 within the suborder Monokonophora Lang, 1956 (as Sphyrapidae). However, the suborder and superfamily were not accepted and Metapseudoidea was synonymised with Apseusoidea Leach, 1814 by Sieg (1984).

More than 300 sphyrapodid specimens were collected during a recent survey of the shallow mud sandy seabed from the southern coast of South Korea. Their examination revealed the presence of a new species in a new genus belonging to the subfamily Sphyrapodinae.

## Materials and methods

The materials were obtained from the sandy bottom off Wando Island on the southern coast of South Korea: (34°31.1'N; 128°33.2'E at a depth of 41 m) in October 2015 using an epi-sledge net. The specimens were extracted by filtering the substrates through a 350 μm sieve and the residue from each sieve was preserved in a 99% alcohol solution. Later, the animals were identified and counted in the laboratory. The specimens were dissected under a dissection microscope (Nikon SMZ745T) in CMC-10 aqueous mounting medium (Masters, Wood Dale, IL, USA), mounted on slides and then sealed with high-quality nail varnish. Drawings were generated using a differential interference contrast microscope (Nikon Y-IM) that was equipped with a drawing tube. The total body length was measured from the tip of the rostrum to the pleotelson apex in the dorsal view. Scale bars are given in mm. The morphological terminology follows Larsen (2003). The type and other materials examined were deposited in the Marine Biodiversity Institute of Korea (MABIK), Seocheon, South Korea.

## Systematics

Order Tanaidacea Dana, 1849

Suborder Apseudomorpha Sieg, 1980

Superfamily Apseudoidea Leach, 1814

Family Sphyrapodidae Guţu, 1980

Subfamily Sphyrapodinae Guţu, 1980

**Genus** *Wandogarida* gen. n.

<http://zoobank.org/C75030C0-3F54-4156-B4BD-00A5D666E67F>

Figs 1–9

**Generic diagnosis.** Rostrum narrow and prominently extended. Carapace wider than long. Pereonite 3 with lateral apophyses. Pleonites each with a ventral spur and pointed epimera. Pleotelson with slight distal extension. Antennule inner flagellum biarticulate; peduncle article 1 in males with a vertical row of rough denticles and groove. Antenna 8-articulate, without squama. Mandible without palp; molar with distal setulose setae and spinose cutting edge. Maxillule with uniarticulate palp. Pereopods 2–4 propodus with ventral seta. Uropod exopod 3-articulate.

**Etymology.** The name refers to Wando, a port city near the type locality and *garida* from the Greek γαίδα, meaning “shrimp” (feminine).

**Type species.** *Wandogarida canalicula* sp. n.

**Remarks.** *Wandogarida* gen. n. is classified in the subfamily Sphyrapodinae following Guţu (1980), Larsen (2005) and Bamber and Marshall (2013), with a definition based on the following morphological features: 1) the rostrum is prominently extended anteriorly; 2) the pereonites are all wider than long; 3) the antennule has a short inner flagellum with 1–2 articles; 4) the mandible is without a palp; 5) the maxillule is with or without a palp; and 6) the pereopod 1 and cheliped are with exopod.

The subfamily is now composed of five genera: *Ansphyrapus* Guţu, 2001, *Poligarida* Bamber & Marshall, 2013, *Sphyrapoides* Guţu & Iliffe, 1998 and *Sphyrapus* Sars, 1882, including the new genus *Wandogarida*.

*Wandogarida* resembles *Poligarida* in the absence of an antennal squama, antennule with a biarticulate inner flagellum, pereonite 3 with anterolateral pointed apophyses and outer flagellum of the male antennule with fringes of aesthetascs. However, *Wandogarida* can be differentiated from *Poligarida* by the following: in both sexes, the number of antenna articles is different (8 vs. 7); the maxillule has a uniarticulate palp (vs. absence); the carpus and propodus of pereopods 2–3 and propodus of the pereopod 4 have ventral spiniform setae (vs. absence); in females, the mandible molar has several setulose distal setae and sharp, spinose distal margin (vs. with distal setae and simple distal edge); in males, sexual dimorphism exists in the antennule article 1 with a vertical row of rough denticles and concave distolateral margin, in reduced and simplified mandibles, maxillule, maxilla and maxilliped endite, in the larger and more robust cheliped and in the shape of ventral margin of the pereopod 1 dactylus, while it exists only in the antennule, cheliped and pereopod 1 in *Poligarida*.

### Key to the genera of the subfamily Sphyrapodinae

- 1        Antennule inner flagellum with 0–1 articles..... *Sphyrapus*
- Antennule inner flagellum with 1–2 articles..... 2
- 2        Antenna with squama ..... *Sphyrapoides*
- Antenna without squama..... 3
- 3        Maxillule with palp..... 4
- Maxillule without palp..... *Wandogarida* gen. n.
- 4        Pereopod 6 with many long setae along basis, merus and carpus..... *Ansphyrapus*
- Pereopod 6 without many long setae along basis, merus and carpus ... *Poligarida*

### *Wandogarida canalicula* sp. n.

<http://zoobank.org/BBEE7CE0-F2C4-42A4-ABD6-DD3D12A836EE>

Figs 1–9

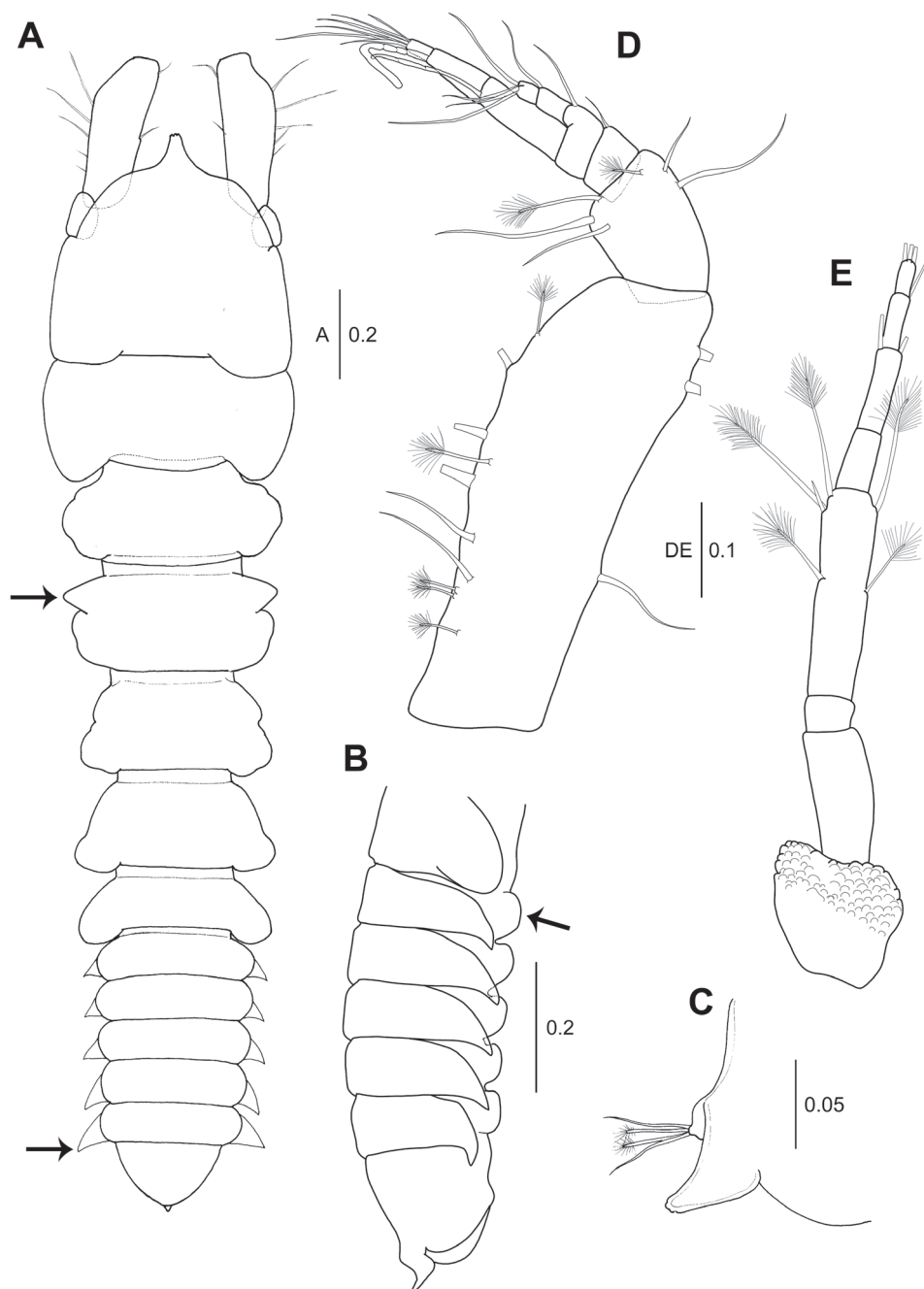
**Type-specimens.** **Holotype:** (MABIK CR00240685) female dissected and mounted on five slides. **Allotype:** (MABIK CR00240686) male dissected and mounted on five slides; from the same locality as the holotype. **Paratypes:** Four females partly dissected on one slide (MABIK CR00240687) and in 3 vials (MABIKCR00235359–MABIKCR00235361); from the same locality as the holotype. Four males partly dissected on one slide (MABIK CR00240688) and in 2 vials (MABIKCR00235362, MABIKCR00235363); from the same locality as for holotype.

**Type-locality.** Wando, South Korea (North West Pacific), 34°31.02'N, 128°33.11'E, mud-sandy bottom at a depth of 41 m.

**Etymology.** The specific name is derived from Latin *canalicula*, meaning a groove and refers to the conspicuous groove formed on the distal margin of the male antennule.

**Descriptions.** **Female (with oostegites).** Body (Fig. 1A), dorsoventrally flattened, holotype 2.4 mm long, 4.3 times as long as wide. Cephalothorax 20.6 % of body length, slightly wider than long, gradually widening posteriorly, proximal margin with conspicuous extended rostrum with or without small protrusions. Eyes well developed, without pigmentation. Pereonites each with different shape. Pleonites each with pointed epimera. Pleotelson gradually tapered.

*Pereon* (Fig. 1A): 53 % of body length. Pereonite 1 not fused to cephalothorax, 0.6 times as long as cephalothorax, half as long as wide, posterolateral margins rounded and distally extended. Pereonite 2 medially swollen, slightly shorter than pereonite 1, 0.4 times as long as wide. Pereonite 3 subequal length of pereonite 2, half as long as wide, anterolateral margin with apophysis (arrowed), mid-lateral margin swollen. Pereonite 4 subtrapezoidal, as long as pereonite 2, 0.6 times as long as wide, mid to posterolateral margin gradually widening. Pereonite 5 as long as pereonite 4, half as long as wide, posterolateral margin rounded and slightly extended. Pereonite 6 shortest, 0.4 times as long as wide, posterolateral margin rounded and extended.



**Figure 1.** *Wandogarida canalicula* gen. n., sp. n., holotype, female: **A** habitus, dorsal view, upper arrow indicating apophysis of pereonite 3 and lower arrow indicating epimera of pleonite 5 **B** pleon, lateral view, arrow indicating hyposphenium of pleonite 1 **C** pleotelson, lateral view **D** antennule **E** antenna. Scale bars are given in mm.



*Pleon* (Fig. 1A, B): 20.2 % of body length, 1.1 times as long as wide, pleonites each with pointed epimera, of which pleonite 5 is largest (arrowed) and rounded ventral hyposphenium (arrowed in Fig. 1B). Pleotelson (Fig. 1A–C): distally tapered, 6.6 % of total length, distally with two broom setae and two simple setae of almost equal length (Fig. 1C).

*Antennule* (Fig. 1D): Peduncle article 1 robust, 57 % of total length, 2.9 times as long as wide, with three simple setae on inner margin and five simple setae and five broom setae on outer margin. Article 2 0.3 times as long as article 1, 1.5 times as long as wide, with two simple setae and one broom seta each on outer and inner distal margin. Article 3 0.3 times as long as article 2, with one simple inner distal seta. Article 4 slightly shorter than article 3, with one simple inner distal seta. Inner flagellum biarticulate: article 1 with one distal simple seta and article 2 with four distal simple setae. Outer flagellum 3-articulate, each article gradually shortened: article 1 with one aesthetasc; article 2 with one aesthetasc and; article 3 shortest, with six distal simple setae.

*Antenna* (Fig. 1E): 8-articulate, slender and shorter than antennule. Article 1 distally wider and covered with scale-like ornamentations. Articles 2, 3 and 5 naked. Article 4 longest, outer margin each with one broom seta medially and distally, inner margin with one medial and two distal broom setae and one distal simple seta. Article 6 with two distal simple setae. Article 7 with one distal simple seta. Article 8 as long as article 3, with three distal simple setae. Proportional lengths of articles 16.8: 18.0: 5.1: 27.9: 8.4: 11.3: 7.6: 4.9.

*Labrum* (Fig. 2A): Sub-rectangular, distal margin covered with numerous setules.

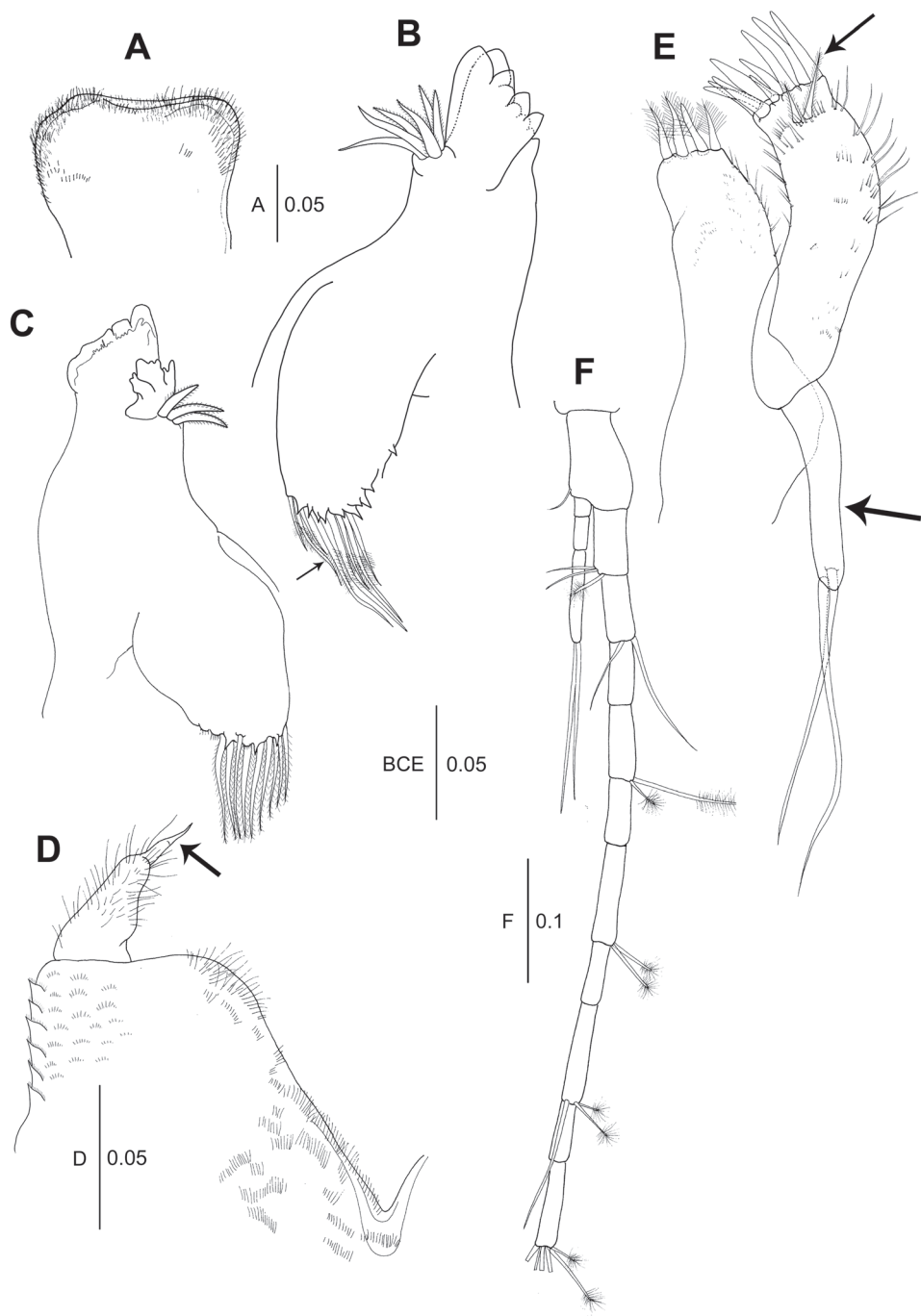
*Left mandible* (Fig. 2B): Incisor with six prominent denticles distally; lacinia mobilis with four distal denticles; setal row with six setulose spiniform setae; molar distally tapered, with sharp, spinose distal edge and eight long setulose setae (arrowed). *Right mandible* (Fig. 2C) incisor with three irregular distal denticles; setal row with one tripartite seta, which is substantially larger than others and three setulose spiniform setae; lacinia mobilis absent; molar similar to that of left mandible. Palp absent.

*Labium* (Fig. 2D): Lobe with setulate spines and microtrichia along outer margin and ornamented with setules along inner margin. Palp covered with setules and one distal spine (arrowed).

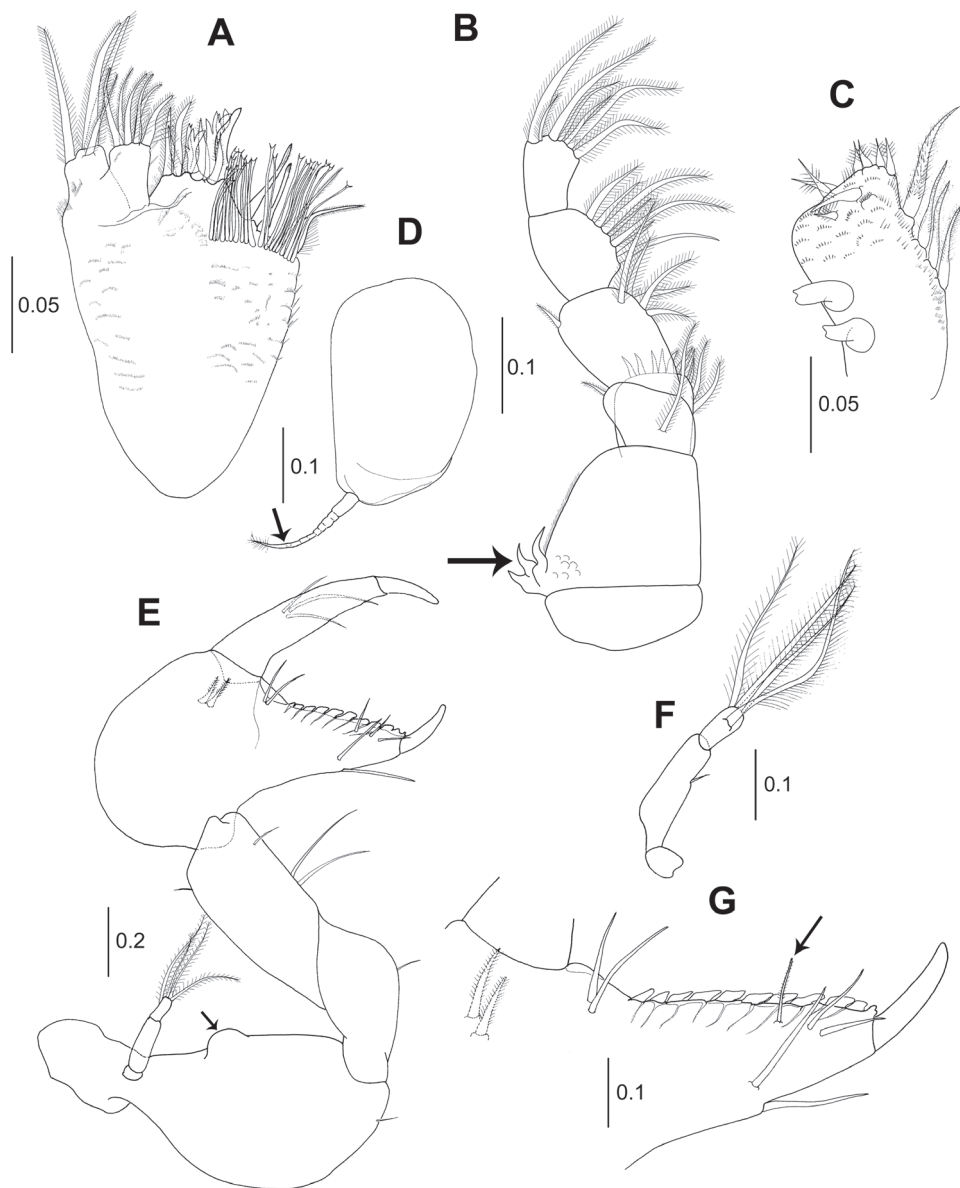
*Maxillule* (Fig. 2E): Inner endite with four setulose setae on distal margin; outer margin ornamented with somewhat long setules. Outer endite with nine distal spiniform setae, two subdistal setulose spiniform setae and setules. Palp uniarticulate (arrowed), with one subterminal and one terminal simple setae.

*Maxilla* (Fig. 3A): Outer lobe of movable endite with four setulose setae. Inner lobe of movable endite with six setulose setae. Outer lobe of fixed endite with six setulose spiniform setae and four setulose tripartite spiniform setae. Inner lobe of fixed endite with four setulose spiniform setae on distal margin and 20 simple bifid tipped setae.

*Maxilliped* (Fig. 3B, C): Coxa naked. Basis with three curved robust spines on outer proximal margin (arrowed). Palp article 1 with one short seta on outer distal margin and one subdistal setulose seta on medial margin. Article 2 longer than wide, with one distal setulose seta on outer margin and five subdistal setae on inner margin. Article 3



**Figure 2.** *Wandogarida canalicula* gen. n., sp. n., holotype, female: **A** labrum **B** left mandible, arrow indicating eight distal setulose setae on molar **C** right mandible **D** labium, arrow indicating distal spine of palp **E** maxillule, upper arrow indicating two subdistal setulose spiniform setae on outer endite and lower arrow indicating uniarticulate palp **F** uropod. Scale bars are given in mm.



**Figure 3.** *Wandogarida canalicula* gen. n., sp. n., holotype, female: **A** maxilla **B** maxilliped, arrow indicating three curved spines on basis **C** maxilliped endite **D** epignath, arrow indicating long wrinkled seta on distal margin **E** cheliped, arrow indicating dorsomedial protrusion on basis **F** cheliped exopodite **G** fixed finger of cheliped, arrow indicating setulose seta near cutting edge. Scale bars are given in mm.

with seven setulose setae along inner margin. Article 4 with seven distal setulose setae. Endite (Fig. 3C) with four stout setulose spiniform setae on inner margin, two coupling hooks on outer margin, six setulose spiniform setae on distal margin, two setu-

lose spiniform setae on subdistal margin and several microtrichia. Epignath (Fig. 3D) rounded, with long distally setulose and wrinkled seta on distal margin (arrowed).

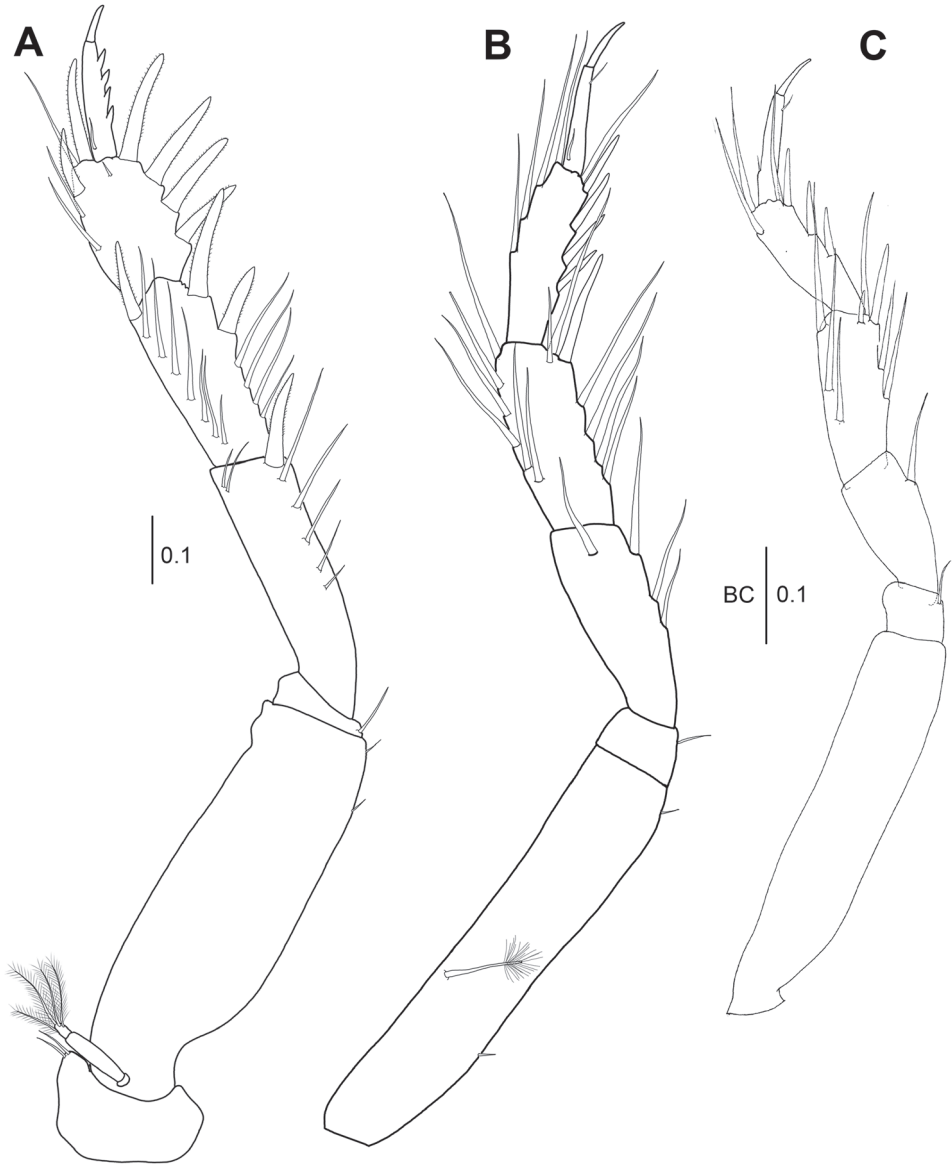
*Cheliped* (Fig. 3E–G): Basis rounded and with dorsomedial protrusion (arrowed), 1.9 times as long as wide, with one ventrodistal simple seta; exopod 3-articulate, with four plumose setae on distal margin (Fig. 3F). Merus ventrally rounded, shorter than basis, with ventromedial simple seta. Carpus sub-rectangular, with three simple setae on ventral margin and one subdistal simple seta on dorsal margin. Propodus longer than basis, merus and carpus, with two simple setae and two setulose spiniform setae near insertion of dactylus. Fixed finger with ten blunt denticles along cutting edge, one setulose seta (arrowed in Fig. 3G) and two simple setae on inner margin and two simple setae on ventral margin. Dactylus with three simple setae on inner medial margin, cutting edge smooth.

*Pereopod 1* (Fig. 4A): Larger than pereopods 2–6, spiniform setae ornamented with small setules bilaterally. Coxa with two dorsodistal simple setae. Basis robust, three times as long as wide, with two short simple setae on ventrodistally. Exopod 3-articulate, distal article with four distal plumose setae. Ischium compact, with one simple ventrodistal seta. Merus 0.6 times as long as basis, 3.3 times as long as wide, with five ventral simple setae and one ventrodistal spiniform seta and two dorsodistal simple setae. Carpus 0.8 times as long as merus, 2.3 times as long as wide, ventral margin with four slender simple setae and two setulose spiniform setae and dorsally with seven simple slender setae and one setulose spiniform seta. Propodus with four strong spiniform setae on ventral margin, one short subdistal simple seta and two distal spiniform setae and three simple setae along dorsal margin. Dactylus and unguis combined subequal to propodus. Dactylus stout, with four pointed denticles along ventral margin and one simple slender seta on subproximal margin; unguis 0.3 times as long as dactylus.

*Pereopod 2* (Fig. 4B): Basis 4.8 times as long as wide, with two ventral short simple setae and one dorsal broom seta. Ischium with one ventrodistal simple seta. Merus with three simple ventral setae and one subdistal simple seta. Carpus slightly shorter than merus, with six simple setae and one spiniform seta on ventral margin and six simple setae along dorsal margin. Propodus slightly shorter than merus, with four spiniform setae and one distal simple seta along ventral margin and four simple setae along dorsal margin. Dactylus and unguis combined 0.8 times as long as propodus, with one dorsoproximal simple seta and one ventrodistal simple seta. Unguis 0.4 times as long as dactylus.

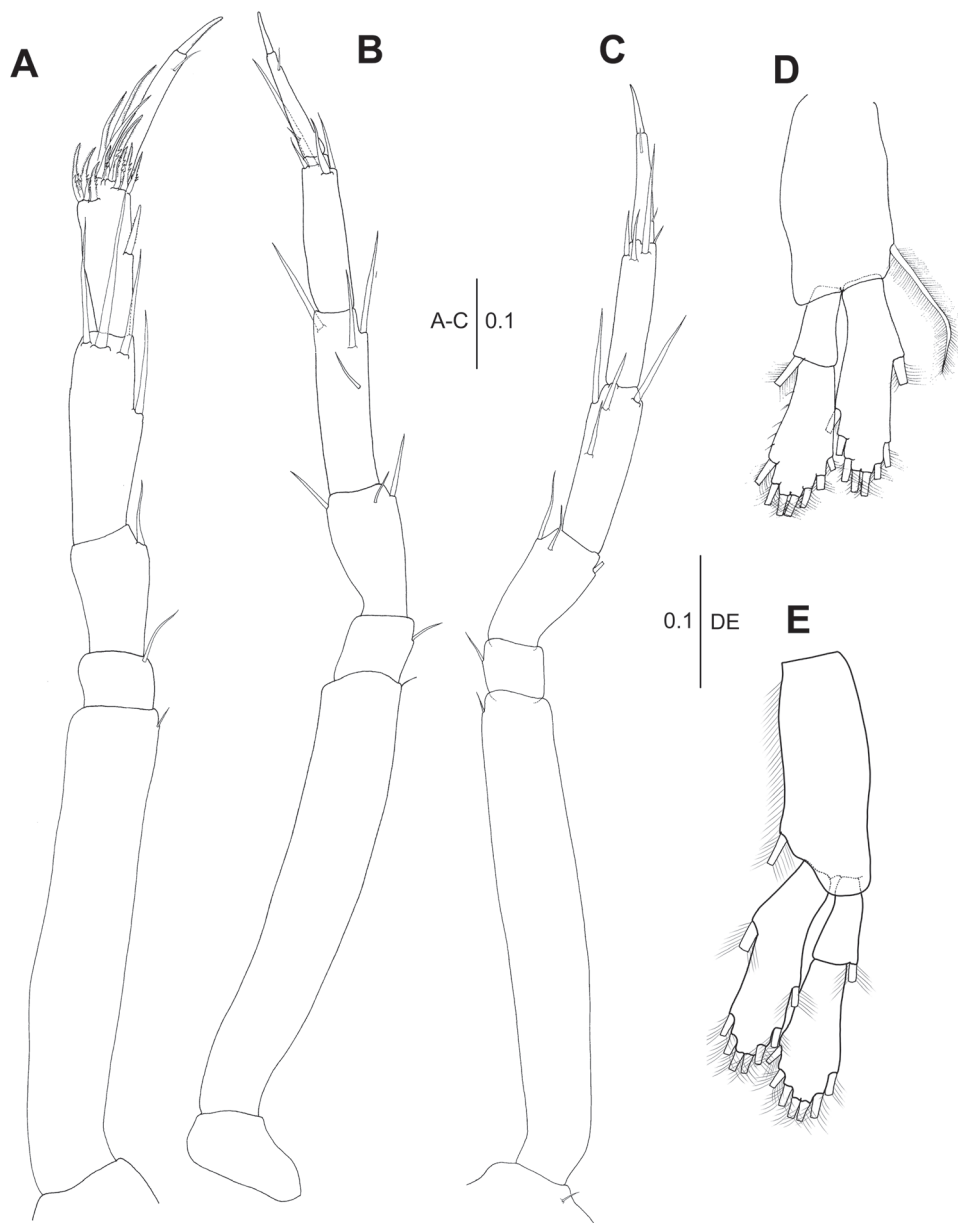
*Pereopod 3* (Fig. 4C): Basis shorter than that of pereopod 2, 4.8 times as long as wide, with one ventrodistal simple seta. Ischium with one ventrodistal simple seta. Merus 0.3 times as long as basis, with one ventro-subdistal simple seta. Carpus 1.2 times as long as merus, with three simple setae and two spiniform setae of unequal length on ventral margin and three simple setae along dorsal margin. Propodus as long as merus, with two simple setae on dorsodistal margin and three spiniform setae and one distal simple seta on ventral margin. Dactylus and unguis combined 1.2 times as long as propodus. Unguis 0.4 times as long as dactylus.

*Pereopod 4* (Fig. 5A): Basis subequal length of pereopod 2, 5.5 times as long as wide, with one simple ventrodistal seta. Ischium with one simple ventrodistal seta.



**Figure 4.** *Wandogarida canalicula* gen. n., sp. n., holotype, female: **A** pereopod 1 **B** pereopod 2 **C** pereopod 3. Scale bars are given in mm.

Merus 0.2 times as long as basis, 1.6 times as long as wide, with one simple seta on ventro-subdistal margin. Carpus 1.6 times as long as merus, 2.7 times as long as wide, with one midventral simple seta and three distal simple setae. Propodus 0.8 times as long as carpus, with one setulose spiniform seta on midventral margin and twelve serrated setae on distal margin. Dactylus and unguis combined 1.3 times as long as propodus. Unguis 0.4 times as long as dactylus.



**Figure 5.** *Wandogarida canalicula* gen. n., sp. n., holotype, female: **A** pereopod 4 **B** pereopod 5 **C** pereopod 6 **D** pleopod **E** male, pleopod. Scale bars are given in mm.

*Pereopod 5* (Fig. 5B): Basis shorter than that of pereopod 2, 5.6 times as long as wide. Ischium similar to that of pereopod 4. Merus as long as pereopod 4, 0.3 times as long as basis, 1.8 times as long as wide, with one distal simple seta each on ventral and dorsal margin. Carpus 1.5 times as long as merus, 3.6 times as long as wide, with three

simple setae on distal and subdistal margins. Propodus 0.8 times as long as carpus, 3.6 times as long as wide, with four simple setae on distal margin. Dactylus and unguis subequal length of propodus. Unguis similar to that of pereopod 4.

*Pereopod 6* (Fig. 5C): Basis 4.4 times as long as wide. Ischium like that of pereopod 5. Merus 0.3 times as long as basis, 1.8 times as long as wide, with three subdistal setae. Carpus 1.3 times as long as merus, with three simple setae subdistally and one simple seta medially. Propodus 0.9 times as long as carpus, with four simple setae on distal margin. Dactylus and unguis 1.2 times as long as propodus. Unguis 0.3 times as long as dactylus.

*Pleopods 1–5* (Fig. 5D): Alike. Biramous. Basal article 1.9 times as long as wide, with one plumose seta on inner distal margin. Endopod shorter than exopod, with one plumose seta on inner medial margin and seven distal and outer plumose setae. Endopod biarticulate, article 1 with one outer distal plumose seta; article 2 with seven distal plumose setae.

*Uropod* (Fig. 2F): Basal article 1.5 times as long as wide, with simple seta on distal margin. Exopod 3-articulate, article 3 1.7 times as long as articles 1 and 2 combined, with two plumose setae on tip of article 3. Endopod 10-articulate, distal article with four simple setae and two broom setae.

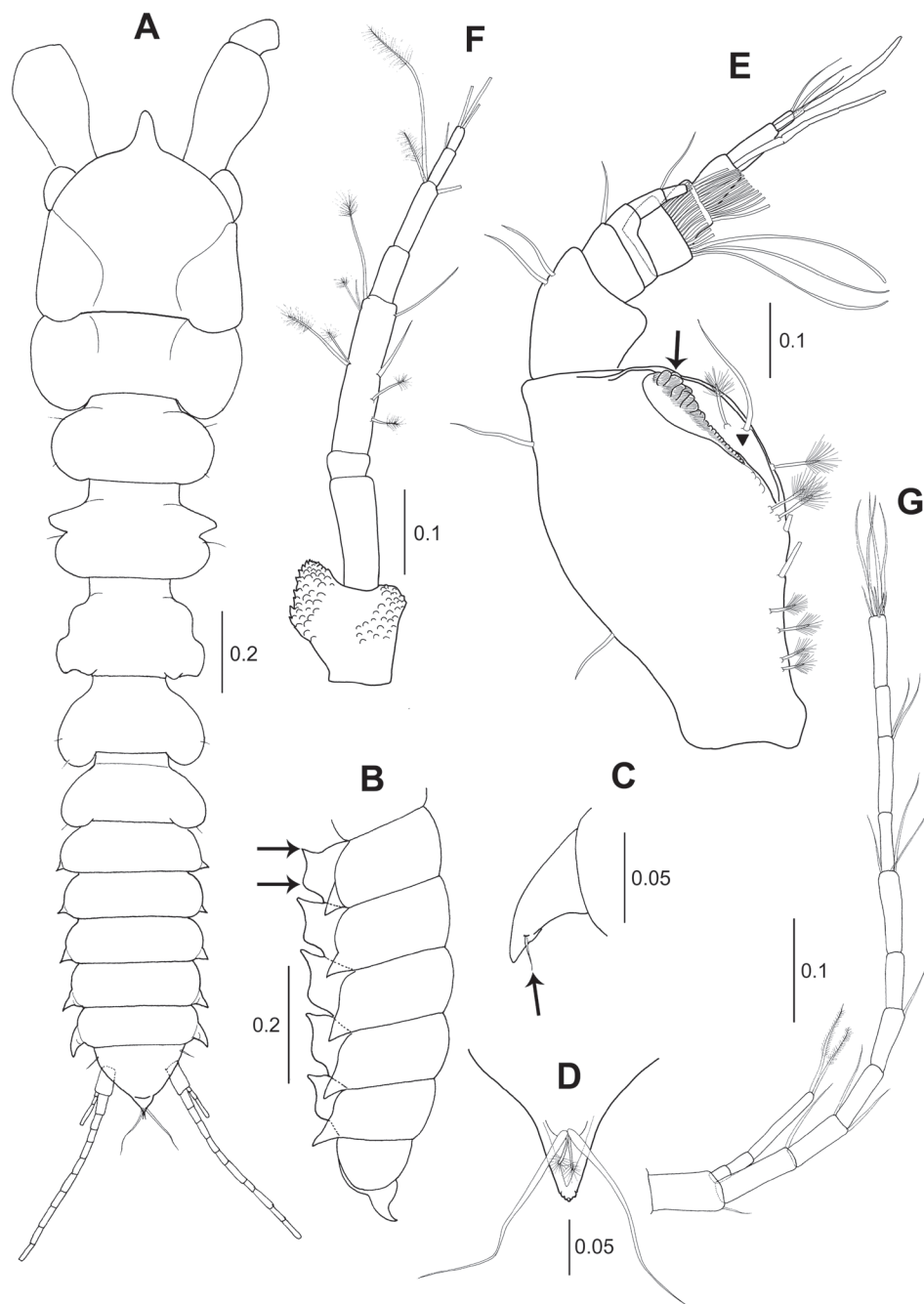
**Male.** Body (Fig. 6A), dorsoventrally flattened, 2.4 mm long, 4.7 times as long as wide, pereonites each with different shape. Cephalothorax 20.5 % of body length, as long as wide, anterior margin with conspicuous elongate, rounded rostrum. Eyes well developed, without pigmentation.

*Pereon* (Fig. 6A): Wider than long, 50.3 % of body length. Pereonite 1 0.4 times as long as cephalothorax, 0.4 times as long as wide, lateral margin rounded and posteriorly extended. Pereonite 2 laterally rounded, 1.15 times as long as pereonite 1, half as long as wide. Pereonite 3 slightly longer than pereonite 2, 0.6 times as long as wide, anterolateral margin with pointed apophysis. Pereonite 4 subequal length of pereonite 3, 0.7 times as long as wide. Pereonite 5 0.8 times as long as pereonite 4, half as long as wide, posterolateral margin rounded and posterior margin extended. Pereonite 6 shortest of all pereonites, half as long as wide.

*Pleon* (Fig. 6A–D): 22 % of body length. Pleonites subequal in length, each with anteriorly pointed and posteriorly rounded ventral hyposphenium (arrowed in Fig. 6B). Epimera pointed and gradually becoming larger posteriorly, with one simple seta (arrowed in Fig. 6C). Pleotelson (Fig. 6A, B, D): 7 % of body length, as long as pereonite 6, with two simple setae on lateral margin, two broom setae and two long simple setae of unequal length on posterior margin.

*Antennule* (Fig. 6E): Peduncle article 1 longer and more robust than other articles, outer distal margin with projection having row of rough denticles (arrowed) and mid to distal surface with deep groove between projection and smooth margin (marked by inverted triangle), 47 % of total length, 1.7 times as long as wide, with eight broom setae and three simple setae on outer margin and two simple setae on inner margin. Article 2 about 0.4 times as long as article 1, with two simple setae on inner margin. Article 3 about 0.4 times as long as article 2, 0.6 times as long as wide, with one inner





**Figure 6.** *Wandogarida canalicula* gen. n., sp. n., allotype, male: **A** habitus, dorsal view **B** pleonites and pleotelson, lateral view, arrows indicating upper and lower margins of hyposphenium of pleonite 1 **C** epimera of pleonite, arrow indicating simple seta **D** distal extension of pleotelson, ventral view **E** antennule, arrow indicating projection having row of rough denticles and inverted triangle indicating deep groove between projection and smooth margin **F** antenna **G** uropod. Scale bars are given in mm.

distal simple seta. Article 4 shorter than article 3, naked. Inner flagellum biarticulate: article 1 with inner distal simple seta and article 2 with three distal simple setae. Outer flagellum 5-articulate: articles 1 and 2 wider than long, with fringes of aesthetascs distally; article 3 with one aesthetasc; article 4 longest, with one aesthetasc; article 5 shortest, with three simple setae.

*Antenna* (Fig. 6F): Similar to those of female. Proportional lengths of articles 15.7: 19.3: 4.2: 26.6: 9.1: 14.6: 6.3: 4.2.

*Labrum* and *labium* almost equal to those of female.

*Left mandible* (Fig. 7B): Incisor terminally pointed, with small denticles. Lacinia mobilis distally pointed. Setal row with four small setae (arrowed). Molar subtriangular and reduced compared to female. *Right mandible* (Fig. 7A): incisor with distal denticles. Setal row with vertical row of denticles and one pointed seta. Molar similar to that of left mandible.

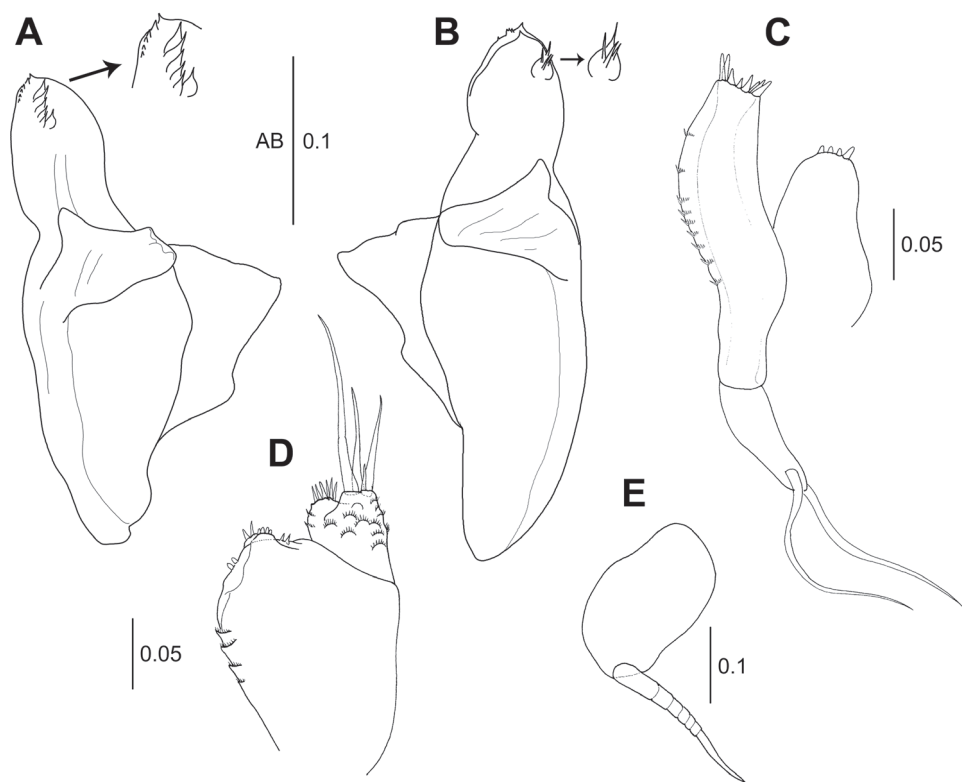
*Maxillule* (Fig. 7C): Reduced and simplified compared to that of female. Inner endite with four short simple spiniform setae. Outer endite with eight distal simple spiniform setae. Palp with one subdistal and one distal simple setae.

*Maxilla* (Fig. 7D): Movable endite with microtrichia on surface: outer lobe with four simple setae; inner lobe with six short simple setae. Fixed endite reduced and simplified: outer lobe with seven short simple setae; inner lobe very reduced, with two short simple setae.

*Maxilliped* (Fig. 8A, B): Palp articles similar to those of female. Endite (Fig. 8B) simplified: distal and subdistal margins with six short simple spiniform setae, inner margin with two coupling hooks and outer margin with several spines. *Epignath* (Fig. 7E): Smaller than that of female, distal seta longer than lobe.

*Cheliped* (Fig. 8C, C', D): Basis rounded, 1.5 times as long as wide, with one ventrodistal seta; exopodite similar to that of female. Merus with one simple seta and three processes on midventral margin (enlarged in Fig. 8C'). Carpus similar to that of female. Propodus 2.5 times as long as carpus, setation equal to that of female. Fixed finger with one ventral and one medial simple setae, cutting edge with three simple setae and several denticles and blunt processes. Dactylus as long as fixed finger, with three simple setae on inner medial margin and several blunt processes and denticles along cutting edge.

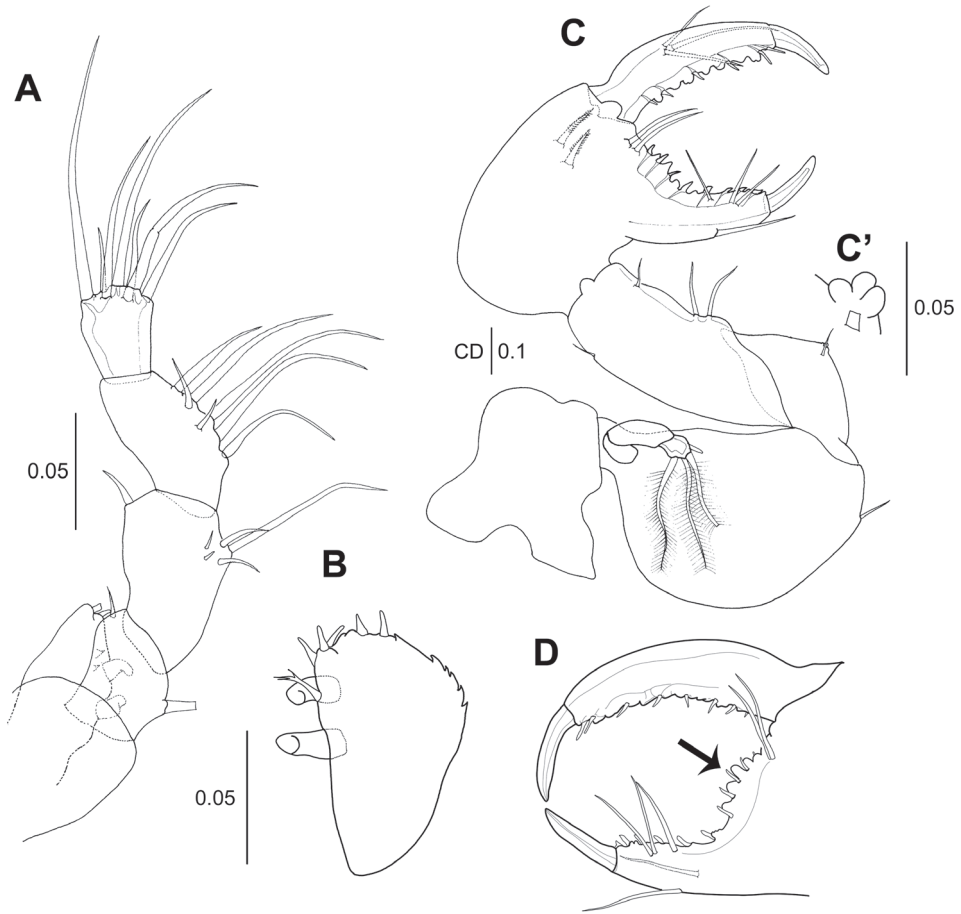
*Pereopod 1* (Fig. 9A): Basis 2.7 times as long as wide, with one ventrodistal simple seta. Ischium with one ventrodistal simple seta. Merus 0.7 times as long as basis, 2.3 times as long as wide, with two dorsodistal simple setae, one ventrodistal spiniform seta and four ventral simple setae. Carpus 0.7 times as long as merus, 2.3 times as long as wide, with two ventrodistal spiniform setae, three ventral simple setae, one dorsodistal spiniform seta and seven dorsal simple setae. Propodus 0.7 times as long as carpus, 1.8 times as long as wide, with four ventral spiniform setae, two ventral simple setae near insertion of dactylus, two dorsodistal spiniform setae and four dorsal simple setae. Dactylus and unguis combined 1.3 times as long as propodus. Dactylus with three short setae along ventral margin and one sub-proximal seta on dorsal margin. Unguis third length of dactylus.



**Figure 7.** *Wandogarida canalicula* gen. n., sp. n., allotype, male: **A** right mandible **B** left mandible **C** maxillule **D** maxilla **E** epignath. Scale bars are given in mm.

*Pereopod 2* (Fig. 9B): Basis 5.2 times as long as wide, with one ventrodistal simple seta on ventral margin and one dorsomedial broom seta. Ischium compact, with one ventrodistal simple seta. Merus half as long as basis, 3.2 times as long as wide, with two ventral simple setae. Carpus slightly shorter than merus, with five ventral simple setae and four dorsal simple setae. Propodus as long as carpus, with three spiniform setae and one distal simple seta on ventral margin and three simple setae and one broom seta on dorsal margin. Dactylus and unguis combined 1.2 times as long as propodus. Dactylus similar to that of female. Unguis half as long as dactylus.

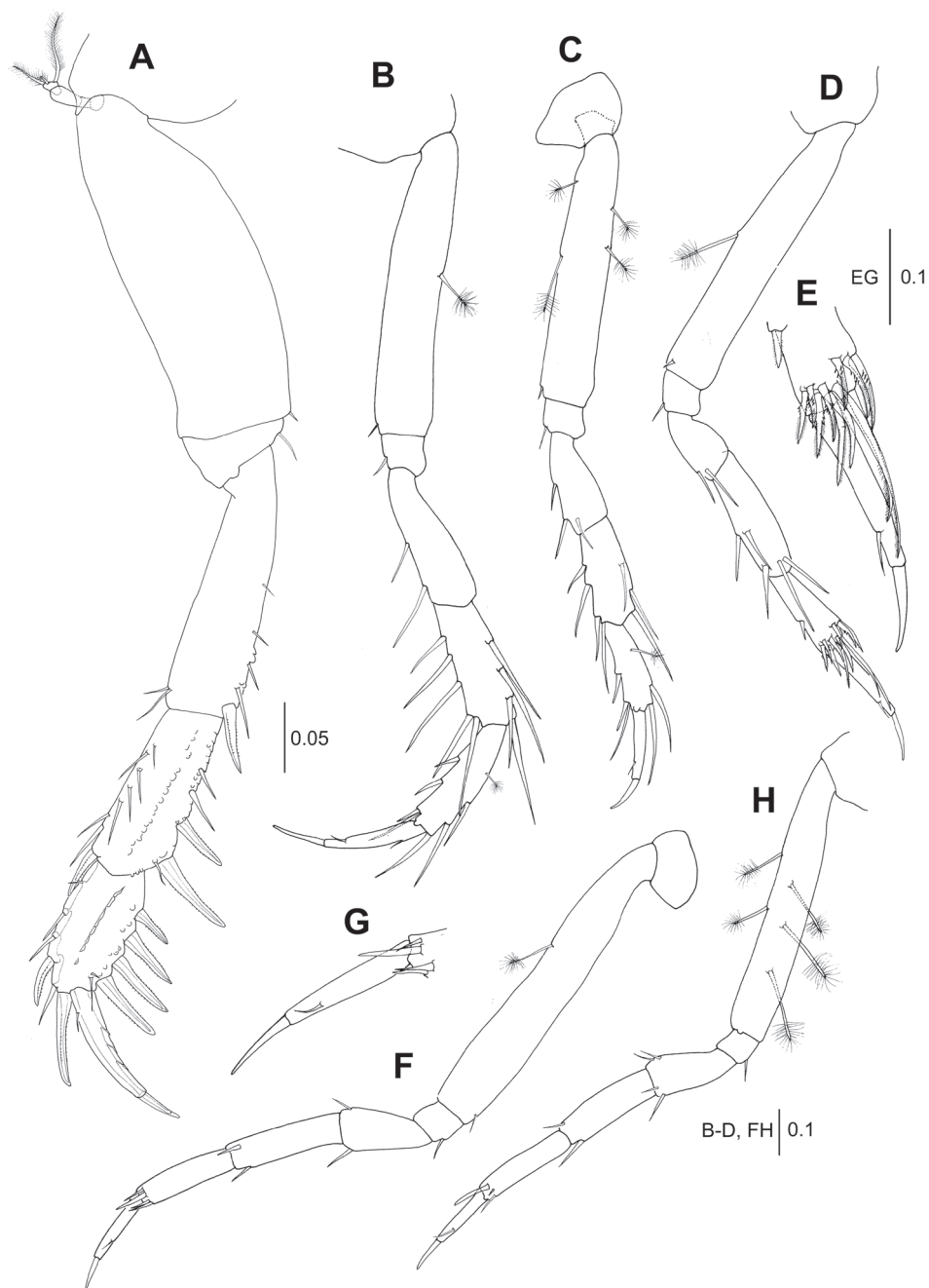
*Pereopod 3* (Fig. 9C): Basis shorter than that of pereopod 2, with two broom setae each on dorsal and ventral margin and one ventrodistal simple seta. Ischium similar to that of pereopod 2. Merus 0.4 times as long as basis, with three ventral simple setae. Carpus as long as merus, with four ventral simple setae and three dorsal simple setae. Propodus slightly shorter than carpus, with two ventral spiniform setae, one ventrodistal simple seta and two dorsodistal simple setae and one dorsal broom seta. Dactylus and unguis combined 1.3 times as long as propodus. Unguis 0.3 times as long as dactylus.



**Figure 8.** *Wandogarida canalicula* gen. n. sp. n., allotype, male: **A** maxilliped **B** maxilliped endite **C** cheliped **C'** processes on the cheliped merus **D** cheliped, fixed finger and dactylus, arrow indicating denticle on cutting edge. Scale bars are given in mm.

*Pereopod 4* (Fig. 9D, E): Basis almost as long as that of pereopod 2, 5.9 times as long as wide, with one ventrodistal simple seta and one ventromedial broom seta. Ischium similar to that of pereopod 3. Merus 0.2 times as long as basis, with two ventrodistal simple setae. Carpus 1.7 times as long as merus, three times as long as wide, with two ventromedial simple setae and three distal simple setae. Propodus 0.8 times as long as carpus, 2.8 times as long as wide, with one ventromedial spiniform seta and eleven distal serrated spiniform setae. Dactylus and unguis combined 1.3 times as long as propodus. Unguis half as long as dactylus.

*Pereopod 5* (Fig. 9F, G): Basis longer than that of pereopod 4, 5.8 times as long as wide, with one ventrodistal simple seta and one dorsomedial broom seta. Ischium similar to that of pereopod 4. Merus 0.2 times as long as basis, twice as long as wide,



**Figure 9.** *Wandogarida canalicula* gen. n., sp. n., allotype, male: **A** pereopod 1 **B** pereopod 2 **C** pereopod 3 **D-E** paratype, male **D** pereopod 4 **E** pereopod 4, distal margin of propodus, dactylus and unguis **F-H** allotype, male **F** pereopod 5 **G** pereopod 5, dactylus and unguis **H** pereopod 6. Scale bars are given in mm.

with two distal simple setae. Carpus 1.4 times as long as merus, 3.6 times as long as wide, with two distal simple setae. Propodus slightly shorter than carpus, 3.6 times as long as wide, with four distal simple setae. Dactylus and unguis combined 1.1 times as long as propodus. Unguis half as long as dactylus.

*Pereopod 6* (Fig. 9H): Basis shorter than that of pereopod 5, 6.4 times as long as wide, with five broom setae and one ventrodistal simple seta. Merus 0.3 times as long as basis, with two ventral and two dorsal setae distally. Carpus 1.3 times as long as merus, 3.1 times as long as wide, with one ventral and one dorsal seta distally. Propodus subequal length of carpus, 4.3 times as long as wide, with three distal simple setae. Dactylus and unguis combined as long as propodus. Unguis half as long as dactylus.

*Pleopod* (Fig. 5E): Similar to that of female.

*Uropod* (Fig. 6G): Basal article 1.7 times as long as wide, with simple seta on distal margin. Exopod 3-articulate, article 3 twice as long as articles 1 and 2 combined, with two plumose setae on tip of article 3. Endopod 10-articulate, distal article with nine simple setae.

**Remarks.** To check the morphological variation with size, ten specimens (1.64–2.73 mm) were partly dissected and the cheliped, the pereopod 1 and a ventral hyposphenium of pleonite were examined for different sizes and some variations were found: 1) in the cheliped, the dactylus and fixed finger of the males were modified in size: in the cheliped of the large sized males (over 2 mm), the cutting edge of the dactylus is extended and strongly curved and the processes on the proximal margin are prominently developed; 2) the number and size of setae on the pereopod 1 propodus increased with size; 3) a ventral hyposphenium of the male pleonite is modified from a rounded shape to a subrectangular shape, with body length reaching over 2 mm.

## Discussion

Sexual dimorphism within the genera *Ansphyrapus* and *Sphyrapoides* affects only their chelipeds, while in *Sphyrapus* and *Poligarida*, it affects their antennule, cheliped and pereopod 1. However, *Wandogarida canalicula* gen. n., sp. n. exhibits a greater level of sexual dimorphism: 1) the female body is wider than males, 2) in the antennule, the distal surface of the article 1 is round and naked in females, while that of the male has a vertical row of rough denticles and a deep groove; the outer flagellum of males is 5-articulate and the first and second articles have fringes of aesthetascs, but that of the female is 3-articulate and bears only one aesthetasc each on the first and second articles, 3) in the mandible, the incisor and lacinia mobilis of the left mandible of the female have distal denticles, but those of the male are distally pointed and naked and the setae of the setal row are shorter and more slender than those of the female. The setal row of the right mandible also differs (a row of five denticles and a small pointed seta in the male vs. a robust tripartite seta and three setulose setae in the female), 4) in the maxillule, the distal setae on the outer and inner endites of males are shorter than those of females, 5) the setae on the maxilla of males are

diminished in size, as compared to those of the female, 6) the maxilliped endite of the male lacks inner setae and the distal margin has six naked and reduced spiniform setae, while that of the female has four plumose setae along the inner margin and eight distal spiniform setae, 7) in the cheliped, the basis, fixed finger and dactylus show dimorphism: the basis of the male is more robust than in the female, the cutting edge of the fixed finger has slender and pointed denticles and processes, but that of the female has distally broad denticles; the dactylus of the male has several denticles and processes along the cutting edge, but that of the female is naked and 8) the pereopod 1 of the male is longer than that of the female and the dactylus also shows a different shape in the both sexes: the ventral margin has small denticles in the male but spines in the female.

These results in the sexual dimorphism and morphological variation with body size shown in *W. canalicula* can be used as an important tool to divide easily and precisely males and females of the sphyrapodid species and upgrade our understanding how their life cycle or morphological function adapts to diverse environments.

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## References

- Anderson G (2013) Tanaidacea-Thirty years of scholarship. v.1.1, May, 2013.
- Băcescu M (1978) Contribution to the knowledge of Monokonophora (Crustacea: Tanaidacea) from the NW of the Indian Ocean. *Memoriile Sectiunii Stiintifice. Series 4*(1): 197–220.
- Bamber RN (2000) Additions to the apseudomorph tanaidaceans (Crustacea: Peracarida) of Hong Kong. In: Morton B (Ed.) *The Marine Flora and Fauna of Hong Kong and Southern China IV. Proceedings of the Tenth International Marine Biological workshop: The Marine Flora and Fauna of Hong Kong and Southern China*. Hong Kong, 2–26 April 1998 Hong Kong University Press, Hong Kong, 37–52.
- Bamber RN, Marshall DJ (2013) Tanaidaceans from Brunei III. A new genus and two new species of shallow-water sphyrapodids (Crustacea: Peracarida: Tanaidacea) from the South China Sea. *Species Diversity* 18: 255–267. <https://doi.org/10.12782/sd.18.2.255>
- Błażewicz-Paszkowycz M, Bamber RN (2012) The shallow-water Tanaidacea (Arthropoda: Malacostraca: Peracarida) of the Bass Strait, Victoria, Australia (other than the Tanaidae). *Memoirs of Museum Victoria* 69: 1–235. <https://doi.org/10.24199/j.mmv.2012.69.01>
- Lang K (1956) Neotanaidae nov. fam., with some remarks on the phylogeny of the Tanaidacea. *Arkiv för Zoologi* 9: 469–475.
- Larsen K (2003) Proposed new standardized anatomical terminology for the Tanaidacea (Peracarida). *Journal of Crustacean Biology* 23: 644–661. <http://dx.doi.org/10.1651/C-2363>



- Larsen K (2005) Deep-sea Tanaidacea (Peracarida) from the Gulf of Mexico. Crustacean Monographs 5: 1–381. <http://dx.doi.org/10.1017/S0025315411000221>
- Larsen K (2012) A new family, genus and species of apseudomorphan Tanaidacea (Crustacea: Peracarida) from the Caribbean Sea. Journal of the Marine Biological Association of the United Kingdom 92(5): 1089–1093. <http://dx.doi.org/10.1017/S0025315411000221>
- Leach WE (1814) Crustaceology. In: Brewster's Edinburgh Encyclopaedia 7: 383–437.
- Guşu M (1980) *Pseudosphyrapus*, a new genus of a new family (Sphyrapidae) of Monokonophora (Crustacea, Tanaidacea). Travaux du Muséum National d'Histoire naturelle "Grigore Antipa" 22(2): 393–400.
- Guşu M (1981) A new contribution to the systematics and phylogeny of the suborder Monokonophora (Crustacea, Tanaidacea). Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa" 23: 81–108.
- Guşu M (2001) *Ansphyrapus*, a new genus of the family Sphyrapidae (Crustacea: Tanaidacea, Apseudomorpha). Travaux du Museum d'Histoire naturelle "Grigore Antipa" 43: 79–84.
- Guşu M, Iliffe TM (1998) *Sphyrapoides bicornis*, new genus and new species from a submarine escarpment in the Bahamas, and some remarks on Sphyrapinae (Crustacea, Tanaidacea). Travaux du Muséum National d'Histoire naturelle "Grigore Antipa" 40: 205–219.
- Sars GO (1882) Revision af gruppen: Isopoda Chelifera med charakteristik af nye herhen hørende arter og slægter. Archiv for Mathematik og Naturvidenskab 7: 1–54. <http://biodiversitylibrary.org/page/29996315>
- Sieg J (1980) Sind die Dikonophora eine polyphyletische Gruppe? Zoologischer Anzeiger 205(5–6): 401–416.
- Sieg J (1984) Neuere Erkenntnisse zum Natürlichen System der Tanaidacea. Eine phylogenetische Studie. Zoologica (Stuttgart) 136: 1–132.

# Two new species of the genus *Hessebius* Verhoeff, 1941 from China (Lithobiomorpha, Lithobiidae)

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## Abstract

Two new species, *Hessebius luquensis* **sp. n.** and *Hessebius ruoergaiensis* **sp. n.**, are described based on material from Qinghai-Tibet Plateau. A key to the Chinese species of *Hessebius* is presented. The partial mitochondrial cytochrome c oxidase subunit I (COI) barcoding gene was amplified and sequenced for nine individuals of both species and the dataset was used for molecular phylogenetic analysis and genetic distance determination.

## Keywords

COI, China, *Hessebius*, Lithobiidae, Taxonomy, the Qinghai-Tibet Plateau

## Introduction

*Hessebius* was created by Verhoeff (1941) to receive two Turkish species and was substantiated by Eason (1981). Its main character is the massive expansion and projection of the dorsolateral ridge of the female gonopod, according to Zaleskaja (1978) and Eason (1981).

Presently, 13 species are known (Pei et al. 2010; Volkova 2016), mainly in Palearctic region including Central Asian (Kirghizistan, Tagikistan, Turkmenistan, Kazakhstan), southern Russia, Middle East (Iran, Iraq, Armenia, Syria, Palestine), westward up to Anotalia (Toros, including the Greek southern Sporades), Cyprus, and north-east Africa (Egypt, Libya), some of which, especially those from Central Asia, were recorded only from few localities (Zapparoli 2003).

The centipedes of China have been poorly studied. Up to now, three species of *Hessebius* have been recorded (Ma et al. 2014): *H. jangtseanus* (Verhoeff, 1942) distributed in Sanshenggou, Wolong Town, Wenchuan Country, Aba City, Sichuan Province; *H. longispinipes* Ma, Pei & Zhu, 2009 recorded in Xinjiang Uyghur Autonomous Region (Barkor country, Hami City) and *H. multiforaminis* Pei, Ma, Zapparoli & Zhu, 2010 recorded in Tibet Autonomous Region (Pulan country, Pulan Town, Ali City). Considering the geographic distribution of the species of *Hessebius* in China, their main habitat preference seems to be steppes, deserts or sub-deserts, and they are all seem to be native species. The known localities of *Hessebius* in China are shown in Figure 1.

## Materials and methods

**Specimen collection and preparation:** The specimens were all collected by hand, preserved in 95 % alcohol, and deposited in the collections of Northwest Institute of Plateau Biology, Chinese Academy of Sciences. Characters were examined using SZ61 Olympus stereoscope and took pictures with a SX-3 (Shanghai optical instrument factory) camera. Terminology for external anatomy follows Bonato et al. (2010). Each specimens are numbered from 1 to 12 according to collection quantity and prefix with the abbreviation of the locality. The abbreviations used are:

**T, TT** tergite, tergites;

**S, SS** sternite, sternites;

**C** coxa;

**Tr** trochanter;

**P** prefemur;

**F** femur;

**Ti** tibia;

**a** anterior;

**m** median;

**p** posterior;

**D** dorsal;

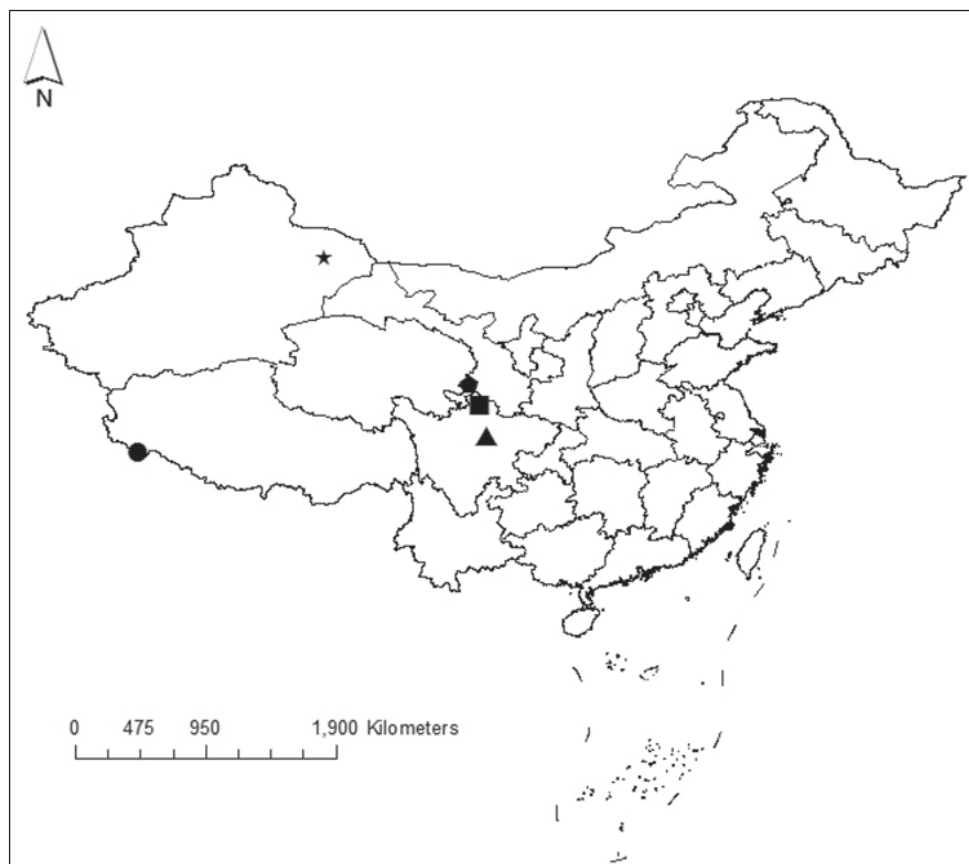
**V** ventral;

**To** Tömösváry's organ;

**LQ** Luqu;

**REG** Ruoergai.

**DNA extraction and sequencing protocols:** Standard DNA extraction and amplification methods were performed. Total DNA was extracted from a single leg removed from each specimen samples using MicroElute Genomic DNA kit (OMEGA), after overnight incubation at 65 °C. Polymerase chain reactions (PCRs) were conducted using Mastercycler pros PCR (Eppendorff) in total reaction volumes of 39-μL volumes containing 5–60 ng template DNA, 1μL; ddH<sub>2</sub>O 28μL; 10×Buffer 5μL (Takara, Dalian,



**Figure 1.** Distribution of *Hessebius* Verhoeff, 1941 in China. Symbols denote localities and species. Star = Xinjiang Uygur *H. longispinipes* Ma, Pei and Zhu, 2009; circle = Tibet *H. multiforaminis* Pei, Ma, Zapparoli and Zhu, 2010; triangle = Sichuan *H. jangtseanus* (Verhoeff, 1942); pentagon = Luqu *H. luquensis* sp. n.; square = Sichuan *H. ruoergaiensis* sp. n.

China); 0.5mm/L dNTPs 2.5μL (Takara, Dalian, China); 5U/μL Taq polymerase 0.5μL (Takara, Dalian, China); Forward Primer 1μL; Reverse Primer 1μL (synthesized by Sangon Biotech from Shanghai). An 686 bp fragment of COI was amplified using the primers LCO 1490/LCO 2198 (Edgecombe et al., 2002). PCR was performed as follows: initial denaturing at 95 °C for 10 min; followed by 35 cycles of 95 °C for 30 s, 44 °C for 30 s, and 72 °C for 90 s and a final extension at 72 °C for 10 min. The PCR products were purified using a purification kit (DC28106 250 Preps, QIAGEN, GERMANY). Sequencing reactions were implemented using ABI Prism BigDye™ Terminator Cycle Sequencing Ready Reaction Kit on ABI 3730XL sequencer, with the PCR primers.

The GenBank accession numbers of all nine new sequences were MG515155-MG515163 (*Hessebius* COI). Sequence identities were confirmed with BLAST searches (Altschul et al. 1997). In order to eliminate indicators of nuclear mitochondrial pseudogenes (numts), such as indels, stop codons, and double peaks in sequence chromatograms,

the whole dataset was translated into amino acids using the ‘invertebrate’ code in MEGA6 (Tamura et al. 2013) ; internal stop codons were absent in our dataset; gaps were absent.

**Phylogenetic analyses:** The sequences were aligned with Clustal X2.0 (Chenna et al. 2003). The aligned sequences were edited using the program BioEdit 7.0.9.0 (Hall 1999) by hand. The substitution model selection was implemented in jModelTest 2.1.4 (Darriba et al. 2012), the TIM2+G model was selected for all datasets by likelihood ratio tests either under the Akaike Information Criterion (AIC 14337.6710) or under the Bayesian Information Criterion (BIC 14617.1521). Topology was reconstructed under the TIM2+G model of nucleotide evolution in MrBayes. Bayesian inference (BI) was used to generate a phylogenetic hypothesis of the DNA haplotypes. BI was performed in MrBayes 3.2 (Ronquist and Huelsenbeck 2003) with 3,000,000 generations, sampling trees every 300 generations. Two independent runs each with four simultaneous Monte Carlo Markov chains (MCMC) were carried out. The first 25 % of generations were discarded as ‘burn-in’. The convergence of chains was confirmed until average standard deviation of split frequency is below 0.01 (0.008300) and the potential scale reduction factor (PSRF) is close to 1.0 for all parameters. In phylogenetic analysis *Anopsobius neozelanicus* Silvestri, 1909 was used as outgroup.

**Distance analysis:** The analysis involved 27 nucleotide sequences (Appendix 1). Codon positions included were 1st+2nd+3rd. All ambiguous positions were removed for each sequence pair. There were a total of 632 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). All pair-wise intra- and inter-specific distances were produced to evaluate species divergence in *Hessebius*.

## Taxonomic accounts

**Class Chilopoda Latreille, 1817**

**Order Lithobiomorpha Pocock, 1895**

**Family Lithobiidae Newport, 1844**

**Subfamily Lithobiinae Newport, 1844**

**Genus *Hessebius* Verhoeff, 1941**

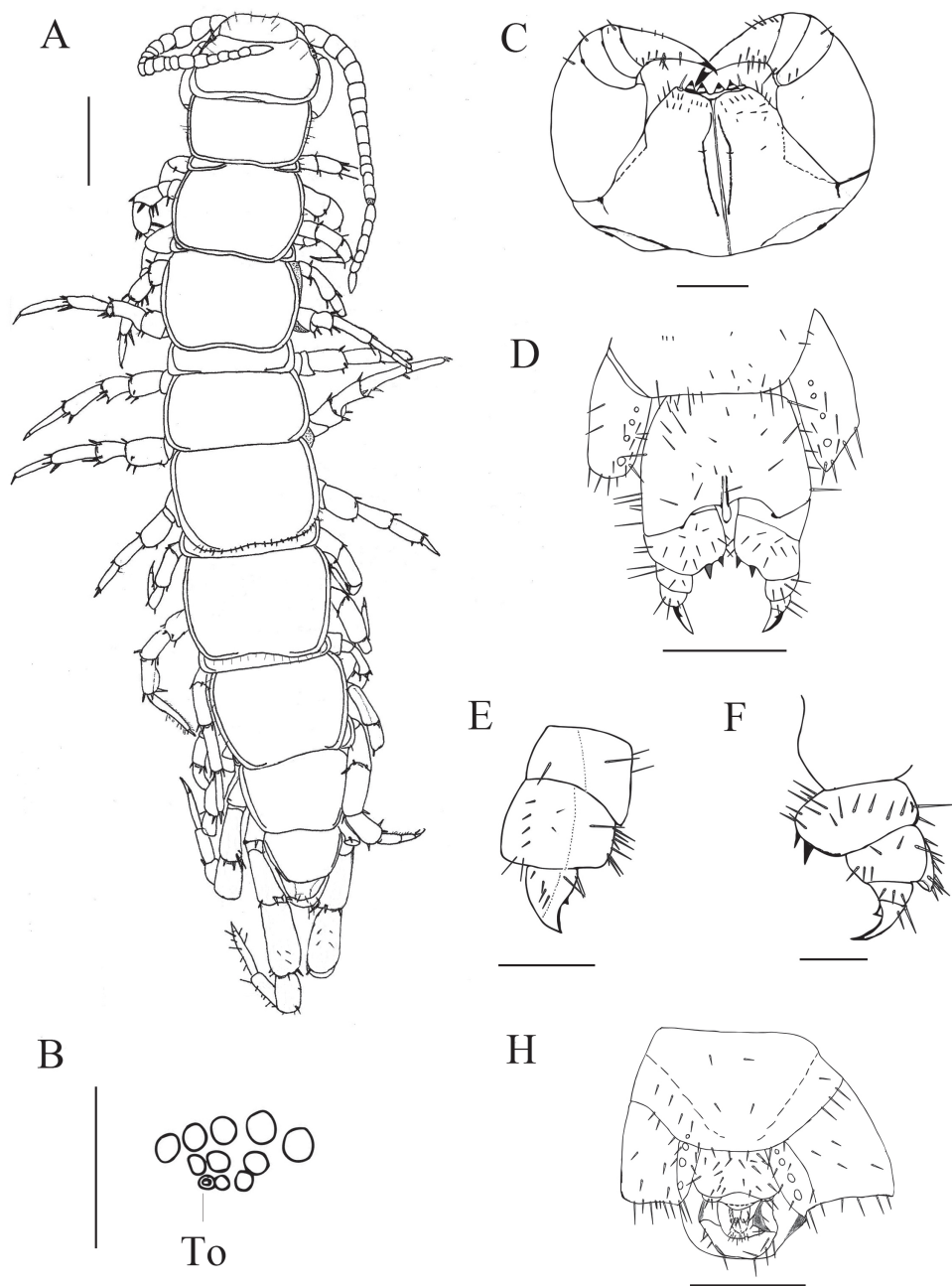
***Hessebius luquensis* sp. n.**

<http://zoobank.org/9D93BD0E-90DE-4516-8C8C-BFF5BA0530CB>

**Type data.** Holotype: female numbered LQ 8 (Fig. 2A–F), body length 10.4 mm, from Luqu County, the Gannan Prefecture, Gansu province, China, 34.75647°N, 102.57245°E, 13 May 2012, 3192 meters above sea level, leg. Gonghua Lin, Weiping Li. Paratypes: 8 females, 2 males, same data as holotype.

**Habitat.** Specimens were collected under stones along roadside on steppes from Luqu.

**Etymology.** The name is derived from the locality Luqu where the species is discovered. Luqu country is situated in the eastern edge of the Tibetan Plateau standing on the junction of Gansu, Qinghai and Sichuan Provinces.



**Figure 2.** *Hessebius luquensis* sp. n., holotype, female: **A** dorsal view, scale 1 mm **B** left ocelli and Tömösváry's organ (To), scale 250 µm; **C**, forcipular coxosternite, ventral view, scale bar 1 mm **D** posterior segments and gonopods, internal view, scale bar 500 µm **E** right gonopod, dorsal view, scale bar 250 µm **F**, right gonopod, ventral lateral view, scale bar 250 µm. Paratype, LQ 9, male **G** posterior segments and gonopods, internal view, scale bar 500 µm.

**Diagnosis.** Body length 8.5–12.3 mm; head slightly widened; antennae of 20 antennomeres; 7–10 ocelli arranged in three rows; Tömösváry's organ oval, almost equal in size to neighboring ocelli (Fig. 2B); lateral margins of forcipular coxosternite slanting; anterior margin with 2 + 2 sharp teeth and with setiform porodonts; tergites without triangular posterolateral process, a line of setae along posterior border of TT 8 and 10; legs 14 and 15 thicker than anterior ones in both sexes; a dorsal furrow on the tibia of legs 14–15 on male; coxal pores 3–6, round, arranged in one row; female gonopods with two moderately long, bullet-shaped spurs, the second article of the female gonopods having a massive process; terminal claw of the third article simple, with a small triangular protuberance on basal ventral side; male gonopods short and small.

**Description.** Holotype (female), *body* 10.4 mm long, cephalic plate 1.3 mm width, 1.2 mm length.

*Colour* (based on specimens in 95 % ethanol): antennomeres yellow; tergites pale yellow, with brown margin; cephalic plate and T 1 brown; pleural region and sternites pale yellow; distal part of forcipules dark brown, maxillipede coxosternum and S 15 yellow; legs pale yellow with gray hue, pretarsal claw dark brown.

*Cephalic plate* smooth, convex, slightly longer than wide; short to long setae scattered along the marginal ridge of the cephalic plate; setae on head shield symmetrically arranged, three pairs between antennocellar and transverse suture, two pairs behind these sutures; frontal marginal ridge with clear transverse suture; projection of lateral marginal conspicuously discontinuous; posterior marginal ridge slightly concave without median thickening.

Ocelli (Fig. 2B): translucent with dark pigment, 1 + 4, 3, 2 ocelli on each cephalic plateau, arranged in three rows. The posterior ocellus is the biggest, seriate ocelli smaller. Tömösváry's organ oval, nearly the same size as the adjacent ocelli, not remote, situated ventrally on anterolateral margin of cephalic pleurite.

Antennae length 3.46 mm, extending back to anterior margin of T 5, consisting of 20 elongate antennomeres covered with dense pale setae. The basal two articles enlarged, then elongate and tapering. The first article wider than long, the second article has the equal width to length, terminal article approximately 2.5 times length to width. Setation: the first two articles has fewer setae than succeeding articles especially on anterior side, then increasing, till 6 or 7, the density become constant.

Forcipular coxosternites subtrapezoidal, coxosternite with narrow, straight or slightly recurved dental margin; 2+2 teeth on dental margin, small, blunt knobs with independent sclerotization from coxosternite; porodont setiform towards its apex, much stouter than a seta at its base; no shoulders lateral to the porodont; 3 to 4 long setae along the slope, some setae scattered on ventral side of coxosternite.

Tergites smooth, T1 narrower than head and T3, subrectangular; on TT 8 and 10 there is a line of setae along posterior borders; posterior margin of TT. 1, 3, 5, 8, 10, 12, 14 a little concave, of TT. 6, 7 straight; posterior angles of all tergites rounded without triangular projections; marginal ridge narrow, entire on TT 1, 3 and 5, interrupted posteriorly on TT 7, 8, 10, 12, and 14 (Fig. 2A); tiny setae inserted in pores



scattered very sparsely over the surface, more setae on anterior and posterior angles of each tergite.

Sternites trapeziform, setae scattered very sparsely on the surface; four pairs of long bristles approximately symmetrical on the anterior corner and margin, one pair on the posterior corner; among long bristles there's small pairs of short bristles; short to long setae along posterior border, in some individuals SS 13, 14, 15 more dense.

Legs: tarsal articulations only visible with shallow ventral suture on 1<sup>st</sup> to 11<sup>th</sup>, distinct on 12<sup>th</sup> and 13<sup>th</sup>, well-defined on legs 14 and 15; leg pairs 14 and 15 thicker and longer with sparse setae in contrast to legs 1–13; pretarsus of legs 1–13 with a slightly curved, long, principal claw and smaller and thinner anterior and posterior accessory spines, anterior accessory spines long and slender, 0.33–0.5 the length of principal claw, posterior one stouter, 0.25 the length of principal claw, forming slightly larger angles with tarsal claws; accessory apical spines on the 14th vestigial, absent on the 15th; abundant glandular pores on surfaces of femur, tibia and tarsus of legs 14 and 15; short to moderately long setae scattered over the surface of legs 1–13, latter half of tarsi generally more setose with two rows of setae along ventral side, fewer setae on legs 14–15.

14<sup>th</sup> and 15<sup>th</sup> legs: swollen, 15th leg 30% of body-length, tarsus 1 4.3 times longer than wide, tarsus 2 48% length of tarsus on leg 15. Data on the leg plectrotaxy are compiled in Table 1. In the male the 14th and 15th tibia has a dorsal sulcus extending along its whole length.

Coxal pores on legs 12–15, circular; inner pores smaller. Distance between pores 2–3 times bigger than diameter of pore; formula 4, 4, 4, 4. Coxal pores set in a shallow groove arranged in a row with short to long setae scattered over the surface of apophysis.

Female S15 generally trapeziform, straight posteromedially; sternite of genital segment well sclerotised, wider than long; sternite of genital segment with posterior margin moderately concave between condyles of gonopods, except for a small, median approximately circular bulge, distal lightly sclerotised; short to long setae scattered over the surface of genital segment and lateral margins.

Female gonopods divided into three articles, the first article moderately broad, bearing 11–17 short to moderately long setae, arranged in three rows; the first article

**Table 1.** *Hessebius luquensis* sp. n. leg plectrotaxy; letters in brackets indicate variable spines.

Legs	Ventral					Dorsal				
	C	Tr	P	F	Ti	C	Tr	P	F	Ti
1			mp	amp	am			a(p)	a(p)	ap
2–5			mp	amp	am			ap	ap	ap
6–9			(a)mp	amp	am			ap	ap	ap
10			(a)mp	amp	am			amp	ap	ap
11			amp	amp	am			amp	ap	ap
12–13			amp	amp	am	a		amp	(a)p	ap
14		m	amp	amp	a	a		amp	(a)p	(a)p
15		m	amp	am	(a)	a		amp	p	(p)

also bearing 2+2 moderately long, bullet-shaped spurs, inner spur slightly smaller and more anterior than the outer (Fig. 2D); the second article with 6 setae arranged in one rows (Fig. 2F); dorsolateral ridge of second article with a massive expansion projecting distally over the base of the third article (Fig. 2F), six short blunt spines along the dorsolateral ridge, one on the ventral side of dorsodistal projection (Fig. 2F); three moderately long setae on third article; dorsolateral setae one on the first article, eight on the second article four of which short and blunt and four on the third (Fig. 2E); one long dorsomedial setae on each article (Fig. 2E); terminal claw simple, slender and sharp, having small triangular protuberance on ventral side (Fig. 2D).

Male S15: subsemicircular, well chitinized, long setae scattered sparsely over its surface and posterior margins. Male genitalia: first genital sternite wider than long, well chitinized; posterior margin quite deeply concave between the gonopods, no bulge medially; 24 short to medium setae scattered sparsely over its surface and at lateral margins, second genital sternite with abundance seta; gonopod of a single small article with 2 seta on its surface, apically slightly chitinized, flat (Fig. 2H).

**Variations.** The length of the body (from anterior to posterior) range from 8.5 mm (LQ 9) to (LQ 12) 12.3 mm. Colour of body from pale yellow to yellow brown to ferruginous. Ocelli 1 + 4, 3, 2 or 1 + 4, 2, 1 or 1 + 3, 2, 1 on the cephalic plateau. Coxal pores 4444, 4443, 5466, 6466, 5555, 4555 in female; 4444 in male. 15<sup>th</sup> legs of LQ 9 (♂): length of each of the three distal articles of the 15<sup>th</sup> legs in comparison with their own diameter, 15<sup>th</sup> tibiae: 0.76 mm/0.31 mm = 2.45x; 15<sup>th</sup> tarsus 1: 0.69 mm/0.22 mm = 3.14x; 15<sup>th</sup> tarsus 2: 0.62–0.13 mm/0.06 mm = 4.77x.

**Remarks.** The female of *H. luquensis* sp. n. is mostly similar to *Hessebius longispinipes* Ma, Pei and Zhu, 2009, but can be readily distinguished by the following characters: more antennomeres (20 + 20, vs. 18 + 18 in *Hessebius longispinipes*), more ocelli in three rows, a bulge exists near the base of the porodont; 14<sup>th</sup> accessory spines present, apical claw of female gonopods with triangular protuberance only on the ventral side and the apex of the male gonopod flat versus hemisphere in *H. longispinipes*.

### *Hessebius ruoergaiensis* sp. n.

<http://zoobank.org/46B3B393-F3C3-4D09-981E-6AC8E3136E77>

**Etymology.** The name of the species is from the type locality.

**Holotype.** ♀, numbered REG 11, China, North of Sichuan province, Ruoergai County, 33.397°N, 103.201°E, 14. V. 2012, under stones on steppe, at 3588 m above sea level, leg. Gonghua Lin, Weiping Li. Paratypes: 6 ♂, 3 ♀, same data as holotype.

**Diagnosis.** Body length 9.2–10.0 mm; antennae composed of 19–20 antennomeres; 7–10 dark ocelli on each side; Tömösváry's organ ovate to round, larger to the adjoining ocelli; 2+2 triangular sharp prosternal teeth; porodonts long and strong, lateral to lateral tooth; posterior angles of all tergites round; legs 14 and 15 thicker than anterior ones; coxal pores 3–5, ovate to round, arranged in one row; female gonopods with 2 bullet-shaped spurs, the second article of the female gonopods extend-

ing backwards bearing 5 lateral spines; terminal claw of the third article simple, with inconspicuous triangular ventral accessory denticles; male gonopods short and small with 2 long setae.

**Description.** Holotype (female). Body 9.2 mm length. Cephalic plate 1.0 mm length, 1.2 mm width.

Colour: body pale yellow; antennae and distal part of forcipules brown; cephalic plate, TT 1, 2 dark and median and posterior parts of TT 3–14 dark forming a line; pleural region and SS pale yellow with dark hue; legs pale yellow with dark hue excluding tarsus yellow.

Antennae: 41.6% of body-length with 20 moderately elongate articles; the basal one wider than long; the 8, 9, 10, 11 elongate; the ultimate one is three times longer than wide. Abundance setae scattered on the surfaces of from the first to the last.

Cephalic plate wider than long, with clear transverse suture; median furrow on cephalic plate absent; lateral margin discontinuous, posterior margin slightly concave; moderately long setae scattered along marginal ridge and cephalic plate (Fig. 3A).

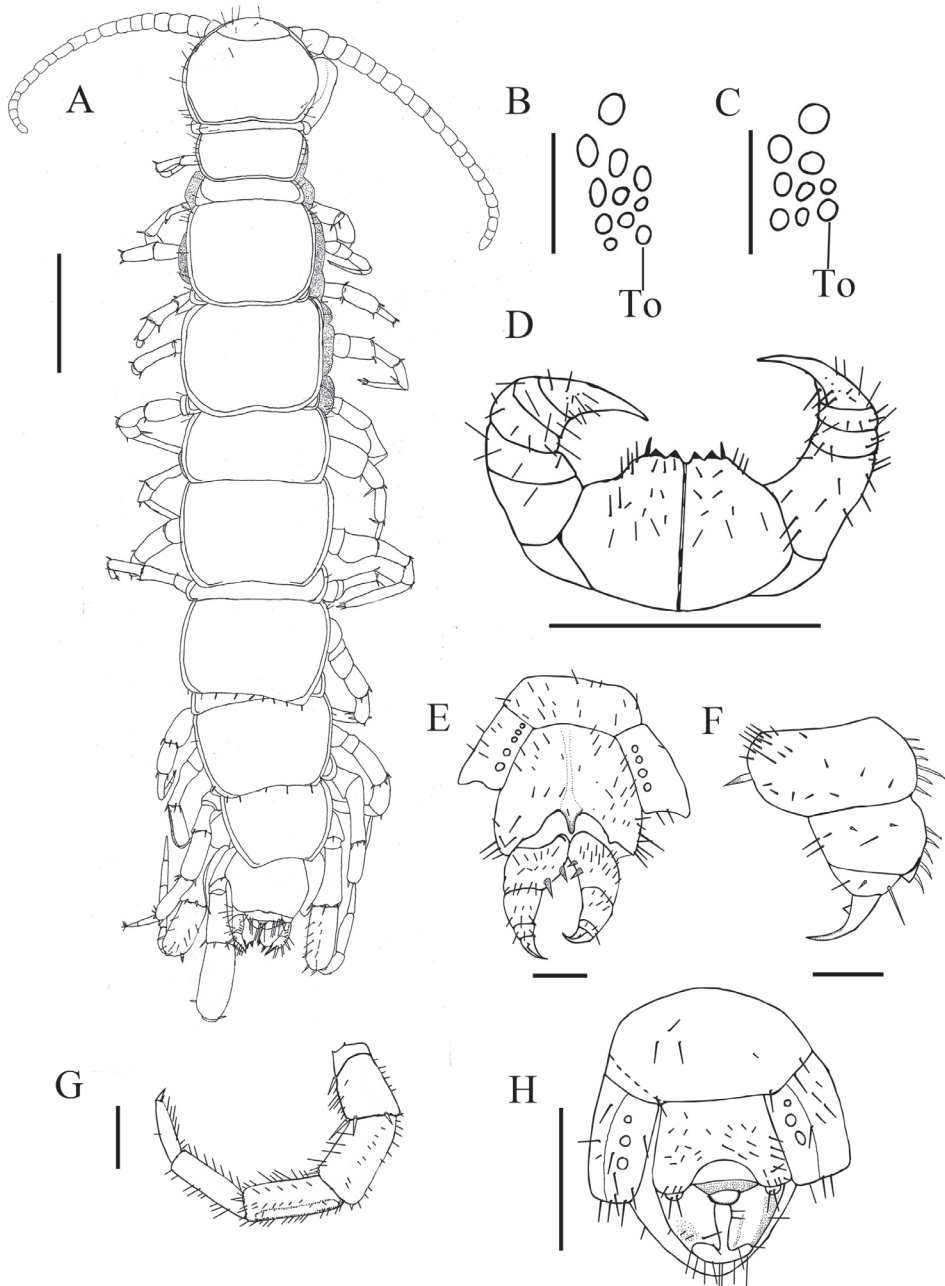
Two posterior large ocelli and eight smaller ocelli arranged in three rows (Fig. 3B). Tömösváry's organ ovate, larger than the adjoining ocelli, some distance from the adjoining ocelli, situated on ventral side of cephalic pleurite.

Prosternum: subtrapezoidal coxosternite with narrow, straight dental margin; 2+2 subtriangular teeth as extensions of the coxosternite teeth; median incision "U" shaped; long and strong setiform porodonts; lateral borders without shoulders; pretarsal section of forcipules slightly longer than tarsal section; 3 lines of short setae and 1 moderately long setae arrange on ventral side of coxosternite (Fig. 3D).

Tergites smooth, angulation of posterolateral corners of tergites all rounded without triangular projections; T1 narrower than head and T3, concave transverse; all tergites with lateral margins; TT 1, 3 and 5 with complete posterior margins, TT 7, 8, 10, 12, and 14 incomplete; posterior margins of TT 3, 5, 8, 10, and 12 a little concave (Fig. 3A), T14 gently concave, TT1, 7, and 9 transverse, tergite of intermediate segment weakly convex. Short to long setae sparsely dispersed along lateral borders and posterior corners, a band of setae on TT 10, 12 (Fig. 3A).

Sternites smooth, S1 subsquare, SS 2–14 trapeziform, posterolateral narrower than anterolateral. One to three pairs of setae symmetrical on anterior corners; one pair of setae on posterolateral margins; a few setae distributed along posterior margins; a band of setae on anterior margins of SS 2–7.

Legs: tarsal articulation on anterior pairs of legs fused on dorsal side of leg, distinct ventrally from 1<sup>st</sup> to 13<sup>th</sup>, on 14<sup>th</sup> and 15<sup>th</sup> leg divided into basitarsus and distitarsus; pretarsus claws moderately long, curved ventrally on all legs; anterior and posterior accessory spines present from the 1<sup>st</sup> to the 14th leg, only posterior accessory spines on the 15th leg; anterior accessory spines long and straight, nearly half of length of the main claw, posterior accessory claws strong and curved, nearly a third length of the main claw; Legs 14–15 (Fig. 3A) thickened. Numerous short to long setae fairly evenly distributed on all sections along legs. Legs' plectrotaxy as in Table 2. In male, one comparatively obvious furrow on the dorsal side of the tibia of legs 14 and 15 (Fig. 3G).



**Figure 3.** *Hessebius ruoergaiensis* sp. n., holotype, female: **A** dorsal view, scale bar 1 mm **B** left ocelli and Tömösváry's organ (To), scale bar 250 µm **D** forcipular coxosternite, ventral view, scale bar 1 mm **E** right gonopod, dorsal view, scale bar 250 µm **C, F–H** paratype, C, REG1, female: left ocelli and Tömösváry's organ (To), scale bar 250 µm **F** REG5, female: right gonopod, ventral lateral view, scale bar 250 µm **G** REG6, male: male left leg 15, dorsal lateral view, scale bar 500 µm **H** REG10, male: posterior segments and gonopods, internal view, scale bar 500 µm.

**Table 2.** Plectrotaxy of *Hessebius ruoergaiensis* sp. n., the holotype and paratypes. Letters in parentheses indicate variable spines.

Legs	Ventral					Dorsal				
	C	Tr	P	F	Ti	C	Tr	P	F	Ti
1			(m)p	am	am			ap	a(p)	a(p)
2–9			mp	amp	am			ap	ap	ap
10			mp	amp	am			(a)mp	ap	ap
11–12			(a)mp	amp	am	(a)		amp	ap	ap
13			(a)mp	amp	am	(a)		amp	(a)p	ap
14		m	amp	amp	am	a		amp	(a)p	(p)
15		m	amp	am	a(m)	a		amp	p	

15<sup>th</sup> legs: approx. one third of body-length. Leg 15 basitarsus 129% length of distitarsus; basitarsus 84% length of tibia; tibia 2.6 times longer than maximal width, basitarsus 3.6 times, distitarsus 3.2 times. Basitarsus nearly the same length of distitarsus on leg 14.

Glandular pores: on the ventral side of femur tibia and tarsus of 14<sup>th</sup> and 15<sup>th</sup> legs only.

Coxal pores: on legs 12–15; set in shallow groove; the inner one smaller, circular, separated from one another by their own diameter or less; 5,5,5,5/5,5,5,4 (holotype) or 4444 in females; 4443, 3444 or 3333 in males.

Female: S15 subtrapeziform with short to long setae covered. The first genital sternite bears approx. 48 setae, posterior margin of which moderately embayed between gonopod articulations. Two long conical spurs on the female gonopod, the proximal ones smaller (Fig. 3E); Claw of female gonopod with small triangular ventral accessory denticles (Fig. 3F); five stronger and curved spines like thorn on distinct dorsodistal projection (Fig. 3F); 15 or 16 setae arranged in three rows on basal article of gonopod, six long setae on second article, 3 long setae on third (Fig. 3E).

Comparatively long setae distribute on male first genital sternite with fewer setae near S 15; posterior median margin of the first genital sternite deeply concave between gonopods; male gonopod short with two setae sometimes retracted from tergite of first genital sternite (Fig. 3H).

**Variations.** body 9.2–10.0 mm long, cephalic plate 0.9–1.2 mm wide, 0.9–1.2 mm long; 1+3, 2, 1—1+4, 3, 2 ocelli (Fig. 3B, C); Leg 15: basitarsus 129–138% length of distitarsus, basitarsus 84–94% length of tibia; tibia 2.6–2.8 times longer than maximal width, basitarsus 3.6–3.9 times, distitarsus 3–3.2 times.

**Remarks.** *Hessebius ruoergaiensis* sp. n. is very similar to *Hessebius jangseanus*: the number of ocelli of both species are overlapped, but *H. ruoergaiensis* has fewer ocelli, no more than ten; fewer coxal pores in *H. ruoergaiensis*, no more than five; the distribution of accessory claw on the legs is the same in both species; however, the tibia of the 14<sup>th</sup> and 15<sup>th</sup> leg of *H. ruoergaiensis* have dorsal sulcus which is absent in *H. jangseanus*; the plectrotaxy of legs also similar but different.

### Key to species of the genus *Hessebius* in China

- |   |   |                          |
|---|---|--------------------------|
| 1 | Antennomeres 17 + 17 – 19 + 19, commonly 18 + 18.....   | <i>H. longispinipes</i>  |
| – | Antennomeres 20 + 20.....   | 2                        |
| 2 | Posterior accessory spinies present on the I5th leg.....  | 3                        |
| – | Posterior accessory spinies absent on the I5th leg .....  | 4                        |
| 3 | Dorsal sulci on 14th and I5th leg absent.....   | <i>H. jangtseanus</i>    |
| – | Dorsal sulci on 14th and I5th leg present.....  | <i>H. ruoergaiensis</i>  |
| 4 | Apical claw of female gonopods simple and broad .....   | <i>H. multiforaminis</i> |
| – | Apical claw of female gonopods sharp and long with small triangular protuberance on ventral side..... | <i>H. luquensis</i>      |

### Molecular analysis

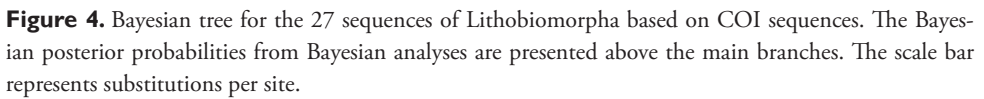
The monophyly of both *Hessebius luquensis* sp. n. and *Hessebius ruoergaiensis* sp. n. is well supported with bootstrap values of 90 and 100 respectively (Fig. 4). A sister clade of *Hessebius luquensis* sp. n. and *Hessebius ruoergaiensis* sp. n. is also supported (67) (Fig. 4).

The number of base differences per site from between sequences are shown in Appendix 2. Intraspecific uncorrected p-distances range up to 6.65 % within *Hessebius luquensis* sp. n. and 0.2 % in *Hessebius ruoergaiensis* sp. n. Interspecific mean p-distance between *Hessebius luquensis* sp. n. and *Hessebius ruoergaiensis* sp. n. range is 17.3 %. Lowest interspecific distances excluding between the two new species are between *Hessebius luquensis* sp. n. and *Lithobius (Ezembius) giganteus* Sseliwanoff, 1881 (15.7 %) and highest between *Lithobius variegatus rubriceps* Newport, 1845 and *Lamyctes inermipes* Silvestri, 1897 (25.6 %). Uncorrected p-distances to the outgroup ranges from 17.6 % to 22.6 % (Appendix 2).

### Discussion

Both molecular analysis (Fig. 4) and morphology support that *Hessebius luquensis* sp. n. and *H. ruoergaiensis* sp. n. form new species and that the relationship is genetically close. The two species are morphologically similar, but can be readily distinguished using COI.

Generally speaking, in Lithobiomorpha, intraspecific distances are less than 10 %, while distances between species ranges often more than 10%. Sometimes the distance between the species from the same genus are larger than from different genus, such as 17.3 % (interspecific mean p-distance between *Hessebius luquensis* sp. n. and *Hessebius ruoergaiensis* sp. n.) vs 15.7 % (interspecific distances between *Hessebius luquensis* sp. n. and *Lithobius (Ezembius) giganteus* Sseliwanoff). This may indicate that each species have been evolved independently its habitat for a long time. Morphologically similar species, for instance species from the same genus, also have high similarity in gene that the branches which they represented joined together shown in phylogenetic tree (St. Clair and Visick 2010).





## Acknowledgements

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## References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research* 25: 3389–3402. <https://doi.org/10.1093/nar/25.17.3389>
- Bonato L, Edgecombe GD, Lewis JGE, Minelli A, Pereira LA, Shelley RM, Zapparoli M (2010) A common terminology for the external anatomy of centipedes (Chilopoda). *ZooKeys* 69: 17–51. <https://doi.org/10.3897/zookeys.69.737>
- Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic acids research* 31: 3497–3500. <https://doi.org/10.1093/nar/gkg500>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772–772. <https://doi.org/10.1038/nmeth.2109>
- Eason EH (1981) On some new and little-known southern Asiatic species of Lithobiidae (Chilopoda: Lithobiomorpha). *Insect Systematics & Evolution* 12: 327–338. <https://doi.org/10.1163/187631281794709755>
- Edgecombe GD, Giribet G (2003) A new blind *Lamyctes* (Chilopoda: Lithobiomorpha) from Tasmania with an analysis of molecular sequence data for the *Lamyctes-Henicops* Group. *Zootaxa* 152: 1–23. <https://doi.org/10.11646/zootaxa.152.1.1>
- Edgecombe GD, Giribet G, Wheeler WC (2002) Phylogeny of Henicopidae (Chilopoda: Lithobiomorpha): a combined analysis of morphology and five molecular loci. *Systematic Entomology* 27: 31–64. <https://doi.org/10.1046/j.0307-6970.2001.00163.x>
- Giribet G, Edgecombe GD (2006) Conflict between Datasets and Phylogeny of Centipedes: An Analysis Based on Seven Genes and Morphology. *Proceedings Biological Sciences* 273: 531–538. <https://doi.org/10.1098/rspb.2005.3365>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic acids symposium series*. Information Retrieval Ltd., c1979-c2000, London, 95–98.
- Ma HQ, Pei SJ, Hou XJ, Zhu TG, Wu DY, Gai YH (2014) An annotated checklist of Lithobiomorpha of China. *Zootaxa* 3847: 333–358. <https://doi.org/10.11646/zootaxa.3847.3.2>
- Ma HQ, Pei SJ, Zhu MS (2009) A new species of the Genus *Hessebius* Verhoeff, 1941 (Lithobiomorpha: Lithobiidae). *Entomologicaal News* 20: 195–200. <https://doi.org/10.3157/021.120.0211>

- Murienne J, Edgecombe GD, Giribet G (2010) Including secondary structure, fossils and molecular dating in the centipede tree of life. *Molecular Phylogenetics & Evolution* 57: 301–313. <https://doi.org/10.1016/j.ympev.2010.06.022>
- Pei SJ, Ma HQ, Zapparoli M, Zhu MS (2010) A review of the Chinese species of *Hessebius* Verhoeff, 1941 (Chilopoda: Lithobiomorpha: Lithobiidae). *Zootaxa* 100(2631): 51–61.
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- St Clair C, Visick J (2010) Exploring bioinformatics: A project-based approach. Jones and Bartlett, Burlington.
- Stoev P, Komerički A, Akkari N, Liu S, Zhou X, Weigand A, Hostens J, Hunter C, Edmunds S, Porco D, Zapparoli M, Georgiev T, Mietchen D, Roberts D, Faulwetter S, Smith V, Penev L (2013) *Eupolybothrus cavernicolus* Komerički & Stoev sp. n. (Chilopoda: Lithobiomorpha: Lithobiidae): the first eukaryotic species description combining transcriptomic, DNA barcoding and micro-CT imaging data. *Biodiversity Data Journal* 1: e1013. <https://doi.org/10.3897/BDJ.1.e1013>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution* 30: 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Verhoeff KW (1941) Asyanin zoogeografiyasi ve hayvan sistematige hakkinda. *Asiatische Beiträge. II. Türkische Chilopoden*. Istanbul Universitesi Orman Fakültesi Dergisi 6 B: 85–117.
- Verhoeff KW (1942) Chilopoden aus innerasiatischen Hochgebirgen. *Zoologischer Anzeiger* 137: 35–52.
- Voigtländer K, Iorio E, Decker P, Spelda J (2017) The subgenus *Monotarsobius* in the Iberian Peninsula with a description of a new pseudo-cryptic species from Northern Spain revealed by an integrative revision of *Lithobius crassipes* L. Koch, 1862 (Chilopoda, Lithobiomorpha, Lithobiidae). *Zookeys* 681: 1–38. <https://doi.org/10.3897/zookeys.681.12942>
- Volkova JS (2016) A new species of the genus *Hessebius* Verhoeff, 1941 from the Lower Volga (Myriapoda, Chilopoda, Lithobiomorpha, Lithobiidae). *Spixiana* 39: 83–88.
- Zalesskaja NT (1978) Identification book of the lithobiomorph centipedes of the USSR (Chilopoda: Lithobiomorpha). Moscow, Nauka Publication House, 212 pp. [In Russian]
- Zapparoli M (2003) The present knowledge on the European fauna of Lithobiomorpha (Chilopoda). *Bulletin of the British Myriapod and Isopod Group* 19: 20–41

## Appendix I

Species used for CO1 sequence analysis, sequence references, GenBank accession numbers, vouchers, and localities. ZSM = Zoologische Staatssammlung München, Germany; AM KS = vouchers in Australian Museum prefix; MCZ = Australian Museum, Sydney, Australia; SMNG = Senckenberg Museum of Natural History, Frankfurt, Germany.

Morph species name	Sequence reference	GenBank accession No.	Voucher No.	Locality
Lithobiidae				
Lithobiinae				
<i>Lithobius</i> ( <i>Monotarsobius</i> ) <i>crassipes</i>	(Voigtländer et al. 2017)	MF123710.1	SMNG VNR 17281-1	France,
<i>Lithobius</i> (L.) <i>forficatus</i>	(Voigtländer et al. 2017)	MF123702	SMNG VNR 17150-2	Germany
<i>Lithobius variegatus rubriceps</i>	(Murienne et al. 2010)	AF334311	DNA100283	Spain
<i>Lithobius</i> (L.) <i>castaneus</i>	(Murienne et al. 2010)	HM453305	DNA103939	
<i>Lithobius</i> ( <i>Ezembius</i> ) <i>giganteus</i>	(Murienne et al. 2010)	HM453306	DNA101089	
<i>Lithobius holsti</i>	(Murienne et al. 2010)	HM453307	DNA102106	
<i>Australobius scabrior</i>	(Giribet and Edgecombe 2006)	DQ201428		
Ethopolyinae				
<i>Eupolybothrus tridentinus</i>	(Stoev et al. 2013)	JN269950.1	BC ZSM MYR 00430	Croatia
<i>Bothropylus xanti</i>	(Murienne et al. 2010)	HM453308	Bmultide	
Henicopidae				
Anopsobiinae				
<i>Anopsobius neozelanicus</i>	(Edgecombe et al. 2002)	AF334313.1	AM KS 57958	New Zealand
Henicopinae				
Henicopini				
<i>Henicops maculatus</i>	(Edgecombe and Giribet 2003)	AF334316.1	AM KS57962	Australia
<i>Lamyctes coeculus</i>	(Edgecombe and Giribet 2003)	AF334315.1	DNA100288	Australia
<i>Lamyctes emarginatus</i>	(Voigtländer et al. 2017)	KX442654.1	ZSM-JSP120527-016	Germany
<i>Lamyctes inermipes</i>	(Edgecombe and Giribet 2003)	AY214425.1	DNA100478	Argentina
<i>Lamyctes hellyeri</i>	(Edgecombe and Giribet 2003)	AY214428.1	DNA100639	Australia
<i>Paralamyctes</i> ( <i>P.</i> ) <i>harrisi</i>	(Edgecombe et al. 2002)	AF334320	AM KS 57971	New Zealand
<i>P. (Thingathingia) validus</i>	(Edgecombe et al. 2002)	AF334330	AM KS 57969	New Zealand
Zygethobiini				
<i>Esastigmatobius japonicus</i>	(Edgecombe et al. 2002)	AF334332	MCZ 28612	Japan



[illegible]

# Two new species of the bamboo-feeding planthopper genus *Bambusiphaga* Huang & Ding from China (Hemiptera, Fulgoromorpha, Delphacidae)

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<http://zoobank.org/BB1F5D25-C803-43A5-B983-5E9B6CA424E9>

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## Abstract

Two new species of the bamboo-feeding genus *Bambusiphaga* Huang & Ding, 1979, *B. yingjiangensis* sp. n. and *B. ventroprocessa* sp. n., are described and illustrated from Yunnan and Hainan, south China. A key to species of the genus are given. A map showing the geographic distribution of the two new species is also provided.

## Keywords

Bamboo planthopper, distribution, Fulgoroidea, Homoptera, Oriental region, taxonomy

## Introduction

The bamboo-feeding planthopper genus *Bambusiphaga*, belonging to the tribe Tropidocephalini (Delphacidae, Delphacinae), was established by Huang and Ding (1979) (type species: *B. nigropunctata* Huang & Ding, 1979). To date, 25 species are recognized in the genus. Among them, 23 species are distributed in China (Huang et al. 1979; Kuoh et al. 1980; Ding 1982; Ding and Hu 1982; Asche 1983; Ding et al. 1986; Yang and Yang 1986; Qin and Yuan 1999; Chen and Li 2000; Chen et al. 2000; Qin

et al. 2006; Chen and Liang 2007; Hou and Chen 2010; Yang and Chen 2011; Qin et al. 2012). Chen and Liang (2007) proposed 20 species of the genus in seven groups: *nigropunctata*, *citricolorata*, *lacticolorata*, *fascia*, *maculata*, *wangmoensis*, and *mirostylis* respectively. Yang and Chen (2011) provided the checklist of 24 species of the genus.

Species of *Bambusiphaga* feed exclusively on bamboo (Bambusoideae). Some of them, such as *B. furca* Huang & Ding, *B. citricolorata* Huang & Tian, *B. taiwanensis* (Muir), *B. lacticolorata* Huang & Ding, *B. maculata* Chen et al. and *B. luodianensis* Ding, are of economic significance since these species have large populations in bamboo fields (Huang et al. 1979; Ding et al. 1986; Yang and Yang 1986; Yang et al. 1999; Chen et al. 2000; Liu and Chen 2008; Zheng and Chen 2013a, b). Specimens have been collected on the leaves of several genera of bamboo, including *Bambusa*, *Dendrocalamus*, *Gelidocalamus*, *Sinocalamus*, *Neosinocalamus*, and *Phyllostachys* (Huang et al. 1979; Ding and Hu 1982; Ding et al. 1986; Yang and Yang 1986; Chen and Li 2000; Chen et al. 2000; Ding 2006; Chen and Liang 2007; Zheng and Chen 2013a, b).

Herein, two new species of *Bambusiphaga*, *B. yingjiangensis* sp. n. and *B. ventroprocessa* sp. n., are described and illustrated from Yunnan and Hainan respectively.

## Materials and methods

Dry male specimens were used for the description and illustration. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. Color pictures for adult habitus were obtained by KEYENCE VHX-1000 system. The genital segments of the examined specimens were macerated in 10% KOH and drawn from preparations in glycerin jelly using a Leica MZ 12.5 stereomicroscope. Illustrations were scanned with Canon CanoScan LiDE 200 and imported into Adobe Photoshop 6.0 for labeling and plate composition.

Terminology of morphological and measurements follow Yang and Yang (1986), Chen and Liang (2007), and the morphological terminology of female genitalia follows Bourgoin (1993). Measurements of body length equal the distance between the apex of vertex and tip of tegmen. All measurements are in millimeters (mm).

The type specimens of the new species are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (IEGU).

## Taxonomy

### *Bambusiphaga* Huang & Ding, 1979

*Bambusiphaga* Huang & Ding, 1979: 170; Asche 1983: 211; Ding and Tian 1983 (in Kuoh et al. 1983): 49; Yang and Yang 1986: 37; Wang and Ding 1996: 22; Ding et al. 1999: 441; Ding 2006: 126; Chen and Liang 2007: 504; Hou and Chen 2010: 392; Yang and Chen 2011: 51.



**Type species.** *Bambusiphaga nigropunctata* Huang & Ding, 1979, by original designation.

**Diagnosis.** For the diagnosis and relationships of *Bambusiphaga* see Yang and Chen (2011: 51), Hou and Chen (2010: 392) and Chen and Liang (2007: 504).

**Host plants.** Bamboo.

**Distribution.** Oriental region, with highest species diversity in China.

### Key to species of genus *Bambusiphaga* (male)

(Modified from Yang and Chen 2011 and Qin et al. 2012)

- 1 Vertex dark brown or with blackish brown markings ..... 2
- Vertex without any markings ..... 3
- 2 Vertex yellowish brown, basal compartment with a black oval spot in middle part; anal segment without a process, pygofer without medioventral processes (Huang et al. 1979: figs 2, 4) ..... *B. nigropunctata*
- Vertex dark brown, basal compartment of vertex without a black oval spot; anal segment with a very long process that surpasses base of genital styles; pygofer with conjugated medioventral processes (Chen and Liang 2007: figs 46, 49)..... *B. pianmaensis*
- 3 Mesonotum with blackish brown markings ..... 4
- Mesonotum without blackish brown markings ..... 12
- 4 Pronotum with blackish brown markings on lateral areas..... 5
- Pronotum without blackish brown markings on lateral areas ..... 11
- 5 Forewings with basal 1/3 black or with black markings at basal half..... 6
- Forewings with a large irregular pale brown stripe along transverse vein hence bending along posterior margin to apex (Fig. 8)..... *B. yingjiangensis* sp. n.
- 6 Forewings with basal 1/3 black ..... 7
- Forewings with large black markings at base ..... 10
- 7 Anal segment without a process on ventral margin (Yang and Chen 2011: fig. 6)..... *B. kunmingensis*
- Anal segment with a very long process on ventral margin..... 8
- 8 Anal spiny process at left lateroapical angle of anal segment..... 9
- Anal spiny process at right lateroapical angle of anal segment (Hou and Chen 2010: fig. 14)..... *B. basifusca*
- 9 Pygofer with a medioventral process; aedeagus with two apical processes (Qin et al. 2012: figs 12, 16–17) ..... *B. taibaishana*
- Pygofer without medioventral process; aedeagus without apical processes (Ding and Hu 1987: figs 1, 3) ..... *B. fascia*
- 10 Forewings with a large black marking at base; anal segment with a long process on ventral margin (Chen et al. 2000: figs 3–4) ..... *B. maculata*
- Forewings with two large black markings at base; anal segment without process on ventral margin (Figs 29, 31)..... *B. ventroprocessa* sp. n.

- 11 Forewings somewhat reddish orange, costal margin blackish brown; genital styles relatively broad and short (Huang et al. 1980: figs 8c, 8f) ..... ***B. nigromarginata***
- Forewings somewhat yellowish brown, costal margin yellowish brown; genital styles relatively slender (Yang and Yang 1986: figs 20C, 20E; Miur 1917: fig. 44) ..... ***B. taiwanensis***
- 12 Anal segment with a process on ventral margin ..... **13**
- Anal segment without a process on ventral margin ..... **17**
- 13 Pygofer with a medioventral process (Muir 1919: fig. 3) ..... ***B. bakeri***
- Pygofer without a medioventral process ..... **14**
- 14 Anal segment with the process on ventral margin very long, reaching ventral margin of pygofer ..... **15**
- Anal segment with the process on ventral margin very short ..... **16**
- 15 Genital styles with a process at base, apex rounded (Ding et al. 1986: figs 1 (5–6) ) ..... ***B. jinghongensis***
- Genital styles without a process at base, apex forked (Huang et al. 1979: fig. 18) ..... ***B. mirostylis***
- 16 Tegula with apical 1/2 blackish brown; pygofer with hind margin produced at an acute angle medially; genital styles slender; aedeagus without phallobase (Ding and Hu 1982: figs 1–4) ..... ***B. huangi***
- Tegula fully yellowish brown; pygofer with hind margin not produced medially; genital styles broad and short; aedeagus with developed phallobase (Chen and Li 2000: figs 11, 13, 15–16) ..... ***B. wangmoensis***
- 17 Pygofer with a spine on ventral margin ..... **18**
- Pygofer without a spine on ventral margin ..... **20**
- 18 Genital style with an inversed spine on caudal side near apex which is as long as 1/5 of genital style; aedeagus with three spines subapically (Yang and Chen 2011: figs 20–22) ..... ***B. yangi***
- Genital style with an angular or tooth-like process on caudal side near apex; aedeagus without spines subapically ..... **19**
- 19 Genital styles asymmetrical, right one shorter than left one, without tooth-like process subapically on caudal side; aedeagus with an inversed process on right side near apical 1/3 (Miur 1919: fig. 8) ..... ***B. singaporensis***
- Genital styles symmetrical; aedeagus without any processes (Ding 1982: figs 3, 5) ..... ***B. luodianensis***
- 20 Genital styles with a finger-like process at base ..... **21**
- Genital styles without a finger-like process at base ..... **22**
- 21 Genital styles with a finger-like process subapically; aedeagus curved in middle (Chen and Liang 2007: figs 20–22) ..... ***B. maolanensis***
- Genital styles with a lamellate process subapically; aedeagus almost straight (Hou and Chen 2010: figs 9–10) ..... ***B. hainanensis***
- 22 Genital styles forked apically ..... **23**
- Genital styles not forked apically ..... **25**

- 23 Frons longer at middle line than wide at widest part, about 2.0: 1; basocaudal portion of genital styles in profile produced at a right angle (Yang and Yang 1986: figs 22B, 22H) ..... ***B. membranacea***
- Frons longer at middle line than wide at widest part, about 2.5: 1; basocaudal portion of genital styles in profile not produced at a right angle ..... **24**
- 24 Median portion of genital styles granulate (Huang et al. 1979: figs 8–11) ..... ***B. furca***
- Median portion of genital styles not granulate (Aschi 1983: fig. 4) ..... ***B. lynchi***
- 25 Ventral margin of anal segment incised medially; genital styles short, lamellate (Huang et al. 1979: fig. 20) ..... ***B. lacticolorata***
- Ventral margin of anal segment not incised medially; genital styles slender .... **26**
- 26 Apex of vertex obviously broadened, frons widest at base; apex of genital styles without small teeth; aedeagus short and stout (Huang et al. 1979: fig. 17) ..... ***B. similis***
- Apex of vertex not broadened, frons widest at apex; apex of genital styles with several small teeth; aedeagus relatively long (Huang et al. 1979: figs 13–15) ..... ***B. citricolorata***

***Bambusiphaga yingjiangensis* sp. n.**

<http://zoobank.org/416EAEAC-A8BF-4D2F-975E-09CE63A54E0E>

Figs 1–21

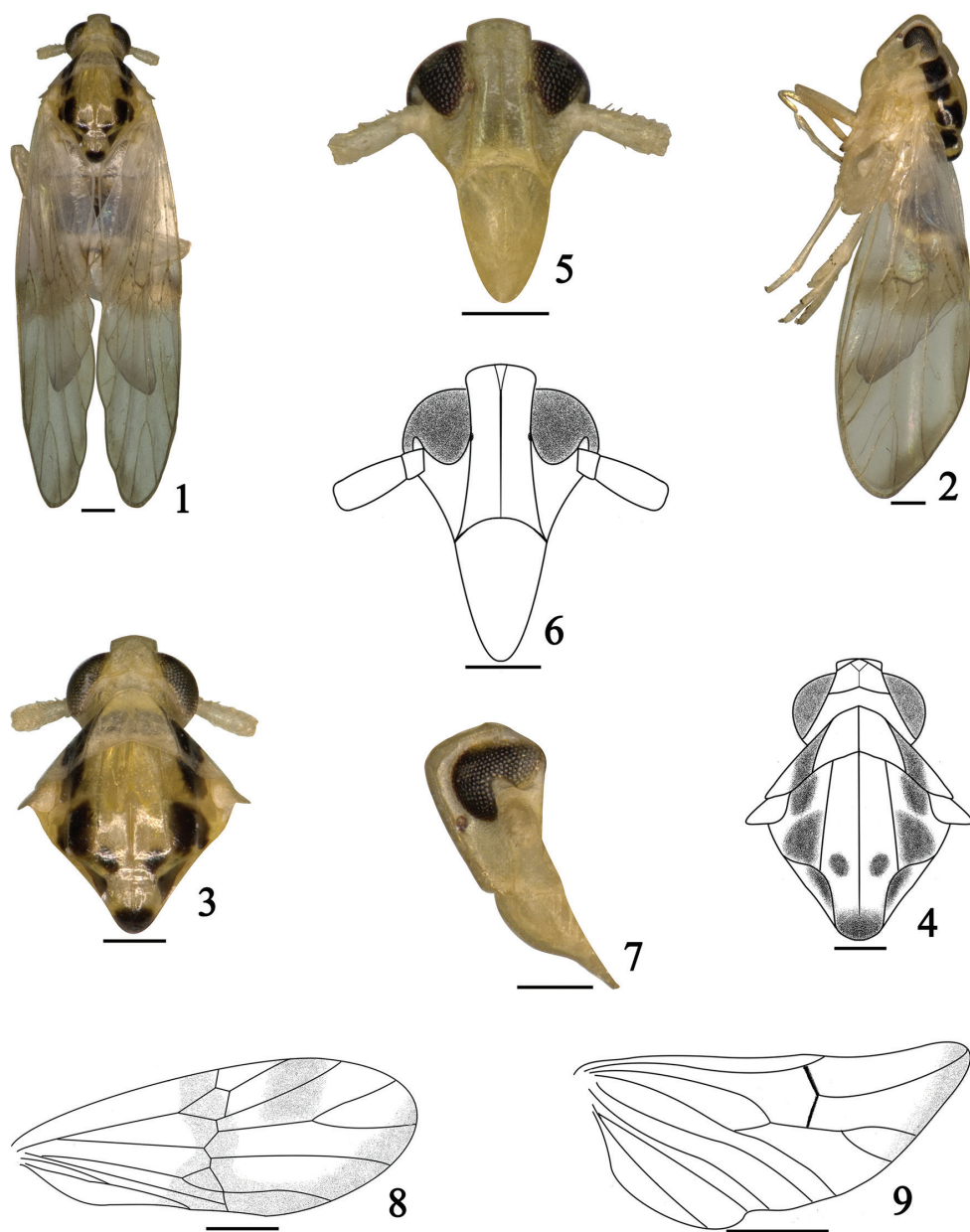
**Type material.** Holotype: ♂, **China:** Yunnan Province, Yingjiang County (97°56'E, 24°41'N), on bamboo, 17 Aug. 2015, X.-S. Chen and L. Yang; paratypes, 5♂♂, 23♀♀, same data as holotype.

**Etymology.** This new species is named after the type locality, Yingjiang, Yunnan Province in China.

**Measurements.** Body length (from apex of vertex to tip of forewings): male 3.2–3.4 mm (N = 6); female 3.6–3.9 mm (N = 23); forewings length: male 2.5–2.7 mm (N = 6); female 3.2–3.5 mm (N = 23).

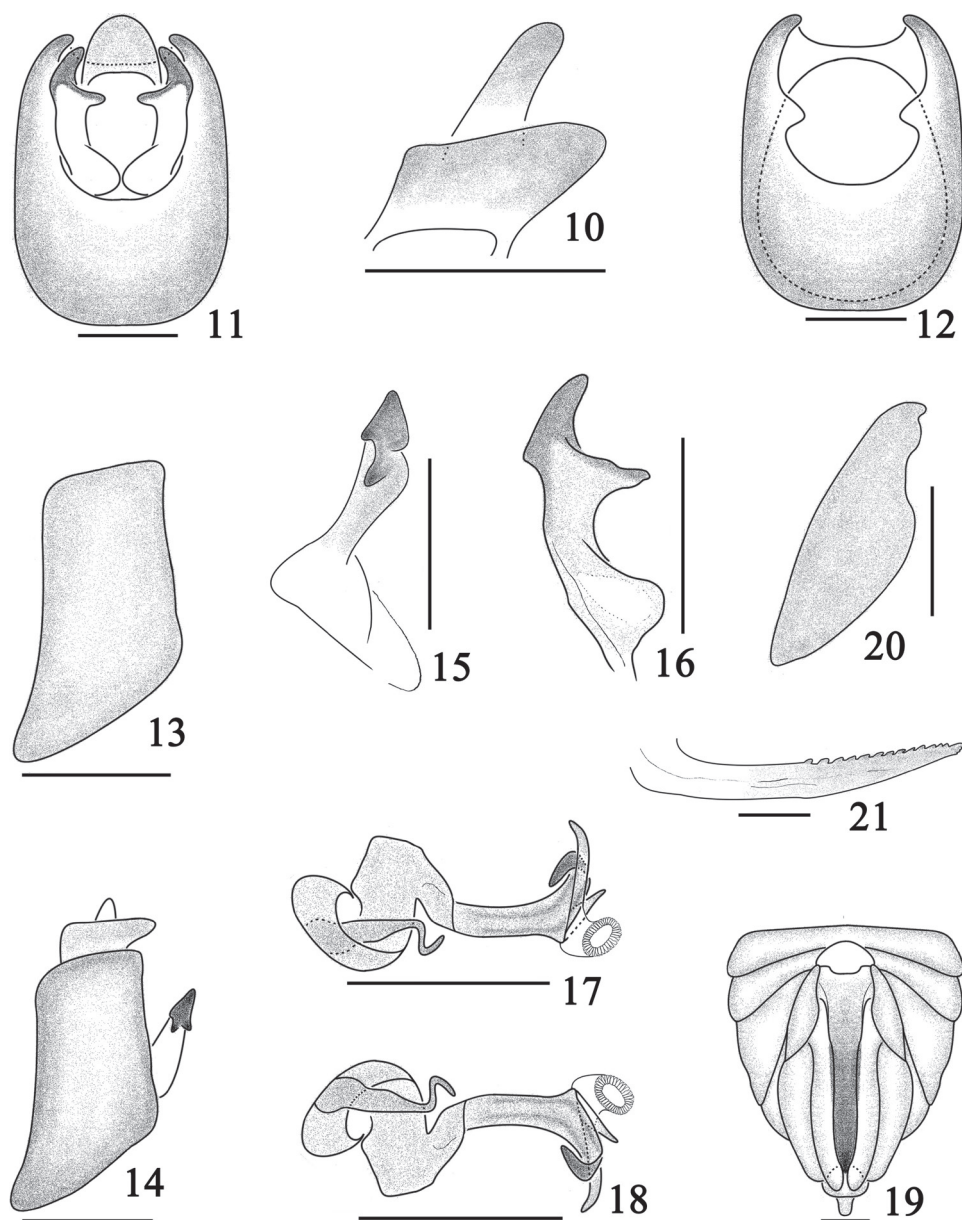
**Diagnosis.** The salient features of the new species include the following: pronotum and mesonotum with blackish brown markings (Figs 3–4); forewings with a large irregular pale brown stripe along transverse vein hence bending along posterior margin to apex (Fig. 8); aedeagus with phallobase, apical 1/4 with three branches (Figs 17–18); genital styles with apical forked (Figs 15–16).

**Description.** *Coloration.* General color light yellow with dark brown markings (Figs 1–2). Vertex, frons, genae, clypeus and antennae light yellow (Figs 1–7). Eyes reddish brown, ocelli red (Figs 5, 7). Pronotum (Figs 3–4) light yellow to yellowish white, outside of each lateral carina with a large dark brown marking. Mesonotum (Figs 3–4) light yellow, outside of each lateral carina with two large dark brown markings, middle area with two small bilateral dark brown markings at apical 1/3, the scutellum with apex dark brown. Forewings (Fig. 8) hyaline, with a large irregular pale brown stripe



**Figures 1–9.** *Bambusiphaga yingjiangensis* sp. n. **1** Male habitus, dorsal view **2** Same, lateral view **3–4** Head and thorax, dorsal view **5–6** Face **7** Frons and clypeus, lateral view **8** Forewing **9** Hindwing. Scale bars **1–7** 0.2 mm; **8–9** 0.5 mm.

along transverse vein hence bending along posterior margin to apex, another large pale brown transverse marking from vein  $Rs+M_1$  to apex of vein  $Sc_2$ . Hindwings (Fig. 9) with a pale brown longitudinal stripe along apical margin.



**Figures 10–21.** *Bambusiphaga yingjiangensis* sp. n. **10** Anal segment, lateral view **11** Male genitalia, posterior view **12** Pygofer, posterior view **13** The same, lateral view **14** Male genitalia, lateral view **15** Genital style, lateral view **16** Same, posterior view **17** Aedeagus, right lateral view **18** Same, left lateral view **19** Female genitalia, posterior view **20** Gonocoxa VIII, posterior view **21** Gonapophysis IX. Scale bars 0.2 mm.

*Head and thorax.* Vertex with anterior margin broadly rounded, lateral and submedian carinae distinct, ratio width at base to width at apex 1.4, ratio of length to width at base 0.5 (Figs 3–4). Frons with ratio of length at midline to width at widest part 2.2,



widest at apex, median carina forked at base (Figs 5–6). Base of postclypeus as wide apex of frons (Figs 5–6). Antennae with basal segment long equal to wide, shorter than second segment (0.4: 1), two segments together reaching to frontoclypeal suture (Figs 5–6). Pronotum with ratio length in midline to length of vertex 1.7 (Figs 3–4). Mesonotum 2.5 times as long as vertex and pronotum combined in middle line (Figs 3–4). Forewings (Fig 8) longer in middle line than broad at widest part (2.6: 1), apical margin rounded.

**Male genitalia.** Pygofer (Figs 12–13) without medioventral process, opening longer than wide in posterior view (Fig. 12), dorsal margin shorter than ventral margin in lateral view (Fig. 13). Aedeagus (Figs 17–18) with phallobase process small and simple, arising from base of aedeagus, with basal 1/2 thick, apical 1/2 thin, S-shaped; phallus complex, apical 1/4 with three branches—the left one curved, directed basad, the middle one small and straight, and the right one, longest slightly curved and directed ventrad; gonopore located at apical 1/4 of phallus, node-like. Genital styles (Figs 15–16) long, with two processes forked at apical 1/3 (Fig. 16), with apex in profile triangular, a large tooth-like located at middle of subapex, directed basad (Fig. 15). Anal segment (Figs 10–11) short, ring-like, without processes, ventral margin convex medially in posterior view (Fig. 11).

**Female genitalia.** Female pygofer (Fig. 19) with gonocoxa VIII moderately large. Ovipositor (Fig. 19) overpassing the pygofer. Gonangulum large, apex blunt, connected gonocoxa VIII. Gonapophyses IX (Fig. 21) curved basally, straight and narrowing apically, dorsal margin with apical 1/2 serrated, ventral margin with three small teeth near the tip.

**Host plant.** Bamboo.

**Distribution.** Southwest China (Yunnan) (Fig. 40).

**Remarks.** This new species resembles *B. nigropunctata* Huang & Ding, 1979, but differs from the latter by: lateral areas of pronotum and mesonotum with several dark brown markings (without dark brown marking in *nigropunctata*); genital styles forked apically (genital styles not forked apically in *nigropunctata*); aedeagus with three branches subapically (aedeagus with two branches apically in *nigropunctata*).

This new species is also similar to *B. taiwanensis* (Muir, 1917) and can be distinguished by: lateral areas of pronotum and mesonotum with several dark brown markings (without dark brown marking in *taiwanensis*); genital styles forked apically (genital styles not forked apically in *taiwanensis*); aedeagus with three branches without tooth (aedeagus with several small teeth on dorsal and lateral sides of the main branch in *taiwanensis*).

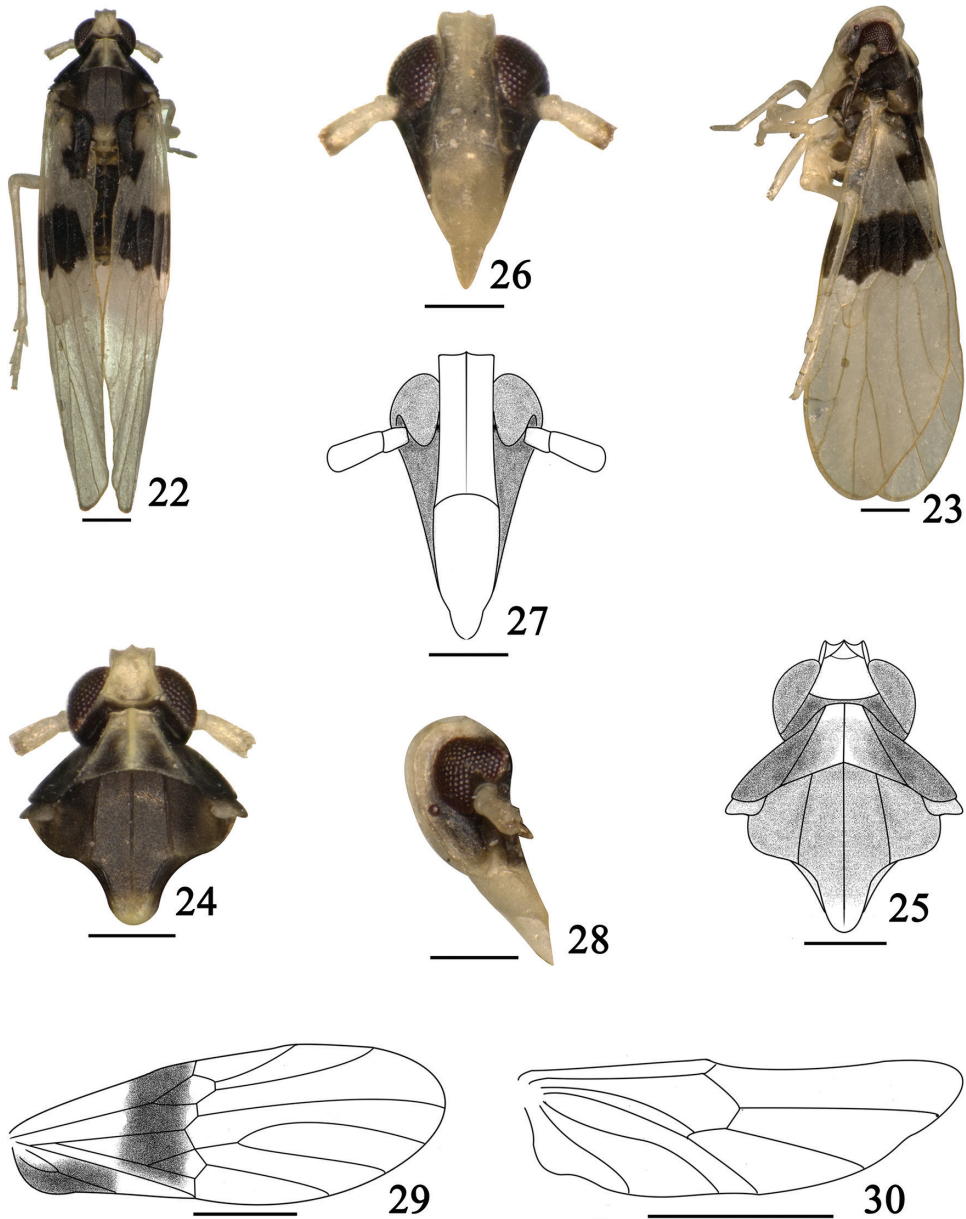
Based on the characters of male genitalia, this species should belong to the *nigropunctata* group.

***Bambusiphaga ventroprocessa* sp. n.**

<http://zoobank.org/3AC6766B-DD2C-4242-9CD9-0EA1702D180D>

Figs 22–39

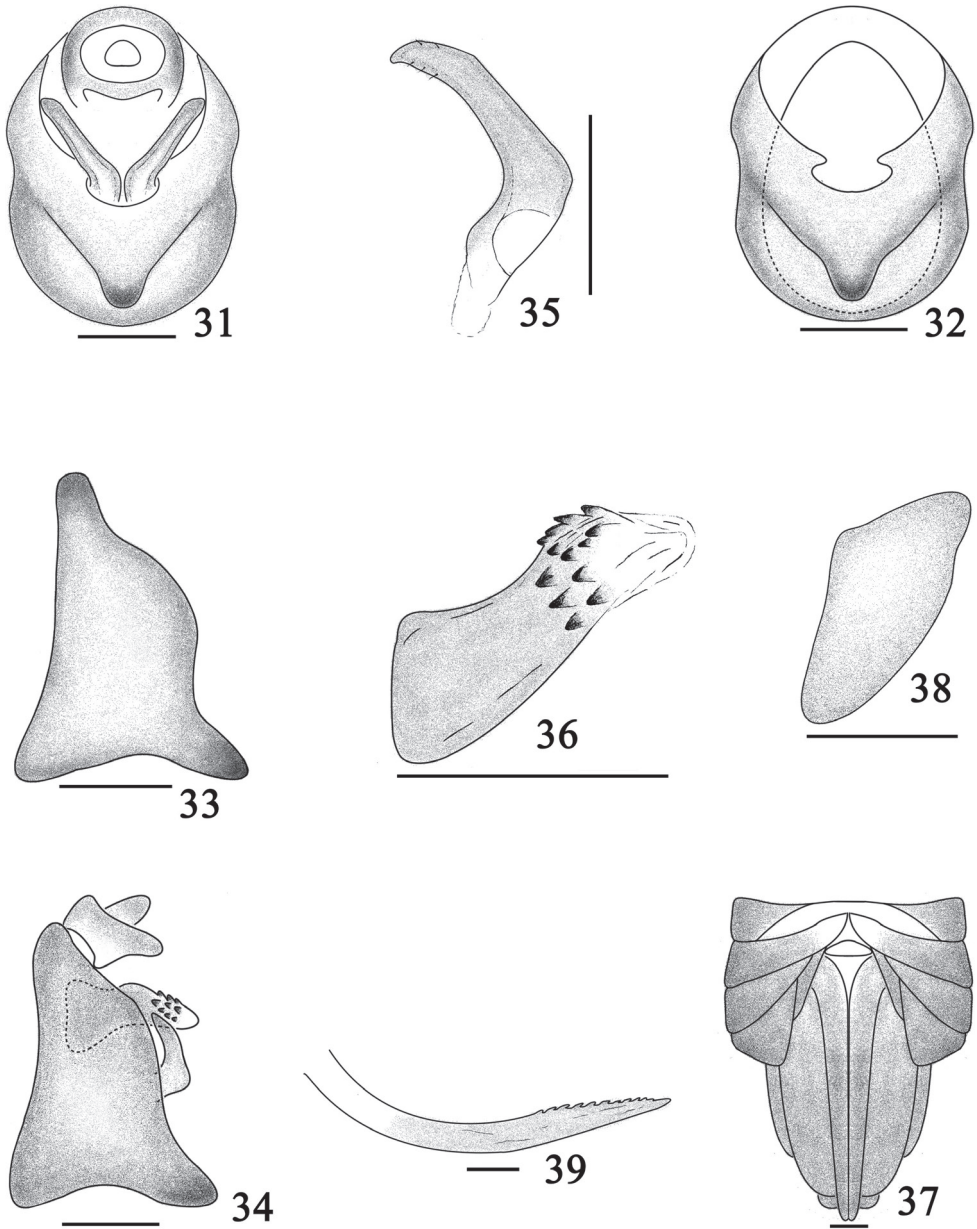
**Type material.** Holotype: ♂, **China:** Hainan Province, Lingshui County (110°01'E, 18°30'N), on bamboo, 16 Apr. 2017, H.-X. Li; paratypes, 3♂♂, 10♀♀, same data as holotype.



**Figures 22–30.** *Bambusiphaga ventroprocessa* sp. n. **22** Male habitus, dorsal view **23** Same, lateral view **24–25** Head and thorax, dorsal view **26–27** Frons and clypeus **28** Same, lateral view **29** Forewing **30** Hindwing. Scale bars **22–28** 0.2 mm; **29–30** 0.5 mm.

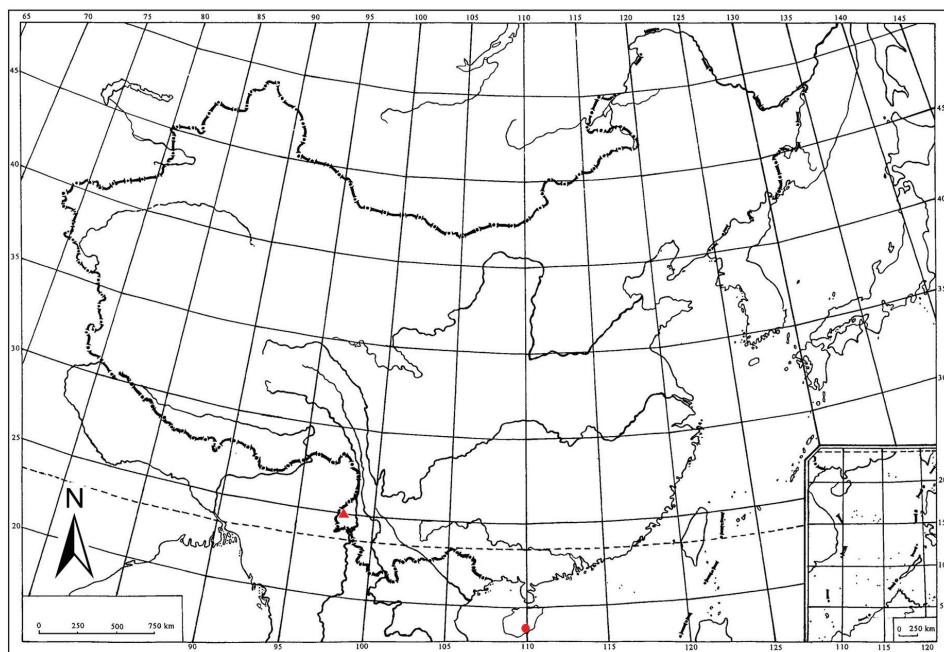
**Etymology.** The specific name is a combination of the Latin word *venter* (truncated, with o- connecting vowel), meaning belly, ventral; and the Latin word *processus*, meant in the modern biological sense of a projection or appendage, truncated with the feminine termination *-a*.





**Figures 31–39.** *Bambusiphaga ventroprocessa* sp. n. **31** Male genitalia, posterior view **32** Pygofer, posterior view **33** Same, lateral view **34** Male genitalia, lateral view **35** Genital style, posterior view **36** Aedeagus **37** Female genitalia, posterior view **38** Gonocoxa VIII, posterior view **39** Gonapophysis IX. Scale bars 0.1 mm.

**Measurements.** Body length (from apex of vertex to tip of forewings): male 2.4–2.6 mm (N = 4); female 2.4–2.7 mm (N = 10); forewings length: male 2.0–2.2 mm (N = 4); female 2.0–2.3 mm (N = 10).



**Figure 40.** Geographic distributions of two new *Bambusiphaga* species in China: *B. yingjiangensis* sp. n. (▲); *B. ventroprocessa* sp. n. (●).

**Diagnosis.** The salient features of the new species include the following: forewings with two large black markings at base (Fig. 29); pygofer with medioventral process large and inverted (Fig. 32); aedeagus with numerous inverted spines at apical 1/2 (Fig. 36).

**Description.** *Coloration.* General color yellowish white to black (Figs 22–30). Vertex, frons, clypeus, antennae and legs yellowish white. Genae black brown. Eyes and ocelli brownish red (Figs 26, 28). Pronotum (Figs 24–25) black, disc with anterior 1/3 between lateral carinae and median carina yellowish white. Mesonotum (Figs 24–25) blackish brown, apex of scutellum yellowish white. Forewings (Fig. 29) with two large dark brown markings at basal area.

*Head and thorax.* Vertex (Figs 24–25) with anterior margin angled convex medially, Y-shaped carina with stalk absent, ratio of length to width at base 0.9, ratio width at base to width at apex 1.4. Frons (Figs 26–27) with ratio of length in middle line to width at widest 2.6, widest at apex, median carina simple and obscure apically. Clypeus (Figs 26–27) with width at base as same as frons at apex. Antennae (Figs 26–27) with basal segment subequal to broad, shorter than second segment (1.0: 3.0), reaching to frontoclypeal suture. Pronotum (Figs 24–25) with carinae distinct, lateral carinae attaining hind margin, length in midline as long as vertex. Mesonotum (Figs 24–25) with lateral carinae straight, subparallel, attaining hind margin, median carina obscured apically, ratio length to pronotum and vertex combined in middle line 1.3. Forewings (Fig. 29) with ratio of length in middle line to width at widest part 2.5, apical margin rounded. Hindwings (Fig. 30) elongate.



**Figure 41.** Adult of *Bambusiphaga yingjiangensis* sp. n. resting on leaf of bamboo. Photograph by X.-S. Chen.

**Male genitalia.** Pygofer in posterior view (Fig. 32) with medioventral process large and inversed, opening longer than wide, lateral margins sinuate; in lateral view (Fig. 33) dorsal margin shorter than ventral margin distinctly, posterior margin concave. Aedeagus (Fig. 36) stout, tubular, apical 1/2 with numerous inversed spines. Genital styles (Fig. 35) moderately long, tapering apically. Anal segment (Fig. 31) short, ring-like, ventral margin without process.

**Female genitalia.** Female pygofer (Fig. 37) with gonocoxa VIII moderately large. Ovipositor (Fig. 37) overpassing apical margin of pygofer distinctly. Gonangulum with apical margin blunt, connected gonocoxa VIII. Gonapophyses IX (Fig. 39) long and large, curved and directed basad, apex sharp, dorsal margin with apical 1/2 serrated.

**Host plant.** Bamboo.

**Distribution.** South China (Hainan) (Fig. 40).

**Remarks.** This species is similar to *B. kunmingensis* Yang & Chen, 2011, but can be distinguished by the basal area of forewing with two dark brown markings (forewing with basal 1/3 full dark brown in *kunmingensis*); the medioventral process of pygofer large (without medioventral process in *kunmingensis*); the aedeagus without phallobase (phallobase arising from base of aedeagus, as long as aedeagus in *kunmingensis*).

This new species is also similar to *B. basifusca* Hou & Chen, 2010, but can be distinguished by the ventral margin of anal segment without process (ventral margin





**Figures 42–43.** Host plant of *Bambusiphaga ventroprocessa* sp. n. **42** View of the area where the specimens of *B. ventroprocessa* sp. n. were captured, in Lingshui (Hainan, China). **43** View of the plant. Photograph by H.-X. Li.

of anal segment with a long process in *basifusca*); the ventral margin of pygofer with a medioventral process (ventral margin of pygofer with three medioventral processes in *basifusca*); and the aedeagus without phallobase (aedeagus with phallobase in *basifusca*).

Based on the characters of male genitalia, this species should belong to the *kunmingensis* group.

## Acknowledgments

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## References

- Asche M (1983) *Bambusiphaga lynchi* nov. spec., a new delphacid from northeastern Himalaya, and some contributions to the genus *Bambusiphaga* Huang and Ding, 1979 (Homoptera Auchenorrhyncha Fulgoromorpha Delphacidae). Marburger Entomologische Publicationen 1(8): 197–210.
- Bourgoin T (1993) Female genitalia in Hemiptera Fulgoromorpha, morphological and phylogenetic data. Annales de la Société Entomologique France 29(3): 225–244.
- Chen XS, Li ZZ (2000) Descriptions of two new species of Delphacidae attacking bamboo from Guizhou Province, China (Homoptera: Delphacidae). Acta Zootaxonomica Sinica 25(2): 178–182.
- Chen XS, Li ZZ, Jiang SN (2000) Descriptions of two new species of Delphacidae attacking bamboo from China (Homoptera: Fulgoroidea). Scientia Silvae Sinicae 36(3): 77–80.

- Chen XS, Liang AP (2007) Revision of the Oriental genus *Bambusiphaga* Huang & Ding (Hemiptera: Fulgoroidea: Delphacidae). Zoological Studies 46(4): 503–519.
- Ding JH (1982) Two new species of the tribe Tropidocephalini (Homoptera: Delphacidae). Journal Nanjing Agricultural College 4: 42–45.
- Ding JH, Hu GW (1982) A new species of the genus *Bambusiphaga* from Yunan (Homoptera: Delphacidae). Acta Entomologica Sinica 25(4): 443–444.
- Ding JH, Yang LF, Hu CL (1986) Descriptions of new genera and species of Delphacidae attacking bamboo from Yunnan Province, China. Acta Entomologica Sinica 29(4): 415–425.
- Hou XH, Chen XS (2010) Oriental bamboo planthoppers: two new species of the genus *Bambusiphaga* (Hemiptera: Fulgoroidea: Delphacidae) from Hainan Island, China. Florida Entomologist 93(3): 391–397. <https://doi.org/10.1653/024.093.0311>
- Huang CL, Tian LX, Ding JH (1979) A new genus and some new species of Delphacidae attacking bamboos in China. Acta Zootaxonomica Sinica 4(2): 170–181.
- Kuoh CL, Huang CL, Tian LX, Ding JH (1980) New species and new genus of Delphacidae from China. Acta Entomologica Sinica 23(4): 413–426.
- Liu MH, Chen XS (2008) Occurrence and harm of planthoppers in bamboo grove in Guiyang. Guizhou Agricultural Science 36(1): 87–89.
- Qin DZ, Liu TT, Lin YF (2012) A new species in the *Bambusiphaga fascia* group (Hemiptera: Fulgoroidea: Delphacidae) from Shaanxi, China, with a key to all species in the group. Acta Zootaxonomica Sinica 37(4): 777–780.
- Qin DZ, Yuan F (1999) One new species of the genus *Bambusiphaga* (Homoptera: Delphacidae) from China. Entomotaxonomia 21(1): 33–35.
- Qin DZ, Zhang YL, Ding JH (2006) A taxonomic study of the genus *Bambusiphaga* (Hemiptera, Fulgoroidea, Delphacidae). Acta Zootaxonomica Sinica 31(1): 148–151.
- Yang L, Chen XS, Chen HM (1999) Notes on planthoppers infesting bamboo in Guizhou. Journal of Mountain Agriculture and Biology 18(3): 154–161.
- Yang L, Chen XS (2011) The Oriental bamboo-feeding genus *Bambusiphaga* Huang & Ding, 1979 (Hemiptera: Delphacidae: Tropidocephalini): a checklist, a key to the species and descriptions of two new species. Zootaxa 2879: 50–59.
- Yang JT, Yang CT (1986) Delphacidae of Taiwan (I) Asiracinae and the tribe Tropidocephalini (Homoptera: Fulgoroidea). Taiwan Museum Special Publication 6: 1–79.
- Zheng WB, Chen XS (2013a) Taxonomic study on nymphs of genus *Bambusiphaga* (I). Guizhou Agricultural Sciences 41(1): 87–89.
- Zheng WB, Chen XS (2013b) Taxonomic study on nymphs of genus *Bambusiphaga* (II). Guizhou Agricultural Sciences 41(3): 50–53.

# Amphibians and reptiles of C. E. Miller Ranch and the Sierra Vieja, Chihuahuan Desert, Texas, USA

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## Abstract

We report the occurrence of 50 species of amphibians and reptiles recently collected on C. E. Miller Ranch and the Sierra Vieja in the Chihuahuan Desert of Texas, USA and describe their perceived distribution and abundance across various habitat associations of the region. Our recent surveys follow intense, historic sampling of amphibians and reptiles from this region in 1948. Of the 50 species detected in recent surveys, six were not collected in 1948 and an additional three species documented in 1948 have yet to be detected in a 14-year period of recent surveys. Combining data from both historic and recent surveys, a total of 53 species of amphibians and reptiles are known from the ranch (11 amphibians, 42 reptiles). Land stewardship and conservation practices have likely contributed to the persistence of the majority of these species through time. Additionally, we discuss the status of amphibians and reptiles not collected during recent surveys and comment on potential species that have not yet been detected.

## Keywords

Amphibia, Checklist, Herpetological diversity, Land stewardship, Long-term survey, Reptilia, Species composition, Voucher collections

## Introduction

The Chihuahuan Desert is a large arid region in North America that is home to high levels of biodiversity and is considered among the world's most valuable ecoregions (Olson and Dinerstein 1998). This ecoregion occupies most of north-central Mexico where it is positioned on the Mexican Plateau and bounded on the east by the Sierra Madre Oriental and on the west by the Sierra Madre Occidental (Schmidt 1979). In the United States, the Chihuahuan Desert extends into southeastern Arizona, southern New Mexico, and most of the Trans-Pecos region of Texas (Ricketts et al. 1999).

Situated in the Chihuahuan Desert, the Sierra Vieja (also referred to as the Tierra Vieja, Vieja Mountains: Gannett 1904; Sierra Vieja Range: Jameson and Flury 1949, Philips and Thornton 1949; Sierra Tierra Viejas: York 1949) is a low-elevation mountain range (1500–1900 m) located north and east of the Rio Grande in western Jeff Davis and Presidio counties, Texas, USA that is approximately 65 km in length and primarily composed of igneous rock (York 1949). The Sierra Vieja serve as an important barrier between the Rio Grande Plain to the west and the Valentine Plain to the east, with its steep, incised canyons providing protection and habitat in an otherwise open terrain. Outside of Blair (1940), Schmidt and Smith (1944), and Schmidt and Owens (1944), little was known about the vertebrates of this region until a large expedition led by W. F. Blair from the University of Texas at Austin travelled to the Sierra Vieja to conduct biological surveys of plants and vertebrates from June–July 1948. This expedition collected a large number of specimens, all of which were deposited into the Texas Natural History Collections (now Biodiversity Collections; vertebrates) and the TEX Herbarium (now Billie L. Turner Plant Resources Center; plants) at The University of Texas at Austin and served as the foundation for four papers describing the flora and fauna of the area (mammals: Blair and Miller 1949; amphibians and reptiles: Jameson and Flury 1949; birds: Phillips and Thornton 1949; plants: York 1949). Over 1700 amphibian and reptile specimens were collected and vouchered during the 38-day survey, primarily by A. L. Carroll, T. M. Burke, D. L. Jameson, A. G. Flury, and W. F. Blair, with the assistance of 18 other undergraduate and graduate students who were part of the collecting party, providing a baseline sampling of species that occurred in this area in 1948. Additionally, at that time, many of these specimens were among the first to be collected for several species (i.e., *Bogertophis subocularis*, *Lampropeltis alterna*, *Trimorphodon wilkinsonii*; Jameson and Flury 1949).

Here we report the results from herpetological surveys conducted from 2004–2017 at the same locality that was sampled in 1948, C. E. Miller Ranch. We compare differences in species composition from 1948 to recent surveys, provide potential explanations for these patterns, and discuss future species that may yet be encountered. Additionally, we highlight the importance of land stewardship in maintaining amphibian and reptile diversity in the Chihuahuan Desert ecoregion.



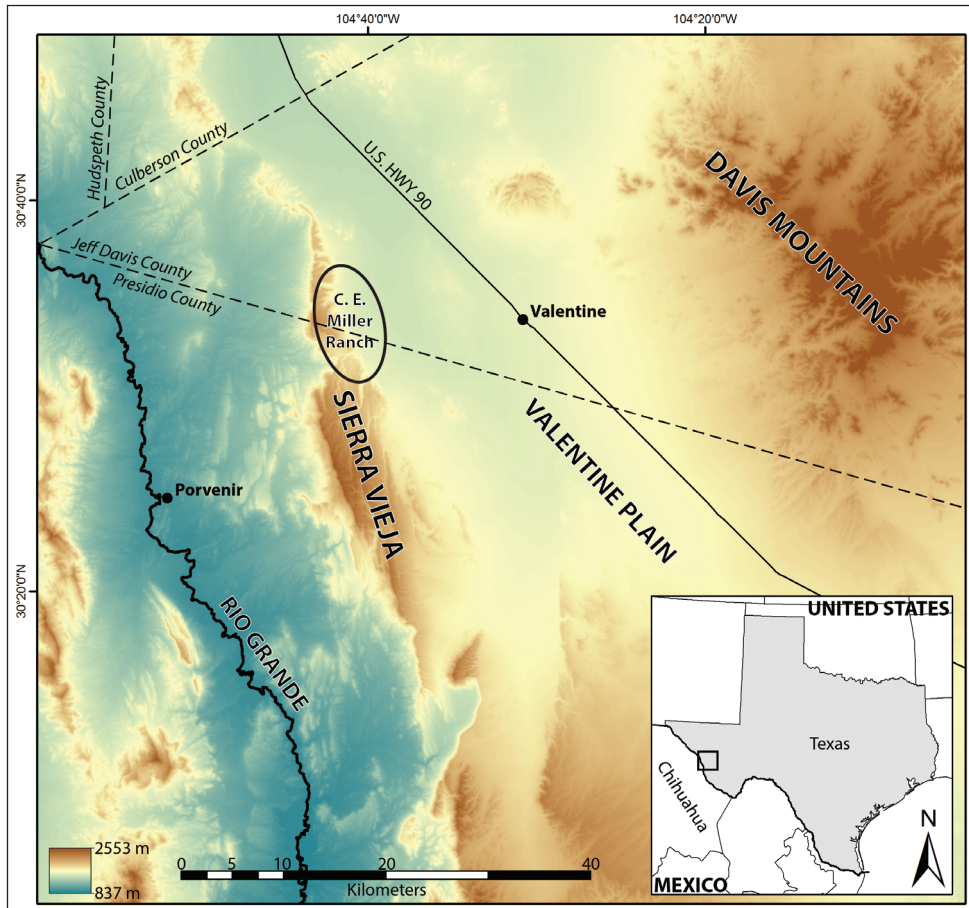
## **Methods**

### **Study site**

C. E. Miller Ranch is located in western Jeff Davis and Presidio counties in west Texas and occurs in the Chihuahuan Desert ecoregion (Figure 1). This ranch is approximately 13,400 ha and has been a working cattle ranch for over 90 years. Additionally, the landowners have been awarded conservation and land stewardship awards for their efforts in maintaining wildlife habitat, promoting conservation, and partnering with various agencies and organizations (Klepper 2003). Much of the geology, climate, and vegetation of this region has previously been described by Baker (1927), Hinckley (1947), and York (1949). Large canyons in the Sierra Vieja empty out onto the Valentine Plain with wide alluvial fans occurring, suggesting a history of flash flooding and rock and sediment transport. Primary aquatic habitats on the ranch include a series of springs and pools that occur in canyons in the Sierra Vieja, temporary to semi-permanent pools in Wild Horse Draw on the Valentine Plain, and a series of semi-permanent and permanent dugout ponds that are water sources for livestock and wildlife (Fig. 2). Ojos Viejitas or Canyon Springs (Brune 1981; Fig. 2G), located near the eastern end of ZH Canyon, is one the most significant and isolated water sources available across the length of the Sierra Vieja, forming a series of permanent pools of varying depth for ca. 250 m. Annual rainfall for C. E. Miller Ranch averages between 35–38 cm/year (C. Miller, unpublished data); average temperatures for Valentine (ca. 20 km to the east) range from 32–34 °C in the summer and 15–18 °C in the winter (www.usclimatedata.com).

### **Habitat associations**

The Sierra Vieja biotic district is located in the Chihuahuan biotic province and is subdivided into two life belts: 1) the Roughland belt which comprises the Sierra Vieja and 2) the Plains belt which comprises the Valentine Plain lowlands that occur between the Sierra Vieja and the Davis Mountains to the east (Dice 1943; York 1949). York (1949) further described and quantified vegetation associations that occur within these life belts in the Sierra Vieja, including seven vegetation associations from the Plains belt (catclaw-cedar, catclaw-tobosa, tobosa-grama, creosote bush-catclaw-blackbrush, mesquite-huisache-blackbrush, yucca-blackbrush-grama, and blackbrush-creosote bush; Fig. 2) and six vegetation associations from the Roughland belt (stream bed, catclaw-grama, grama-bluestem, rock bluff, huisache-lechuguilla, and lechuguilla-beargrass; Fig. 2). The most abundant vegetation associations within the Plains belt at the ranch were the tobosa-grama and catclaw-tobosa associations and the most abundant in the Roughland belt was the lechuguilla-beargrass association (York 1949).



**Figure 1.** Map of C. E. Miller Ranch and the Sierra Vieja in the Chihuahuan Desert of Texas, USA. Major geographic features of the area, county boundaries, roads, and towns are included. Oval boundary around C. E. Miller Ranch is approximate.

## Data collection

An amphibian and reptile survey was conducted from 3 June–9 July 1948 as part of a large expedition to further understand the biodiversity of the Chihuahuan Desert (Jameson and Flury 1949). Our recent amphibian and reptile surveys were conducted from 2004–2017, with one to six sampling trips per year during the months of May–October. Surveys for amphibians and reptiles were conducted opportunistically at both day and night by hiking through canyons and along trails, searching under rocks, debris, and cover objects, trapping and seining aquatic habitats, and driving roads looking for road-killed, thermoregulating, or actively moving amphibians and reptiles. Recent collections were primarily made by the authors, though additional individuals occasionally participated in surveys as well. Additionally, in



**Figure 2.** Photos of representative habitats present at C. E. Miller Ranch and the Sierra Vieja. **A** Valentine Plain, tobosa-grama association **B** Valentine Plain, mesquite-huisache-blackbrush association **C** Valentine Plain, creosote bush-catclaw-blackbrush association **D** Sierra Vieja, stream bed association **E** Sierra Vieja, catclaw-grama association **F** Sierra Vieja, grama-bluestem association **G** Sierra Vieja, Ojos Viejitas or Canyon Springs, and **H** Valentine Plain, 96 Tank. Photos by DRD.

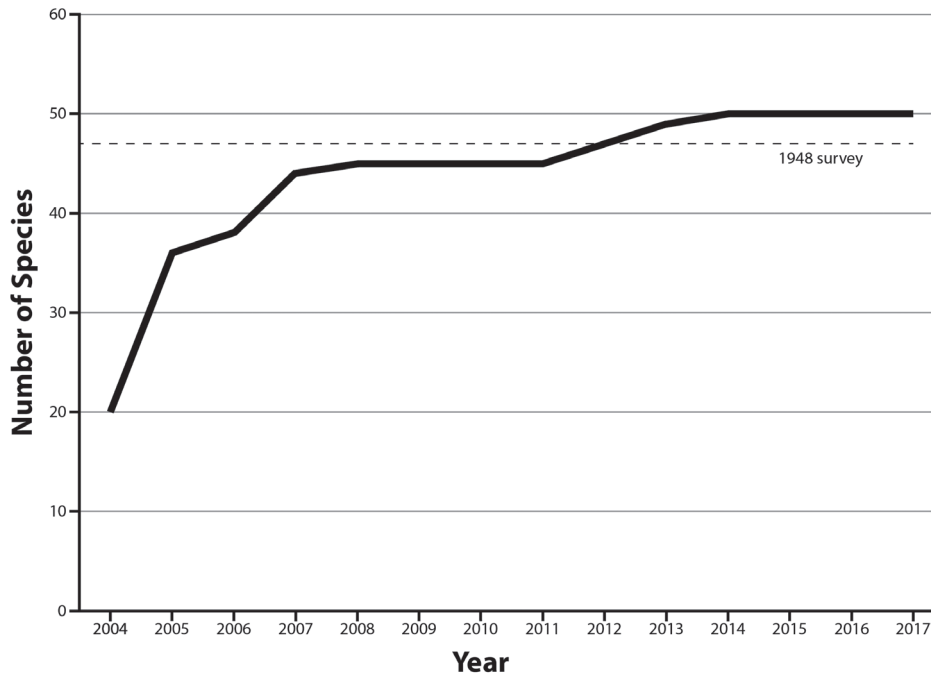


May 2007, the Texas Herpetological Society spring field meet was held at C. E. Miller Ranch, in which over 25 amateurs and professionals participated in survey efforts over three days.

Voucher specimens of all species that were encountered were collected and deposited at the Biodiversity Collections (formerly the Texas Natural History Collections) at The University of Texas at Austin or at the Biodiversity Research and Teaching Collections (formerly the Texas Cooperative Wildlife Collections) at Texas A&M University. Specimens from the 1948 survey were all deposited at the Biodiversity Collections. Specimens from recent surveys were collected under a Texas Parks and Wildlife Department Scientific Collecting Permit issued to TJL (#SPR-1097-912); collections were performed under University of Texas IACUC protocol AUP-2015-00106 (and earlier versions of this protocol). Individuals were collected, then euthanized with aqueous chlorotone or benzocaine (amphibians) or via injection of sodium pentobarbital (reptiles). Tissue samples (muscle or liver) were collected from specimens and individuals were then fixed in 10% buffered formalin for a minimum of 48 h, then transferred to 70% ethanol for long-term storage. Species identifications primarily follow those outlined by Crother (2017) and Devitt et al. (2008). Additionally, we recognize the genera *Bufo* (Pauly et al. 2009), *Syrrophus* (Lynch 1970; T. Devitt, pers. comm.), *Rana* (Yuan et al. 2016), and *Masticophis* (Myers et al. 2017), as well as the species *Salvadora deserticola* (Bogert 1945, 1985).

## Results

A total of 315 specimens comprising 10 species of amphibians and 40 species of reptiles were collected during surveys from 2004–2017 (Fig. 3; Tables 1, 2; Appendix 1). This total was in contrast to the nine species of amphibians and 38 species of reptiles encountered during the survey conducted almost 70 years previous (Jameson and Flury 1949). Of the 50 total species encountered during recent surveys, there were 10 species of anurans, two species of turtles, 14 species of lizards, and 24 species of snakes (Figs 4–7). From 2004–2008, 45 of the 50 species (90%) had been documented on the ranch (Fig. 3). New species were not documented until 2012–2014, when the final five species were collected, despite no noticeable shifts or change in survey effort (Fig. 3). Additionally, six species were collected in 2007 (Fig. 3), four of which correspond to collecting efforts from individuals participating in the Texas Herpetological Society spring field meet. Three species that were collected in 1948 were not encountered during recent surveys: Western Tiger Salamander (*Ambystoma mavortium*), Gray-banded Kingsnake (*Lampropeltis alterna*), and Eastern Patch-nosed Snake (*Salvadora grahamiae*; Tables 1, 2). Six species (two anurans and four snakes) were collected from the study site in recent surveys that were not collected in 1948: Great Plains Toad (*Bufo cognatus*), Texas Toad (*Bufo speciosus*), Chihuahuan Hook-nosed Snake (*Gyalopion canum*), Desert Kingsnake (*Lampropeltis splendida*), Plains Black-headed Snake (*Tantilla nigriceps*), and New Mexico Threadsnake (*Rena dissecta*; Tables 1, 2). In total, 11 species



**Figure 3.** Species accumulation curve for species encountered during recent survey years at C. E. Miller Ranch from 2004–2017. Values indicate total number of species known at the end of each year. The dashed line represents the total number of species detected during the 1948 survey of the site.

**Table 1.** Amphibian species from C. E. Miller Ranch and the Sierra Vieja. Species presence during the historic (1948) and recent (2004–2017) surveys are indicated. × = vouchered individuals present, \* = species heard, but not collected.

Order	Family	Species Name	Common Name	1948	2004–2017
Anura	Bufonidae	<i>Bufo cognatus</i>	Great Plains Toad		×
		<i>Bufo debilis</i>	Chihuahuan Green Toad	×	×
		<i>Bufo punctatus</i>	Red-spotted Toad	×	×
		<i>Bufo speciosus</i>	Texas Toad		×
	Eleutherodactylidae	<i>Syrhophus marnockii</i>	Cliff Chirping Frog	*	×
	Hylidae	<i>Hyla arenicolor</i>	Canyon Treefrog	×	×
	Microhylidae	<i>Gastrophryne olivacea</i>	Western Narrow-mouthed Toad	×	×
	Ranidae	<i>Rana berlandieri</i>	Rio Grande Leopard Frog	×	×
	Scaphiopodidae	<i>Scaphiopus couchii</i>	Couch’s Spadefoot	×	×
		<i>Spea multiplicata</i>	Mexican Spadefoot	×	×
Caudata	Ambystomatidae	<i>Ambystoma mavortium</i>	Western Tiger Salamander	×	

of amphibians (10 frogs, 1 salamander) and 42 species of reptiles (2 turtles, 14 lizards, 26 snakes) have been documented from C. E. Miller Ranch. Species accounts discussing the status and distribution of these species on the ranch are included below.

**Table 2.** Reptile species from C. E. Miller Ranch and the Sierra Vieja. Species presence during the historic (1948) and recent (2004–2017) surveys are indicated. × = vouchered individuals present, # = vouchered individual collected in 1947, but not seen in 1948.

Order	Family	Species Name	Common Name	1948	2004–2017
Testudines	Emydidae	<i>Terrapene ornata</i>	Ornate Box Turtle	×	×
	Kinosternidae	<i>Kinosternon flavescens</i>	Yellow Mud Turtle	×	×
Squamata	Crotaphytidae	<i>Crotaphytus collaris</i>	Eastern Collared Lizard	×	×
	Gekkonidae	<i>Coleonyx brevis</i>	Texas Banded Gecko	×	×
	Phrynosomatidae	<i>Cophosaurus texanus</i>	Greater Earless Lizard	×	×
		<i>Holbrookia maculata</i>	Common Lesser Earless Lizard	×	×
		<i>Phrynosoma cornutum</i>	Texas Horned Lizard	×	×
		<i>Phrynosoma modestum</i>	Round-tailed Horned Lizard	×	×
		<i>Sceloporus cowlesi</i>	Southwestern Fence Lizard	×	×
		<i>Sceloporus poinsettii</i>	Crevice Spiny Lizard	×	×
		<i>Urosaurus ornatus</i>	Ornate Tree Lizard	×	×
	Scincidae	<i>Plestiodon obsoletus</i>	Great Plains Skink	×	×
		<i>Plestiodon tetragrammus</i>	Four-lined Skink	×	×
	Teiidae	<i>Aspidoscelis exsanguis</i>	Chihuahuan Spotted Whiptail	×	×
		<i>Aspidoscelis inornata</i>	Little Striped Whiptail	×	×
		<i>Aspidoscelis tessellata</i>	Common Checkered Whiptail	×	×
	Colubridae	<i>Bogertophis subocularis</i>	Trans-Pecos Ratsnake	×	×
		<i>Diadophis punctatus</i>	Ring-necked Snake	×	×
		<i>Gyalopion canum</i>	Chihuahuan Hook-nosed Snake		×
		<i>Heterodon kennerlyi</i>	Mexican Hog-nosed Snake	×	×
		<i>Hypsiglena jani</i>	Chihuahuan Nightsnake	×	×
		<i>Lampropeltis alterna</i>	Gray-banded Kingsnake	×	
		<i>Lampropeltis splendida</i>	Desert Kingsnake		×
		<i>Masticophis flagellum</i>	Coachwhip	×	×
		<i>Masticophis taeniatus</i>	Striped Whipsnake	×	×
		<i>Pituophis catenifer</i>	Gophersnake	×	×
		<i>Rhinocheilus lecontei</i>	Long-nosed Snake	×	×
		<i>Salvadora deserticola</i>	Big Bend Patch-nosed Snake	×	×
		<i>Salvadora grahamiae</i>	Eastern Patch-nosed Snake	×	
		<i>Sonora semiannulata</i>	Western Groundsnake	×	×
		<i>Tantilla hobartsmithi</i>	Smith's Black-headed Snake	×	×
		<i>Tantilla nigriceps</i>	Plains Black-headed Snake		×
		<i>Thamnophis cyrtopsis</i>	Black-necked Gartersnake	×	×
		<i>Thamnophis marcianus</i>	Checkered Gartersnake	×	×
		<i>Trimorphodon wilkinsonii</i>	Chihuahuan Lyresnake	×	×
	Leptotyphlopidae	<i>Rena dissecta</i>	New Mexico Threadsnake		×
		<i>Rena humilis</i>	Western Threadsnake	×	×
	Viperidae	<i>Crotalus atrox</i>	Western Diamond-backed Rattlesnake	×	×
		<i>Crotalus lepidus</i>	Rock Rattlesnake	×	×
		<i>Crotalus ornatus</i>	Eastern Black-tailed Rattlesnake	×	×
		<i>Crotalus scutulatus</i>	Mohave Rattlesnake	#	×
		<i>Crotalus viridis</i>	Prairie Rattlesnake	#	×

## Species accounts

### Class Amphibia, Order Anura

### Family Bufonidae

#### *Bufo cognatus* Say, 1823

#### Great Plains Toad

Five individuals were collected during recent surveys, primarily from the tobosa-grama and catclaw-tobosa associations on the Valentine Plain. Previously, *B. cognatus* was not known from the ranch. The failure to detect this species on the ranch in 1948 despite seemingly favorable environmental conditions (i.e., heavy rains the preceding week) may suggest that this species has increased its range or abundance in the area. *Bufo cognatus* appears to be less common in the area than *B. speciosus*, though both species occupy similar habitats in the tobosa-grama association.

#### *Bufo debilis* Girard, 1854

#### Chihuahuan Green Toad (Fig. 4A)

Fourteen individuals were collected during recent surveys, most from the tobosa-grama and catclaw-tobosa associations on the Valentine Plain. This species appears to be common in the area and is frequently observed and heard calling in small ephemeral pools in the tobosa-grama association or along Wild Horse Draw after rains. *Bufo debilis* was frequently encountered and appeared similarly abundant during the 1948 survey (Jameson and Flury 1949).

#### *Bufo punctatus* Baird & Girard, 1852

#### Red-spotted Toad (Fig. 4B)

Fifteen individuals were collected during recent surveys, all from rocky canyons in the Sierra Vieja and from where these canyons empty out onto the Valentine Plain. This species appears to be common in these rocky habitats and was primarily found in the stream bed and catclaw-cedar associations, similar to that in the 1948 survey (Jameson and Flury 1949). *Bufo punctatus* was previously reported from dugout cattle tanks in the tobosa-grama association (Jameson and Flury 1949); however, we only detected individuals in the Valentine Plain near the mouths of Sierra Vieja canyons.

#### *Bufo speciosus* Girard, 1854

#### Texas Toad (Fig. 4C)

Ten specimens were collected from the Valentine Plain during recent surveys and represent one of the two amphibians previously unknown from the ranch. The failure to





**Figure 4.** Photos in life of species of anurans collected from C. E. Miller Ranch. **A** Chihuahuan Green Toad (*Bufo debilis*) **B** Red-spotted Toad (*Bufo punctatus*) **C** Texas Toad (*Bufo speciosus*) **D** Cliff Chirping Frog (*Syrphophus marnockii*) **E** Canyon Treefrog (*Hyla arenicolor*) **F** Western Narrow-mouthed Toad (*Gastrophryne olivacea*) **G** Couch's Spadefoot (*Scaphiopus couchii*), and **H** Mexican Spadefoot (*Spea multiplicata*). Photos by DRD.

detect this species on the ranch in 1948 despite seemingly favorable environmental conditions (i.e., heavy rains the preceding week; Jameson and Flury 1949) may suggest that this species has increased its range or abundance in the area. *Bufo speciosus* appears to be more common than *B. cognatus* in the area, though both species occupy similar habitats in the tobosa-grama association.

### **Family Eleutherodactylidae**

*Syrrhophus marnockii* Cope, 1878

Cliff Chirping Frog (Fig. 4D)

A single individual was collected in the Sierra Vieja during recent surveys (Owen et al. 2014). This species was frequently heard calling during the 1948 survey on rainy nights, but no individuals were collected. During recent surveys, this species was frequently heard calling in canyons in the Sierra Vieja, and individuals were observed only twice, once in the stream bed association and once in the rock bluff association.

### **Family Hylidae**

*Hyla arenicolor* Cope, 1866

Canyon Treefrog (Fig. 4E)

Seven individuals were collected during recent surveys, all from the Sierra Vieja, except one individual that was collected at Glidewell Pond in the Valentine Plain. These results are similar to those reported in Jameson and Flury (1949), with this species being fairly common along springs and pools in the stream bed association that line the canyons in the Sierra Vieja. The individual from Glidewell Pond likely moved down from the Sierra Vieja and through the catclaw-cedar association that occurs along the mouths of canyons and through the catclaw-tobosa association to Glidewell Pond. This species is also periodically found around the irrigated landscape of the ranch headquarters, ca. 1.5 km east of the Sierra Vieja.

### **Family Microhylidae**

*Gastrophryne olivacea* Hallowell, 1857

Western Narrow-mouthed Toad (Fig. 4F)

Seven specimens were collected during recent surveys. Most of these specimens came from dugout ponds in the Valentine Plain near the eastern slopes of the Sierra Vieja, but one individual was collected in the stream bed association in Box Canyon, which

suggests that individuals may move into the canyons from the Valentine Plain on occasion. Unlike our detection of *G. olivacea* primarily from the Valentine Plains, Jameson and Flury (1949) indicated *G. olivacea* were collected in roughly equal numbers from both the Valentine Plain and Sierra Vieja.

### Family Ranidae

#### *Rana berlandieri* Baird, 1859

Rio Grande Leopard Frog

Six specimens were collected during recent surveys: five from the Sierra Vieja and one from the Valentine Plain. Within the Sierra Vieja, this species can be found in the stream bed association along springs, spring runs, and pools. Jameson and Flury (1949) reported *R. berlandieri* in equal abundance in both the Sierra Vieja and the Valentine Plain; however, despite extensive surveys around suitable habitat in the Valentine Plain (e.g., dugout ponds that occur along the eastern edge of the Sierra Vieja), only a single individual has been observed in a dugout pond along Wild Horse Draw.

### Family Scaphiopodidae

#### *Scaphiopus couchii* Baird, 1854

Couch's Spadefoot (Fig. 4G)

Nine specimens were collected during recent surveys, with seven collected in the Valentine Plain and two collected from the Sierra Vieja. Despite individuals being collected in both the Plains and Roughland life belts, this species is infrequently encountered in the Sierra Vieja. Jameson and Flury (1949) reported individuals only from the Valentine Plain. Individuals occasionally may disperse into Sierra Vieja canyons, though breeding activity appears to exclusively take place in shallow, ephemeral pools that fill after heavy rains in the tobosa-grama and catclaw-tobosa associations of the Valentine Plain.

#### *Spea multiplicata* (Cope, 1863)

Mexican Spadefoot (Fig. 4H)

Sixteen specimens were collected during recent surveys, all from the Valentine Plain. Jameson and Flury (1949) reported individuals collected from the Valentine Plain, but also collected individuals from the stream bed and catclaw-tobosa associations of the

Sierra Vieja. Those individuals collected in the Sierra Vieja likely represent individuals dispersing into the canyons from the Valentine Plain. This species appears common, especially after mid- to late-summer rains and is frequently found in shallow, ephemeral pools in the tobosa-grama vegetation association in the Valentine Plain.

**Class Amphibia, Order Caudata**  
**Family Ambystomatidae**

*Ambystoma mavortium* Baird, 1850  
Western Tiger Salamander

This species was not detected during recent surveys despite extensive survey effort in potential habitats (e.g., dugout ponds in the Valentine Plain) and is presumed locally extirpated. In 1948, a large lot (172 individuals) of larval specimens was collected from a single dugout pond in the tobosa-grama association on the eastern side of the Sierra Vieja.

**Class Reptilia, Order Testudines**  
**Family Emydidae**

*Terrapene ornata* (Agassiz, 1857)  
Ornate Box Turtle (Fig. 5A)

Three specimens were collected during recent surveys, all from the Valentine Plain. This species likely occurs throughout the various associations in the Valentine Plain as suggested in Jameson and Flury (1949), but has been most frequently observed in the tobosa-grama and catclaw-tobosa associations during recent surveys.

**Family Kinosternidae**

*Kinosternon flavescens* (Agassiz, 1857)  
Yellow Mud Turtle (Fig. 5B)

Twenty-five specimens were collected in recent surveys as part of a separate project studying the natural history of this species in the region. All specimens were taken from either natural or artificial ponds in the Valentine Plain. These turtles seem to be abundant at these sites and are occasionally found in temporary, ephemeral pools after heavy rains. Many of these sites occur in the tobosa-grama, catclaw-tobosa, and catclaw-cedar associations, similar to localities reported in Jameson and Flury (1949).





**Figure 5.** Photos in life of species of turtles and lizards collected from C. E. Miller Ranch. **A** Ornate Box Turtle (*Terrapene ornata*) **B** Yellow Mud Turtle (*Kinosternon flavescens*) **C** Eastern Collard Lizard (*Crotaphytus collaris*) **D** Texas Banded Gecko (*Coleonyx brevis*) **E** Common Lesser Earless Lizard (*Holbrookia maculata*) **F** Texas Horned Lizard (*Phrynosoma cornutum*) **G** Round-tailed Horned Lizard (*Phrynosoma modestum*), and **H** Southwestern Fence Lizard (*Sceloporus cowlesi*). Photos by DRD.

**Class Reptilia, Order Squamata**

**Family Crotophytidae**

***Crotaphytus collaris*** (Say, 1823)

Eastern Collared Lizard (Fig. 5C)

Three specimens were collected in recent surveys: two from the Sierra Vieja and one from the Valentine Plain. Within the Sierra Vieja, *C. collaris* has been observed in the stream bed and catclaw-grama associations and in the Valentine Plain it has been observed in the rocky areas near the eastern slopes of the Sierra Vieja. Jameson and Flury (1949) reported *C. collaris* from rocky areas in similar habitat associations in roughly equal proportions in both the Sierra Vieja and Valentine Plain.

**Family Gekkonidae**

***Coleonyx brevis*** Stejneger, 1893

Texas Banded Gecko (Fig. 5D)

Seven specimens were collected in recent surveys: six from the Sierra Vieja and one from the Valentine Plain near the mouth of ZH Canyon. All of these specimens from the Sierra Vieja were collected in the stream bed association, and the single individual from the Valentine Plain was taken in the catclaw-cedar association where large rocks and boulders are present. Jameson and Flury (1949) reported this species in rocky areas from both the Sierra Vieja and the Valentine Plain, in roughly equal proportions.

**Family Phrynosomatidae**

***Cophosaurus texanus*** Troschel, 1852

Greater Earless Lizard

Fifteen specimens were collected during recent surveys, all from areas along the eastern slopes of the Sierra Vieja and nearby areas in the Valentine Plain. This species appears common in rocky habitats where it is frequently encountered perching on top of large rocks. Within the Sierra Vieja, individuals have been encountered in the stream bed and catclaw-grama associations, and in the Valentine Plain, individuals were primarily encountered in the creosote bush-catclaw-blackbrush association. Jameson and Flury (1949) reported *C. texanus* from similar habitat associations, however, the majority of their specimens collected from the Valentine Plain were taken in the catclaw-cedar association.

***Holbrookia maculata*** Girard, 1851

Common Lesser Earless Lizard (Fig. 5E)

Six specimens were collected during recent surveys, all from the tobosa-grama association on the Valentine Plain. Individuals appeared abundant in these sandy habitats. Jameson and Flury (1949) reported individuals from additional habitat associations, though the majority of specimens they collected were taken from the catclaw-tobosa association.

***Phrynosoma cornutum*** (Harlan, 1825)

Texas Horned Lizard (Fig. 5F)

Six specimens were collected during recent surveys, all from the Valentine Plain. *Phrynosoma cornutum* appears widespread throughout most habitats in the Valentine Plain, especially in the tobosa-grama and catclaw-tobosa associations, similar to reports in Jameson and Flury (1949). Despite declines in the abundance of this species throughout its range (Price 1990), it still remains common throughout this study site.

***Phrynosoma modestum*** Girard, 1852

Round-tailed Horned Lizard (Fig. 5G)

Four specimens were collected during recent surveys from both the Sierra Vieja and Valentine Plain. Individuals appear to be abundant in the rocky habitats along the eastern slopes of the Sierra Vieja where they have been detected in the stream bed and catclaw-grama associations. Additionally, this species was found in the rockier portions of the catclaw-tobosa and creosote bush-catclaw-blackbrush associations in the Valentine Plain. Jameson and Flury (1949) reported *P. modestum* from additional habitats in the Valentine Plain, though they only cite a single specimen from the Sierra Vieja.

***Sceloporus cowlesi*** Lowe & Norris, 1956

Southwestern Fence Lizard (Fig. 5H)

Seven specimens were collected during recent surveys, all from the Valentine Plain. Individuals were primarily encountered in the catclaw-tobosa and tobosa-grama associations where they were commonly observed on vertical structure (e.g., fence posts, yuccas, catclaw), similar to habitat associations reported in Jameson and Flury (1949). Although Jameson and Flury (1949) reported a single individual from the Sierra Vieja, we observed no individuals there during our recent surveys.



***Sceloporus poinsettii*** Baird & Girard, 1852

Crevise Spiny Lizard

Only a single photo voucher of this species exists from recent surveys from an abandoned stone structure in Fort Holland in the Sierra Vieja. During historic surveys this species was frequently encountered in rock crevices throughout stream bed, catclaw-grama, and rock bluff associations in the Sierra Vieja (Jameson and Flury 1949). Despite extensive survey efforts in the canyons of the Sierra Vieja, no other individuals have been collected.

***Urosaurus ornatus*** (Baird & Girard, 1852)

Ornate Tree Lizard (Fig. 6A)

Nine specimens were collected during recent surveys, all from the catclaw-grama association in the Sierra Vieja. This species was frequently encountered at Fort Holland where individuals would be observed on the walls and rafters of buildings. Jameson and Flury (1949) reported *U. ornatus* from additional habitat associations in the Sierra Vieja, but also in rocky areas in the catclaw-cedar association along the eastern edge of the Sierra Vieja in the Valentine Plain.

**Family Scincidae**

***Plestiodon obsoletus*** Baird & Girard, 1852

Great Plains Skink (Fig. 6B)

Six specimens were collected in recent surveys from both the Sierra Vieja and Valentine Plain. Individuals primarily occur in the catclaw-grama and stream bed associations in the Sierra Vieja and the catclaw-cedar and catclaw-tobosa associations in the Valentine Plain where large rocky areas exist, similar to locations reported in Jameson and Flury (1949).

***Plestiodon tetragrammus*** Baird, 1859

Four-lined Skink (Fig. 6C)

Four specimens were collected in recent surveys, all from the Sierra Vieja. This species is infrequently encountered and all occurrences of individuals have been reported from the stream bed and catclaw-grama associations, similar to locations listed in Jameson and Flury (1949).



**Figure 6.** Photos in life of species of lizards and snakes collected from C. E. Miller Ranch. **A** Ornate Tree Lizard (*Urosaurus ornatus*) **B** juvenile Great Plains Skink (*Plestiodon obsoletus*) **C** juvenile Four-lined Skink (*Plestiodon tetragrammus*) **D** Chihuahuan Spotted Whiptail (*Aspidoscelis exsanguis*) **E** Trans-Pecos Ratsnake (*Bogertophis subocularis*) **F** Ring-necked Snake (*Diadophis punctatus*) **G** Chihuahuan Nightsnake (*Hypsiglena jani*), and **H** Coachwhip (*Masticophis flagellum*). Photos by DRD.

## **Family Teiidae**

*Aspidoscelis exsanguis* (Lowe, 1956)  
Chihuahuan Spotted Whiptail (Fig. 6D)

Sixteen specimens were collected in recent surveys, from both the Valentine Plain and the Sierra Vieja, where this species appears to be more abundant. Jameson and Flury (1949) reported that this species was primarily in the Sierra Vieja and in rocky regions of the Valentine Plain along the eastern edge of the Sierra Vieja; however, we have collected this species in areas dominated by sandy soils ca. 6 km east of the Sierra Vieja in the Valentine Plain. Within the Sierra Vieja, this species is frequently observed in the catclaw-grama association.

*Aspidoscelis inornata* (Baird, 1859)  
Little Striped Whiptail

Fifteen specimens were collected in recent surveys, from both the Sierra Vieja and Valentine Plain. Jameson and Flury (1949) reported that this species appeared to be restricted to the Valentine Plain. While individuals do appear to be more abundant in the catclaw-tobosa association in the Valentine Plain, numerous individuals were taken in catclaw-grama association surrounding Fort Holland.

*Aspidoscelis tessellata* (Say, 1823)  
Common Checkered Whiptail

Eight specimens were collected in recent surveys, from both the Sierra Vieja and Valentine Plain. This species has been encountered in many different habitats, including tobosa-grama, catclaw-cedar, stream bed, and catclaw-grama associations. Jameson and Flury (1949) also collected *A. tessellata* from the Valentine Plain and Sierra Vieja, though encountered individuals in additional habitat associations within both life belts.

## **Family Colubridae**

*Bogertophis subocularis* (Brown, 1901)  
Trans-Pecos Ratsnake (Fig. 6E)

Two individuals were collected during recent surveys, one from the Sierra Vieja and one from the Valentine Plain. Within the Sierra Vieja, one individual was collected in

the stream bed association at the mouth of ZH Canyon. Within the Valentine Plain, one individual was collected from the vicinity of the ranch headquarters in the catclaw-tobosa association. Individuals have been found throughout the Sierra Vieja (stream bed, catclaw-grama, and rock bluff associations) and Valentine Plain (catclaw-tobosa and catclaw-cedar) adjacent to the Sierra Vieja. Six individuals were observed during a one-week span in June 2007, including three found while walking through ZH and Box canyons between 2100–0000 h. Two of those individuals were found climbing in vegetation (*Quercus* sp. and *Acacia greggi*; Pauly and LaDuc 2008); an additional animal was found 1 m above the ground in a catclaw on a subsequent survey. Jameson and Flury (1949) reported *B. subocularis* from similar habitats in both the Sierra Vieja and Valentine Plain.

***Diadophis punctatus*** (Linnaeus, 1766)

Ring-necked Snake (Fig. 6F)

Two individuals were collected from the ranch during recent surveys, with our first specimen being found nine years into our survey. One individual was found in the stream bed association in Box Canyon in the Sierra Vieja and the second individual was found at the ranch headquarters in the catclaw-tobosa association of the Valentine Plain. An additional specimen was collected crossing a dirt road in the yucca-tobosa association, just east of the ranch, which may suggest that *D. punctatus* has a broader range in the Valentine Plain. None of the specimens encountered possessed a nuchal ring. Jameson and Flury (1949) reported this species solely from the stream bed association in the Sierra Vieja.

***Gyalopion canum*** Cope, 1860

Chihuahuan Hook-nosed Snake

A single specimen was collected during recent surveys and represents a new species for the ranch that was not detected in 1948. The female snake was collected just after midnight in June 2012 in the catclaw-grama association between the rocky bluffs and stream bed of Fox Hollow in the Sierra Vieja. Because this species is represented by this single individual, we consider this species among the most cryptic species of snake at the ranch.

***Heterodon kennerlyi*** Kennicott, 1860

Mexican Hog-nosed Snake

Three individuals were collected during recent surveys from both the Valentine Plain and the Sierra Vieja. Several individuals have been observed in the catclaw-tobosa and



tobosa-grama associations in the Valentine Plain. One individual was collected in a peculiarly sandy portion of the catclaw-grama association in the Sierra Vieja (adjacent to Fort Holland), suggesting that this species may be tied to soils rather than vegetation associations on the ranch. Jameson and Flury (1949) reported *H. kennerlyi* only from the Valentine Plain, and hypothesized this species was restricted to the associations therein.

***Hypsiglena jani*** (Dugès, 1865)  
Chihuahuan Nightsnake (Fig. 6G)

Four specimens were collected during recent surveys from both the Valentine Plain and the Sierra Vieja. This species is likely distributed across the entire ranch and most of the vegetation associations. Individuals have been collected under debris in the catclaw-tobosa association, crawling in the open in the stream bed association, or across roads at night in the yucca-tobosa association. One individual collected at night just east of the property had recently ingested two *Aspidoscelis inornata*, representing a new maximum prey/predator mass ratio (Davis and LaDuc 2017). Jameson and Flury (1949) report that *H. jani* appeared to be restricted to rocky areas, though our observations of individuals have occurred in habitats devoid of rocks.

***Lampropeltis alterna*** (Brown, 1901)  
Gray-banded Kingsnake

This species has not been encountered during the recent surveys, despite exhaustive surveys over a decade at the same locality where the single female snake was collected in July 1948: the stream bed and catclaw-grama associations at the mouth of Fox Hollow in the Sierra Vieja. Further, this habitat is similar to the area around Fort Holland and ZH Canyon that has been intensively sampled, producing no specimens for the past 14 years.

***Lampropeltis splendida*** (Baird & Girard, 1853)  
Desert Kingsnake

This species was not encountered on the ranch in 1948, but multiple individuals have been seen in recent surveys in the tobosa-grama and catclaw-tobosa associations in the Valentine Plain, where *L. splendida* is likely restricted. A gravid female was collected in June 2005 under debris (tobosa-grama association) and laid five eggs, which all subsequently hatched. Two hatchlings were preserved as vouchers; the remaining hatchlings and the female were later released. Another individual was collected at 2 Section Tank in the tobosa-grama association and two additional individuals were observed closer to the Sierra Vieja in the rocky catclaw-tobosa association of the Valentine Plain.

***Masticophis flagellum*** (Shaw, 1802)

Coachwhip (Fig. 6H)

Five specimens were collected during recent surveys from the Sierra Vieja and Valentine Plain. Jameson and Flury (1949) commented that *M. flagellum* being more abundant in the Valentine Plain than the Sierra Vieja, though we observed this species in roughly equal proportions between these two regions. The three animals from the Sierra Vieja were collected from the catclaw-grama association surrounding Fort Holland, the stream bed of ZH Canyon, and the lechuguilla-beargrass association on the mesa above Fort Holland. From the Valentine Plain, one animal was collected at the mouth of ZH Canyon in the cedar-catclaw association and another from the catclaw-tobosa alluvial plain east of the ranch house. This species is likely widely distributed across the ranch and in most habitat associations.

***Masticophis taniatus*** (Hallowell, 1852)

Striped Whipsnake (Fig. 7A)

A single individual was collected in a funnel trap at Fort Holland in the catclaw-grama association of the Sierra Vieja. One individual was captured while it was drinking from the stone tank at the mouth of ZH Canyon (catclaw-cedar association), but not collected. Other individuals have been seen but not collected due to the speed of the snakes and the dense nature of both the vegetation and the collectors. This species is likely found across all of the rocky associations of the Sierra Vieja, as well as those associations in the Valentine Plain along the eastern border of the Sierra Vieja. Similarly, Jameson and Flury (1949) reported that *M. taniatus* was collected only from the Sierra Vieja in rocky habitat associations.

***Pituophis catenifer*** (Blainville, 1835)

Gophersnake

Despite only two specimens having been collected during recent surveys, this species has been seen (and not collected) from multiple areas across the property and is likely widely distributed among most habitat associations. The two specimens collected were each found adjacent to a human dwelling within the catclaw-tobosa association of the Valentine Plain; several additional snakes have been found near these dwellings, likely attracted because of the availability of small prey associated with farms and ranches (i.e., rodents, chickens). Individuals have also been caught, marked, and released from the catclaw-grama association in Box Canyon in the Sierra Vieja, crossing several roads through the catclaw-tobosa alluvial fans, and near human structures in the tobosa-grama association in the Valentine Plain. Jameson and Flury (1949) similarly remarked on the broad distribution of *P. catenifer* across the ranch in both the Valentine Plain and Sierra Vieja.



**Figure 7.** Photos in life of species of snakes collected from C. E. Miller Ranch. **A** Striped Whipsnake (*Masticophis taeniatus*) **B** Long-nosed Snake (*Rhinocheilus lecontei*) **C** Big Bend Patch-nosed Snake (*Salvadora deserticola*) **D** Black-necked Gartersnake (*Thamnophis cyrtopsis*) **E** Chihuahuan Lyresnake (*Trimorphodon wilkinsonii*) **F** Western Diamond-backed Rattlesnake (*Crotalus atrox*) **G** Eastern Black-tailed Rattlesnake (*Crotalus ornatus*), and **H** Mohave Rattlesnake (*Crotalus scutulatus*). Photos by DRD.



***Rhinocheilus lecontei*** Baird & Girard, 1853

Long-nosed Snake (Fig. 7B)

Only a single specimen was collected during recent surveys in the Valentine Plain. This individual was found at night while it was crossing the road within the catclaw-tobosa association. Despite extensive surveys, *R. lecontei* has not been encountered in the Sierra Vieja in recent years. Jameson and Flury (1949) reported on four individuals collected from the ranch: three from the catclaw-grama association in the Sierra Vieja and one from the catclaw-tobosa association of the Valentine Plain.

***Salvadora deserticola*** Schmidt, 1940

Big Bend Patch-nosed Snake (Fig. 7C)

This species has been collected during recent surveys in both the Valentine Plain and the Sierra Vieja. Two specimens were collected in and directly adjacent to the Sierra Vieja: one was collected in the catclaw-cedar association at the mouth of ZH Canyon and the other within catclaw-grama association at Fort Holland. Two additional specimens were collected from the Valentine Plain: one in the catclaw-tobosa association just east of the ranch house and the other in the tobosa-grama association east of Wild Horse Draw. This widespread distribution across the property was similar to that reported in Jameson and Flury (1949).

***Salvadora grahamiae*** Baird & Girard, 1853

Eastern Patch-nosed Snake

No specimens of this species have been seen or collected during the recent surveys, though several were collected in 1948 (Jameson and Flury 1949). This species was restricted to rocky habitats in the Sierra Vieja and Valentine Plain during the 1948 surveys, but despite many hours of work in these rocky areas, particularly ZH and Box canyons, the only *Salvadora* that has been collected has been *S. deserticola*.

***Sonora semiannulata*** Baird & Girard, 1853

Western Groundsnake

Four specimens were collected in recent surveys: three from the catclaw-grama association around Fort Holland in the Sierra Vieja and a single specimen from the tobosa-grama association in the Valentine Plain. This species is probably more widely distributed than our collections might indicate. Jameson and Flury (1949) reported a single individual from the catclaw-grama association in the Sierra Vieja.

***Tantilla hobartsmithi*** Taylor, 1936

Smith's Black-headed Snake

This species has been found in both the Valentine Plain and the Sierra Vieja. Within the Valentine Plain, two specimens were collected under debris within the catclaw-tobosa association and a third specimen was collected under a board at 96 Tank within the creosote bush-catclaw-blackbrush association along the eastern slopes of the Sierra Vieja. In the Sierra Vieja, one individual was collected at Fort Holland in the catclaw-grama association. Jameson and Flury (1949) reported collecting a single individual near the ranch house in the tobosa-grama association of the Valentine Plain.

***Tantilla nigriceps*** Kennicott, 1860

Plains Black-headed Snake

Two specimens were collected during recent surveys, both in the sandy tobosa-grama association in the Valentine Plain. One individual was collected under debris near a storage building while the second was found at night moving on the ground. Jameson and Flury (1949) did not detect this species during previous surveys.

***Thamnophis cyrtopsis*** (Kennicott, 1860)

Black-necked Gartersnake (Fig. 7D)

Eight individuals were collected during recent surveys, all from the Sierra Vieja, except one that was collected away from the main canyons at 96 Tank in the creosote bush-catclaw-blackbrush association of the Valentine Plain. This species is most commonly encountered in close proximity to the springs and pools in the stream bed association in the Sierra Vieja but has also been collected in the catclaw-grama association surrounding Fort Holland. Jameson and Flury (1949) reported *T. cyrtopsis* from similar locations.

***Thamnophis marcianus*** (Baird & Girard, 1853)

Checkered Gartersnake

Six individuals were collected during recent surveys, all from the tobosa-grama, catclaw-tobosa, and creosote bush-catclaw-blackbrush associations in the Valentine Plain. Within these habitats, it is usually found in close proximity to water, whether permanent or ephemeral tanks. One dead and rotting individual was found in a large pool in ZH Canyon in the Sierra Vieja (not vouchered), which may suggest that *T. marcianus* is more widespread across the property than *T. cyrtopsis*. *Thamnophis marcianus* was reported to be found in similar habitats in Jameson and Flury (1949).

***Trimorphodon wilkinsonii*** Cope, 1886

Chihuahuan Lyresnake (Fig. 7E)

Three individuals were collected from the stream bed association of the Sierra Vieja (Box Canyon, Cottonwood Canyon, and ZH Canyon), and one additional animal was captured, bled, and released following injection of a microchip. Two of these animals were found on the ground in the stream bed association and the other two snakes were found climbing in vegetation, 1–3 m above ground (Davis et al. 2008). An additional animal was photographed (not vouchered) by the ranch owners at the ranch house in 2015, ca. 1 km east of the Sierra Vieja in the catclaw-grama association, suggesting that *T. wilkinsonii* occurs in rocky areas of the Valentine Plain. A single individual was collected during previous surveys, a snake found in the stream bed association in the Sierra Vieja (Jameson and Flury 1949).

**Family Leptotyphlopidae*****Rena dissecta*** (Cope, 1896)

New Mexico Threadsnake

Two individuals were collected from the stream bed association in the Sierra Vieja (Cottonwood Canyon and ZH Canyon) during recent surveys, and this species was not found in the 1948 survey. Because two individuals of *R. humilis* were found in the stream bed association in 1948 (Jameson and Flury 1949), the two recently collected specimens of *R. dissecta* now provide evidence that both species of *Rena* live in close sympatry in the Sierra Vieja.

***Rena humilis*** (Baird & Girard, 1853)

Western Threadsnake

A single individual was collected from the ranch during recent surveys in the catclaw-grama association at Fort Holland in the Sierra Vieja. *Rena humilis* are rarely encountered, though we agree with Jameson and Flury (1949) in that this species may be found throughout the Sierra Vieja as well as rocky associations in the Valentine Plain.

**Family Viperidae*****Crotalus atrox*** Baird & Girard, 1853

Western Diamond-backed Rattlesnake (Fig. 7F)

Nine individuals of this common species were collected during recent surveys. In descending order of encounter frequency, individuals of *C. atrox* were collected and observed throughout the catclaw-tobosa, tobosa-grama, catclaw-cedar, and creosote

bush-catclaw-blackbrush vegetation associations on the Valentine Plain; a single specimen was collected in the Sierra Vieja at Fort Holland in the catclaw-grama vegetation association. Jameson and Flury (1949) commented on the scarcity of *C. atrox* on the ranch during the 1948 survey, finding individuals only in the creosote bush-catclaw-blackbrush association of the Valentine Plain.

***Crotalus lepidus*** (Kennicott, 1861)

Rock Rattlesnake

Only two individuals have been seen since 2005: one adult male was collected in May 2007 from the catclaw-grama association of the Sierra Vieja, and another animal escaped a collector in a south facing talus slope in ZH Canyon in June 2005. This is most infrequently observed species of rattlesnake on the ranch, despite being a commonly encountered animal in the Davis Mountains (ca. 64 air km to the east) and the Indio (Eagle) Mountains (ca. 32 air km to the northwest; TJL, unpubl. data). Jameson and Flury (1949) reported *C. lepidus* from both the catclaw-grama and stream bed associations of the Sierra Vieja.

***Crotalus ornatus*** Hallowell, 1854

Ornate Black-tailed Rattlesnake (Fig. 7G)

This is the mostly commonly encountered rattlesnake in the Sierra Vieja. Only two individuals have been vouchered, but four additional animals were captured, marked, and released. This species was found in the stream bed and catclaw-grama associations surrounding Fort Holland and both Box and ZH canyons in the Sierra Vieja. *Crotalus ornatus* was also found in the catclaw-tobosa association and the alluvial fans spreading east from the mountains on the Valentine Plain. Habitat associations where we have found *C. ornatus* are similar to those reported in Jameson and Flury (1949).

***Crotalus scutulatus*** (Kennicott, 1861)

Mohave Rattlesnake (Fig. 7H)

This species is commonly encountered in the sandier catclaw-tobosa and tobosa-grama associations on the Valentine Plain and we have collected sixteen specimens over the course of our recent surveys. W. F. Blair collected a single specimen from an earlier trip to the ranch in July 1947 from the tobosa-gram association of the Valentine Plain (Jameson and Flury 1949), but no individuals were collected as part of the 1948 survey. The consistent number of individuals encountered across our recent survey efforts would suggest population numbers are greater now than in the 1940s, despite any noticeable shifts in vegetative distribution or composition over the last 70 years. An instance of climbing in this species was noted at the ranch by Davis and Cardwell (2017).

***Crotalus viridis*** (Rafinesque, 1818)

## Prairie Rattlesnake

This species is infrequently encountered on the ranch, with only three observed on the ranch property (one vouchered) in 2007. Two additional specimens were collected from the main dirt road leading into the ranch from Valentine (once in 2009, another in 2014). All observations of *C. viridis* are restricted to the sandy soils of the tobosagrama or yucca-grama associations. C. E. Miller, Jr. collected a single specimen from an earlier trip to the ranch in June 1947 from the tobosagrama association in the Valentine Plain (Jameson and Flury 1949), but no individuals were collected as part of the 1948 survey.

**Discussion**

The species composition of amphibians and reptiles encountered during recent years has remained remarkably similar to that observed during the historic 1948 survey. Out of the 47 species of amphibians and reptiles detected in 1948, 44 species (93.6%) have been collected during recent surveys, and many of the localities where specimens were collected in 1948 remain similar to localities where species have been collected in recent years. These results highlight the critical importance of land stewardship in maintaining species diversity at this study site. Additionally, six of the 50 species (12%) encountered in recent years were not collected during the 1948 survey. One of these species, *Lampropeltis splendida*, was collected in areas near, but just off, the study site in 1948, and two anuran species, *Bufo cognatus* and *B. speciosus*, were collected in 1948 along the Rio Grande near Porvenir, ca. 20 km (air) west of the Sierra Vieja. The remaining three species (*Gyalopion canum*, *Tantilla nigriceps*, and *Rena dissecta*) were never encountered in 1948. All of these six species that were undetected in 1948 occur in this region and should have been expected to occur at the study site, but may not have been encountered due to unfavorable environmental conditions from June–July 1948, their cryptic nature, or existed in low abundances making detection difficult. Jameson and Flury (1949) reported that heavy rains fell on the ranch the week prior to their surveys. Both *B. cognatus* and *B. speciosus* occupy grassland habitats and use ephemeral pools as breeding habitats similar to *Scaphiopus couchii* and *Spea multiplicata*, which were collected in abundance. Therefore it is difficult to understand why *B. cognatus* and *B. speciosus* were not previously detected on the ranch, and it may be possible that both species have increased their abundance since 1948. Additionally, our recent surveys have resulted in the first specimen of *Syrrhophus marnockii* collected in the Sierra Vieja (Owen et al. 2014). *Syrrhophus marnockii* has previously been heard calling in canyons in the Sierra Vieja, but collection attempts have been unsuccessful over the last 60 years (Jameson and Flury 1949; TJL, unpublished data).

Over the 14 years that we have been surveying this site for amphibians and reptiles, only three species that were detected in 1948 have yet to be encountered: *Ambys-*

*toma mavortium*, *Lampropeltis alterna*, and *Salvadora grahamiae*. The exact reasons for the failure to detect these species is unknown. *Ambystoma mavortium* was collected in abundance from an artificial, dugout pond surrounded by tobosa-grama association in 1948. Additional voucher specimens of *A. mavortium* exist from 1947 (TNHC 1102: lot of 100 specimens) and 1949 (TNHC 8920) from C. E. Miller Ranch, and most were collected from a series of small ponds surrounding the 1948 locality. Many of these specimens collected in 1947 (TNHC 1102) have reduced external gill size and show the beginnings of adult patterning, indicating that these individuals do undergo metamorphosis to terrestrial adults. The multiple localities where *A. mavortium* were found suggests that a meta-population may have once existed on the ranch and in the Valentine Plain. The landowners have reported seeing this species in ponds and crossing roads as recently as the 1970's (Miller Family, pers. comm.), and suggest that a series of droughts may have resulted in the local extirpation of this species. Detailed survey work for *A. mavortium* in west Texas is lacking, but this species has recently been detected in the Davis Mountains to the east (iNaturalist 3956528; <http://www.inaturalist.org/observations/3956528>). It is possible that a series of high rainfall years in succession may allow individuals to disperse back to C. E. Miller Ranch. Failure to detect *L. alterna* is attributed to its cryptic nature. A single *L. alterna* (TNHC 4181) was collected in 1948 from Fox Canyon in the Sierra Vieja and targeted surveys for this species in canyons within the Sierra Vieja have been unsuccessful in locating this species. Unfortunately, we cannot explain our failure to encounter *S. grahamiae* during recent surveys. Seven specimens of *S. grahamiae* (TNHC 3153, 3369, 3563, 3834, 3924, 3993, 4264) were collected in 1948, but extensive diurnal and crepuscular surveys across multiple associations within the Sierra Vieja did not produce a single specimen. We suggest that future surveys should focus on sampling within canyons for *L. alterna* and the plateaus on top of the Sierra Vieja for *S. grahamiae*.

Future surveys may still detect new species from this study site. For example, both the Glossy Snake (*Arizona elegans*) and the Western Massasauga (*Sistrurus tergeminus*) have been found in the Valentine Plain between the Sierra Vieja and the Davis Mountains. The nearest known occurrences of both *A. elegans* (TNHC 95847) and *S. tergeminus* (Sul Ross State University [SRSU] 6616) are ca. 23 km and 28 km respectively (measured from the C. E. Miller Ranch Headquarters), to the east along U.S. Hwy 90. Several other additional species of reptiles can be found ca. 45 km to the east in the Davis Mountains and ca. 18 km to the west along the Rio Grande that have not been detected in the Sierra Vieja and at the study site. Species present in the Davis Mountains that have not been detected in the Sierra Vieja include Greater Short-horned Lizard (*Phrynosoma hernandesi*), North American Racer (*Coluber constrictor*), Milksnake (*Lampropeltis triangulum*), Baird's Ratsnake (*Pantherophis bairdi*), Trans-Pecos Black-headed Snake (*Tantilla cucullata*), and Copperhead (*Agkistrodon contortrix*). Species present along the Rio Grande but not found in the Sierra Vieja include the Marbled Whiptail (*Aspidoscelis marmorata*), Big Bend Spotted Whiptail (*A. scalaris*), Common Side-blotched Lizard (*Uta stansburiana*), Great Plains Ratsnake (*Pantherophis emoryi*), Spiny Softshell (*Apalone spinifera*), and Mexican Plateau Slider (*Trachemys gaigeae*). At

our study site, suitable habitat for some of these species (e.g., *A. spinifera*, *T. gaigeae*) does not occur, though suitable habitats for many of these other species do appear to be present and match nearby habitats where these species can be found. Instead, the primary constraint for these species is the lack of corridors to connect proximate populations to the Sierra Vieja and the study site. The Valentine Plain between the Sierra Vieja and the Davis Mountains serves as a barrier to impede the movement of montane species and the Sierra Vieja themselves, along with the rim rock on the western edge of the range, serve as a barrier to impede the movement of flatland species between the Rio Grande and the Valentine Plain (York 1949).

The involvement of amateurs and professionals during the 2007 Texas Herpetological Society (THS) spring field meet helped to generate records of previously reported and new species of amphibians and reptiles from C. E. Miller Ranch. Yearly field meets conducted by the THS functionally serve as bioblitzes, rapid assessments of biodiversity for a given area. Specifically, four new species of reptiles were collected during the THS field meet: *Holbrookia maculata*, *Trimorphodon wilkinsonii*, *Rena dissecta*, and *Crotalus lepidus*. Many additional vouchers of previously known species collected during this trip also helped record species from additional habitat associations. While no systematic effort was made to voucher photographs, observations of amphibians and reptiles from the THS field meet, and additional trips to the ranch by various individuals, have been posted to the Herps of Texas project on iNaturalist (<https://www.inaturalist.org/projects/herps-of-texas>). Although none of these photographic records represented new species occurrences for the property, we recognize the significance of these records in confirming the presence, distribution, and persistence of the C. E. Miller Ranch herpetofauna. The creation of focused taxon- or locality-based projects on repositories such as iNaturalist could be crucial in identifying rarely seen or even new taxa (Deutsch et al. 2017). While we concede there are limitations of photographic records when compared to voucher-based surveys, we encourage the incorporation of citizen science observations with standard voucher-based surveys and collections and further acknowledge the important contributions such photographs can provide to traditional surveys (e.g., Spear et al. 2017).

In sum, our recent surveys from 2004–2017 have been successful in detecting the vast majority of species previously detected from this study site in 1948, though we have been unable to detect three species. While we are unable to determine the exact reasons, we believe that two of these species (*Lampropeltis alterna*, *Salvadora grahamiae*) are likely still present at this site, but their cryptic nature has precluded their detection; the third species, *Ambystoma mavortium*, is presumed to be locally extirpated. Additionally, our recent surveys have been able to detect six species that were previously undetected during the 1948 survey. These six species were likely present at the study site in 1948, but their cryptic nature, low abundance, or unfavorable environmental conditions prevented their detection. Follow-up surveys like ours are important to document changes in species diversity or assemblage through time, even though the exact causal relationships between change in diversity and factors influencing this change (i.e., land management decisions, climate change) remain uncertain. With high levels of amphibian and reptile



diversity in the Chihuahuan Desert (Jones et al. 2016), our results highlight the importance of land stewardship and environmentally conscious land management decisions on maintaining the diversity of amphibians and reptiles in the region.

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## References

- Baker CL (1927) Exploratory geology of a part of southwestern Trans-Pecos Texas. University of Texas Bulletin 2745: 1–70.
- Blair WF (1940) A contribution to the ecology and faunal relationships of the mammals of the Davis Mountain region, southwestern Texas. Miscellaneous Publications, Museum of Zoology, University of Michigan 46: 1–39.
- Blair WF, Miller CE Jr (1949) Mammals of the Sierra Vieja region, southwestern Texas, with remarks on the biogeographic position of the region. Texas Journal of Science 1: 67–92.
- Bogert CM (1945) Two additional races of the patch-nosed snake, *Salvadora hexalepis*. American Museum Novitates 1285: 1–14.
- Bogert CM (1985) [Report on sibling species now recognizable in the genus *Salvadora*]. Snake Systematics Newsletter 3: 1.
- Brune G (1981) Springs of Texas, Volume I. Branch-Smith, Inc., Fort Worth, Texas, 566 pp.
- Crother BI (Ed.) (2017) Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. SSAR Herpetological Circular 43: 1–102.
- Davis DR, Cardwell MD (2017) *Crotalus scutulatus* (Mohave Rattlesnake). Arboreality and climbing behavior. Herpetological Review 48: 670–671.
- Davis DR, LaDuc TJ (2017) *Hypsiglena jani* (Chihuahuan Nightsnake). Diet. Herpetological Review 48: 613.
- Davis DR, Box B, Tibbitts TJ (2008) *Trimorphodon vilkinsonii* (Chihuahuan Lyresnake) Arboreal Behavior. Herpetological Review 39: 102.

- Devitt TJ, LaDuc TJ, McGuire JA (2008) The *Trimorphodon biscutatus* (Squamata: Colubridae) species complex revisited: a multivariate statistical analysis of geographic variation. *Copeia* 2008: 370–387. <https://doi.org/10.1643/ch-07-045>
- Dice LR (1943) The biotic provinces of North America. University of Michigan Press, Ann Arbor, Michigan, 78 pp.
- Deutsch C, Bilenca B, Agostini G (2017) In search of the horned frog (*Ceratophrys ornata*) in Argentina: complementing field surveys with citizen science. *Herpetological Conservation and Biology* 12: 664–672.
- Gannett H (1904) A Gazetteer of Texas. Second edition. United States Geological Survey Bulletin 224, 177 pp.
- Hinckley LC (1947) Contrasts in the vegetation of the Sierra Tierra Vieja in Trans-Pecos Texas. *American Midland Naturalist* 37: 162–178. <https://doi.org/10.2307/2421650>
- Jameson DL, Flury AG (1949) The reptiles and amphibians of the Sierra Vieja Range of southwestern Texas. *Texas Journal of Science* 1: 54–79.
- Jones LLC, Halama KJ, Lovich RE (2016) Habitat Management Guidelines for Amphibians and Reptiles of the Southwestern United States. Partners in Amphibian and Reptile Conservation, Technical Publication HMG-5, Birmingham, Alabama, 193 pp.
- Klepper ED (2003) On higher ground. *Texas Parks and Wildlife Magazine* 61: 31–33.
- Lynch JD (1970) A taxonomic revision of the leptodactylid frog genus *Syrrhophus* Cope. University of Kansas Publications, Museum of Natural History 20: 1–45. <https://doi.org/10.5962/bhl.part.2809>
- Myers EA, Burgoon JL, Ray JM, Martínez-Gómez JE, Matías-Ferrer N, Mulcahy DG, Burbrink FT (2017) Coalescent species tree inference of *Coluber* and *Masticophis*. *Copeia* 105: 642–650. <https://doi.org/10.1643/CH-16-552>
- Olson DM, Dinerstein E (1998) The Global 200: a representation approach to conserving the Earth's most biologically valuable ecoregions. *Conservation Biology* 12: 502–515. <https://doi.org/10.1046/j.1523-1739.1998.012003502.x>
- Owen JD, Marshall TL, Davis DR (2014) Geographic distribution: USA, Texas, Jeff Davis Co.: *Eleutherodactylus (Syrrhophus) marnockii* (Cliff Chirping Frog). *Herpetological Review* 45: 652.
- Pauly GB, LaDuc TJ (2008) *Bogertophis subocularis* (Trans-Pecos Ratsnake). *Arboreality. Herpetological Review* 40: 93.
- Pauly GB, Hillis DM, Cannatella DC (2009) Taxonomic freedom and the role of official lists of species names. *Herpetologica* 65: 115–128. <https://doi.org/10.1655/08-031R1.1>
- Phillips HW, Thornton WA (1949) The summer resident birds of the Sierra Vieja Range in southwestern Texas. *Texas Journal of Science* 1: 101–131.
- Price AH (1990) *Phrynosoma cornutum*. *Catalog of American Amphibians and Reptiles* 469: 1–7.
- Ricketts TH, Dinerstein E, Olson DM, Loucks CJ, Eichbaum W, DellaSalla D, Kavanagh K, Hedao P, Hurley PT, Carney KM, Abell R, Walters S (1999) Terrestrial Ecoregions of North America: a Conservation Assessment. Island Press, Washington D.C., 415 pp.
- Schmidt KP, Owens DW (1944) Amphibians and reptiles of northern Coahuila, Mexico. *Zoological Series of Field Museum of Natural History* 29: 97–115.
- Schmidt KP, Smith TF (1944) Amphibians and reptiles of the Big Bend region of Texas. *Zoological Series of Field Museum of Natural History* 29: 75–96.

- Schmidt Jr. RH (1979) A climatic delineation of the 'real' Chihuahuan Desert. *Journal of Arid Environments* 2: 243–250.
- Spear DM, Pauly GB, Kaiser K (2017) Citizen science as a tool for augmenting museum collection data from urban areas. *Frontiers in Ecology and Evolution* 5: 86. <https://doi.org/10.3389/fevo.2017.00086>
- York CL (1949) The physical and vegetational basis for animal distribution in the Sierra Vieja Range of southwestern Texas. *Texas Journal of Science* 1: 46–62.
- Yuan Z-Y, Zhou W-W, Chen X, Poyarkov Jr. NA, Chen H-M, Jang-Liaw N-H, Chou W-H, Matzke NJ, Iizuka K, Min M-S, Kuzmin SL, Zhang YP, Cannatella DC, Hillis DM, Che J (2016) Spatiotemporal diversification of the true frogs (genus *Rana*): a historical framework for a widely studied group of model organisms. *Systematic Biology* 65: 824–842. <https://doi.org/10.1093/sysbio/syw055>

## Appendix I

Voucher specimens from C. E. Miller Ranch in Jeff Davis and Presidio counties, Texas, USA collected during recent surveys from 2004–2017. Numbers in parentheses indicate the number of specimens collected. TCWC = Biodiversity Research and Teaching Collections, Texas A&M University (formerly Texas Cooperative Wildlife Collections); TNHC = Biodiversity Collections, The University of Texas at Austin (formerly Texas Natural History Collections).

- Bufo cognatus* (5): TNHC 67036, 67037, 67353, 67354, 95841.
- Bufo debilis* (14): TCWC 89827; TNHC 67333–67337, 89580, 89581, 89583, 89887, 95852, 97394, 97481, 99572.
- Bufo punctatus* (15): TNHC 67325–67327, 67375–67377, 89589, 89590, 95850, 97122, 97123, 98914, 99589, 99590, 104419.
- Bufo speciosus* (10): TNHC 67034, 67035, 67381, 68912, 98920, 99580, 104397–104400.
- Syrrhophus marnockii* (1): TNHC 92230.
- Hyla arenicolor* (7): TNHC 67029–67031, 67403, 89446, 95843, 97033.
- Gastrophryne olivacea* (7): TNHC 65363, 65364, 67324, 68907, 97488, 194414, 104415.
- Rana berlandieri* (6): TCWC 88229; TNHC 67032, 67033, 89606, 89607, 104420.
- Scaphiopus couchii* (9): TNHC 67328, 67329, 68908, 89611, 89663, 91983, 95859, 97410, 99574.
- Spea multiplicata* (16): TNHC 67338–67344, 68909–68911, 89445, 89889, 97128, 104416–104418.
- Terrapene ornata* (3): TNHC 92309, 99558, 99559.
- Kinosternon flavescens* (25): TNHC 65365, 65366, 66970–66972, 69375–69378, 89642, 95868, 95869, 97118–97120, 99560–99562, 99583, 99584, 104401–104404, 104450.

- Crotaphytus collaris* (3): TNHC 66883, 66884, 104393.  
*Coleonyx brevis* (7): TNHC 66997–67000, 67347, 89591, 99587.  
*Cophosaurus texanus* (15): TNHC 67005–67008, 89592, 89593, 98915–98917, 98919, 98921–98924, 98926.  
*Holbrookia maculata* (6): TNHC 67018, 68818, 85316, 85317, 89599, 89600.  
*Phrynosoma cornutum* (6): TNHC 66889, 68809, 85350, 85351, 89579, 95840.  
*Phrynosoma modestum* (4): TNHC 66885, 68817, 89603, 89604.  
*Sceloporus cowlesi* (7): TNHC 67014, 67015, 89614–89617, 99592.  
*Sceloporus poinsettii* (1): TNHC 100867  
*Urosaurus ornatus* (9): TCWC 89836–89838; TNHC 67019–67022, 89621, 89622.  
*Aspidoscelis exsanguis* (16): TCWC 88247, 89833–89835, 91583, 92136; TNHC 67009–67013, 67016, 67017, 89584, 89585, 89659.  
*Aspidoscelis inornata* (15): TCWC 88238, 88239, 89832; TNHC 67023–67028, 89586, 89587, 89662, 97361, 97393, 104396.  
*Aspidoscelis tessellata* (8): TNHC 66886–66888, 89501, 89502, 89588, 89660, 89661.  
*Plestiodon obsoletus* (6): TCWC 89841; TNHC 67001, 67002, 85236, 89509, 89623.  
*Plestiodon tetragrammus* (4): TCWC 91823; TNHC 67003, 67004, 67348.  
*Bogertophis subocularis* (2): TNHC 66576, 99599.  
*Diadophis punctatus* (2): TNHC 89595, 97115.  
*Gyalopion canum* (1): TNHC 89597.  
*Heterodon kennerlyi* (3): TNHC 66582, 89598, 104421.  
*Hypsiglena jani* (4): TCWC 91824; TNHC 66598, 66600, 89601.  
*Lampropeltis splendida* (4): TNHC 66595, 66596, 89496, 89582.  
*Masticophis flagellum* (5): TNHC 66577, 66579, 97125, 97126, 99585.  
*Masticophis taeniatus* (1): TNHC 66581.  
*Pituophis catenifer* (2): TNHC 66752, 89846.  
*Rhinocheilus lecontei* (1): TNHC 89515.  
*Salvadora deserticola* (4): TNHC 66714, 66715, 89608, 89609.  
*Sonora semiannulata* (4): TNHC 66591–66594.  
*Tantilla hobartsmithi* (4): TCWC 89846; TNHC 66741–66743.  
*Tantilla nigriceps* (2): TNHC 89679, 89694.  
*Thamnophis cyrtopsis* (8): TCWC 92430; TNHC 66716, 66717, 85444, 85445, 85869, 89618, 89619.  
*Thamnophis marcianus* (6): TNHC 66590, 85450, 89620, 99579, 104405, 104451.  
*Trimorphodon wilkinsonii* (3): TNHC 66487, 66513, 89913.  
*Rena dissecta* (2): TNHC 66486, 67346.  
*Rena humilis* (1): TNHC 68780.  
*Crotalus atrox* (9): TCWC 88252; TNHC 65741, 66497, 66540–66542, 89886, 99576, 104394.  
*Crotalus lepidus* (1): TNHC 100146.  
*Crotalus ornatus* (2) TNHC 66543, 89706.  
*Crotalus scutulatus* (17): TCWC 93138; TNHC 66531–66533, 66537–66539, 66881, 66882, 68735, 68736, 89648, 89782, 89855, 95871, 97398, 97399.  
*Crotalus viridis* (2): TNHC 66528, 97121.

# DKey software for editing and browsing dichotomous keys

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## Abstract

Despite advances in computer technology and the increasing availability of multiple-access taxonomic keys, traditional dichotomous keys remain the most often used taxonomic identification tools. On the other hand, there seems to be a lack of an editor of dichotomous keys, which is both freely available and easy to use. The DKey software was developed in order to alleviate this problem. A taxonomic key in text format can be imported to the software in order to edit it. Various editing options are possible, including: moving couplets, removing couplets, combining keys and renumbering keys. The software can output the key either in the traditional text format, ready for publication in a scientific journal, or in hypertext linked format, which makes identification faster and easier, due to the fact that pointers can be clicked in order to move to the next couplet. The DKey software should be useful for both taxonomic experts creating keys and those who use them for identification. The DKey software is freely available and open source.

## Keywords

key, single-access key, software, taxonomic identification of species

## Introduction

The identification of species is often based on dichotomous keys (also called single-access keys or pathway keys) (Quicke 1993, Walter and Winterton 2007). They are a series of consecutively numbered couplets. Each couplet consists of two parts called leads. At the end of each lead, there is a reference, which can be either a number pointing to another couplet or a text indicating the name of a species or another taxonomic group. The two types of references are called “pointer” and “endpoint”, respectively. The identification based on a dichotomous key starts at couplet number one and it is stepwise. At each step, the user reads both leads of a couplet and chooses one that better fits the identified individual. If the chosen lead is associated with a pointer, then the user goes to another couplet, which is the next step of the identification. The identification is finished when the chosen couplet is associated with an endpoint.

Originally, dichotomous keys were constructed by taxonomic experts without any support from computer technology. However, when computers became more widespread, computer programs for the automated construction of taxonomic keys were developed (Pankhurst 1971, Dallwitz 1974, Quicke 1993). Computerized key construction software requires a data matrix consisting of a table with information about multiple characters of each taxon in the key. The key is constructed automatically by a computer algorithm; however, there is often the need for some intervention from the user to improve it. The data matrix can also be used for the construction of a free-access key (also called a multi-access key) (for review see Walter and Winterton 2007, Hagedorn et al. 2010, Cerretti et al. 2012). In this type of taxonomic key, there is no fixed sequence of identification steps. Instead, the user chooses characters from the data matrix that are easily available. This attitude can be preferred when some characters are not available in the identified specimen.

Multiple-access keys have many advantages (Dallwitz et al. 2000). In those keys not only qualitative but also quantitative characters can be used. Moreover, tolerance to errors is higher than in dichotomous keys. Despite their advantages multiple-access keys have not become overwhelmingly popular among taxonomists and manmade dichotomous keys remain the most often used identification tool (Quicke 1993, Walter and Winterton 2007). The preparation of the data matrix and, in particular, the fine tuning of the process of key construction requires some specialist knowledge. On the other hand, most taxonomists have limited interest in computer technology and sophisticated software can intimidate them. In consequence, many taxonomists design dichotomous keys without the use of any dedicated computer software, except a text editor. Creating a key in this way is time-consuming. A relatively simple task of numbering couplets is tedious and error prone. This task can be repeated many times when preparing the key, as any removal or addition of a couplet requires renumbering of some couplets and pointers. This problem is particularly acute in the case of large keys consisting of hundreds of couplets. There seems to be a lack of an editor of dichotomous keys, which is freely available and easy to use.



The aim of the project was to develop the DKey software, which would assist taxonomists in the preparation of dichotomous identification keys. A taxonomic key in text format can be easily imported to the software in order to edit it. The software can output the key either in traditional text format, ready for publication in a scientific journal, or hypertext linked format, which makes identification faster and easier. The DKey software is freely available and open source.

## **Methods**

DKey was developed in the C++ programming language. It is based on the QT framework. At the moment, executables are available for the Windows operating system (<http://drawwing.org/dkey>). However, users of macOS and Linux can obtain the source code and build executables for their operating system, as QT is a cross-platform framework. In future access to DKey should be equally easy for all three operating systems. The source code of the software can be downloaded from the GitHub (<https://github.com/DrawWing/DKey>). DKey is open source and it is licensed under GNU General Public License, version 3.

DKey allows for reticulation, which means that a couplet can be reached over more than one path and a single taxon can be associated with more than one endpoint. The only restrictions are that the couplet numbers need to be unique, they need to start with 1, they need to be consecutive, each couplet need to be reached from couplet 1 and it needs to refer either to an existent other couplet or an endpoint. The software does not create the key automatically; it is a responsibility of the taxonomic expert to create couplets and to arrange them in an optimal way. In order to test the software, I have used the existing key to the genera of Agathidinae (Hymenoptera, Braconidae) (Sharkey et al. 2009).

## **Results**

### **Description of the software**

The DKey software has a graphical user interface. In the main window, the taxonomic key is displayed in a table (Fig. 1A) where one row corresponds to one couplet. There are three columns. In the first column, there is the couplet number; in the second column, the first lead; and finally, in the third column, the second lead. The table can be used to edit the key. It is possible to insert, remove, copy, cut and paste couplets. Those editing operations can be made only within one key. If two keys need to be combined, the “append” option should be used. Then, the content of the appended file is added at the end of the currently edited key.

When couplets are inserted, removed, moved or copied, the key is not renumbered automatically and its consecutive numbering can be temporally broken. The user decides

A

Sharkey2009Agathidinae.dk.xml - DKey


File Edit Debug View Help

No.	First lead	Second lead
1	Forewing venation greatly reduced, last abscissa (segment) of RS vein completely absent (Fig. 1a). - Aneurobracon Brues, 1930	Forewing more complete, last abscissa of RS vein present though sometimes weak (Fig. 1b). - 2
2	Fore and mid claws simple (Fig. 2c). - Bassus Fabricius, 1804	Fore and mid claws cleft (Fig. 2a) or with a basal lobe (Fig. 2b). - 3
3	Fore and mid claws cleft (Fig. 2a). - 4	Fore and mid claws with a basal lobe (Fig. 2b). - 13
4	Hind trochantellus with one or two distinct carinae (Fig. 3a). - 5	Hind trochantellus lacking carinae (Fig. 3b). - 8


B

Key browser - DKey

13. RS+M vein of forewing mostly or entirely absent (Fig. 12a); notauli present but not necessarily complete (Fig. 12aa).



13. RS+M vein of forewing present and complete (Fig. 12b); notauli absent (Fig. 12bb).  
**Earinus Wesmael, 1837**



Path

- 3. Fore and mid claws with a basal lobe (Fig. 2b).
- 2. Fore and mid claws cleft (Fig. 2a) or with a basal lobe (Fig. 2b).
- 1. Forewing more complete, last abscissa of RS vein present though sometimes weak (Fig. 1b).

Remaining endpoints

- Agathis Latreille, 1804 - 17
- Braunsia Kriechbaumer, 1894 - 16
- Camptothlipsis Enderlein, 1920 - 14
- Earinus Wesmael, 1837 - 13
- Lytopylus Förster, 1862 - 16
- Therophilus Wesmael, 1837 - 17

Excluded endpoints

- Amputostypus Sharkey, gen. n. - 6
- Aneurobracon Brues, 1930 - 1
- Bassus Fabricius, 1804 - 2
- Biroia Szépligeti, 1900 - 9
- Coccygidium Saussure, 1892 - 5
- Cremonops Förster, 1862 - 11

**Figure 1.** An example of using the DKey software based on the key to the genera of Agathidinae (Sharkey et al. 2009). The key was imported to the DKey software (A) and displayed in an interactive key browser (B).

when the renumbering occurs. This makes it easier to keep track of changes made. Moreover, the renumbering can take a noticeable time in the case of large keys. The renumbering should be done before the key is exported or displayed in the key browser.

There is a validation tool allowing to find logical errors in the key. Among others it is verified if the couplet numbering is unique and consecutive, if the pointers are valid and if each couplet has at least one reference in other couplets. Moreover, a warning is generated whenever reticulation occurs in the key. The reticulation means that the same endpoint occurs in the key more than once or a single couplet is referred by more than one couplet. A key created with DKey can be saved in a XML file which allows relatively easy import to other software. Moreover, in this format international characters can be safely exchanged between operating systems.

DKey can format the key in various ways. For publication in a scientific journal, the key can be saved in rich text format. On the other hand, for making the key available online, it should be saved in hypertext linked format. In this format, pointers can be clicked in order to move to the next couplet. Moreover, a key browser (Fig. 1B) can be used on a local computer to make identification more user-friendly. In the key browser, only one couplet is displayed at any time. The key browser consists of five windows. The two top windows contain the two leads of the couplet and images associated with the leads. If a lead is associated with a pointer, there is a link to the pointed couplet. The three bottom windows of the key browser contain: the path, the remaining endpoints and the excluded endpoints. The path, or history of identification, contains a list of couplets leading to the current couplet. The remaining endpoints contain a list of taxa, which can be reached from the current couplet. The excluded endpoints contain list of taxa, which cannot be reached from the current couplet. At the beginning of identification, when the first couplet is displayed, the path window and the excluded endpoint window are empty; on the other hand, the remaining endpoint window contains all the taxa covered by the key. By following the steps of the identification and visiting couplets, the lists in the path window and the excluded endpoint window are growing and the list in the remaining endpoint window is shrinking until there are only two endpoints.

Identification using a key browser is easier because the user moves from couplet to couplet by clicking a chosen lead. It is also possible to go back to the earlier stages of identification by clicking on the list of steps leading to current couplet. Moreover, the software searches the leads for the keyword "Fig.", and if found, it looks for the presence of an image file in the same directory. If the image is found, it is displayed in the key browser next to the lead in which it is referenced. This simple mechanism is sufficient for the integration of images into the key; there is no need for a manual linking of images with couplets.

## **Getting started**

The preparation of a new key can be started from scratch using the "new key" option. Then, the first dummy couplet, which needs to be edited, is created. In order to edit the couplet it should be double clicked. More couplets can be added using the option "insert couplet below" (for details see Suppl. material 1).

Taxonomic keys are often developed by the modification of older keys created by another expert. Therefore, the import of an existing key in text format is an important part of the software. Traditional dichotomous taxonomic keys are usually formatted in a consistent way (Quicke 1993); therefore, they can be analyzed by computer software in order to extract various information. In the language of computer technology, the analysis is called "parsing". The DKey software parses the key in text format in order to extract all relevant information, including: number of the couplet, leads, pointers

and endpoints. In order to facilitate the import, the user should place leads in separate lines and place a tab character in each line before pointers or endpoints. The process of import is not interactive and incorrect preparation of the imported file will result in incorrect key structure. However, the described earlier validation can be used to detect those errors. If the errors are present the user should edit the imported file and repeat the import.

In order to illustrate the import, the key to the genera of Agathidinae (Sharkey et al. 2009) was saved as a plain text file and small adjustments facilitating the import to the DKey software were made (for details see Suppl. material 2). The modifications included adding a tab character before each reference (both endpoint and pointer), adding the keyword “Fig.” before each figure number and removing unwanted line breaks within each lead. The modified text file was imported to the DKey software. In the imported key some errors were detected because there was one couplet (number two) with three leads. It was converted to two couplets with two leads in order to be compatible with the dichotomous key. Finally the key was renumbered in order to correct it for the added couplet in which the number was not consecutive (Fig. 1A, B).

## Discussion

The DKey software fills the gap for an easy-to-use and free editor of conventional dichotomous taxonomic keys. The main advantage of the DKey software is its wide availability. It can be downloaded free of charge and used by both scientists as well as a wider audience. The price of commercial software, for example Lucid Phoenix (Anonymous 2017), can be prohibitive for some enthusiasts of taxonomy. Not only is the DKey software free of charge, but its source code is also widely available. Therefore, it can be developed in the future by a larger group of programmers who can correct and improve it. In consequence, the software can survive longer. Many projects related to biodiversity informatics vanished or stopped to be developed when the funding has finished or when the original developer lost interest in the further improvement of the software. In the case of open source software, there is a chance that programmers, other than the author, will continue the work on it.

There were other attempts to develop free and open source software for taxonomic keys. Open Key Editor (Martellos et al. 2010, van Spronsen et al. 2010) can be used to create user-friendly taxonomic keys available online or on mobile devices. This software is open source; however, the license under which it is distributed is relatively restrictive (Hagedorn et al. 2011). The installation of Open Key Editor can be difficult for most taxonomists, as it requires a web server, the creation of an SQL database and a manual modification of the configuration files.

There is also a wide range of freely available software, which can be used for the creation of online identification keys, including: ActKey (Brach and Song 2005), ARPHA Writing Tool (Smith et al. 2013), KeyBase (Thiele and Klazenga 2016), WE-BiKEY (Attigala et al. 2016). The purpose of this software is to make identification

easier. Taxonomic keys published in scientific journals and books are formatted for efficient printing and page layout. This formatting can create problems for the key users, as images are usually on a different page than the couplets referring to them. In order to minimize those problems, dichotomous keys can be implemented as a webpage or a computer program. In this form, the key is more user-friendly and the identification time can be markedly reduced because the user does not need to flip pages to find the next couplet or an image (Wright et al. 1995, Farr 2006, Burkmar 2014). However, the process of preparing the computer-based keys can be time-consuming because it requires the preparation of a data matrix or rewriting the key. On the other hand, DKey allows a relatively easy import of existing keys and images. A user of a traditional printed key can scan it, convert it to text and import it to the DKey software in order to make identification faster and more flexible. Moreover, taxonomic experts who use the software to create a key for scientific publication do not need to invest any more time or effort to create a computer-based version of the key.

In contrast to other free software, DKey is focused not only on the creation of computer based keys but also provides many editing options, which are not available elsewhere, including: import from text, export to text, combining keys, moving and copying couplets. The DKey software is particularly useful in the case of large taxonomic keys consisting of hundreds or thousands of couplets. Nowadays, the identification of large taxonomic groups involves using many small keys. There are separate keys for families, genera, species groups and species. The smaller keys are easier to manage by the taxonomic experts who created them because their renumbering is less time-consuming. On the other hand, this solution is less user-friendly because the user needs to find the key to the next taxonomic level. DKey can be easily used to combine many smaller keys into one very large one.

Taxonomists are encouraged to publish their datasets and allow others to re-use them in the future (Penev et al. 2008, 2009). The current recommendations (Penev et al. 2009) are related mainly to interactive keys based on data matrices and there is no standard format for dichotomous keys. The later keys are usually presented in two main formats: bracketed and indented (Quicke 1993). Within each of those formats, there are many variants with various punctuation marks around couplet numbers. This variation hinders the process of parsing a key in text format by a computer software. The use of standard formatting would make the exchange of information easier. The format in which the key is saved by the DKey software can become such a standard.

## **Conclusion**

The DKey software is an easy-to-use and freely available dichotomous taxonomic key editor. It can be used for importing existing keys, editing them and exporting them in various formats. Some of the formats are suitable for publication in scientific journals; others make identification easier and faster. The DKey software should be useful for both taxonomic experts creating keys and those who use them for identification.

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## References

- Anonymous (2017) Lucid Phoenix. <http://www.lucidcentral.com/en-us/software/lucidphoenix.aspx> [November 20, 2017]
- Artigala L, Silva NID, Clark LG (2016) Simple Web-based interactive key development software (WEBiKEY) and an example key for Kuruna (Poaceae: Bambusoideae). *Applications in Plant Sciences* 4: 1500128. <https://doi.org/10.3732/apps.1500128>
- Brach AR, Song H (2005) ActKey: a Web-based interactive identification key program. *Taxon* 54: 1041–1046. <https://doi.org/10.2307/25065490>
- Burkmar R (2014) The shifting paradigm of biological identification. Fields Studies Council, Shrewsbury, 37 pp.
- Cerretti P, Tschorsnig H-P, Lopresti M, Di Giovanni F (2012) MOSCHweb—a matrix-based interactive key to the genera of the Palaearctic Tachinidae (Insecta, Diptera). *ZooKeys* 205: 5–18. <https://doi.org/10.3897/zookeys.205.3409>
- Dallwitz MJ (1974) A flexible computer program for generating identification keys. *Systematic Biology* 23: 50–57. <https://doi.org/10.1093/sysbio/23.1.50>
- Dallwitz MJ, Paine TA, Zurcher EJ (2000) Principles of interactive keys. Web-based document <http://delta-intkey.com>. Available from: <http://delta-intkey.com/www/interactivekeys.pdf> [September 21, 2017]
- Farr DF (2006) On-line keys: more than just paper on the web. *Taxon* 55: 589–596. <https://doi.org/10.2307/25065636>
- Hagedorn G, Mietchen D, Morris RA, Agosti D, Penev L, Berendsohn WG, Hobern D (2011) Creative Commons licenses and the non-commercial condition: Implications for the re-use of biodiversity information. *ZooKeys* 150: 127–149. <https://doi.org/10.3897/zookeys.150.2189>
- Hagedorn G, Rambold G, Martellos S (2010) Types of identification keys. In: Nimis PL, Lebbe RV (Eds), *Tools for Identifying Biodiversity: Progress and Problems*. Edizioni Università di Trieste, Trieste, 59–64.
- Martellos S, Van Spronsen E, Seijts D, Aloy NT, Schalk P, Nimis P (2010) User-generated content in the digital identification of organisms: the KeyToNature approach. *International Journal of Information and Operations Management Education* 3: 272–283. <https://doi.org/10.1504/IJIOME.2010.033550>
- Pankhurst RJ (1971) Botanical keys generated by computer. *Watsonia* 8: 357–368.
- Penev L, Erwin T, Sues H, Engel M, Agosti D, Pyle R, Ivie M, Assmann T, Henry T, Miller J, Casale A, Lourenco W, Golovatch S, Fagerholm H, Taiti S, Alonso-Zarazaga M, van Nieukerken E (2008) ZooKeys, unlocking Earth's incredible biodiversity and building a



- sustainable bridge into the public domain: From “print-based” to “web-based” taxonomy, systematics, and natural history. *ZooKeys* 1: 1–7. <https://doi.org/10.3897/zookeys.1.11>
- Penev L, Sharkey M, Erwin T, van Noort S, Buffington M, Seltmann K, Johnson N, Taylor M, Thompson F, Dallwitz M (2009) Data publication and dissemination of interactive keys under the open access model. *ZooKeys* 21: 1–17. <https://doi.org/10.3897/zookeys.1.11>
- Quicke DL (1993) Principles and techniques of contemporary taxonomy. Chapman & Hall, Glasgow, 311 pp. <https://doi.org/10.1007/978-94-011-2134-7>
- Sharkey MJ, Yu DS, van Noort S, Seltmann K, Penev L (2009) Revision of the Oriental genera of Agathidinae (Hymenoptera, Braconidae) with an emphasis on Thailand including interactive keys to genera published in three different formats. *ZooKeys* 21: 19–54. <https://doi.org/10.3897/zookeys.21.271>
- Smith V, Georgiev T, Stoev P, Biserkov J, Miller J, Livermore L, Baker E, Mietchen D, Couvreur T, Mueller G, Dikow T, Helgen KM, Frank J, Agosti D, Roberts D, Penev L (2013) Beyond dead trees: integrating the scientific process in the Biodiversity Data Journal. *Biodiversity Data Journal* 1: e995. <https://doi.org/10.3897/BDJ.1.e995>
- van Spronsen E, Martellos S, Seijts D, Schalk P, Nimis PL (2010) Modifiable digital identification keys. In: Nimis PL, Lebbe RV (Eds) *Tools for Identifying Biodiversity: Progress and Problems*. Edizioni Università di Trieste, Trieste, 127–131.
- Thiele K, Klazenga N (2016) KeyBase-teaching old keys new tricks. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation* 25: 3–4.
- Walter DE, Winterton S (2007) Keys and the crisis in taxonomy: extinction or reinvention? *Annual Review of Entomology* 52: 193–208. <https://doi.org/10.1146/annurev.ento.51.110104.151054>
- Wright JF, Morse DR, Tardivel GM (1995) An investigation into the use of hypertext as a user interface to taxonomic keys. *Bioinformatics* 11: 19–27. <https://doi.org/10.1093/bioinformatics/11.1.19>

## Supplementary material I

### Video tutorial describing how to build a simple taxonomic key using DKey software

Author: Adam Tofilski

Data type: Multimedia.

Explanation note: Use any video player which supports MP4 format in order to see the tutorial.

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Link: <https://doi.org/10.3897/zookeys.735.21412.suppl1>

## **Supplementary material 2**

### **Video tutorial describing how to import a taxonomic key to DKey software**

Author: Adam Tofilski

Data type: Multimedia.

Explanation note: Use any video player which supports MP4 format in order to see the tutorial.

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