# Non-destructive morphological observations of the fleshy brittle star, Asteronyx loveni using micro-computed tomography (Echinodermata, Ophiuroidea, Euryalida) 

Masanori Okanishi', Toshihiko Fujita ${ }^{2}$, Yu Maekawa ${ }^{3}$, Takenori Sasaki ${ }^{3}$<br>I Faculty of Science, Ibaraki University, 2-1-1 Bunkyo, Mito, Ibaraki, 310-8512 Japan 2 National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki, 305-0005 Japan 3 University Museum, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo, 113-0033 Japan

Corresponding author: Masanori Okanishi (mokanishi@tezuru-mozuru.com)

Academic editor: Y. Samyn \| Received 6 December 2016 \| Accepted 23 February 2017 | Published 27 March 2017
http://zoobank.org/58DC6268-7129-4412-84C8-DCE3C68A7EC3
Citation: Okanishi M, Fujita T, Maekawa Y, Sasaki T (2017) Non-destructive morphological observations of the fleshy brittle star, Asteronyx loveni using micro-computed tomography (Echinodermata, Ophiuroidea, Euryalida). ZooKeys 663: 1-19. https://doi.org/10.3897/zookeys.663.11413


#### Abstract

The first morphological observation of a euryalid brittle star, Asteronyx loveni, using non-destructive Xray micro-computed tomography ( $\mu \mathrm{CT}$ ) was performed. The body of euryalids is covered by thick skin, and it is very difficult to observe the ossicles without dissolving the skin. Computed tomography with micrometer resolution (approximately $4.5-15.4 \mu \mathrm{~m}$ ) was used to construct 3D images of skeletal ossicles and soft tissues in the ophiuroid's body. Shape and positional arrangement of taxonomically important ossicles were clearly observed without any damage to the body. Detailed pathways inside the vertebral ossicles, lateral arm plates, and arm spines for passage of nerves and water vascular structures were observed. Inter-vertebral muscles were also observed. Forms and 3D arrangements of many important taxonomical characters of the euryali xonomy of the class Ophiu ds were scrutinized by $\mu \mathrm{CT}$ in high enough resolution for taxonomic description of ophiuroids.


## Keywords

Anatomy, Asteronychidae, computed tomography, Euryalida, soft tissue, taxonomy

## Introduction

The class Ophiuroidea (phylum Echinodermata) is globally distributed, ranging from the equator to polar regions, and from the intertidal zone to the greatest depths (Stöhr et al. 2012; O'Hara et al. 2014). Ophiuroidea is the most diverse class in Echinodermata, comprising approximately 2100 species (Stöhr et al. 2012). Among the ophiuroids, the order Euryalida, one of two orders of superorder Euryaophiurida, comprises 186 valid species (Okanishi and Fujita 2013; O'Hara et al. 2017), which accounts for about $10 \%$ of the species of Ophiuroidea.

The ophiuroid skeleton is composed of numerous small ossicles whose shapes and sizes have been intensively used for taxonomy of the class Ophiuroidea (e.g., Fell 1960; Smith et al. 1995; Stöhr et al. 2012). Shapes and arrangements of their superficial ossicles, such as oral plates, adoral shields, and arm plates can be relatively easily observed in the order Ophiurida and superorder Ophintegrida which are the ophiuroids other than euryalids, through their thin integuments, but the order Euryalida has a thick skin on their body surface which conceals the ossicles and impairs their external observation (Baker 1980). In Euryalida, presence/absence or density of superficial external ossicles, and length of very conspicuous radial shields have been used as taxonomic characters (e.g., Lyman 1882; Döderlein 1911; Matsumoto 1917), but they cannot usually be observed without removal of their skin. These characters can vary with growth, and different developmental stages of the same species have been mistakenly described as a different species (e.g., Astrothorax waitei, see Baker 1980; Asteronyx loveni, see Stöhr 2005). Recently, many taxonomists have intentionally removed the skin and observed the various ossicles directly, using the shape and size of each external ossicle, and their arrangements, presence/absence of adoral shields, and layered structure of radial shields as taxonomic characters in Euryalida (e.g., Stöhr 2005; Okanishi and Fujita 2011).

To remove the skin in Euryalida, a solution of sodium hypochlorite has been used which dissolves the epidermis (e.g., Mortensen 1933; Stöhr 2011). As a result, some morphological characters are lost. For example, although intact parts were left after the dissection as ophiuroids are pentaradial, part of the external ossicles and skin of the paratypes of Asterostegus sabineae and Squamophis lifouensis have been lost when treated with bleach to reveal the deeper embedded ossicles (Stöhr 2011; Okanishi and Fujita 2014).

The ossicles which are deep inside the body have been used for higher-level taxonomic characters of Ophiuroidea (e.g., Matsumoto 1917; Smith et al. 1995). Matsumoto (1917) discussed that shapes and numbers of peristomial plates were diagnostic characters to distinguish his four orders of Ophiuroidea, Chilophiurida, Gnathophiurida, Laemophiurida and Phrynophiurida. The peristomial plates have hardly ever been observed or described by subsequent workers, because destructive dissection of the disc is required for their observation. For example, the disc of the holotype of Astrophyton annulatum Matsumoto, 1912 has been split into two halves (see Fujita 2006: Fig. 3D; the picture available also on http://umdb.um.u-tokyo.ac.jp/DDoubutu/invertebrate/ophiuroidea/type.html). Such a destructive method increases the risk of loss of characters, for example jaws, and many taxonomists refrain from applying
destructive methods to specimens. As a consequence not only the peristomial plates, but also oral and dental plates as well as genital plates, and other internal disc ossicles have been observed and described in the type specimens of only a limited number of ophiuroid species.

Micro-computed tomography ( $\mu \mathrm{CT}$ ) is a non-destructive imaging technique using X-ray. This method allows rapid creation of three dimensional (3D) morphological and anatomical images at $\mu \mathrm{m}$ scale resolution of biological materials. The output data can then be analyzed with virtual dissection and with rotation optionally, so that 3D arrangements of complex combinations of materials can be recognized (Faulwetter et al. 2013). Micro-CT technology can be used for dried and wet biological specimens (e.g., ethanol preserved and formalin fixed specimens) and is suitable for imaging of hard materials, such as calcareous skeletons (e.g., Ziegler 2012). While this has been a popular analytical method in paleontology (e.g., Hamada et al. 1991; Hendrickx et al. 2006; Tafforeau et al. 2006; Sutton 2008), the application of $\mu$ CT to morphology and anatomy of extant invertebrate taxa began only recently (Golding and Jones 2006; Greco et al. 2008; Golding et al. 2009; Heim and Nickel 2010; McPeek et al. 2011; Ziegler et al. 2011; Ziegler 2012; Faulwetter et al. 2013; Kohtsuka 2014; Sentoku et al. 2015; Landschoff and Griffiths 2015).

In the Ophiurida and Ophintegrida, $\mu \mathrm{CT}$ observation has been applied to Ophiocomina nigra (Ziegler et al. 2011), Ophiomastix mixta and Ophiarachnella gorgonia (Kohtsuka 2014) and these authors showed horizontal sections and 3D reconstruction images. These images provide evidence that shapes and arrangements of various ossicles can be clearly illustrated non-destructively. Recently, high resolution 3D visualization was performed to observe brooding behavior in three brittle stars, Amphiura capensis, Amphipholis squamata and Ophioderma wahlbergii (Landschoff and Griffiths 2015; Landschoff et al. 2015; Du Plessiss et al. 2015). They successfully observed the positions and postures of brooded juveniles by 3D construction of CT images, but their descriptions were not sufficient for anatomical and/or taxonomical studies. Until now no $\mu \mathrm{CT}$ observations have been performed on species in the order Euryalida.

Asteronyx loveni Müller \& Troschel, 1842 is a very fleshy brittle star and it is very difficult to study the skeletal ossicles embedded in its thick skin. To study skeletal elements of this species, destructive anatomical dissection and dissolution of skin have been employed (e.g., Mortensen 1912; Matsumoto 1917). In the present study, for the first time, we applied $\mu \mathrm{CT}$ scanning to the Euryalida, using Asteronyx loveni to non-destructively observe ossicle morphology at an enough resolution for taxonomic description. We focused our analysis on the shape and arrangements of external ossicles, oral plates and adoral shields because they have scarcely been observed or described in euryalids but are well described for most species of Ophiurida and Ophintegrida as useful taxonomic characters especially from external views. We observed a single vertebral ossicle and illustrated pathways of radial nerve canals and radial water canals in the ossicle, which have never previously been described. Additionally, we tried to observe soft tissues such as muscles, which have not previously been observed by $\mu \mathrm{CT}$ scanning.

## Materials and methods

## Sample preparations

Applying $\mu \mathrm{CT}$ to an entire specimen, an arm fragment from a second specimen, and an isolated vertebra of Asteronyx loveni.

Two specimens of Asteronyx loveni deposited in the National Museum of Nature and Science, Japan (NSMT), were selected for examination (Table 1). The entire body of a specimen (NSMT E-6986, disc diameter 5.8 mm ) and a basal part of an arm of another specimen (NSMT E-5638, disc diameter 10.7 mm ) were air-dried for $\mu \mathrm{CT}$ study. A vertebral ossicle was isolated from the latter specimen by immersion in domestic bleach (approximately 5\% sodium hypochlorite solution), washed in deionized water, and dried in air for digital microscope observation and $\mu \mathrm{CT}$ observation. We examined shapes of a variety of skeletal ossicles and their positional arrangements, internal structures of a vertebra and soft tissues.

Morphological terminology follows Stöhr et al. (2012). Some additional terms for euryalid ophiuroids follow Byrne (1994), Stewart (2000), and Okanishi and Fujita (2014). Especially, we here provide explanation about superficial ossicles of euryalid ophiuroids. Recently, both "epidermal ossicles" and "external ossicles" have been used for those ossicles in descriptions of euryalid ophiuroids (e.g., Okanishi and Fujita 2014; Okanishi 2017). However, "epidermal ossicles" may not be suitable because epidermis is frequently lost in echinoderms. Therefore, we use "external ossicles" for the superficial ossicles in this study.

## $\mu \mathrm{CT}$ observation and 3D reconstructions

A ScanXmate B100TSS110 $\mu$ CT (Comscantecno Co., Ltd.) was used at the University Museum, The University of Tokyo, Japan. Parameters of scanning are shown in Table 2.

3D reconstruction employed Molcer version 1.32 (http://www.white-rabbit. $\mathrm{jp} /$ molcer.html) using image stacks of virtual sections. The single section images were selected by using imageJ software 1.48 ver. (Figs 3B-D; 5B-G; 6D-O). Two kinds of 3D reconstructive images were created by Molcer 1.32: volume rendering and surface rendering. The rendering technique is the computer algorithm used to transform serially acquired CT image data into 3D images. The volume and surface rendering techniques project the 3D data into the 2 D viewing plane from the desired point of view (Rodt et al. 2006). Volume rendering examines the intensity of the objects and the rendering images show all projected materials including internal structures. The surface rendering treats the isosurface from the voxel data. This technique created 3D images composed of polygons, and use sharply shading to show the location of a light source. Thus the surface rendered images only show surficial information for each object, but they enable us to recognize the forms of the materials more clearly. Both surface and volume rendering images were created

Table I. Sampling information of the two examined specimens of Asteronyx loveni.

| Catalog <br> Number | Locality | Water <br> Depth (m) | Date |
| :---: | :---: | :---: | :---: |
| NSMT E-6986 | East China Sea, southwestern Japan, <br> $26^{\circ} 56.30^{\prime} \mathrm{N}, 127^{\circ} 37.00^{\prime} \mathrm{E}$ | 648 | June 1, 2011 |
| NSMT E-5638 | Off Miyako, northeastern Japan, 39 $20.19^{\prime} \mathrm{N}$, <br> $142^{\circ} 51.39^{\prime} \mathrm{E} ;-39^{\circ} 19.22^{\prime} \mathrm{N}, 142^{\circ} 49.17^{\prime} \mathrm{E}$ | $1709-1737$ | November 6, 2007 |

Table 2. Scanning parameters of $\mu \mathrm{CT}$ for the observations of Asteronyx loveni.

| Observed <br> specimen | Source <br> voltage <br> $(\mathbf{k V})$ | Source <br> current <br> $(\boldsymbol{\mu} \mathbf{A})$ | Exposure time <br> for 1 frame $(\mathbf{s e c})$ | Total number <br> of frames | Total time <br> for scanning <br> $(\mathbf{m i n})$ | Detector size <br> $(\mathbf{p i x e l})$ | Resolution <br> $(\boldsymbol{\mu m})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Entire body | 80 | 155 | 1.0 | 1,500 | 25 | $1,024 \times 1,012$ | 15.440 |
| Basal part of <br> an arm | 100 | 100 | 1.2 | 1,200 | 16 | $1,024 \times 1,012$ | 13.759 |
| Isolated <br> vertebral arm <br> ossicle | 75 | 43 | 0.4 | 1,200 | 50 | $1,024 \times 1,012$ | 4.459 |

from the same set of scans. All section images were non-destructively obtained by using "virtual dissection" mode of Molcer version 1.32. Management and storage of CT data was implemented in Morphobank (project 2440, http://www.morphobank.org/; O'Leary and Kaufman 2012). This "project" is to store the images of this paper and not public one.

## Microscopic observations

The specimens were also examined by digital microscopes after $\mu \mathrm{CT}$ observations. The entire specimen and a part of arm were observed with a Keyence VHX 1000. The separated vertebral ossicles were observed and photographed with Keyence VHX D510 using a SEM mode.

## Embedding 3D images into PDFs

Three supplementary PDFs with embedded surface were prepared rendering images of the entire specimen of NSMT E-6986 (Suppl. material 1), a basal portion of an arm (Suppl. material 2) and a vertebra (Suppl. material 3). Molcer version 1.32 and Geomagic Sculpt version V2016.0.38 (3D Systems, Inc.: http://www.geomagic.com/en/ legal/patents-sensable) were used in preparing the 3D data in STL and U3D format, respectively. "Interactive object tool" of Acrobat XI Pro (Adobe System Inc.) was used to embed the images into PDFs.

## Results

Entire specimen (NSMT E-6986). The overall morphology of almost all ossicles was clearly visible in volume rendered images and surface rendered images (e.g., Figs 1A-E, G, H; 2; 3A-C; Suppl. material 1). Details of each ossicle were more clearly visible on surface rendered images than volume rendered images (Fig. 1B, C). In the aboral view of the surface rendered images, radial shields are clearly observed, not reaching to the disc center (Fig. 1E). Radial shields are clearly multilayered (Fig. 1H). The outer edges of radial shields are located on the abradial side of the 4th vertebra (Fig. 1D, E, G, H). Peristomial plates are observed on the aboral side of each oral frame, situated on the aboral side of the 1st vertebra and oral plates, and are oblong, twice as wide as long (Fig. 1F). Smaller additional peristomial plates are present between the larger peristomial plates, variable in shape and one-third to one-fourth of the peristomial plates in length (Fig. 1F). External ossicles in the skin on the aboral disc are circular or oblong and spheroid-shaped. The diameter of external ossicles are larger in the center of disc and smaller in the peripheral disc, except some large ones in the interradial area (Fig. 1E, H), which are separated from each other.

In the surface rendered image of the oral side of the disc, the outer edges of the radial shields are articulated with the outer edges of the adradial genital plates on the abradial side of the 4 th vertebra (Fig. 2B, D). Adradial and abradial genital plates are bar like and latter is much smaller. Adradial and abradial genital plates are articulated on the abradial side of the 3rd vertebra (Fig. 2D). Adoral shields are rectangulare parallelepiped (Fig. 2B), and connect to the first lateral arm plates (Fig. 2B, D). Oral plates are triangular prism, slightly pointed to proximo-oral side, and in contact with each other on the midline of each jaw (Fig. 2B). One small circular madreporite is located on the distal side of the adoral shields (Fig. 2B, D). Spearhead-shaped teeth are situated on the top of the jaw (Fig. 2B, F). Five to six teeth form a vertical row on dental plate, and another parallel row of two or three teeth is also formed in three of five jaws (Fig. 2F). The length of teeth decreases from aboral toward oral side (Fig. 2F). Two or three granule-like oral papillae present on the lateral side of oral plates, and they are not acute, granule-like (Fig. 2F).

Section images are obtained non-destructively (e.g., Fig. 3A, B) and show the positional arrangement of the ossicles, the internal structure of vertebrae, dental plates, and oral plates (Fig. 3B-D). Oral plates are in contact with the first vertebra (Fig. 3B) and the adoral shields are located on the oral side of the oral plates (Fig. 3B). Oblong dental plates are observed on top of the oral plates (Fig. 3B, C). All dental plates are partly vertically fragmented and a vertical crack is observed in one dental plate (Fig. 3B). The adradial genital plates are in contact with the abradial genital plates on the abradial side of the third vertebra (Fig. 3B). An adradial muscular depression is observed on the oral plates (Fig. 3D). External skin and stomach are observed (Fig. 3B-D).

From the surface rendered images, soft tissues such as tentacles are not observed in any $\mu \mathrm{CT}$ images (Fig. 2A-D) and tentacle pore depressions are observed to be formed by lateral arm plate and ventral arm plate (Fig. 2B, D). First to third tentacle pores


Figure I. Microscopic ( $\mathbf{A}, \mathbf{D}, \mathbf{G}$ ), $\mu \mathrm{CT}$ volume rendered $(\mathbf{B})$ and $\mu \mathrm{CT}$ surface rendered $(\mathbf{C}, \mathbf{E}, \mathbf{F}, \mathbf{H})$ images of the entire body of Asteronyx loveni (NSMT E-6986). A-C whole animal, oral view. Black arrow heads indicate longitudinal dorsal midlines of vertebrae and white arrows indicate external ossicles embedded in skin $\mathbf{D}, \mathbf{E}$ whole animal, aboral view $\mathbf{F}$ mouth frame, virtually dissecting aboral view, peristomial plates are colored green $\mathbf{G}, \mathbf{H}$ disc, aboral view. White arrow heads indicate external ossicles and black arrows indicate positions where components of plate shaped ossicles of the radial shields are layered. Oral side of bodies were virtually dissected (H). Abbreviations: RS, radial shields; V, vertebra.
have no arm spine and fourth or more distal tentacle pores have one, ovoid arm spine (Fig. 2D). The arm spines are approximately half the length of the corresponding arm segment. First ventral arm plate is conspicuous on the third arm segment, ellipse and


Figure 2. Microscopic $(\mathbf{A}, \mathbf{C}, \mathbf{E})$ and $\mu \mathrm{CT}$ surface rendered $(\mathbf{B}, \mathbf{D}, \mathbf{F})$ images of the entire body of Asteronyx loveni (NSMT E-6986). A, B a part of disc, oral lateral view C, D basal part of an arm, oral lateral view $\mathbf{E}$ oral disc, oral view. An arrow in $\mathbf{E}$ indicates the orientation of observation of $\mathbf{F} ; \mathbf{F}$ two jaws, lateral view. The other jaws are virtually dissected. Parallel teeth are indicated by numeral with apostrophe or double apostrophe. Abbreviations: ABG, abradial plate; ADG, adradial plate; ASH, adoral shield; ASP, arm spine; GSL, genital slit; LAP, lateral arm plate; M, madreporite; OPA, oral papilla; OPL, oral plate; RS, radial shield; TC, tentacle; TO, tooth; TP, tentacle pore; V, vertebra; VAP, ventral arm plate.


Figure 3. Micro CT surface rendered (A) and 2D section (B-D) images of the entire body of Asteronyx loveni (NSMT E-6986). A oral view, section planes of figures B-D are shown by dotted tetragons B disc and basal part of an arm, horizontal section, a white arrow head indicates vertical crack of a dental plate, a black arrow head indicates the indistinct border of a 1 st vertebrae and an oral plate $\mathbf{C}$ disc and basal part of an arm, vertical section of central disc $\mathbf{D}$ disc and basal part of an arm, vertical section of abradial disc. Abbreviations: AMD, adradial muscular depression; ABG, abradial plate; ADG, adradial plate; ASH, adoral shield; DP, dental plate; ES, external skin; LAP, lateral arm plate; OPL, oral plate; RNC, radial nerve canal; RS, radial shield; RWC, radial water canal; S, stomach; TO, tooth; V, vertebra; VAP, ventral arm plate.


Figure 4. Microscopic $(\mathbf{A}), \mu \mathrm{CT}$ volume rendered $(\mathbf{B})$ and surface rendered (C, D) images of basal part of arm (7-13th arm segments) of Asteronyx loveni (NSMT E-5638). Oral view. Upper and lower sides indicate distal and basal orientation, respectively. Oral side is virtually dissected in $\mathbf{D}$ Inter-vertebral muscles are colored purple in $\mathbf{D}$ Black arrow heads in $\mathbf{B}$ and $\mathbf{D}$ indicate identical vertebra, and ST and AS labels also indicates the identical characters in $\mathbf{A}, \mathbf{B}$ and $\mathbf{C}$. Abbreviations: ASP, arm spine; LAP, lateral arm plate; ST, secondary teeth.
flat (Fig. 2B, D). From fourth arm segment, rudimentary ventral arm plates are observed and they decreased in size distally.

Stereom structure of the ossicles are not observed but difference in density of ossicles, which depends on the volume of stereom interstices is recognized by volume rendered images (Fig. 1B). For example, the longitudinal median line of vertebrae is more whitish than the other parts of the vertebrae (Fig. 1B). This indicates that stereom with reduced pores and interstices are smaller, and higher density in the median part of vertebrae (Figs 1B; 6D-O). In 2D section images, internal canals of vertebrae are observed (Fig. 3B, C).

Arm specimen (NSMT E-5638). Position of articulation for arm spines of lateral arm plates and detailed shapes of arm spines are observed on volume rendered images without any virtual dissections (Fig. 4A, B), but not on surface rendered images. In the latter case, dried and shrunken thick skin on the arm is detected by X-ray (see also Figs 4D; 5A; Suppl. material 2). The thick skin conceals surface features of the ossicles. Lateral arm plates, arm spines, and vertebrae are observed by 2 D section images (Fig. 5B-G). Lateral arm plates are located on the oral lateral side of vertebrae, bar-like, but slightly curved to around the vertebra, approximately twice long wide (Fig. 5B-G). Arm spines are hook-shaped with several (two to five) secondary teeth (Fig, 4B). External ossicles are observed in the skin on the aboral side, small, granuleshaped (Fig. 5B). Inter-vertebral muscles are observed by virtual dissection of surface rendered images (Figs 4D; 5B, C, E, F).

Two pairs of canals are observed inside vertebrae: radial nerve canals and radial water canals (Figs 3B, C; 5B-G). In lateral arm plates, up to five nerve canals are observed on the oral side of each lateral arm plate (Fig. 5D, E, G). The number of arm spines corresponded to the number of nerve canals (Fig. 5B, D, E, G).


Figure 5. Micro CT surface rendered $(\mathbf{A})$ and 2D section (B-G) images of the basal part of arm (7-13th arm segments) of Asteronyx loveni (NSMT E-5638). A basal lateral view. Section planes of figures B-G are shown by dotted tetragons $\mathbf{B}$ vertical longitudinal section, adradial side of the arm $\mathbf{C}, \mathbf{D}$ horizontal longitudinal section, aboral view $\mathbf{C}$ is set on more aboral side than $\mathbf{D}$ horizontal longitudinal section, oral view E-G transverse vertical sections from basal $(\mathbf{E})$ to distal $(\mathbf{G})$ arm, basal view. Vertebrae, lateral arm plates, arm spines and muscles are colored red, blue, yellow and purple, respectively. Red and blue arrow heads $(\mathbf{B}-\mathbf{G})$ indicate radial nerve canals and radial water canals, respectively. Arrows indicate the orientations (ab, aboral; ba, basal; dis, distal; or, oral). Abbreviations: E, epidermis; EO, external ossicle.

Isolated vertebra (NSMT E5638). Moderate resolution ( $4.5 \mu \mathrm{~m}$ ) images are obtained for the isolated vertebra (Fig. 6B-O, Suppl. material 3). Stereom structure is clearly observed in surface rendered images (Fig. 6B). The resolution of the surface rendered images is equivalent to that of SEM images (Fig. 6A). Detailed morphology of radial nerve canals and radial water canals are observed (Fig. 6D-O). A pair of radial water canals opened into the basal part of the oral groove of the vertebra (Fig. 6C; Suppl. material 3), and a radial water canal connected to a depression for tube feet opens on the oral lateral side of the vertebra (Fig. 6D-I). A pair of radial nerve canals opens in a distal position to the oral groove of the vertebra (Fig. 6C). The radial nerve canals extend to the distal side and dead-ended inside the vertebra (Fig. 6K-O).


Figure 6. SEM $(\mathbf{A}), \mu \mathrm{CT}$ surface rendering $(\mathbf{B}, \mathbf{C})$ and 2 D section ( $\mathbf{D}-\mathbf{O}$ ) images of the isolated vertebral ossicles of Asteronyx loveni (NSMT E-5638). A, B basal view, arrow heads indicate oral groove C oral view D-O transverse vertical sections from basal (D) to distal (O) arm, basal view. Arrow heads indicate radial water canals (D-I) and radial nerve canals (K-O). Arrows indicate the orientations (ab, aboral; ba, basal; dis, distal; or, oral). Abbreviations: RNC, pore of radial nerve canal; RWC, pore of radial water canal.

## Discussion

In the present study, the shapes, numbers, and arrangement of various ossicles of $A s$ teronyx loveni were successfully observed by $\mu$ CT (Figs 1H, 2D, 3, 4B and 5; Suppl. materials 1, 2). The shape, size and arrangement of external ossicles were recently employed as species-level diagnostic characters for euryalids (e.g., Okanishi and Fujita 2009; Okanishi and Fujita 2011), however to examine these characters, once ossicles were extracted by bleaching, their positional relationships to each other could not be obtained. These characteristics of external ossicles were simultaneously observed by $\mu \mathrm{CT}$ without the need to extract them destructively (Fig. 1E, H). Their shape was circular, the size differed depending on the position in the disc, and they were slightly separated from one another (Fig. 1G, H).

Presence of adoral shields is an important diagnostic character of the genus Asteronyx (Stöhr 2005). Adoral shields of Asteronyx were observed by dissolving the surrounding skin in previous studies (e.g., Mortensen 1912; Stöhr 2005), but they were clearly observed non-destructively in this study (Fig. 2B, D; Suppl. material 1). Two types of ossicle components of radial shields have been known for Euryalida. Astrocharidae has monolayer radial shields and the other families have multilayer radial shields (Okanishi et al. 2011). These shield characters had not previously been recognized without removing the skin with external ossicles of the disc (e.g., Okanishi and Fujita 2009; Stöhr 2011). In this study, $\mu \mathrm{CT}$ observation clearly showed that Asteronyx loveni has multilayer radial shields and one radial shield is composed of at least eight plates (Fig. 1H).

Moreover, shapes and 3D positional relationships of radial shields, adradial and abradial genital plates, and the shapes and number of peristomial plates were also successfully observed (Figs 1F; 2A, B). These characters have been considered important for the identification of (sub)orders of Ophiuroidea (e.g., Matsumoto 1917; Smith et al. 1995), but they have been examined for only a very small number of species, and only by destructive dissection. Additional small plates between the peristomial plates were here observed for the first time in Ophiuroidea (Fig. 1F). The above taxonomically important characters of the ossicles have not been confirmed for many type specimens of Ophiuroidea but this study showed they can be easily observed by $\mu \mathrm{CT}$ scanning. This study has shown that $\mu \mathrm{CT}$ is a powerful tool for species- to order-level taxonomy in Ophiuroidea as Ziegler (2012) suggested. Considering that specimens remain non-dissected following $\mu \mathrm{CT}$ observations (Figs 1A, D, G; 2A, C, E; 4A; 6A), this tool is ideal for observing type specimens.

Recently, the micromorphology of the ossicle surface (e.g., articulation forms of lateral arm plates) have become heavily used as taxonomic characters of ophiuroids (e.g., Stöhr et al. 2008; Stöhr and Muths 2010; Okanishi and Fujita 2009, 2011, 2013; Martynov 2010; Thuy and Stöhr 2011; Gondim et al. 2015; Thuy and Stöhr 2016). On the other hand, internal structures of ossicles, such as radial water canals and radial nerve canals in vertebrae, have scarcely been observed and their taxonomic significance has never been discussed. In this study, radial water canals and radial nerve canals in the vertebra, as well as nerve canals in lateral arm plates and arm spines, were observed (Figs 3B, D; 5B-G; 6D-O). The resolution of $\mu$ CT images in this study (ca. $4.5-15.5 \mu \mathrm{~m}$ ) was
high enough to observe the density of interstices of stereom structure, and the detailed pathway of radial water canals and radial nerve canals were recognized (Fig. 6D-O). Martynov et al. (2015) observed the canals by serial cross-sections of resin embedded specimens. However, this method is time-consuming and destructive. In the present setting, the total time required for scanning and reconstruction of 3 D images ranged from 16 to 50 minutes, much shorter than the time used for cross-sectioning methods.

In this study, inter-vertebral muscles of the dried specimen were observed along with its ossicles (Figs 4D; 5B, C, E, F), which is the first observation of ophiuroid muscles by $\mu \mathrm{CT}$. Muscles might be increased in density by shrinking when dried, making them detectable by X-ray.

The most novel and remarkable aspects of this study is that complete morphological information of all fundamental ossicles of the order Euryalida was successfully obtained from $\mu \mathrm{CT}$ observations. Micro CT observation has increased the number of available taxonomic characters, which have hardly ever been observed and/or never explored. These taxonomic characters obtained in Euryalida may be compared to those in the order Ophiurida and the superorder Ophintegrida which should accelerate future taxonomic study of the class Ophiuroidea.

## Acknowledgements

We wish to express our sincere gratitude to Dr. David Pawson of the Smithsonian Institution, National Museum of Natural History for his critical reading of the earlier manuscript and constructive comments. The material for this study was collected by R/Vs Tansei-Maru of JAMSTEC and T/S Nagasaki-Maru of the Nagasaki University. Thanks are also extended to Dr. Eri Katayama of the National Museum and Nature and Science and Dr. Yoichi Ezaki of the Osaka City University for their assistance in study and photography of the specimens with VHX D5 10 and VHX 1000, and Dr. Asuka Sentoku of the University of Queensland for her helpful comments on image processing and on preparation of figure plates.

This work was financially supported by grants from the Research Institute of Marine Invertebrates (Tokyo, Japan), from the Japan Science Society (Tokyo, Japan), from the Japan Society for the Promotion of Science (Research fellowships for Young Scientists No. 22506, Scientific Research (C) Nos. 22570104 and 25440226, Grants-in-Aid for challenging Exploratory Research No. 15K14589), and from the Director General of National Museum of Nature and Science, Japan. Financial support was also obtained from Hideki Aso, Takashi Hamaji, Akiko Iijima, Ayumi Irisawa, Masakazu Jimbo, Yuji Kamiya, Hideo Konami, Hiroyuki Kurokawa, Ayako Matsuda, Takamasa Mikami, Toshiharu Mitsuhashi, Morichika Miyazaki, Hironobu Muragaki, Haruyo Nakayama, Masaru Nakano, Kazunori Okubo, Yoshiko Ooiwa, Hirotaka Osawa, Tsuyoshi Sakamoto, Ryosuke Shibato, Emiko Shishido, Masataka Shishido, Hitomi Suto, Syotaro Suzuki, Yumeko Taguchi, Yuki Tokuda, Hiroko Uchida, Chie Yamaura, Koga Yohei and Yuki Yoshimine, via 'academist', crowd funding site for scientific research.

## References

Baker AN (1980) Euryalinid Ophiuroidea (Echinodermata) from Australia, New Zealand and the south-west Pacific Ocean. New Zealand Journal of Zoology 7: 11-83. https://doi.org/ 10.1080/03014223.1980.10423763

Byrne M (1994) Ophiuroidea. In: Harrison FW, Chia F-S (Eds) Microscopic Anatomy of Invertebrates, Vol. 14. Echinodermata. Wiley-Liss, New York, 247-343.
Döderlein L (1911) Über japanische und andere Euryalae. Abhandlungen der Bayerischen Akademie der Wissenschaften 2: 1-123.
Du Plessis A, Griffith CL, Landschoff J (2015) 3-dimensional microCT reconstructions of brooding brittle stars. GigaScience Database. https://doi.org/10.5524/100130
Faulwetter S, Vasileiadou A, Kouratoras M, Dailianis T, Arvanitidis C (2013) Micro-computed tomography: Introducing new dimensions to taxonomy. Zookeys 263: 1-45. https:// doi.org/10.3897/zookeys. 263.4261
Fell HB (1960) Synoptic keys to the genera of Ophiuroidea. Zoology Publications from Victoria University of Wellington 26: 1-44.
Fujita T (2006) Tokyo-Daigaku Sogo Hakubutsukan Shozou Kumohitode Hyohon ni Tsuite. The University Museum, the University of Tokyo, material reports 62: 135-150. [In Japanese]
Golding RE, Jones AS (2006) Micro-CT as a novel technique for 3D reconstruction of molluscan anatomy. Molluscan Research 27: 123-128.
Golding RE, Ponder WF, Byrne M (2009) Three-Dimensional reconstruction of the odontophoral cartilages of Caenogastropoda (Mollusca: Gastropoda) using Micro-CT: Morphology and phylogenetic significance. Journal of Morphology 270: 558-587. https://doi. org/10.1002/jmor. 10699
Gondim AE, Dias TLP, Christoffersen ML, Stöhr S (2015) Redescription of Hemieuryale pustulata von Martens, 1867 (Echinodermata, Ophiuroidea) based on Brazilian specimens, with notes on systematics and habitat association. Zootaxa 3925: 341-360. https://doi. org/10.11646/zootaxa.3925.3.2
Greco M, Jones A, Spooner-Hart R, Holford P (2008) X-ray computerized microtomography (MicroCT): A new technique for assessing external and internal morphology of bees. Journal of Apicultural Research 47: 286-291. https://doi.org/10.1080/00218839.2008 .11101476
Hamada T, Tateno S, Suzuki N (1991) Three dimensional reconstruction of fossils with Xray CT and computer graphics. Scientific Papers of the College of Arts and Sciences, the University of Tokyo 41: 107-118.
Heim I, Nickel M (2010) Description and molecular phylogeny of Tethya leysae sp. nov. (Porifera, Demospongiae, Hadromerida) from the Canadian Northeast Pacific with remarks on the use of microtomography in sponge taxonomy. Zootaxa 2422: 1-21.
Henderickx H, Cnudde V, Masschaele B, Dierick M, Vlassenbroeck J, Van-Hoorebeke L (2006) Description of a new fossil Pseudograpus (Pseudoscorpiones: Pseudogarypidae) with the use of X-ray micro-CT to penetrate opaque amber. Zootaxa 1305: 41-50.
Kohtsuka H (2014) Roles of the technical staffs at Misaki Marine Biological Station. Taxa Proceedings of the Japanese Society of Systematic Zoology 36: 24-32. [In Japanese]

Landschoff J, Du Plessis A, Griffiths CL (2015) A dataset describing brooding in three species of South African brittle stars, comprising seven high-resolution, micro X-ray computed tomography scans. GigaScience 4: 52. https://doi.org/10.1186/s13742-015-0093-2
Landschoff J, Griffith CL (2015) Three-dimensional visualization of brooding behavior in two distantly related brittle stars from South African waters. African Journal of Marine Science 37(4): 533-541. https://doi.org/10.2989/1814232X.2015.1095801
Lyman T (1882) Report on the Ophiuroidea dredged by H.M.S. Challenger during the years 1873-1876. Report on the scientific result of the voyage of H.M.S. Challenger during the years 1873-1876 Zoology 5: 1-386.
Märkel K, Röser U (1985) Comparative morphology of echinoderm calcified tissue: Histology and ultrastructure of ophiuroid scales (Echinodermata, Ophiuroida). Zoomorphology 105: 197-207. https://doi.org/10.1007/BF00312157
Martynov A (2010) Reassessment of the classification of the Ophiuroidea (Echinodermata), based on morphological characters. I. General character evaluation and delineation of the families Ophiomyxidae and Ophiacanthidae. Zootaxa 2697: 1-154.
Martynov A, Ishida Y, Irimura S, Tajiri R, O’Hara T, Fujita T (2015) When ontogeny matters: a new Japanese species of brittle star illustrates the importance of considering both adult and juvenile characters in taxonomic practice. PLoS ONE 10(10): e0139463. https://doi. org/10.1371/journal.pone. 0139463
Matsumoto H (1917) A monograph of Japanese Ophiuroidea, arranged according to a new classification. Journal of the College of Science, Imperial University of Tokyo 38: 1-408.
McPeek MA, Symes LB, Zong DM, McPeek CL (2011) Species recognition and patterns of population variation in the reproductive structures of a damselfly genus. Evolution 65: 419-428. https://doi.org/10.1111/j.1558-5646.2010.01138.x
Mortensen T (1912) Über Asteronyx loveni M. Tr. Zeitschrift für wissenschaftliche Zoologie 101: 264-289.
Mortensen T (1933) Studies of Indo-Pacific euryalids. Videnskabelige Meddelelser fra Naturhistorisk Forening i København 96: 1-75.
O'Hara TD, Hugall AF, Thuy B, Moussalli A (2014) Phylogenetic resolution of the class Ophiuroidea unlocks a global microfossil record. Current Biology 24: 1874-1879. https:// doi.org/10.1016/j.cub.2014.06.060
O’Hara TD, Hugall AF, Thuy B, Stöhr S, Martynov AV (2017) Restructuring higher taxonomy using broad-scale phylogenomics: The living Ophiuroidea. Molecular Phylogenetics and Evolution 107: 415-430. https://doi.org/10.1016/j.ympev.2016.12.006
Okanishi M, Fujita T (2009) A new species of Asteroschema (Echinodermata: Ophiuroidea: Asteroschematidae) from southwestern Japan. Species Diversity 14: 115-129.
Okanishi M, Fujita T (2011) A taxonomic review of the genus Astrocharis Koehler (Echinodermata: Ophiuroidea: Asteroschematidae) with a description of a new species. Zoological Science 28: 148-157. https://doi.org/10.2108/zsj. 28.148
Okanishi M, O’Hara TD, Fujita T (2011) Molecular phylogeny of the order Euryalida (Echinodermata: Ophiuroidea), based on mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution 61: 392-399. https://doi.org/10.1016/j.ympev.2011.07.003

Okanishi M, Fujita T (2013) Molecular phylogeny based on increased number of species and genes revealed more robust family-level systematics of the order Euryalida (Echinodermata: Ophiuroidea). Molecular Phylogenetics and Evolution 69: 566-580. https://doi. org/10.1016/j.ympev.2013.07.021
Okanishi M, Fujita T (2014) A taxonomic review of the genus Asterostegus (Echinodermata: Ophiuroidea: Euryalidae). European Journal of Taxonomy 76: 1-18.
Okanishi M (2017) A taxonomic review of the genus Astrohelix Döderlein, 1930 including the synonymy of the subgenus Asteroporpa (Astromoana) Baker, 1980 to Astrohelix. Zootaxa 4227(4): 543-553. https://doi.org/10.11646/zootaxa.4227.4.4
O’Leary MA, Kaufman SG (2012) MorphoBank 3.0: Web application for morphological phylogenetics and taxonomy. http://www.morphobank.org [Accessed in 2016 June 8]
Rodt T, Bartling SO, Zajaczek JE, Vafa MA, Kapapa T, Majdani O, Krauss JK, Zumkeller M, Matthies H, Becker H, Kaminsky J (2006) Evaluation of surface and volume rendering in 3D-CT of facial fractures. Dentomaxillofacial Radiology 35: 227-231. https://doi. org/10.1259/dmfr/22989395
Sentoku A, Morisaki H, Matsumoto S, Ohno R, Tomiyama T, Ezaki Y (2015) Internal skeletal analysis of the clonial azooxanthellate scleractinian Dendrophyllia cribrosa using microfocus X-ray CT images: Underlying basis for its rigid and highly adaptive colony structure. Journal of Structural Biology 189: 37-43. https://doi.org/10.1016/j.jsb.2014.11.002
Smith AB, Paterson GLJ, Lafay B (1995) Ophiuroid phylogeny and higher taxonomy: Morphological, molecular and palaeontological perspectives. Zoological Journal of the Linnean Society 114: 213-243. https://doi.org/10.1111/j.1096-3642.1995.tb00117c.x
Stewart B (2000) Anatomical features of the euryalid snake star Astrobrachion constrictum (Ophiuroidea; Asteroschematidae). Invertebrate Biology 119: 222-233. https://doi. org/10.1111/j.1744-7410.2000.tb00009.x
Stöhr S (2005) Who's who among baby brittle stars (Echinodermata: Ophiuroidea): postmetamorphic development of some North Atlantic forms. Zoological Journal of the Linnean Society 143: 543-576. https://doi.org/10.1111/j.1096-3642.2005.00155.x
Stöhr S, Conand C, Boissin E (2008) Brittle stars (Echinodermata: Ophiuroidea) from La Réunion and the systematic position of Ophiocanops Koehler, 1922. Zoological Journal of the Linnean Society 153: 545-560. https://doi.org/10.1111/j.1096-3642.2008.00401.x
Stöhr S, Muths G (2010) Morphological diagnosis of the two genetic lineages of Acrocnida brachiate (Echinodermata: Ophiuroidea) with description of a new species. Journal of the Marine Biological Association of the United Kingdom 90(4): 831-843. https://doi. org/10.1017/S0025315409990749
Stöhr S (2011) New records and new species of Ophiuroidea (Echinodermata) from Lifou, Loyalty Islands, New Caledonia. Zootaxa 3089: 1-50.
Stöhr S, O'Hara TD, Thuy B (2012) Global diversity of brittle stars (Echinodermata: Ophiuroidea). PLoS ONE 7(3): e31940. https://doi.org/10.1371/journal.pone. 0031940
Sutton MD (2008) Tomographic techniques for the study of exceptionally preserved fossils. Proceedings of the Royal Society B-Biological Science 275: 1587-1593. https://doi. org/10.1098/rspb.2008.0263

Tafforeau P, Boistel, R, Boller E, Bravin A, Brunet M, Chaimanee Y, Cloetens P, Feist M, Hoszowska J, Jaeger JJ, Kay RF, Lazzari V, Marivaux L, Nel A, Nemoz C, Thibault X, Vignaud P, Zambler, S (2006) Applications of X-ray synchrotron microtomography for non-destructive 3D studies of paleontological specimens. Applied Physics A 83: 195-202. https://doi.org/10.1007/s00339-006-3507-2
Thuy B, Stöhr S (2011) Lateral arm plate morphology in brittle stars (Echinodermata: Ophiuroidea): new perspectives for ophiuroid micropalaeontology and classification. Zootaxa 3013: 1-47.
Thuy B, Stöhr S (2016) A new morphological phylogeny of the Ophiuroidea (Echinodermata) accords with molecular evidence and renders microfossils accessible for cladistics. PLoS ONE 11(5): e0156140. doi:10.1371/journal.pone. 0156140
Ziegler A, Kunth M, Mueller S, Bock C, Pohmann R, Schröder L, Faber C, Giribet G (2011) Application of magnetic resonance imaging in zoology. Zoomorphology 130: 227-254. https://doi.org/10.1007/s00435-011-0138-8
Ziegler A (2012) Broad application of non-invasive imaging techniques to echinoids and other echinoderm taxa. Zoosymposia 7: 53-70.

## Supplementary material I

## Figure S1

Authors: Masanori Okanishi, Toshihiko Fujita, Yu Maekawa, Takenori Sasaki
Data type: 3D model
Explanation note: The interactive 3D model of $\mu \mathrm{CT}$ surface rendering images of the entire body of Asteronyx loveni (NSMT E-6986). This image can be activated by clicking on the image in Adobe Acrobat Reader (version 8 or higher) and can be rotated, moved and magnified.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

## Supplementary material 2

## Figure S2

Authors: Masanori Okanishi, Toshihiko Fujita, Yu Maekawa, Takenori Sasaki
Data type: 3D model
Explanation note: The interactive 3D model of $\mu$ CT surface rendering images of the basal part of an arm of Asteronyx loveni (NSMT E-5638).
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

## Supplementary material 3

Figure S3
Authors: Masanori Okanishi, Toshihiko Fujita, Yu Maekawa, Takenori Sasaki
Data type: 3D model
Explanation note: The interactive 3D model of $\mu \mathrm{CT}$ surface rendering images of the isolated vertebral ossicles of Asteronyx loveni (NSMT E-5638).
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

# Molecular assessment of three species of Anilocra (Isopoda, Cymothoidae) ectoparasites from Caribbean coral reef fishes, with the description of Anilocra brillae sp. n . 

Rachel L. Welicky', Kerry A. Hadfield', Paul C. Sikkel ${ }^{1,2}$, Nico J. Smit ${ }^{1}$<br>I Water Research Group, Unit for Environmental Sciences and Management, Potchefstroom Campus, NorthWest University, Private Bag X6001, Potchefstroom, 2520, South Africa 2 Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, AR, 72467, USA<br>Corresponding author: Rachel L. Welicky (Rachel.Welicky@nwu.ac.za)

Academic editor: P.B. Araujo | Received 6 December 2016 | Accepted 13 March 2017 | Published 27 March 2017
http://zoobank.org/DCDEFE31-8F34-431C-9136-A149DBD486AD
Citation: Welicky RL, Hadfield KA, Sikkel PC, Smit NJ (2017) Molecular assessment of three species of Anilocra (Isopoda, Cymothoidae) ectoparasites from Caribbean coral reef fishes, with the description of Anilocra brillae sp. n. ZooKeys 663: 21-43. https://doi.org/10.3897/zookeys.663.11415


#### Abstract

A morphological review and molecular characterization of Anilocra haemuli Bunkley Williams \& Williams, 1981, were completed using specimens collected from Haemulon flavolineatum Desmarest, 1823 (French grunt) and Epinephelus guttatus Linnaeus, 1758 (red hind). Molecular and morphological data suggest that the isopods parasitizing H. flavolineatum and $E$. guttatus are different species. The specimens collected from E. guttatus are recognized as a new species, Anilocra brillae sp. n. Differences between Anilocra brillae sp. n. and $A$. haemuli include but are not limited to the pleonites $1-3$ of $A$. brillae $\mathbf{s p}$. $\mathbf{n}$. being wider than 4-5 and 4-5 subequal, whereas the pleonites 1-2 of $A$. haemuli are wider than $3-5$, and $3-5$ are subequal. The seventh pereopod of $A$. brillae $\mathbf{s p}$. $\mathbf{n}$. is proportionally larger, has more robust setae, and the setae are distributed more extensively over the articles when compared to $A$. haemuli. Additionally, this study provides the first genetic characterization of three Anilocra spp. from the Caribbean, and is based on mitochondrial cytochrome c oxidase subunit gene (COI) for $A$. haemuli from $H$. flavolineatum, A. brillae sp. n. from E. guttatus, and A. chromis Bunkley Williams \& Williams, 1981 from Chromis multilineata Guichenot, 1853.


## Keywords

Anilocra haemuli, Anilocra chromis, brown chromis, Caribbean, coral reef, Cymothoidae, fish ectoparasite, French grunt, Isopoda, molecular analysis, new species, parasite, red hind, taxonomy

## Introduction

In the past half-century, taxonomic studies on the fish parasitic isopod genus Anilocra Leach, 1818, have reported nine species from the Caribbean (Bunkley Williams and Williams 1981) and 12 species from Australia (Bruce 1987). This genus of parasite parasitizes the external surfaces of marine fish hosts that inhabit subtropical, tropical, and temperate waters (Smit et al. 2014). Host specificity of species of Anilocra is highly variable, such that different Caribbean Anilocra have been identified as family, genus, and species specific (i.e. Bunkley Williams and Williams 1981, Bruce 1987). For example, Anilocra holocentri Bunkley Williams \& Williams, 1981 has been reported only to infest Holocentrus adscensionis Osbeck, 1765, whereas Anilocra chaetodontis Bunkley Williams \& Williams, 1981 has been reported to infest four members of the genus Chaetodon Linnaeus, 1758. Anilocra baemuli Bunkley Williams \& Williams, 1981 is the only Caribbean species reported to infest fishes from two families: Haemulidae and Serranidae. Anecdotal accounts from both parasitologists and ecologists suggest that records of $A$. haemuli from Haemulids and Serranids may in fact be two species given the differences in the biology and ecology of these host fishes.

To evaluate this claim a review of Anilocra haemuli morphology using specimens from both the Haemulidae and Serranidae families is warranted. The original description of $A$. haemuli was published before molecular approaches were used to aid in confirming the morphological classification of organisms. In the original description, careful attention was taken to describe $A$. haemuli as type specimens were collected from the same host and locality (Bunkley Williams and Willams 1981). Nevertheless, multiple morphologically similar species of Anilocra may have been identified as $A$. haemuli because there was no other method to verify if these specimens represented multiple species.

An increasing number of ecological studies are using Anilocra to understand trophic level dynamics (Roche et al. 2013, Binning et al. 2014), and $A$. haemuli infestation has been associated with altering H. flavolineatum behavior and condition (Welicky and Sikkel 2014, 2015, Welicky et al. in press). To facilitate future ecological and evolutionary studies on Anilocra-host interactions, the identity of Anilocra haemuli is here validated using both a morphological redescription and a molecular analysis.

## Materials and Methods

## Specimen collection

In August 2016, Epinephelis guttatus Linnaeus, 1758, (family Serranidae) ( $\mathrm{n}=8$ ) parasitized by a cymothoid isopod of the genus Anilocra were collected by free-divers using a modified cast net (Sikkel et al. 2004, 2006, Welicky et al. 2013) from Guana Island, British Virgin Islands (BVI). The Anilocra specimens were removed from host fish
using forceps and then stored in $80 \%$ ethanol. Anilocra haemuli from H. flavolineatum Desmarest, 1823, (family Haemulidae) (St. John, USVI, $\mathrm{n}=4$, 2011; $\mathrm{n}=2$, 2012; n = 1, 2013; Guana Island, BVI, $\mathrm{n}=1,2012 ; \mathrm{n}=2$, 2013; St. Thomas, USVI, $\mathrm{n}=2$ ) were collected in a similar manner as part of other studies, and initially frozen and then preserved in $80 \%$ ethanol. To include a third and more morphologically distinct Anilocra sp., Anilocra chromis Bunkley Williams \& Williams, 1981, infesting Chromis multilineata Guichenot, 1853 (St. John USVI, $\mathrm{n}=8,2012-2013$ ) were also collected. These were collected in a similar manner to those of $A$. haemuli from $H$. flavolineatum.

## Molecular analysis

Of the specimens collected, genomic DNA was extracted from eight Anilocra from $E$. guttatus, seven $A$. haemuli from H. flavolineatum, and eight $A$. chromis from $C$. multilineata using a rapid DNA extraction method as described in the KAPA Express Extract Kit (Kapa Biosystems, Cape Town, South Africa). Polymerase chain reactions (PCR) were used to amplify a 710 basepair fragment of the mitochondrial cytochrome c oxidase subunit gene (COI) using the primer sets LCO 1490 and HCO 2198 (Folmer et al. 1994). PCR was performed using $12.5 \mu \mathrm{l}$ Thermo Scientific DreamTaq PCR master mix $(2 \times)(2 \times$ DreamTaq buffer, 0.4 mM of each dNTP , and 4 mM MgCl 2$)$, $1.25 \mu \mathrm{l}$ of each primer, $1 \mu \mathrm{DNA}$, and $9 \mu \mathrm{l}$ of PCR-grade nuclease free water (Thermo Scientific, Vilnius, Lithuania). Total volume per reaction was $25 \mu \mathrm{l}$, and PCR reactions were conducted using a ProFlex ${ }^{\text {TM }}$ PCR thermal cycler (Applied Biosystems by Life Technologies). Reactions were amplified under the following PCR conditions: Stage $1,94^{\circ} \mathrm{C}$ for 5 min , Stage 2,36 cycles of $94^{\circ} \mathrm{C}$ for $30 \mathrm{~s}, 47^{\circ} \mathrm{C}$ for $50 \mathrm{~s}, 72^{\circ} \mathrm{C}$ for 2 min , and Stage $3,72^{\circ} \mathrm{C}$ for 10 min . PCR products were sent to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) for purification and sequencing in both directions. Obtained sequences were assembled, and chromatogram-based contigs were generated using Geneious Ver. 9.1. Sequences were aligned and trimmed to the length of the shortest sequence using MEGA 7 bioinformatics software program (http://www.megasoftware.net)

Using BLASTn (Basic Local Alignment Search Tool; http://www.ncbi.nlm. nih.gov/blast), the obtained sequences were verified as belonging to the Isopoda. Pair-wise distance (p-distance) using the Kimura 2-parameter model and nucleotide differences were determined in MEGA7. Supplemental comparisons among the sequences of this study and those available for Anilocra sp. from GenBank were also determined. Newly-generated sequences for Anilocra spp. were deposited in GenBank under the accession numbers: A. haemuli: KY562752, KY562753, KY562754, KY562755, KY562756, KY562757, KY562758; A. brillae sp. n.: KY562744, KY562745, KY562746, KY562747, KY562748, KY562749, KY562750, KY562751; A. chromis: KY562736, KY562737, KY562738, KY562739, KY562740, KY562741, KY562742, KY562743.

## Morphological data

Anilocra haemuli from Haemulon flavolineatum and Anilocra from Epinephelus guttatus were examined using material previously collected by Ernest Williams and Lucy BunkleyWilliams during 1976-1977 and 1983 and reported in Bunkley-Williams and Williams (1981). Additionally, specimens from each host were collected using the aforementioned methods as part of other studies conducted in the US Virgin Islands (USVI) and British Virgin Islands (BVI) during 2011-2016. Isopods were processed according to the techniques described in Hadfield et al. (2010, 2013). Descriptions were prepared using DELTA (Descriptive Language for Taxonomy, Coleman et al. 2010) using a general character set for the Cymothoidae (Hadfield et al. 2014, 2016). Ratios and measurements were rounded off to one decimal place and were made using maximum values of the specific measured article. Ratios and measurements were taken from the female ( $\uparrow$ ) and transitional stage (TS) specimens used for the drawings and presented herein as figures. Pleotelson length (TL) and width (W) for all specimens examined are reported. All measurements are reported in milliimeters (mm). Classification follows Brandt and Poore (2003).

## Results

## Molecular analyses

Comparative sequence analysis indicated that there were three distinct species present in the samples based on the host species, A. haemuli from H. flavolineatum, A. chromis from C. multilineata and another undescribed species of Anilocra from E. guttatus. The intraspecific divergence observed within species was as follows: A. haemuli, 1-6 nt ( $0.6 \%$ ); A. sp. n., $1-4$ nt ( $0.3 \%$ ); and $A$. chromis, $1-6$ nt ( $0.7 \%$ ) (Suppl. materials 1 and 2). The interspecific divergence between pairs of Anilocra spp. was as follows: $A$. haemuli and A. sp. n., 12-19 nt (4\%); A. haemuli and A. chromis, 31-37 nt (9\%); and A. chromis and A. sp. n., 31-37 nt (8\%) (Suppl. materials 1 and 2). The interspecific divergence ranged between $104-109$ nt ( $30 \%$ ) for all of our specimens combined and those available on GenBank (Suppl. materials 1 and 2).

## Taxonomy

## Genus Anilocra Leach, 1818

Anilocra Leach, 1818: 348, 350. Desmarest 1825: 306; Edwards 1840: 255; Dana 1853: 747; Schioedte and Meinert 1881: 100; Gerstaecker 1882: 231; Richardson 1905: 25; Hale 1926: 210; Schultz 1969: 153; Kensley 1978: 78; Kussakin 1979: 281; Brusca 1981: 140; Brusca and Iverson 1985: 45. Bruce 1987: 89; Trilles 1975: 303; Trilles 1994: 55; Thatcher and Blumenfeldt 2001: 270.

Canolira Leach, 1818: 350.
Epichthyes Herklots, 1870: 122.

Diagnosis. A detailed diagnosis was given by Bruce (1987).
Type species. The type species for this genus is Anilocra cuvieri Leach, 1818, junior synonym of Anilocra physodes (Linnaeus, 1758) (see Bruce 1987); by subsequent designation (Kussakin 1979).

Leach (1818) described three species: Anilocra cuvieri, Anilocra mediterranea Leach, 1818, and Anilocra capensis Leach, 1818 without designating a type species. A. cuvieri was designated as the type species by Kussakin (1979). Both Anilocra cuvieri and A. mediterranea were synonymized with $A$. physodes (Trilles 1975; Ellis 1981).

Remarks. The body of female Anilocra is dorsally symmetrical and strongly vaulted. The posterior margins of their cephalon are smooth and straight, and the rostrum is more blunt than pointed. The rostrum folds into the area between the antennula bases. The antennula is shorter than the antenna. The posterolateral margins of the pereonites are not produced. Coxae $1-3$ are short, posteriorly rounded and do not form a rounded point posteriorly, whereas coxae 4-6 are longer, less rounded and more elongate than coxae $1-3$, and form a rounded point posteriorly. The pereopods gradually increase in size towards the posterior.

In the Cymothoidae, the external-attaching genera include but are not limited to Anilocra, Nerocila Leach, 1818, Renocila Miers, 1880, Creniola Bruce, 1987, and Pleopodias Richardson, 1910. Anilocra can be distinguished from Nerocila by the posterior margin of the cephalon, which is conspicuously trilobed in Nerocila, whereas the posterior margin of the cephalon of Anilocra is not tri-lobed to weakly tri-lobed. The posterolateral pereonite margins of Nerocila are more produced, elongate and pointed than that of Anilocra. In the Caribbean, some species of Anilocra and Renocila share numerous similarities, but in Anilocra pereopod 6 is shorter in length than pereopod 7, whereas in Renocila pereopods 6 and 7 are of similar length. To date the genera Creniola and Pleopodias have not been reported from the Caribbean.

## Anilocra haemuli Bunkley Williams \& Williams, 1981

Figs 1-4
Part Anilocra haemuli Bunkley Williams and Williams 1981: 1004-1014, figs 4-5; Williams and Williams 1985: 92-95; Bunkley-Williams and Williams 1998: 862-869; Bunkley-Williams et al. 1999: 311-314; Bunkley-Williams et al. 2006: 175-188; Welicky and Sikkel 2014: 1018-1026, 2015: 1437-1446; Welicky et al. in press [specimens from Haemulon flavolineatum]

Type material. Holotype (female, TL, W unknown) subocular region of Haemulon flavolineatum (USNM 184796); allotype (male, TL, W unknown) (USNM 184797); Paratypes (USNM 184798-184805) (Bunkley Williams and Williams 1981). Not examined.

Material examined. All material from the subocular region of Haemulon flavolineatum. (TL, W, Voucher Number) Collected by EH and LB Williams: q (32, 13, AMNH_IZC 250203; 32, 14, AMNH_IZC 250204) Mosquito Island, BVI; $q$ $(30,10)$ West End Enrique Reef, La Parguera, Puerto Rico, 30 Nov 1976; o (30,12, AMNH_IZC 250205) San Cristobal Reef, La Parguera, Puerto Rico, 30 Nov 1976; ¢ (30, 11, AMNH_IZC 250206; 29,14) Mingo Cay, St. John, USVI, 4 Mar 1977; $q$ (32, 13; 34, 14, AMNH_IZC 250207) Lameshur Bay, St. John, USVI, 2 Mar 1977; Q (31, 12, AMNH_IZC 250208) West of buoy site, SE of La Parguera, Puerto Rico, 22 Jan 1977. Collected by PC Sikkel and/or ER Brill: $q(30,11 ; 31,12)$ Cinnamon Bay, St. John, Jun 2011; $q(28,12)$ White Bay, Guana Island, BVI; Jul 2011; $q$ (damaged; 25, 9) St. Thomas, USVI, Jun 2012; $q(25,10)$ White Bay, Guana Island, BVI, Jul 2012; $q(26,11)$ Jumbee Bay, St. John, USVI, Jul 2013; $q(22,9 ; 28,12)$ TS (12, 6) White Bay, Guana Island, BVI, Jul-Aug 2016.

Ovigerous female. Size intact $(29,13)$. Body weakly ovoid, 2-2.6 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5 , most narrow at pereonite 1 , lateral margins mostly ovate posteriorly. Cephalon $0.5-$ 0.7 times longer than wide, visible from dorsal view, weakly trapezoid shaped. Frontal margin rounded to form blunt rostrum or simple, not folded. Eyes oval with distinct margins, one eye width $0.1-0.2$ times width of cephalon; one eye length $0.4-0.5$ times length of cephalon. Pereonite 1 smooth, anterior border straight, anterolateral angle narrowly rounded, not produced. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2-3 wide; with posteroventral angles rounded; 4-7 rounded and curved; not extending past pereonite posterior margin. Pereonites $1-5$ increasing in length and width; 6-7 decreasing in length and width; 1-4 narrower. Pleon with pleonite 1 wider than pleonites $2-5$, visible in dorsal view; pleonites posterior margin 1-3 posteriorly weakly concave, $4-5$ mostly straight. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonite 1 similar in form to pleonite 2. Pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin straight. Pleotelson 0.9 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins convex, posterior margin narrowly rounded.

Antennula consisting of 7-8 articles; peduncle articles 1 and 2 distinct and articulated; article 20.8 times as long as article 1 ; article 30.9 times as long as wide, 0.4 times as long as combined lengths of articles 1 and 2; flagellum with 5 articles, extending to posterior margin of eye. Terminal article with 2 short simple terminal setae. Antenna consisting of 10 articles; article 31.6 times as long as article 2; article 41.2 times as long as wide, 1.5 times as long as article 3; article 51.3 times as long as wide, 1.1 times as long as article 4 ; flagellum with 5 articles, terminal article terminating in 5 short simple setae, extending to middle of pereonite 1. Mandibular molar process ending in an acute incisor; mandibular palp article 3 with 7 simple setae. Maxillula simple with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe with 2 recurved robust setae; mesial lobe with 2 recurved robust setae. Maxilliped weakly segmented, with lamellar oostegite lobe, article 3 with 3 small robust setae.



Figure 2. Anilocra haemuli female ( 23 mm ) A, G-K Anilocra haemuli female ( 25 mm ) B-F: A antenna (left) and antennula (right) B maxilla $\mathbf{C}$ article 3 of maxilliped $\mathbf{D}$ maxillule $\mathbf{E}$ mandible $\mathbf{F}$ maxilliped G-K pleopods 1-5 respectively.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.3 times as long as wide; dactylus stout, 2.7 times as long as propodus, 3.8 times as long as wide. Pereopod 2 propodus 2.1 times as long as wide; dactylus 2.2 as long as propodus. Pereopod 6 basis 2.6 times as long as greatest width; ischium 0.5 times as long as basis; propodus 1.3 times as long as wide; dactylus 2.5 times as long as propodus. Pereopod 7 basis 3.2 times as long as greatest width; ischium 0.7 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion; merus 1.1 times as long as wide, 1.6 times as long as ischium; carpus 1.5 times as long as wide, 0.5 times as long as ischium, without bulbous protrusion; propodus 2.6 times as long as wide, 0.8 times as long as ischium; dactylus slender, 1.8 times as long as propodus, 5.0 times as long as wide. Pereopod 7 with few setae on propodus, carpus, and merus.


Figure 3. Anilocra haemuli transitional stage ( 12 mm ): A dorsal view B pereopod $1 \mathbf{C}$ dorsal view of cephalon $\mathbf{D}$ ventral view of cephalon $\mathbf{E}$ pereopod $7 \mathbf{F}$ dorsal pleotelson $\mathbf{G}$ lateral view.

Pleopods without setae, exopod larger than endopod. Pleopod 1 exopod 1.5 times as long as wide, lateral margin weakly convex, distally narrowly rounded, medial margin weakly oblique, mesial margin weakly convex; endopod 1.6 times as long as wide, lateral margin weakly convex, distally narrowly rounded, mesial margin slightly convex; peduncle twice as wide as long, without retinaculae, pointed projection on lateral margin. Pleopods 2-5 similar to pleopod 1. Pleopods 3-5 endopods proximal borders


Figure 4. Anilocra haemuli transitional stage ( 12 mm ): A antenna (left) and antennula (right) B maxilla $\mathbf{C}$ mandible $\mathbf{D}$ maxilliped $\mathbf{E}$ article 3 of maxilliped $\mathbf{F}$ article 3 of mandibular palp $\mathbf{G}$ maxillule $\mathbf{H}-\mathbf{K}$ pleopods 1-5 respectively.
do not extend below exopod to peduncle, fleshy lobes and medial lobes present. Peduncle lobes absent.

Uropod length equal length of pleotelson; peduncle 0.7-0.9 times longer than rami, lateral margin without setae; rami not extending beyond pleotelson, marginal
setae absent, apices broadly rounded. Endopod apically rounded, 3.1-3.5 times as long as greatest width. Exopod not extending to end of endopod, 3.8-4.4 times as long as greatest width, apically rounded, lateral margin weakly convex, mesial margin weakly convex, terminating without setae.

Transitional stage. Size (12, 6). Similar to female but smaller. Body 2.5 times as long as wide. Antennula bases separated, consisting of 8 articles, extending to posterior margin of eye. Antenna consisting of 10 articles, extending to middle of pereonite 1. Mandibular molar process ending in an acute incisor; mandibular palp article 3 with 11 simple setae. Maxillula simple with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe with 2 recurved robust setae; mesial lobe with 2 recurved robust setae. Maxilliped weakly segmented, with lamellar oostegite lobe, article 3 with 3 small recurved robust setae. Pereopod 7 with few small robust setae on carpus, merus and propodus. Pleopod 2 appendix masculina absent.

Distribution. Off the coast of southern Florida (USA) and throughout the Caribbean (Bunkley Williams and Williams 1981; Welicky et al. 2013, Welicky and Sikkel 2014, 2015, Welicky et al. in press).

Hosts. Known from Haemulon flavolineatum (Desmarest, 1823), H. aurolineatum (Cuvier, 1830), H. carbonarium (Poey, 1860), H. chrysargyreum (Günther, 1859), H. macrostomum (Günther, 1859) H. plumieri (Lacépède, 1801), H. sciurus (Shaw, 1803). Host records previously reported and which should be verified in the future: Cephalopholis cruentaus (Lacepède, 1802; formerly reported and classified as Epinephelus cruentatus, Lacepède, 1802 ), C. fulva (Linnaeus, 1758; formerly reported and classified as Epinephelus fulvus Linnaeus, 1758), Epinephelus guttatus (Linnaeus, 1758), Paranthias furcifer (Valenciennes, 1828), Mycteroperca rubra (Bloch, 1793), M. bonaci (Poey, 1860), and Orthopristis ruber (Cuvier, 1830).

Remarks. The description of $A$. haemuli from H. flavolineatum given above is in agreement with the original description in Bunkley Williams and Williams (1981). We supplement the original species diagnosis by now providing drawings and measurements of the antenna and antennula articles, additional pereopods, and pleopods.

Anilocra haemuli from H. flavolineatum can be distinguished from all other Caribbean species based on the morphological and/or site attachment differences among species that were reported in Bunkley Williams and Williams (1981). Pereopods 2-4 do not swell on the outer margin of the dactyl, thereby excluding it from being Anilocra adudefdufi Bunkley Williams \& Williams, 1981, A. holocanthi Bunkley Williams \& Williams, 1981, A. chaetodontis, or A. partiti Bunkley Williams \& Williams, 1981. In $A$. haemuli, the posterioventral angle of pereonite 6 is slightly produced thereby excluding it from being $A$. holocentri. The The endopod of the uropod of $A$. haemuli extends beyond the posterior end of the exopod, which is not the case in Anilocra chromis or $A$. partiti. Whereas the attachment site of $A$. haemuli is under the eye, A. holocentri and $A$. myripristis Bunkley Williams \& Williams, 1981 attach between the eyes, and $A$. acanthuri Bunkley Williams \& Williams, 1981 attaches under the pectoral fin.

## Anilocra brillae sp. n.

http://zoobank.org/0D6D3D87-D9AD-46E3-B976-9A77D7245E34
Figs 5-8
Part Anilocra haemuli of Bunkley Williams and Williams (1981) [records from Serranidae].
Material examined. All material from the subocular region of Epinephelus guttatus.
Holotype. Ovigerous $q$ ( 38,17 , AMNH_IZC 250209), Lameshur Bay, St. John, $18^{\circ} 18^{\prime} 59^{\prime \prime} \mathrm{N}, 64^{\circ} 43^{\prime} 25^{\prime \prime W}$ W, US Virgin Islands, 2 Mar 1977, coll. EH and LB Williams.

Paratype. Ovigerous + dissected (39, 15, AMNH_IZC 250210), Lameshur Bay, St. John, USVI, 2 Mar 1977 by EH and LB Williams.

Others examined. Collected by EH and LB Williams: q (33, 13, AMNH_IZC 250211; 24, 9) San Cristobal Reef, La Parguera, Puerto Rico 28-29 Jan 1977; of (35, 15, AMNH_IZC 250212; 32, 13 AMNH_IZC 250213) Lameshur Bay, St. John, USVI, 2 Mar 1977; ㅇ (39, 16, AMNH_IZC_250214) Buck Island, St. Thomas, USVI, 5 Mar 1977; ㅇ (34, 15, AMNH_IZC 250215; 25, 10 AMNH_IZC 25016) Laurel Reef, La Parguera, Puerto Rico, 18 May 1977; \& (30,12) Ensenada Honda, Vieques, Puerto Rico, 20 Dec 1983. Collected by PC Sikkel and ER Brill: $\&$ (27, 10; 30, 13; 26, 10; 31, 12; 29, 12; 29, 12; damaged) TS (11,6) White Bay, Guana Island, $18^{\circ} 28^{\prime} 0^{\prime \prime} \mathrm{N}, 64^{\circ} 33^{\prime} 59^{\prime \prime W}$ W, BVI, Jul-Aug 2016.

Ovigerous female. Size $(38,17)$. Body ovoid, $2.1-2.4$ times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5 , most narrow at pereonite 1 , lateral margins mostly posteriorly ovate. Cephalon 0.5-0.7 times longer than wide, visible from dorsal view, trapezoid shaped. Frontal margin rounded to form blunt rostrum, not folded. Eyes oval with distinct margins, one eye width 0.1 times width of cephalon; one eye length $0.5-0.6$ times length of cephalon. Pereonite 1 smooth, anterior border straight, anterolateral angle narrowly rounded, not produced. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2-3 wide with posteroventral angles rounded; 4-7 with narrowly produced point, curved; not extending past pereonite posterior margin. Pereonites 1-5 increasing in length and width; 6-7 decreasing in length and width; 5 and 6 subequal in width, $1-4$ narrower. Pleon with pleonite 1 most wide, visible in dorsal view; pleonites posterior margin smooth, 1-4 posteriorly concave, 5 straight. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonite 1 differ in form to pleonite 4 and 5 , similar to pleonite 2 and 3 . Pleonite 5 equal width to pleonite 4 , not overlapped by lateral margins of pleonite 4, posterolateral angles narrowly rounded, posterior margin straight. Pleotelson 1.1-1.4 times as long as anterior width, dorsal surface smooth, lateral margins convex, posterior margin converging to weak caudomedial point.

Antennula bases separated, shorter than antenna, consisting of 7-9 articles; peduncle articles 1 and 2 distinct and articulated; article 21.5 times as long as article 1 ; article 30.9 times as long as wide, 0.5 times as long as combined lengths of articles 1 and 2 ; flagellum with 4 articles, extending to posterior margin of eye. Terminal arti-


Figure 5. Anilocra brillae sp. n. female holotype ( 38 mm )(AMNH_IZC 250209) A-E Anilocra brillae sp. n. female paratype ( 39 mm ) (AMNH_IZC 250210) F-I: A dorsal view $\mathbf{B}$ lateral view $\mathbf{C}$ dorsal view of cephalon $\mathbf{D}$ pleotelson $\mathbf{E}$ ventral view of cephalon $\mathbf{F}$ pereopod $1 \mathbf{G}$ pereopod $2 \mathbf{H}$ pereopod $6 \mathbf{I}$ pereopod 7 .


Figure 6. Anilocra brillae sp. n. female paratype ( 39 mm ) (AMNH_IZC 250210) A, G-K Anilocra brillae sp. n. female (pleotelson damaged) B-F: A antenna (left) and antennula (right) B maxilla $\mathbf{C}$ article 3 of maxilliped $\mathbf{D}$ maxillule $\mathbf{E}$ mandible $\mathbf{F}$ maxilliped $\mathbf{G}-\mathbf{K}$ pleopods 1-5 respectively.
cle terminating in 1 short simple seta. Antenna comprised of 9-10 articles, peduncle article 31.5 times as long as article 2; article 41.3 times as long as wide, 1.1 times as long as article 3 ; article 51.6 times as long as wide, 1.1 times as long as article 4; flagellum with 4 articles, terminal article with 5 short simple setae, extending to posterior of pereonite 1. Mandibular molar process ending in an acute incisor; mandibular palp article 3 with 8 simple setae. Maxillula simple with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe with 2 recurved robust setae; mesial lobe with 1 recurved robust seta. Maxilliped weakly segmented, with lamellar oostegite lobe, article 3 with 3 recurved robust setae.

Pereopod 1 basis 1.8 times as long as greatest width; ischium 0.23 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.9 times as long as wide; dactylus moderately slender, 1.8 times as long as propodus, 3.7 times as long as wide. Pereopod 2 propodus 1.7 as long as wide; dactylus 2.7 times as long as propodus, 4.9 times as long as wide.


Figure 7. Anilocra brillae sp. n. transitional stage ( 11 mm ): A dorsal view B pereopod $1 \mathbf{C}$ dorsal pleotelson $\mathbf{D}$ pereopod $7 \mathbf{E}$ dorsal view of cephalon $\mathbf{F}$ ventral view of cephalon $\mathbf{G}$ lateral view.

Pereopods gradually increasing in size towards posterior. Pereopod 6 basis 1.7 times as long as greatest width; ischium 0.7 times as long as basis; propodus 1.5 times as long as wide, dactylus 2.3 times as long as propodus, 3.8 times as long as wide. Pereopod 7 basis 3.0 times as long as greatest width; ischium 0.7 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 2.0 times as long as wide, 0.7 times as long as ischium; carpus 1.5 times as long as wide, 0.6 times as long as ischium, without bulbous protrusion; propodus 3.2 times as long as wide, 0.8 times as long as ischium; dactylus moderately slender, 0.9 times as long


Figure 8. Anilocra brillae sp. n. transitional stage ( 11 mm ): A antenna (left) and antennula (right) B maxilla $\mathbf{C}$ mandible $\mathbf{D}$ maxilliped $\mathbf{E}$ article 3 of maxilliped $\mathbf{F}$ article 3 of mandibular palp $\mathbf{G}$ maxillule H-K pleopods 1-5 respectively.
as propodus, 3.5 times as long as wide. Pereopod 7 with many setae on propodus, carpus, and merus.

Pleopods without setae, exopod larger than endopod. Pleopod 1 exopod 1.2 times as long as wide, lateral margin weakly convex, distally narrowly rounded, medial margin weakly oblique, mesial margin weakly convex; endopod 1.8 times as long as wide,
lateral margin weakly convex, distally narrowly rounded, mesial margin slightly convex, peduncle 2.2 times as wide as long, with pointed projection on lateral margin. Pleopods $2-5$ similar to pleopod 1. Pleopods 3-5 endopods proximal borders do not extend below exopod to peduncle, fleshy lobes and medial lobes present. Peduncle lobes absent.

Uropod more than half the length of pleotelson, peduncle 0.7 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices broadly rounded. Endopod apically rounded, 2.2 times as long as greatest width, lateral margin weakly convex, mesial margin weakly convex, terminating without setae. Exopod not extending to end of endopod, 2.6 times as long as greatest width, apically rounded, lateral margin convex, mesial margin weakly convex or weakly concave, terminating without setae.

Transitional stage. Size (11, 6). Similar to female but smaller. Body 2.6 times as long as wide. Antennula bases separated, consisting of 8 articles, extending to middle of eye. Antenna consisting of 10 articles, extending to middle of pereonite 1. Mandibular molar process ending in an acute incisor; mandibular palp article 2 with 2 simple setae, article 3 with 7 simple setae. Maxillula simple with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe with 2 recurved robust setae; mesial lobe with 2 recurved robust setae. Maxilliped weakly segmented, with lamellar oostegite lobe, article 3 with 3 recurved robust setae. Pereopod 7 with several small robust setae on carpus, merus and propodus. Pleopod 2 appendix masculina absent.

Etymology. This species is named in honor of Elizabeth R. Brill for her dedication to studying the ecology of $A$. haemuli, and for collecting many of the $A$. haemuli and A. brillae sp. n. specimens used in this study.

Distribution. Known from St. John and St. Thomas, USVI, Guana Island, BVI, and islands of Puerto Rico, Spanish Virgin Islands. Expected distribution throughout the Caribbean Sea, where fish of the Serranidae family inhabit.

Hosts. Known from Epinephelus guttatus (Linnaeus, 1758).
Remarks. Previously, $A$. brillae sp. n. was identified as $A$. haemuli. Compared to $A$. haemuli, the outer margins of the cephalon and pereonites $1-4$ of $A$. brillae sp. n. form a more pronounced trapezoid shape and the remaining portion of the body is ovoid. A. brillae sp. n has more strongly narrowed pleonites than $A$. haemuli. Pleonites 1-3 of $A$. brillae sp. n. are wider than $4-5$ and $4-5$ are subequal, whereas the pleonites $1-2$ of $A$. haemuli are wider than $3-5$, and $3-5$ are subequal. Pleonite 5 is more posteriorly rounded in $A$. brillae sp. n, but this is somewhat variable among individuals. Another more variable feature is $A$. brillae sp . n . has a more caudomedially pointed pleotelson than $A$. baemuli. Typically, the seventh pereopod of $A$. brillae sp. n. is proportionally larger, has more robust setae, and the setae are distributed more extensively over the articles when compared to $A$. haemuli. The antennula peduncle of $A$. brillae sp. n. is regularly observed as shorter and more robust than that of $A$. haemuli. With respect to attachment, both species infest the subocular region, and if infested by two parasites, one parasite typically attaches under each eye. Infestation by a third $A$. brillae sp. n. on a single host seems to occur with more frequency than tertiary infestation by $A$. hae-
muli on a single host. The third parasite is typically attached between the eyes on the head of the host, or adjacent to one of the other parasites (RLW, pers obs).

Anilocra brillae sp. n. can be distinguished from all other Caribbean species except Anilocra haemuli using the same morphological comparisons described between $A$. haemuli and other Anilocra spp. given in Bunkley Williams and Williams (1981). Additionally, the body of $A$. brillae sp. n. is not expanded and is more elongate compared to the bodies of $A$. holocanth $i$ and $A$. chaetodontis.

## Discussion

The results of this study provide the first reliable COI sequences for species of Anilocra, and confirm that $A$. haemuli from $H$. flavolineatum is morphologically and genetically different than the Anilocra specimens collected from E. guttatus, and are here described as $A$. brillae sp. n. Our morphological data suggest there are two different species given the number of differences consistently observed, and our molecular analyses demonstrate a $4 \%$ difference between $A$. haemuli and $A$. brillae sp. n . This difference is less than half of that observed between $A$. brillae sp. n. and $A$. chromis, which are more conspicuously morphologically different. Our supplemental analyses were conducted utilizing the available Anilocra sp. COI sequences on GenBank, and there was a high level of interspecific divergence of these sequences compared with our dataset. The large differences in interspecific divergence between the specimens of this study and those provided on GenBank may be explained by the fact that the GenBank specimens may have been misidentified or not identified at all, as no morphological identification was described in Ketmaier et al. (2007). Thus, further interspecific comparisons cannot be assessed at this time.

Anilocra spp. have been reported to influence the fitness (Adlard and Lester 2004, Fogelman et al. 2009) and behavior (Meadows and Meadows 2003, Welicky and Sikkel 2015, Welicky et al. in press) of their fish hosts, and Anilocra brillae sp. n. infests E. guttatus, a grouper species that is currently recovering from previously intense fishing pressure (Nemeth et al. 2005). There is limited knowledge on the biotic stressors that influence $E$. guttatus population dynamics, and thus the effects of $A$. brillae sp. n. on E. guttatus should be examined as a potential stressor. Moreover, by studying this hostparasite interaction, further insight into variations in life histories of Anilocra spp. may be gained, if the life cycle of the parasite coincides with that of their host. The only complete description of an Anilocra spp. life cycle is of a species that infests an egg laying/guarding fish species (Adlard and Lester 1995), whereas many Anilocra spp. infest broadcast spawners. Interestingly, $A$. brillae sp. n. infests a fish species that undergoes an annual long distance migration to spawn in an aggregation (Nemeth 2011). Given that Anilocra spp. infection has been reported to influence host swimming performance in some fish (e.g., Binning et al. 2013), A. brillae sp. n. infection may indirectly influence the reproductive success of their hosts.

This study exemplifies that there is an incomplete but growing knowledge of cymothoid life histories, genetics, and morphology, and how these disciplines relate to host-parasite ecology. Continued efforts to conduct studies in these disciplines are necessary to better understand one of the least understood parasite families.

## Acknowledgements

The financial assistance of the National Research Foundation (NRF) of South Africa supported this research and is hereby acknowledged (NRF project IFR2011040100022, NJ Smit, PI). Opinions expressed, and conclusions arrived at, are those of the authors and are not necessarily those of the NRF. Financial assistance for KA Hadfield and RL Welicky from the Claude Leon Foundation of South Africa for this research is acknowledged. A portion of the fieldwork reported herein was supported by the U.S. National Science Foundation (OCE-121615, PC Sikkel, PI) and Puerto Rico Sea Grant (project number R-31-1-14, PC Sikkel, PI). The Falconwood Corporation supported work at Guana Island. Drs. Bert and Lucy Williams are acknowledged for the specimens they collected during 1976-1977 and 1983 that were used in this study. This is the first in a series of publications emanating from the Williams' parasitic isopods collection hosted by the NWU-Water Research Group. We thank C. Cook and E.C. Netherlands for their assistance with the molecular component of this study, and O. Kudlai and C. Baillie for their feedback on the preparation of this manuscript. We also thank the Sikkel lab, the staff of the Virgin Islands Environmental Resource Station, US National Parks Service (St. John), and Guana Island for logistic support, and E.R. Brill for field and collections assistance. This is contribution number 173 from the Center for Marine and Environmental Studies, University of the Virgin Islands and contribution number 179 from the NWU-Water Research Group.

## References

Adlard RD, Lester RJ (1994) Dynamics of the interaction between the parasitic isopod, Anilocra pomacentri, and the coral reef fish, Chromis nitida. Parasitology 109: 311-324. https:// doi.org/10.1017/S0031182000078343
Adlard RD, Lester RJ (1995) The life-cycle and biology of Anilocra pomacentri (Isopoda, Cymothoidae), an ectoparasitic isopod of the coral-reef fish, Chromis nitida (Perciformes, Pomacentridae). Australian Journal of Zoology 43: 271-281. https://doi.org/10.1071/ZO9950271
Binning SA, Barnes JI, Davies JN, Backwell PR, Keogh JS, Roche DG (2014) Ectoparasites modify escape behaviour, but not performance, in a coral reef fish. Animal Behaviour 93: 1-7. https://doi.org/10.1016/j.anbehav.2014.04.010
Binning SA, Roche DG, Layton C (2013) Ectoparasites increase swimming costs in a coral reef fish. Biology Letters 9: 20120927.

Brandt A, Poore GCB (2003) Higher classification of the flabelliferan and related Isopoda based on a reappraisal of relationships. Invertebrate Systematics 17: 893-923. https://doi. org/10.1071/IS02032
Bruce NL (1987) Australian Pleopodias Richardson, 1910, and Anilocra Leach, 1818 (lsopoda: Cymothoidae), crustacean parasites of marine fishes. Records of the Australian Museum 39: 85-130. https://doi.org/10.3853/j.0067-1975.39.1987.166
Brusca RC (1981) A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific. Zoological Journal of the Linnaean Society 73: 117-199. https://doi. org/10.1111/j.1096-3642.1981.tb01592.x
Brusca RC, Iverson EW (1985) A guide to the marine isopod Crustacea of Pacific Costa Rica. Revista de Biologia Tropical 33: 1-77.
Bunkley-Williams L, Williams Jr EH (1981) Nine new species of Anilocra (Crustacea: Isopoda: Cymothoidae) external parasites of West Indian coral reef fishes. Proceedings of the Biological Society of Washington 94: 1005-1047.
Bunkley-Williams L, Williams EH (1998) Ability of Pederson cleaner shrimp to remove juveniles of the parasitic cymothoid isopod, Anilocra haemuli, from the host. Crustaceana 71: 862-869. https://doi.org/10.1163/156854098X00888
Bunkley-Williams L, Williams Jr EH, Bashirullah AK (2006) Isopods (Isopoda: Aegidae, Cymothoidae, Gnathiidae) associated with Venezuelan marine fishes (Elasmobranchii, Actinopterygii). Revista de Biología Tropical 54: 175-188.
Coleman CO, Lowry JK, Macfarlane T (2010) DELTA for beginners. An introduction into the taxonomy software package DELTA. ZooKeys 45: 1-75. https://doi.org/10.3897/ zookeys.45.263
Dana JD (1853) Crustacea, Part 11. United States Exploring Expedition during the years 1838, 1839, 1840, 1841, 1842, under the command of Charles Wilkes, U.S.N. 14: 689-1618.
Desmarest AG (1825) Considérations générales sur la classe des crustacés, et description des espèces de ces animaux, qui vivent dans la mer, sur les côtes, ou dans les eaux douces de la France. F. J. Levrault, Paris, 56 pp.
Edwards HM (1840) Histoire naturelle des Crustacés comprenent l'anatomie la physiologie et la classification de ces animaux. Librairie Encyclopédique de Roret 3: 1840.
Ellis JP (1981) Some type specimens of Isopoda (Flabellifera) in the British Museum (Natural History), and the isopods in the Linnaean Collection. Bulletin of the British Museum (Natural History) 40: 121-128.
Fogelman RM, Kuris AM, Grutter AS (2009) Parasitic castration of a vertebrate: effect of the cymothoid isopod, Anilocra apogonae, on the five-lined cardinalfish, Cheilodipterus quinquelineatus. International Journal for Parasitology. 39: 577-583. https://doi.org/10.1016/j. ijpara.2008.10.013
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 94-299.
Gerstaecker A (1882) Sechste Ordnung. Isopoda-Asseln [Part] In: Bronn HG (Ed.) Klassen und Ordnung des ThierReichs, wissenschaftlich dargestellt in Wort und Bild. Funfter

Band 11. Abtheilung. Gliederfiissler: Arthropoda. Crustacea. (Zweite Hiilfte: Malacostraca) 4, 5, 6, 7, 8. Leiferung, 97-278.
Hadfield KA, Bruce NL, Smit NJ (2010) Redescription of the monotypic genus Cinusa Schioedte and Meinert, 1884 (Isopoda, Cymothoidae), a buccal-cavity isopod from South Africa. Zootaxa 2437: 51-68.
Hadfield KA, Bruce NL, Smit NJ (2013) Review of the fish-parasitic genus Cymothoa Fabricius, 1783 (Isopoda, Cymothoidae, Crustacea) from the south-western Indian Ocean, including a new species from South Africa. Zootaxa 3640: 152-176. https://doi. org/10.11646/zootaxa.3640.2.2
Hadfield KA, Bruce NL, Smit NJ (2016) Redescription of poorly known species of Ceratothoa Dana, 1852 (Crustacea, Isopoda, Cymothoidae), based on original type material. ZooKeys 592: 39-91. https://doi.org/10.3897/zookeys.592.8098
Hadfield KA, Bruce NL, Szinetár C, Smit NJ (2014) Ceratothoa retusa (Schiœdte \& Meinert, 1883) (Isopoda, Cymothoidae), a variable species of fish parasitic marine isopod from the Indian Ocean. Crustaceana 87: 448-462. https://doi.org/10.1163/15685403-00003293
Hale HM (1926) Review of Australian isopods of the Cymothoid group. Transactions of the Royal Society of South Australia 50: 201-234.
Herklots JA (1870) Deux nouveaux genres de Crustacés vivant en parasites sur des poissons. Epichthyes et Ichthyoxenos. Archiv Neerlandaise Sciences Exact et Naturelle 5: 120-137.
Kensley B (1978) Guide to the marine isopods of southern Africa. South African Museum, Cape Town, 173 pp.
Ketmaier V, Joyce DA, Horton T, Mariani S (2007) A molecular phylogenetic framework for the evolution of parasitic strategies in cymothoid isopods (Crustacea). Journal of Zoological Systematics and Evolutionary Research 46: 19-23. https://doi.org/10.1111/j.1439-0469.2007.00423.x
Kussakin OG (1979) Marine and brackish-water isopod Crustacea. Suborder Flabellifera. Academy of Science, U.S.S.R., Leningrad, 470 pp.
Leach WE (1818) Cymothoidées. In: Cuvier F (Ed.) Dictionnaire des Sciences Naturelle 12. Strasbourg \& Paris, Levrault \& Le Normant, 338-354.
Meadows DW, Meadows CM (2003) Behavioral and ecological correlates of foureye butterflyfish, Chaetodon capistratus,(Perciformes: Chaetodontidae) infested with Anilocra chaetodontis (Isopoda: Cymothoidae). Revista de Biología Tropical 51: 77-81.
Nemeth RS (2005) Population characteristics of a recovering US Virgin Islands red hind spawning aggregation following protection. Marine Ecology Progress Series 286: 81-97. https://doi.org/10.3354/meps286081
Nemeth RS (2011) Ecosystem aspects of species that aggregate to spawn. Reef Fish Spawning Aggregations: Biology, Research and Management. Fish and Fisheries Series 35: 21-55.
Richardson H (1905) A monograph on the isopods of North America. Bulletin of the United States National Museum 54: 1-727. https://doi.org/10.5479/si.03629236.54.i
Roche DG, Binning SA, Strong LE, Davies JN, Jennions MD (2013) Increased behavioural lateralization in parasitized coral reef fish. Behavioral Ecology and Sociobiology 67: 1339-1344. https://doi.org/10.1007/s00265-013-1562-1
Schiodte JC, Meinert FR (1883) Symbolae ad monographium cymothoarum crustaceorum familiae. Ill. Saophridae. IV. Ceratothoinae. Naturhistorisk Tidsskrift 13: 281-378.

Schultz GA (1969) The marine isopod crustaceans. Wm. C. Brown Company Publishers, Dubuque, 359 pp.
Sikkel PC, Cheney KL, Côté IM (2004) In situ evidence for ectoparasites as a proximate cause of cleaning interactions in reef fish. Animal Behaviour 68: 241-247. https://doi. org/10.1016/j.anbehav.2003.10.023
Sikkel PC, Schaumburg CS, Mathenia JK (2006) Diel infestation dynamics of gnathiid isopod larvae parasitic on Caribbean reef fish. Coral Reefs 25: 683-689. https://doi.org/10.1007/ s00338-006-0154-1
Smit NJ, Bruce NL, Hadfield KA (2014) Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. International Journal for Parasitology: Parasites and Wildlife 3: 188-197. https://doi.org/10.1016/j.ijppaw.2014.03.004
Thatcher VE, Blumenfeld CL (2001) Anilocra montti sp. n. (Isopoda, Cymothoidae) a parasite of caged salmon and trout in Chile. Revista Brasileira de Zoologia 18: 269-276. https:// doi.org/10.1590/S0101-81752001000500023
Trilles JP (1975) Les Cymothoidae (Isopoda, Flabellifera) des collections du Muséum National d'Histoire Naturelle de Paris. II. Les Anilocridae Schiodte et Meinert, 1881. Genres Anilocra Leach, 1818 et Nerocila Leach, 1818.Bulletin du Muséum National d'Histoire Naturelle, Paris, 3e série, 290. Zoologie 200: 303-340.
Trilles JP (1994) Catalogue mondial des Cymothoidae. Studia Marina 21: 5-288.
Welicky RL, Cheney KL, Coile AM, McCammon A, Sikkel PC (2013) The relationship between lunar periodicity and activity of fish-parasitic gnathiid isopods in the Caribbean. Marine Biology 160: 1607-1617. https://doi.org/10.1007/s00227-013-2213-9
Welicky RL, Sikkel PC (2014) Variation in occurrence of the fish-parasitic cymothoid isopod, Anilocra baemuli, infesting French grunt (Haemulon flavolineatum) in the north-eastern Caribbean. Marine and Freshwater Research 65: 1018-1026. https://doi.org/10.1071/ MF13306
Welicky RL, Sikkel PC (2015) Decreased movement related to parasite infestation in a diel migratory coral reef fish. Behavioral Ecology and Sociobiology 69: 1437-1446. https:// doi.org/10.1007/s00265-015-1956-3
Welicky RL, Demopoulos AWJ, Sikkel PC (in press) Host-dependent differences in resource use associated with Anilocra spp. parasitism in two coral reef fishes, as revealed by stable carbon and nitrogen isotope analyses. Marine Ecology. https://doi.org/10.1111/maec. 12413
Williams LB, Williams EH (1985) Brood pouch release of Anilocra chromis Williams \& Williams (Isopoda, Cymothoidae) a parasite of brown chromis, Chromis multilineatus (Guichenot) in the Caribbean. Crustaceana 49: 92-95. https://doi.org/10.1163/156854085X00251

## Supplementary material I

## Basepair differences of Anilocra spp.

Authors: Rachel L. Welicky, Kerry A. Hadfield, Paul C. Sikkel, Nico J. Smit
Data type: statistical data
Explanation note: The number identifier in the horizontal header column represents the number and corresponding species listed in the vertical column header.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

## Supplementary material 2

Kimura 2-Parameter (K2P) distance of Anilocra spp.
Authors: Rachel L. Welicky, Kerry A. Hadfield, Paul C. Sikkel, Nico J. Smit Data type: statistical data
Explanation note: K2P distance expressed in percent. The number identifier in the horizontal header column represents the number and corresponding species listed in the vertical column header.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

# A survey of five Pireneitega species (Agelenidae, Coelotinae) from China 

Xiaoqing Zhang ${ }^{1,2}$, Zhe Zhao ${ }^{3}$, Guo Zheng ${ }^{1}$, Shuqiang Li ${ }^{3,4}$<br>I College of Life Science, Shenyang Normal University, Shenyang, Liaoning 110034, China 2 Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar 3 Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China 4 University of Chinese Academy of Sciences, Beijing 100049, China<br>Corresponding authors: Guo Zheng (zhengguo@synu.edu.cn); Shuqiang Li (lisq@ioz.ac.cn)

Academic editor: Y. Marusik | Received 29 November 2016 | Accepted 8 March 2017 | Published 27 March 2017
http://zoobank.org/940A0A63-CA92-4007-B2D6-9348112C8428
Citation: Zhang X, Zhao Z, Zheng G, Li S (2017) A survey of five Pireneitega species (Agelenidae, Coelotinae) from China. ZooKeys 663: 45-64. https://doi.org/10.3897/zookeys.663.11356


#### Abstract

Five species of Pireneitega spiders from China are surveyed, of which three are new to science: P. huashanensis  known species are redescribed: P. liansui (Bao \& Yin, 2004) and P. triglochinata (Zhu \& Wang, 1991). The males of $P$. liansui and P. triglochinata (Zhu \& Wang, 1991) are described for the first time. DNA barcodes for five species are documented for future use and as proof of molecular differences between species.


## Keywords

Taxonomy, description, diagnosis, East Asia, Paracoelotes

## Introduction

Coelotinae is the largest subfamily of Agelenidae, with 25 genera and 676 valid species distributed in the Holarctic and southeast Asia (World Spider Catalog 2017). The genus Pireneitega Kishida, 1955 is one of the most species-rich genera of the subfamily.

Thirty-five valid Pireneitega species are distributed from Europe to East Asia (Zhang and Marusik 2016), and 20 were known from China before the current study ( Li and Lin 2016; Zhang et al. 2016).

During the study of Pireneitega spiders from China, five interesting species were found. The goal of this paper is to provide descriptions of three new species and redescriptions of two poorly known species.

## Material and methods

Specimens were examined with a Leica M205C stereomicroscope. Images were captured with an Olympus C7070 wide zoom digital camera ( 7.1 megapixels) mounted on an Olympus SZX12 dissecting microscope. Epigynes and male palps were examined after dissection from the spiders' bodies. The epigyne was cleared by boiling it in $10 \%$ KOH solution before taking photos of the vulva. All measurements were obtained using a Leica M205C stereomicroscope and are given in millimeters. Leg measurements are given as: Total length (femur, patella + tibia, metatarsus, tarsus). Only structures (palp and legs) of the left side of the body are described and measured.

Terminology used for copulatory organ characters in the text and figure legends follows Wang (2002) with some modifications. Abbreviations used in the text and figure legends are: $\mathrm{A}=$ epigynal atrium; $\mathrm{ALE}=$ anterior lateral eye; $\mathrm{AME}=$ anterior median eye; AME-ALE = distance between AME and ALE; AME-AME = distance between AME and AME; ALE-PLE = distance between ALE and PLE; CD = copulatory ducts; $\mathrm{CF}=$ cymbial furrow; $\mathrm{CO}=$ conductor; $\mathrm{d}=$ dorsal; $\mathrm{E}=$ embolus; EB = embolic base; ET = epigynal teeth; $\mathrm{FD}=$ fertilization ducts; $\mathrm{Fe}=$ femur; $\mathrm{H}=$ epigynal hood; $\mathrm{MA}=$ median apophysis; $\mathrm{Mt}=$ metatarsus; $\mathrm{p}=$ prolateral; $\mathrm{PA}=\mathrm{pa}-$ tellar apophysis; $\mathrm{Pa}=$ patella; $\mathrm{PLE}=$ posterior lateral eye; $\mathrm{PME}=$ posterior median eye; $\mathrm{PME}-\mathrm{PLE}=$ distance between PME and PLE; $\mathrm{PME}-\mathrm{PME}=$ distance between PME and PME; $\mathrm{R}=$ receptacle; $\mathrm{r}=$ retrolateral; RTA = retrolateral tibial apophysis; ST = subtegulum; $\mathrm{T}=$ tegulum; $\mathrm{Ta}=$ tarsus; $\mathrm{TC}=$ tip of conductor; $\mathrm{Ti}=$ tibia; v = ventral; VTA = ventral tibial apophysis. References to figures in the cited papers are listed in lowercase (fig. or figs); figures from this paper are noted with an initial capital (Fig. or Figs).

DNA barcodes were obtained for future use: a partial fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced for these 5 species using primers LCO1490-oono (5'-CWACAAAYCATARRGA-TATTGG-3') (Folmer et al. 1994; Miller et al. 2010) and C1-N-2776 (5’-GGA-TAATCAGAATANCGNCGAGG-3') (Simon et al. 1994). For additional information on extraction, amplification and sequencing procedures, see Zhao et al. (2013). All sequences were blasted in GenBank; accession numbers are provided in Table 1.

All specimens (including molecular vouchers) are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing, China.

Table I. Voucher specimen information.

| Species | GenBank accession <br> number | Sequence <br> length | Collection localities (all in China) |
| :--- | :---: | :---: | :---: |
| P. huashanensis sp. n. | KY593329 | 1194bp | Shaanxi Prov.: Huayin Prefecture: Mt. Huashan |
| P. liansui | KY593330 | 1194 bp | Hunnan Prov.: Daoxian Co. |
| P. lushuiensis sp. n. | KY593327 | 1194 bp | Yunnan Prov.: Lushui Co. |
| P. triglochinata | KY593328 | 1194 bp | Sichuan Prov.: Mt. Emei |
| P. xiyankouensis sp. n. | KY5933331 | 1194 bp | Guangxi Prov.: Yizhou City |

## Taxonomy

Genus Pireneitega Kishida, 1955
Pireneitega Kishida, 1955: 11. Type species Amaurobius roscidus L. Koch, 1868 (= P. segestriformis (Dufour, 1820)) from Germany.
Paracoelotes Brignoli, 1982: 348. Type species Coelotes armeniacus Brignoli, 1978 from Turkey.

Diagnosis. Females of Pireneitega can be distinguished from all other coelotine genera by the widely separated epigynal teeth, the large atrium with subparallel margins, and the broad copulatory ducts (Fig. 2A-B); other coelotines usually have a small atrium and copulatory ducts. The males can be distinguished by the small RTA, the distinct median apophysis and the absence of a conductor dorsal apophysis (Fig. 1A-C); other coelotines usually have a broad conductor dorsal apophysis and a reduced or indistinct median apophysis (Zhang and Marusik 2016).

## Pireneitega buashanensis Zhao \& Li, sp. n.

http://zoobank.org/A75AC3BA-9598-4DEA-B235-1485879B4EFB
Figs 1-2, 11
Type material. Holotype $\delta^{\lambda}$ : China: Shaanxi: Huayin Prefecture: Mt. Huashan, Duyukou Village, $34^{\circ} 31^{\prime} 42^{\prime \prime} \mathrm{N}, 110^{\circ} 07^{\prime} 22^{\prime \prime} \mathrm{E}, 530 \mathrm{~m}, 30 . \mathrm{IX} .2013$, Y. Li and J. Liu. Paratypes: $1 \delta^{\lambda}$, same data as holotype; $4 \not \subset 1 \delta^{\lambda}$, same area, $34^{\circ} 32^{\prime} 46^{\prime \prime} \mathrm{N}, 110^{\circ} 07^{\prime} 06^{\prime \prime} \mathrm{E}$, 536 m, 2.X.2016, Z. Zhao and X. Zhang.

Etymology. The specific name refers to the type locality; adjective.
Diagnosis. The male can be distinguished from all other Pireneitega species except P. luniformis (Zhu \& Wang, 1994) by having a tapering conductor tip and longer cymbial furrow. From $P$. luniformis, it can be distinguished by the elongate embolus base and the larger diameter of the conductor's loop, approximately six times the width of the conductor (vs the small embolus base and the small diameter of the conductor's loop in P. luniformis) (Fig. 1; Zhu and Wang 1994: figs 7-8). The female can be distinguished from all other Pireneitega species except $P$. luniformis by having short


Figure I. Palp of Pireneitega huashanensis sp. n., male holotype. A Prolateral view B Ventral view C Retrolateral view. Scale bar: equal for $\mathbf{A}, \mathbf{B}, \mathbf{C}$.
copulatory ducts and long epigynal teeth, subequal to the length of the atrium. From $P$. luniformis, it can be distinguished by the longer septum (vs the short septum in $P$. luniformis) (Fig. 2; Zhu and Wang 1994: figs 5-6).

Description. Male (holotype): Total length 5.09. Carapace 2.40 long, 1.85 wide. Abdomen 2.69 long, 1.75 wide. Eye sizes and interdistances: AME 0.13, ALE 0.18, PME 0.15 , PLE 0.15 ; AME-AME 0.03 , AME-ALE 0.03, PME-PME 0.05 , PME-PLE 0.05 . Leg measurements: I: 8.50 (2.50, 2.75, 2.00, 1.25); II: 7.50 (2.25, 2.25, 1.75, 1.25); III: 6.85 (2.00, 2.10, $1.75,1.00)$; IV: $9.30(2.55,3.00,2.50,1.25)$. Carapace greenish, with black lateral margins, radial grooves indistinct. Abdomen blackish, with yellow herringbone pattern. Palp as in Fig. 1: patellar apophysis short, about four times shorter than tibia; tibia four times shorter than cymbium; VTA long, about $2 / 3$ length of tibia, without pointed tip, extending beyond the tibia; RTA indistinct; cymbial furrow long, more than half the length of cymbium; embolus with broad base, beginning at the $5: 30$ o'clock position.


Figure 2. Pireneitega huashanensis sp. n., female paratype and male holotype. A Epigyne, ventral view B Vulva, dorsal view $\mathbf{C}$ Male habitus, dorsal view $\mathbf{D}$ Female habitus, dorsal view $\mathbf{E}$ Female habitus, ventral view. Scale bars: equal for D, E.

Spination in male:

|  | Fe | Pt | Ti | Mt | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | $3 \mathrm{~d} \mathrm{2p} \mathrm{1r}$ | - | $1 \mathrm{p} 3-3 \mathrm{v}$ | $1 \mathrm{p} 3-3 \mathrm{v}$ | - |
| II | $3 \mathrm{~d} \mathrm{1p} \mathrm{2r}$ | 1 d 1 p 1 r | $2 \mathrm{p} 3-3 \mathrm{v}$ | $3 \mathrm{p} 3-3 \mathrm{v}$ | - |
| III | $3 \mathrm{~d} \mathrm{2p}$ | 1 p 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $2 \mathrm{~d} 5 \mathrm{p} 3 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |
| IV | $3 \mathrm{~d} \mathrm{1r}$ | 1 p 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $4 \mathrm{p} 4 \mathrm{r} 3-3 \mathrm{v}$ | 2 p 2 r |

Female (paratype): Total length 8.5. Carapace 3.5 long, 2.9 wide. Abdomen 5.0 long, 2.9 wide. Eye sizes and interdistances: AME 0.16, ALE 0.20, PME 0.16, PLE 0.16; AME-AME 0.10, AME-ALE 0.05 , PME-PME 0.10 , PME-PLE 0.20 . Leg measurements: I: 10.25 (3.00, 3.50, 2.25, 1.50); II: 8.30 (2.50, 3.00, 1.80, 1.00); III: 8.00 (2.40, 2.75, 1.85, 1.00); IV: 11.55 (3.50, 3.75, 3.00, 1.30). Carapace brown. Abdomen black with yellow spots and herringbone pattern. Epigyne as in Fig. 2A-B: epigynal teeth long; septum long with weakly sclerotized tip; atrium with well delimited posterior margin, about 0.6 times longer than wide, about two times longer and wider than septum; copulatory opening distinct; receptacle long, about three times longer than wide, separated by two diameters; copulatory ducts with three parts, the basal part running from receptacle posteriorly ( $B d$ ), median part running anteriorly ( $M d$ ), and terminal part (Td) running posteriorly and leading to copulatory opening; median part as wide as terminal and two times longer than basal part; median part separated; hoods indistinct.

Spination in female:

|  | Fe | Pt | Ti | Mt | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 3d 2p 1r | - | $1 \mathrm{p} 3-3 \mathrm{v}$ | 1p 3-3v | - |
| II | 3 d 2 p 2 r | 1d 1p | 2p 3-3v | $2 \mathrm{p} 3-3 \mathrm{v}$ | - |
| III | 3 d 2 p 2 r | 2d 1p 1r | $2 \mathrm{~d} 2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $2 \mathrm{~d} 4 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |
| IV | 3 d 1 p 2 r | 1 d 1 p 1 r | 2d 2p 2 r 3-3v | $3 \mathrm{~d} 2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | 2p 2r |

Distribution. Known only from Shanxi (Fig. 11).

## Pireneitega liansui (Bao \& Yin, 2004)

Figs 3-4, 11
Coelotes liansui Bao \& Yin, 2004: 455, figs 1-3 (q). Holotype $q$ from Hunan, Daoxian County, $25^{\circ} 31^{\prime} \mathrm{N}, 111^{\circ} 36^{\prime} \mathrm{E}$. Types lost (originally at College of Life Science, Hunan Normal University).
Pireneitega liansui: Wang \& Jäger 2007: 46 (transfer from Coelotes).
Paracoelotes liansui: Yin et al. 2012: 1020, fig. 528a-c (审).
Material examined. 3q1才, China: Hunan: Daoxian County: Dongzhou Village, $25^{\circ} 31^{\prime} 45^{\prime \prime N}, 111^{\circ} 36^{\prime} 17^{\prime \prime} \mathrm{E}, 168 \mathrm{~m}, 5 . X I .2016$, H. Yang.


Figure 3. Palp of Pireneitega liansui, specimen from Diaoxian. A Prolateral view B Ventral view C Retrolateral view. Scale bar: equal for A, B, C.

Diagnosis. The male can be distinguished from all other Pireneitega species except P. involuta (Wang et al., 1990), by having a narrow embolus base and a long cymbial furrow, more than half the length of the cymbium. From $P$. involuta it can be distinguished by the bifurcate tip of the patellar apophysis (vs a tapering tip in P. involuta) (Fig. 3; Wang et al. 1990: figs 13-15, 18-19).

Description. Female (Fig. 4): Well described by Bao \& Yin (2004: figs 1-3).
Male: Total length 10.0. Carapace 5.0 long, 3.75 wide. Abdomen 5.0 long, 3.25 wide. Eye sizes and interdistances: AME 0.30, ALE 0.30, PME 0.20, PLE 0.20; AMEAME 0.10, AME-ALE 0.10, PME-PME 0.20, PME-PLE 0.25. Leg measurements: I: 15.75 (4.50, 5.00, 4.00, 2.25); II: 14.45 (4.25, 4.50, 3.70, 20); III: 13.55 (4.00, 4.50, 3.30, 1.75); IV: 17.00 ( $5.00,5.50,4.50,2.00$ ). Carapace brown, the radial grooves distinct. Abdomen whitish, with green herringbone pattern. Palp as in Fig. 3: patellar apophysis long, about $1 / 2$ length of tibia; tibia short, about four times shorter than cymbium; VTA long, subequal to the tibial length, without pointed tip, extending beyond the tibia; RTA short, about 1/8 length of VTA; width of conductor about 1/5 of loop diameter; embolus beginning at 6:30 o'clock position.


Figure 4. Pireneitega liansui, specimens from Diaoxian. A Epigyne, ventral view B Vulva, dorsal view C Male habitus, dorsal view D Female habitus, dorsal view E Female habitus, ventral view. Scale bars: equal for $\mathbf{D}, \mathbf{E}$.

Spination in male:

|  | Fe | $\mathbf{P t}$ | $\mathbf{T i}$ | $\mathbf{M t}$ | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 2 d 2 p | - | $3-3 \mathrm{v}$ | $1 \mathrm{r} 3-3 \mathrm{v}$ | - |
| II | 3 d 2 p 2 r | - | $2 \mathrm{p} 3-3 \mathrm{v}$ | $3 \mathrm{p} 3-3 \mathrm{v}$ | - |
| III | 3 d 2 p 1 r | 1 p 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |
| IV | 3 d 2 p 1 r | 1 p 1r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $1 \mathrm{~d} 4 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |

Distribution. Known only from Hunan (Fig. 11).
Remarks. The male of $P$. liansui is described for the first time.

## Pireneitega lushuiensis Zhao \& Li, sp. n.

http://zoobank.org/25A7D65B-EBAD-486E-81D5-2608E74670D8
Figs 5-6, 11
Type material. Holotype $\delta^{\lambda}$ : China: Yunnan: Nujiang Lisu Autonomous Prefecture, Lushui County, Pianma Town, $25^{\circ} 59^{\prime} 52^{\prime \prime} \mathrm{N}, 98^{\circ} 37^{\prime} 53^{\prime \prime} \mathrm{E}, 2257 \mathrm{~m}, 28 . V I .2016$, Y. Li, M. Xu \& M. Hu. Paratypes: 8 q 5 त, same data as holotype; $3 \not \subset 2$ § , Nujiang Lisu Autonomous Prefecture, Lushui County, $25^{\circ} 59^{\prime} 38^{\prime \prime} \mathrm{N}, 98^{\circ} 39^{\prime} 42^{\prime \prime} \mathrm{E}, 2337 \mathrm{~m}$, 29.VI.2016, Y. Li, M. Xu \& M. Hu; 7q, Baoshan Prefecture, Tengchong City, Mangbang Town, Changlinggan Village, $24^{\circ} 58^{\prime} 07^{\prime \prime N}, 98^{\circ} 36^{\prime} 54^{\prime \prime} \mathrm{E}, 2032 \mathrm{~m}, 23 . V I .2013$, Z. Zhao \& J. Liu; 2 q ${ }^{\text {J }}$, Baoshan Prefecture, Tengchong City, Mt. Gaoligong National Park, $24^{\circ} 49^{\prime} 44^{\prime \prime} \mathrm{N}, 98^{\circ} 46^{\prime} 03^{\prime \prime} \mathrm{E}, 2177 \mathrm{~m}, 21-22 . V I .2013$, Z. Zhao and J. Liu; 10 , Baoshan Prefecture, Tengchong City, Mingguang Town, Xinjie, Yunyan Temple, $25^{\circ} 29^{\prime} 19^{\prime \prime N}$ N, $98^{\circ} 32^{\prime} 35^{\prime \prime} \mathrm{E}, 1797 \mathrm{~m}, 28 . X I .2013$, Y. Li \& J. Liu.

Etymology. The specific name refers to the type locality; adjective.
Diagnosis. The male can be distinguished from all other Pireneitega species except $P$. huashanensis and $P$. luniformis, by having a longer cymbial furrow and the arched tip of conductor. From $P$. huashanensis it can be distinguished by the thick tip of the patellar apophysis and the narrow and straight embolus base (vs the thin tip of the patellar apophysis and the elongate embolus base in $P$. huashanensis, and the tapering tip of the patellar apophysis, and the small and nearly triangular embolus base in P. luniformis) (Figs 1, 5; Zhu \& Wang 1994: figs 7-8). The female can be distinguished from all other Pireneitega species except $P$. luniformis by having a blunt tip of the septum and a short receptacle. From $P$. luniformis it can be distinguished by long copulatory ducts, and the median part subequal to the length of receptacle ( $v s$ short copulatory ducts in P. luniformis) (Fig. 6; Zhu \& Wang 1994: figs 5-6).

Description. Male (holotype): Total length 9.50 . Carapace 4.75 long, 3.50 wide. Abdomen 4.75 long, 2.75 wide. Eye sizes and interdistances: AME 0.25, ALE 0.20, PME 0.20, PLE 0.20; AME-AME 0.10, AME-ALE 0.05, PME-PME 0.15, PME-PLE 0.20. Leg measurements: I: 16.25 (4.75, 5.25, 4.00, 2.25); II: 15.00 (4.50, 5.00, 3.50, 2.00); III: 13.00 (4.00, 4.25, 3.00, 1.75); IV: 17.15 (5.00, 5.50, 4.65, 2.00). Carapace yellow with black lateral margins, radial grooves distinct. Abdomen blackish, with gray


Figure 5. Palp of Pireneitega lushuiensis sp. n., male holotype. A Prolateral view B Ventral view C Retrolateral view. Scale bar: equal for A, B, C.
herringbone pattern. Palp as in Fig. 5: patellar apophysis short, about 1/3 length of tibia; tibia short, about $1 / 4$ length of tarsus; VTA subequal to the tibial length, without pointed tip, extending beyond the tibia; RTA short, about $1 / 10$ length of VTA; cymbial furrow long, more than half length of cymbium; width of conductor about $1 / 3$ of loop diameter; embolus with narrow base originating proximally on base of tegulum, beginning at the 6:00 o'clock position.

Spination in male:

|  | Fe | Pt | Ti | $\mathbf{M t}$ | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 2 d 4 p 1 r | - | $1 \mathrm{p} 3-3 \mathrm{v}$ | $3-3 \mathrm{v}$ | - |
| II | 3 d 1 p 1 r | - | $1 \mathrm{r} 3-3 \mathrm{v}$ | $1 \mathrm{r} 3-3 \mathrm{v}$ | - |
| III | 3 d 2 p 1 r | 1 p 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 p |
| IV | 3 d 2 p | 1 d 1 p 1 r | $1 \mathrm{~d} 2 \mathrm{p} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |

Female (paratype): Total length 10.25 . Carapace 4.00 long, 3.25 wide. Abdomen 6.25 long, 3.75 wide. Eye sizes and interdistances: AME 0.25, ALE 0.20, PME 0.20, PLE 0.20; AME-AME 0.10, AME-ALE 0.20, PME-PME 0.20, PME-PLE 0.30. Leg measurements: I: 11.75 (4.00, 4.25, 3.00, 1.50); II: 11.50 (3.50, 4.00, 2.50, 1.50); III: 10.65 (3.35, 3.50, 2.50, 1.30); IV: 14.35 (4.25, 5.00, 3.50, 1.60). Carapace yellow.


Figure 6. Pireneitega lushuiensis sp. n., female paratype and male holotype. A Epigyne, ventral view B Vulva, dorsal view C Male habitus, dorsal view $\mathbf{D}$ Female habitus, dorsal view $\mathbf{E}$ Female habitus, ventral view. Scale bars: equal for $\mathbf{D}, \mathbf{E}$.

Abdomen yellow, with black spots and herringbone pattern. Epigyne as in Fig. 6A-B: epigynal teeth broad and long (subequal to length of atrium); septum long with sclerotized tip; atrium with well delimited posterior margin, about two times wider than long, about 1.4 times longer than septum, about 1.8 times wider than septum; copulatory opening distinct; receptacle short, separated by three diameters; copulatory ducts separated, median part as wide as terminal and two times longer than basal part; hoods distinct.

Spination in female:

|  | Fe | Pt | Ti | $\mathbf{M t}$ | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 3 d 2 p | - | $3-3 \mathrm{v}$ | $3-3 \mathrm{v}$ | - |
| II | 3 d 2 p 2 r | - | $2 \mathrm{p} 3-3 \mathrm{v}$ | $2 \mathrm{p} 3-3 \mathrm{v}$ | - |
| III | 3 d 2 p 2 r | 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 r |
| IV | 3 d 1 p 2 r | 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |

Distribution. Known only from Yunnan (Fig. 11).

## Pireneitega triglochinata (Zhu \& Wang, 1991)

Figs 7-8, 11
Coelotes triglochinatus Zhu \& Wang, 1991: 1, figs 1-4 ( $\uparrow$ only, male mismatched). Holotype $q$ : China: Sichuan: Mt. Emei. Types lost (originally at Jilin University). Coelotes triglochinatus: Song et al. 1999: 388, f. 225W-X, 227J, 228K ( $q$ only, male mismatched).
Pireneitega triglochinata: Wang \& Jäger 2007: 48 (transfer from Coelotes).

Material examined. China: Sichuan: $2 \widehat{\delta}^{\lambda}$, Mt. Emei, Yuanhong Cave, $29^{\circ} 34^{\prime} 08^{\prime \prime} \mathrm{N}$, $103^{\circ} 2^{\prime} 4^{\prime} 32^{\prime \prime} \mathrm{E}, 858 \mathrm{~m}, 29 . \mathrm{IX} .2016$, Z. Zhao \& X. Zhang; 2q50 ${ }^{\text {ºn }}$, Mt. Emei, $29^{\circ} 34^{\prime} 111^{\prime \prime N}, 103^{\circ} 25^{\prime} 36^{\prime \prime} \mathrm{E}, 834 \mathrm{~m}, 29 . I X .2016, ~ Z . ~ Z h a o ~ \& ~ X . ~ Z h a n g . ~$

Diagnosis. The male can be distinguished from all other Pireneitega species except $P$. involuta and $P$. liansui by having a broad conductor, the width of the conductor is about $1 / 5$ of the loop diameter. From $P$. involuta it can be distinguished by the embolus base, beginning at the 6:00 o'clock position (vs beginning at the 6:30 o'clock position in $P$. involuta). From $P$. liansui it can be distinguished by the tapering tip of the patellar apophysis (vs a bifurcate tip in P. liansui). (Figs 3, 7; Wang et al. 1990: figs 13-15, 18-19). The female can be distinguished from all other Pireneitega species except $P$. involuta and $P$. liansui by having a bent and longer receptacle. From $P$. involuta it can be distinguished by a short septum. From $P$. liansui it can be distinguished by narrow epigynal teeth and the tapering tip of the septum (vs broad epigynal teeth and a blunt of septum tip in P. liansui) (Figs 4, 8; Wang et al. 1990: figs 16-17).

Description. Male: Total length 9.25. Carapace 4.75 long, 3.25 wide. Abdomen 4.50 long, 3.50 wide. Eye sizes and interdistances: AME 0.30 , ALE 0.25 , PME 0.20 , PLE 0.20; AME-AME 0.10, AME-ALE 0.10, PME-PME 0.20, PME-PLE 0.20. Leg measurements: I: 15.75 (4.50, 5.25, 4.00, 2.00); II: 14.00 (4.25, 4.50, 3.50, 1.75); III:


Figure 7. Palp of Pireneitega triglochinata, specimen from Mt. Emei. A Prolateral view B Ventral view C Retrolateral view. Scale bar: equal for A, B, C.
12.45 ( $3.75,4.10,3.00,1.60$ ); IV: 16.30 (4.75, 5.25, 4.30, 2.00). Carapace brown, radial grooves indistinct. Abdomen yellow with black herringbone pattern. Palp as in Fig. 7: patellar apophysis long, more than half length of tibia, with tapering tip; tibia short, about the same length as cymbium; VTA subequal to the tibial length, without pointed tip, extending beyond the tibia; RTA short, about $1 / 8$ length of VTA; cymbial furrow short, about $1 / 3$ length of cymbium; width of conductor about $1 / 5$ of loop diameter; embolus with broad base, beginning at the 6:00 o'clock position.

Spination in male:

|  | Fe | Pt | Ti | $\mathbf{M t}$ | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 2 d 2 p 1 r | - | $3-3 \mathrm{v}$ | $3-3 \mathrm{v}$ | - |
| II | 3 d 2 p 2 r | - | 2 p 3 b | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | - |
| III | 3 d 2 p 2 r | 1 p 1 r | $1 \mathrm{~d} 2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | - |
| IV | 3 d 2 p 1 r | $1 \mathrm{~d} 1 \mathrm{p} \mathrm{1r}$ | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 r |

Female: Total length 9.75. Carapace 5.00 long, 4.00 wide. Abdomen 4.75 long, 3.50 wide. Eye sizes and interdistances: AME 0.25, ALE 0.20, PME 0.25, PLE 0.20 ; AME-AME 0.10, AME-ALE 0.10, PME-PME 0.20, PME-PLE 0.25. Leg measurements: I: 14.00 ( $4.50,4.75,3.25,1.50$ ); II: 13.20 (4.20, 4.50, 3.00, 1.50); III: 12.05 (4.00, 4.00, 2.80, 1.25); IV: 14.50 (4.75, 4.75, 3.50, 1.50). Carapace yellow. Abdomen black with yellow spots and herringbone pattern. Epigyne as in Fig. 8A-B: epigy-


Figure 8. Pireneitega triglochinata, specimens from Mt. Emei. A Epigyne, ventral view B Vulva, dorsal view C Male habitus, dorsal view D Female habitus, dorsal view E Female habitus, ventral view. Scale bars: equal for $\mathbf{D}, \mathbf{E}$.
nal teeth narrow and short about 0.9 of atrium length; septum with well sclerotized tip; atrium with weakly delimited posterior margin, about 3.3 times longer than septum, about 1.9 times wider than septum; copulatory opening distinct; receptacle narrow and long, about five times longer than wide, separated by the diameter of receptacle; median part of copulatory ducts as wide as terminal and 1.5 times longer than basal part, median part about three times wider than receptacle; hoods distinct.

Spination in female:

|  | Fe | Pt | Ti | Mt | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | $1 \mathrm{~d} \mathrm{1p} \mathrm{1r}$ | - | $1 \mathrm{p} \mathrm{3-3v}$ | $4-4 \mathrm{v}$ | - |
| II | $3 \mathrm{~d} 2 \mathrm{p} \mathrm{2r}$ | - | $2 \mathrm{p} \mathrm{3-3v}$ | $2 \mathrm{p} 3-3 \mathrm{v}$ | - |
| III | $3 \mathrm{~d} \mathrm{2p} \mathrm{2r}$ | 1 p 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 r |
| IV | $3 \mathrm{~d} \mathrm{2p} \mathrm{1r}$ | 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $5 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 p 1 r |

Distribution. Known only from Sichuan (Fig. 11).
Note. The DNA barcode of the male described here matches that of the female. In the original species description of Coelotes triglochinatus, the female holotype and male 'allotype' were not correctly matched (Wang and Jäger 2007). The male 'allotype' of C. triglochinatus might match the female of other Coelotinae species described from Mt. Emei. Currently, two Coelotinae species described from Mt. Emei are known only by females, they are Draconarius sichuanensis Wang \& Jäger, 2007 and Platocoelotes imperfectus Wang \& Jäger, 2007 (World Spider Catalog 2017).

## Pireneitega xiyankouensis Zhao \& Li, sp. n .

http://zoobank.org/2176DAC7-EF2A-4753-8AEA-153FDE021D35
Figs 9-10, 11
Type material. Holotype : China: Guangxi: Hechi Prefecture: Yizhou City: Xiyankou Village, Mt. Baihu, Xiannvyan, $24^{\circ} 29^{\prime} 17^{\prime \prime N}$, $108^{\circ} 34^{\prime} 02^{\prime \prime} \mathrm{E}, 110 \mathrm{~m}, 11 . X I I .2012$, Z. Chen \& Z. Zhao. Paratypes: $2 q$, same data as holotype; $1 q$, Hechi Prefecture: Donglan County: Sanshi Town: Gongping Village, unnamed cave, $24^{\circ} 21^{\prime} 44$ "N, $107^{\circ} 23^{\prime} 11^{\prime \prime} \mathrm{E}, 383 \mathrm{~m}, 11 . \mathrm{II} .2015$, Y. Li \& Z. Chen; 1q, Hechi Prefecture: Donglan County: Bala Village, unnamed cave, $24^{\circ} 26^{\prime} 37^{\prime \prime} \mathrm{N}, 107^{\circ} 20^{\prime} 50^{\prime \prime} \mathrm{E}, 385 \mathrm{~m}, 18 . I I I .2015$, Y. Li \& Z. Chen; $2 q 1 \delta^{\lambda}$, Hechi Prefecture: Nandan County: Chengguan Town, unnamed cave, $25^{\circ} 02^{\prime} 11^{\prime \prime} \mathrm{N}, 107^{\circ} 25^{\prime} 00^{\prime \prime} \mathrm{E}, 559 \mathrm{~m}, 2 . I I .2015$, Y. Li \& Z. Chen; 1 q1 ${ }^{\AA}$, Chongzuo Prefecture: Daxin County: Fulong Town: Pingliang Village, Banzhongtun, Shuiniu Cave, $22^{\circ} 57^{\prime} 55^{\prime \prime} \mathrm{N}, 107^{\circ} 28^{\prime} 12^{\prime \prime} \mathrm{E}, 248 \mathrm{~m}, 24 . X I I .2012$, Z. Chen \& Z. Zhao; 3 의, Baise Prefecture: Debao County: Yandong Town: Yandong Village, Chuanshan Cave, $23^{\circ} 10^{\prime} 00^{\prime \prime} \mathrm{N}, 106^{\circ} 40^{\prime} 01^{\prime \prime} \mathrm{E}, 596 \mathrm{~m}, 20 . X I I .2012, \mathrm{Z}$. Chen \& Z. Zhao; 1 q, Chongzuo Prefecture: Pingxiang City: Liancheng County, Baiyu Cave, $22^{\circ} 07^{\prime} 44^{\prime \prime} \mathrm{N}$, $106^{\circ} 45^{\prime} 55^{\prime \prime} \mathrm{E}, 326 \mathrm{~m}, 28 . X I I .2012$, Z. Chen \& Z. Zhao; 1 q, Chongzuo Prefecture: Tiandeng County: Dukang Town: Bakong Village, Yuanliutun, entrance to unnamed cave, $23^{\circ} 06^{\prime} 45^{\prime \prime} \mathrm{N}, 107^{\circ} 04^{\prime} 33^{\prime \prime} \mathrm{E}, 457 \mathrm{~m}, 26 . X I I .2012$, Z. Chen \& Z. Zhao.


Figure 9. Palp of Pireneitega xiyankouensis sp. n., male holotype. A Prolateral view B Ventral view C Retrolateral view. Scale bar: equal for A, B, C.

Etymology. The specific name refers to the type locality; adjective.
Diagnosis. The male can be distinguished from all other Pireneitega species except P. involuta, P. liansui and P. triglochinata by having a broad conductor, the width of the conductor about $1 / 5$ of loop diameter. From $P$. involuta it can be distinguished by the bifurcate tip of the patellar apophysis ( $v s$ a tapering tip in $P$. involuta and $P$. triglochinata). From P. liansui it can be distinguished by the short cymbial furrow, about 0.3 times the length of the cymbium ( $v s$ a long cymbial furrow in $P$. liansui, more than half the length of the cymbium) (Figs 3, 7, 9; Wang et al. 1990: figs 13-15, 18-19). The female can be distinguished from all other Pireneitega species except $P$. xinping Zhang, Zhu \& Song, 2002 by having bent and narrow epigynal teeth, a broad atrium and sclerotized tip of the septum. From P. xinping it can be distinguished by a long receptacle, about four times longer than wide ( $v s$ a straight and short receptacle in P. xinping, about two times longer than wide) (Fig. 10; Zhang et al. 2002: figs 7-8).

Description. Male (holotype): Total length 9.60 . Carapace 4.25 long, 3.75 wide. Abdomen 5.35 long, 3.50 wide. Eye sizes and interdistances: AME 0.35, ALE 0.30,


Figure 10. Pireneitega xiyankouensis sp. n., female paratype and male holotype. A Epigyne, ventral view B Vulva, dorsal view C Male habitus, dorsal view D Female habitus, dorsal view E Female habitus, ventral view. Scale bars: equal for $\mathbf{D}, \mathbf{E}$.

PME 0.30, PLE 0.25; AME-AME 0.05, AME-ALE 0.10, PME-PME 0.16, PME-PLE 0.20. Leg measurements: I: 18.85 (5.00, 6.50, 4.85, 2.50); II: 17.25 (4.75, 5.75, 4.50, 2.25); III: 15.70 ( $4.45,5.00,4.25,2.00$ ); IV: 20.35 (5.50, 6.60, 5.75, 2.50). Carapace yellow, radial grooves distinct, with black lateral margins. Abdomen brown with yellow herringbone pattern. Palp as in Fig. 9: patellar apophysis short, about 1/3 length of tibia; tibia short, about 1/4 length of cymbium; VTA subequal to the tibial length, without pointed tip, extending beyond the tibia; RTA short, about 1/8 length of VTA; cymbial furrow short, about $1 / 3$ length of cymbium; conductor broad; embolus with broad base Spination in male:

|  | Fe | Pt | Ti | Mt | Ta |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 2d 2p 2 r | - | $1 \mathrm{p} 3-3 \mathrm{v}$ | 2p 3-3v | - |
| II | 2d 3p 2 r | 1p | $1 \mathrm{p} 3-3 \mathrm{v}$ | $1 \mathrm{p} 1 \mathrm{r} 3-3 \mathrm{v}$ | - |
| III | 2d 2 p 2 r | 2d 1p 1r | $1 \mathrm{~d} 2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $3 \mathrm{~d} 2 \mathrm{p} 4 \mathrm{r} 3-3 \mathrm{v}$ | 1 r |
| IV | 2 d 2 p 1 r | 1d 1p 1r | 2d 2p 3-3v | 1d 1p 3r 3-3v | - |

Female (paratype): Total length 10.90. Carapace 5.13 long, 3.95 wide. Abdomen 5.77 long, 3.75 wide. Eye sizes and interdistances: AME 0.35, ALE 0.35, PME 0.26, PLE 0.26; AME-AME 0.10, AME-ALE 0.10, PME-PME 0.24, PME-PLE 0.28. Leg measurements: I: 15.75 (4.50, 5.50, 4.00, 1.75); II: 14.60 (4.25, 5.10, 3.50, 1.75); III: 13.60 (4.10, 4.50, $3.50,1.50$ ); IV: 16.90 ( $4.75,5.65,4.75,1.75$ ). Carapace yellow. Abdomen brown with yellow spots and herringbone pattern. Epigyne as in Fig. 10A-B: epigynal teeth narrow and long; septum short with weakly sclerotized tip; atrium with well delimited posterior margin, about three times longer than septum, about 1.3 times wider than septum; copulatory opening distinct; receptacle long, separated by three diameters; median part of copulatory ducts as wide as terminal and 1.3 times longer than basal part, median part about two times wider than receptacle; hoods distinct.

Spination in female:

|  | Fe | Pt | $\mathbf{T i}$ | $\mathbf{M t}$ | $\mathbf{T a}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| I | 3 d 2 p 1 r | - | $3-3 \mathrm{v}$ | $3-3 \mathrm{v}$ | - |
| II | 3 d 2 p 2 r | 1 p | $2 \mathrm{p} 3-3 \mathrm{v}$ | $2 \mathrm{p} 1 \mathrm{r} 3-3 \mathrm{v}$ | - |
| III | $3 \mathrm{~d} 2 \mathrm{p} \mathrm{3r}$ | 2 d 1 p 1 r | $1 \mathrm{~d} 2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $4 \mathrm{p} 5 \mathrm{r} 3-3 \mathrm{v}$ | 1 r |
| IV | 3 d 2 p 1 r | 1 p 1 r | $2 \mathrm{p} 2 \mathrm{r} 3-3 \mathrm{v}$ | $2 \mathrm{~d} 2 \mathrm{p} 3 \mathrm{r} 3-3 \mathrm{v}$ | - |

Distribution. Known only from Guangxi (Fig. 11).

## Acknowledgements

The manuscript benefitted greatly from comments by Yuri M. Marusik (Institute for Biological Problems of the North RAS, Russia), Alexander A. Fomichev (Altai State University, Russia), Mykola Kovblyuk (V.I. Vernadsky Taurida National University, the Crimea). Sarah Crews (California Academy of Sciences, USA) kindly checked English of the manuscript. This study was supported by the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (2015CASEABRI005, Y4ZK111B01)


Figure II. Collection localities of five Pireneitega species from China. I P. huashanensis sp. n. 2 P. liansui 3 P. lushuiensis sp. n. 4 P. triglochinata 5 P. xiyankouensis sp. n.
to Shuqiang Li, the National Natural Sciences Foundation of China to Guo Zheng (NSFC-31372224, 31672315) and Shuqiang Li (NSFC-31530067, 31471960).

## References

Bao YH, Yin CM (2004) Two new species of the genus Coelotes from Hunan Province (Araneae, Amaurobiidae). Acta Zootaxonomica Sinica 29: 455-457.
Brignoli PM (1982) On a few spiders from China (Araneae). Bulletin of the British Arachnological Society 5: 344-351.
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.

Kishida K (1955) A synopsis of spider family Agelenidae. Acta Arachnological, Tokyo 14: 1-13. https://doi.org/10.2476/asjaa.14.1
Li S, Lin Y (2016) Species Catalogue of China. Volume 2. Animals. Invertebrates (I), Arachnida: Araneae. Science Press, Beijing, 549 pp.
Miller JA, Carmichael A, Ramirez MJ, Spagna JC, Haddad CR, Řezáč M, Johannesen J, Král J, Wang XP, Griswold CE (2010) Phylogeny of entelegyne spiders: affinities of the family Penestomidae (new rank), generic phylogeny of Eresidae, and asymmetric rates of change in spinning organ evolution (Araneae, Araneoidea, Entelegynae). Molecular Phylogenetics and Evolution 55: 786-804. https://doi.org/10.1016/j.ympev.2010.02.021
Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and Phylogenetics utility of mitochondrial gene sequences and compilation of conserved polymerase chain reaction Primers. Annals of the Entomologcal Society of America 87(6): 651-701. https://doi.org/10.1093/aesa/87.6.651
Song DX, Zhu MS, Chen J (1999) The Spiders of China. Hebei University of Science and Techology Publishing House, Shijiazhuang, 640 pp .
Wang JF, Yin CM, Peng XJ, Xie LP (1990) New species of the spiders of the genus Coelotes from China (Araneae: Agelenidae). In: Spiders in China: One Hundred New and Newly Recorded Species of the Families Araneidae and Agelenidae. Hunan Normal University Press, 172-253.
Wang XP (2002) A generic-level revision of the spider subfamily Coelotinae (Araneae, Amaurobiidae). Bulletin of the American Museum of Natural History 269: 1-150. https://doi. org/10.1206/0003-0090(2002)269<0001:AGLROT>2.0.CO;2
Wang XP, Jäger P (2007) A revision of some spiders of the subfamily Coelotinae F. O. PickardCambridge 1898 from China: transfers, synonymies, and new species (Arachnida, Araneae, Amaurobiidae). Senckenbergiana Biologica 87: 23-49.
World Spider Catalog (2017) World Spider Catalog. Natural History Museum Bern, online at http://wsc.nmbe.ch, version 18.0 [accessed on January 12, 2017]
Yin CM, Peng XJ, Yan HM, Bao YH, Xu X, Tang G, Zhou QS, Liu P (2012) Fauna Hunan: Araneae in Hunan, China. Hunan Science and Technology Press, Changsha, 1590 pp.
Zhang X, Marusik YM (2016) A survey of Pireneitega from Tajikistan (Agelenidae, Coelotinae). ZooKeys 635: 89-107. https://doi.org/10.3897/zookeys.635.10487
Zhang X, Zhao Z, Zheng G, Li S (2016) Nine new species of the spider genus Pireneitega Kishida, 1955 (Agelenidae, Coelotinae) from Xinjiang, China. ZooKeys 601: 49-74. https://doi.org/10.3897/zookeys.601.7893
Zhang ZS, Zhu MS, Song DX (2002) Three new species of the subfamily Coelotinae from Mt. Shennongjia of Hubei Province, China (Araneae: Amaurobiidae). Journal of the Baoding Teachers College 15: 52-55.
Zhao Z, Su TJ, Chesters D, Wang SD, Ho SYW, Zhu CD, Chen XL, Zhang CT (2013) The mitochondrial genome of Elodia flavipalpis Aldrich (Diptera: Tachinidae) and the evolutionary timescale of tachinid flies. PLoS ONE 8:e61814. https://doi.org/10.1371/journal. pone. 0061814
Zhu CD, Wang JF (1991) Six new species of the genus Coelotes from China (Araneae: Agelenidae). Journal of Norman Bethune University of Medical Sciences 17(5): 1-4.
Zhu CD, Wang JF (1994) Seven new species of the genus Coelotes from China (Araneae: Agelenidae). Acta Zootaxonomica Sinica 19: 37-45.

# Qingryllus jiguanshanensis sp. n. from Sichuan, China, the second species of Qingryllus (Orthoptera, Gryllidae) 

Haoyu Liu', Dongxiao Zhang', Fuming Shi'<br>I The Key Laboratory of Zoological Systematics and Application, College of Life Sciences, Hebei University, Baoding 071002, Hebei Province, China<br>Corresponding author: Haoyu Liu (liuhy@aliyun.com)

Academic editor: F. Montealegre-Z | Received 15 December 2016 | Accepted 28 February 2017 | Published 27 March 2017
http://zoobank.org/A49F6346-655E-4295-8982-F53C431EEC15
Citation: Liu H, Zhang D, Shi F (2017) Qingryllus jiguanshanensis sp. n. from Sichuan, China, the second species of Qingryllus (Orthoptera, Gryllidae). ZooKeys 663: 65-70. https://doi.org/10.3897/zookeys.663.11494


#### Abstract

A second species of Qingryllus Chen \& Zheng is described and illustrated, Q. jiguanshanensis sp. n., from Sichuan, China. This new species is similar to Q. striofemorus Chen \& Zheng, 1995, but differs from the latter by the posterior margin of pronotum being distinctly widened, the veins of tegmina yellowish-white only on lateral side of dorsal area, and the epiphallus distinctly widened posteriorly. A distribution map of the species of this genus and habitus photographs of the new species are presented.


## Keywords

Gryllinae, Qingrylus, taxonomy

## Introduction

The genus Qingryllus was established by Chen and Zheng (1995) for Q. striofemorus Chen \& Zheng, 1995, based on female specimens. Li and Zheng (2001) described the male characters of $Q$. striofemorus, with limited distribution in Ningshan (China: Shaanxi), this genus remains monotypic until the present time (Eades et al. 2016).

In our recent study on Gryllinae from Sichuan, China, a new species of Qingryllus was discovered and it is described here under the name of $Q$. jiguanshanensis sp. n. There are two species of Qingryllus, both occurring in China (Figure 1).


Figure I. Distribution map of the genus Qingryllus Chen \& Zheng.

The genus Qingryllus differs from most groups of Gryllinae by the fore tibia without tympanum and tegminal venation similar in both sexes. In Gryllinae, the genus Qingryllus has the greatest similarity to Goniogryllus Chopard, 1936, but differs from the latter by the tegmina, developed hind wings, and hind tibiae with four pairs of dorsal spines on both sides. Goniogryllus is generally without wings or with very short tegmina, and the hind tibiae usually has three dorsal spines (Chen \& Zheng 1995). Meanwhile, the two genera have different microhabitat preferences, with Qingryllus living in trees and shrubs and Goniogryllus living in the litter layer.

## Materials and methods

Specimens examined were collected using sweeping method in trees and shrubs. The type specimens of the new species have been deposited in the Museum, Hebei University, Baoding, China (MHBU).

The male genitalia were dissected and cleared in $10 \% \mathrm{KOH}$ solution. All morphological structures were photographed using a Leica M205A microscope. Images of
multiple layers were stacked using Combine ZM. The distribution map was constructed using the software package ArcGIS 10.2 (ESRI, Redlands, CA, USA), based on localities of the specimens examined for this study and those mentioned in literature (Chen and Zheng 1995; Li and Zheng 2001).

## Taxonomy

## Qingryllus jiguanshanensis sp. n.

http://zoobank.org/6F867825-77BF-4133-97E2-4B331CF3A1DE
Figures 2-4

Type material. Holotype $\widehat{\sigma}^{\lambda}$ : CHINA: Sichuan, Chongzhou, Jiguanshan, Shaoyaogou, 29.V.2016, leg. Fuming Shi. Paratypes: $1 \delta^{\lambda}, 1 q$ : same data as the holotype.

Description. Male: Body medium, slightly small (Figure 2A). Head globular, smooth, with few pubescence. Frontal rostrum slightly obvious, dorsal surface flat, 1.8 times as wide as the scapus; eyes slightly protruding, rounded, located in lateroanterior sides of head; ocelli slightly rounded, median ocellus very small, lateral ocelli distinctly large; third joint of maxillary palpus distinctly long, 1.7 times as long as $4^{\text {th }}$ joint, slightly shorter than $5^{\text {th }}$ joint, $5^{\text {th }}$ joint widened apically, apical margin transversal and arc-shaped; last joint of labial palpus slightly longer than $2^{\text {th }}$ joint, slightly widened at apex, apical margin obtuse. Pronotum transverse, anterior margin slightly concave, nearly as wide as the head, distinctly widened posteriorly, posterior margin nearly straight, 1.3 times as wide as the anterior margin, 2.1 times wider than the length of pronotum; centre of pronotal disc with inconspicuous longitudinal furrow, the lateral sides with crescent impressions, lateral margins angularly rounded bent into paranota; lateral lobes distinctly smooth, without tomenta, anterior lower angles right angled, terminal obtuse, distinctly rising backwards, posterior lower angles wide, rounded. Tegmina slightly overlapping abdominal apex, with several irregular longitudinal veins and numerous transverse veins, forming irregular reticulum. Hind wings distinctly overlapping tegmina. Fore legs smooth, with no tympanum and no impressions on tibiae. Hind tibiae with four pairs of dorsal spines and three pairs of apical spurs on both sides, of which inner, upper and medial apical spurs are equal in length and distinctly longer than lower apical spurs; outer, upper and lower apical spurs slightly shorter than middle ones, nearly equal in length. Dorsal area of first tarsi with 5-8 outer and 4-5 inner spinules.

Supra anal plate (Figure 3D) semicircular, posterior margin broad, round. Subgenital plate rather long, narrowed posteriorly, coniform. Genitalia (Figure 3A-C). Epiphallus slightly longitudinal, anterior margin distinctly concave in middle; middle part of epiphallus narrowest, and distinctly widened anteriorly and posteriorly; apical part of epiphallus with two divided lobes, posterior margin slightly concave bearing setae. Ectoparamers semimembranous and long finger-like, not overlapping posterior margin of epiphallus; medial lobes short and slightly divided at apex. Endoparamers


Figure 2. Habitus of Qingryllus jiguanshanensis sp. n., dorsal view. A male B female.
narrowed, reaching middle part of epiphallus. Apodeme distinctly developed and bend upward at apex.

Female (Figure 2B): Body very similar to that of male. Subgenital plate (Figure 4A) wide and short, narrowed posteriorly, posterior margin nearly straight. Ovipositor (Figure 4 B ) long and straight, near the end not swollen, apex acute.

Colouration. Body black brown to black. Part of $4^{\text {th }}$ and $5^{\text {th }}$ joint of maxillary palpus, superior border of eyes, vein of tegmina on lateral sides and lower sides of hind femur yellowish-white.

Measurements (mm). Male: body 11.2-12.2, length with wings 17.0-17.5, pronotum 1.9-2.0, tegmen 9.5-9.6, hind femur 7.0-7.5; Female: body 11.0, length with wings 16.0 , pronotum 2.0 , tegmen 9.5 , hind femur 7.0 , ovipositor 9.5 .

Diagnosis. This new species is similar to the type species, but differs from the latter by the posterior margin of pronotum being distinctly widened; veins of tegmina yellowish-white only on lateral side of dorsal area (between dorsal and lateral area of tegmina); epiphallus distinctly widened posteriorly. In the type species, the posterior margin of pronotum being slightly widened; basal part of dorsal area of tegmina also yellowish-white; epiphallus distinctly narrowed at apex.


Figure 3. Qingryllus jiguanshanensis sp. n. Male. A-C genitalia (A dorsal view $\mathbf{B}$ ventral view $\mathbf{C}$ lateral view) D supra anal plate, dorsal view.


Figure 4. Qingryllus jiguanshanensis sp. n. Female. A Subgenital plate, ventral view B Ovipositor, lateral view.

Distribution. China (Sichuan).
Etymology. The specific name is derived from its type locality, Jiguanshan (China: Sichuan).

## Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 31201731), Conservation International (Insect Resource Survey in Anzihe Protected Area) and the Natural Science Foundation of Hebei Province (No. C2014201043).

## References

Chen J, Zheng ZM (1995) One new genus and two species of Gryllidae from China (Orthoptera: Grylloidea). Journal of Shaanxi Normal University (Natural Science Edition) 23(2): 72-76. [In Chinese with English Summary]
Eades DC, Otte D, Cigliano MM, Braun H (2016) Orthoptera Species File. Version 5.0/5.0. http://Orthoptera.SpeciesFile.org [accessed 12 August 2016]
Li K, Zheng ZM (2001) The descriptions of the males of Goniogryllus ovalatus and Qingryllus striofemorus (Orthoptera: Grylloidea). Entomotaxonomia 23(3): 165-168. [In Chinese with English Summary]

# The Phanuromyia galeata species group (Hymenoptera, Platygastridae, Telenominae): shining a lantern into an unexplored corner of Neotropical diversity 

Katherine C. Nesheim', Lubomír Masner², Norman F. Johnson ${ }^{\text { }}$<br>I Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 1315 Kinnear Road, Columbus, Ohio, 43212, USA 2 Agriculture and Agri-Food Canada, K. W. Neatby Building, Ottawa, Ontario K1A 0C6, Canada<br>Corresponding author: Norman F. Johnson (johnson.2@osu.edu)

## Academic editor: M. Sharkey \| Received 19 December 2016 | Accepted 3 March 2017 | Published 27 March 2017

## http://zoobank.org/B330445E-8AB6-4200-8D8E-547F7B77F66D

Citation: Nesheim KC, Masner L, Johnson NF (2017) The Phanuromyia galeata species group (Hymenoptera, Platygastridae, Telenominae): shining a lantern into an unexplored corner of Neotropical diversity. ZooKeys 663: 71-105. https://doi.org/10.3897/zookeys.663.11554


#### Abstract

The Phanuromyia galeata species group is delineated and its species richness explored for the first time (Hymenoptera: Platygastridae, Telenominae). Fifteen species are described, all of which are new: Phanuromyia comata Nesheim \& Masner, sp. n. (Brazil), P. constellata Nesheim, sp. n. (Paraguay), P. corys Nesheim \& Masner, sp. n. (Brazil), P. cranos Nesheim \& Masner, sp. n. (Bolivia, Costa Rica, Ecuador, French Guiana), P. cudo Nesheim \& Masner, sp. n. (Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Panama, Peru, Trinidad and Tobago, Venezuela), P. dissidens Nesheim \& Masner, sp. n. (Bolivia, Brazil, French Guiana), P. galeata Nesheim \& Masner, sp. n. (Belize, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Mexico, Peru), P. galerita Nesheim \& Masner, sp. n. (Brazil, Ecuador, French Guiana), P. hjalmr Nesheim, sp. n. (Bolivia, Costa Rica, Ecuador, Paraguay, Venezuela), P. krossotos Nesheim, sp. n. (Ecuador), P. odo Nesheim, sp. n. (Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Mexico, Panama, Peru, Trinidad and Tobago, Venezuela), P. pauper Nesheim, sp. n. (Ecuador, Peru), P. princeps Nesheim, sp. n. (Brazil, Ecuador, French Guiana), P. tonsura Nesheim, sp. n. (Brazil, Colombia, Ecuador, Paraguay, Peru), P. tubulifer Nesheim \& Masner, sp. n. (Brazil, Guyana).


## Keywords

Platygastroidea, parasitoid, species description, key, Neotropical, new species

## Introduction

The subfamily Telenominae (Hymenoptera: Platygastroidea, Platygastridae) is a large group of egg-parasitoid wasps, comprising 905 known species found throughout the world. Traditionally it has been thought to be composed of two major genera, Telenomus Haliday and Trissolcus Ashmead, and a number of small, morphologically distinctive satellite genera. This view of telenomine diversity was largely based on over 180 years of work on the Holarctic fauna, beginning with Haliday (1833). This scheme, however, has struggled to cope with the diversity of species from the world's tropics.

The genus Phanuromyia Dodd was originally described in 1914 on the basis of a single species from southern coastal Queensland, distinguishing it on the basis of the presence of an extruded ovipositor. Dodd later (1916) added a second species from New South Wales. In the years that followed the concept of the genus largely fell into obscurity, being cited only five times in the taxonomic literature for the rest of the century, largely because Dodd's laconic description did little to distinguish the taxon from the many other species in the subfamily and because the original type material in Australia was not studied. Johnson and Musetti (2003) sought to better define the genus, using new characters and a perspective informed by decades of new collections from around the world. Mineo (2006) rejected the recognition of Phanuromyia, basing this on the a priori assertion that the characters used were only appropriate for distinguishing species groups and not genera. Taekul et al. (2014), however, confirmed that Phanuromyia is distinct from Telenomus and, further, expanded the concept to embrace species before placed in the crassiclava group of Telenomus (following Johnson 1984). The data and analyses (Taekul et al. 2014) suggest that Phanuromyia is the sister group of Telenomus + Trissolcus (along with several satellite genera), and the limited host data that are available all indicate that Phanuromyia are egg parasitoids of lanternflies and planthoppers in the families Fulgoridae and Flatidae (Hemiptera: Auchenorrhyncha).

In the Neotropics Phanuromyia is often the most common telenomine genus to be encountered, even surpassing the abundance of Telenomus s.str. However, very few of the species have been formally described. One subset of this diversity is a group of large, elongate, and distinctive species initially recognized by LM as a discrete entity in the fauna of Central and South America: the galeata group. The goals of this paper are to document the diversity of the Phanuromyia galeata group. The contributions of the authors are as follows: K.C. Nesheim: character definition, species group concept development, species concept development, imaging, key development, manuscript preparation; L. Masner: species group concept development, species concept development, key development; N.F. Johnson: species concept development, manuscript preparation, database design and maintenance.

## Materials and methods

This work is based upon specimens deposited in the following collections, with abbreviations used in the text: CNCI, Canadian National Collection of Insects, Ottawa,

Canada; OSUC, C.A. Triplehorn Insect Collection, Columbus, OH. Morphological terminology follows Mikó et al. (2007) and the Hymenoptera Anatomy Ontology (http://portal.hymao.org/projects/32/public/ontology), which is searchable for all morphological terms in this manuscript.

Information management. Holotypes are unambiguously identifiable by means of the unique identifier or the red holotype label. The numbers prefixed with "OSUC" are unique identifiers for the individual specimens. These unique identifiers are associated with their specimens' data in The Ohio State University's Hymenoptera Online database, which can be accessed at http://hol.osu.edu. Searching this database using a specimen's unique identifier will produce all data associated with the specimen. All new species have been prospectively registered with Zoobank as well as the Hymenoptera Name Server (http:// hns.osu.edu).

Tools. Images were created using AutoMontage and Combine ZP extended focus software. All images are archived within The Ohio State University's image database (http://specimage.osu.edu). Species descriptions were generated using a database application, vSysLab (http://vsyslab.osu.edu). This application facilitates the construction of taxon character data matrices, the integration of matrices with our existing taxonomic database, and the exportation of data in a variety of file types which can be used in other programs.

Species concept. We define species as populations with the potential to interbreed (Mayr 1942). Interbreeding populations will develop a gradient of character states within each morphological character, while distinct separations between character states will exist in non-interbreeding populations (Wild 2004); therefore, species delimitations are made by identifying discrete character states within characters that are present across multiple specimens.

## Results

## Phanuromyia Dodd

http://zoobank.org/FDEC083E-2450-477E-B678-82F53B317E22
http://bioguid.osu.edu/xbiod_concepts/600
Phanuromyia Dodd, 1914: 121. Original description. Type: Phanuromyia rufobasalis Dodd, by monotypy and original designation. Kieffer 1926: 16, 131 (description, keyed); Muesebeck and Walkley 1956: 384 (citation of type species); Masner, 1976: 79 (taxonomic status); Johnson 1991: 211 (description); Johnson 1992: 564 (catalog, catalog of world species); Johnson and Musetti 2003: 139 (description, synonymy, list of included species); Taekul et al. 2014: 30 (diagnosis, phylogenetic relationships within Telenominae); Veenakumari and Mohanraj 2014: 135, 146 (key to species of India, distribution).
Issidotelenomus Pélov, 1975: 89. Original description. Type: Issidotelenomus obscuripes Pélov, by original designation. Kozlov and Kononova 1983: 137 (junior synonym of Telenomus Haliday); Johnson and Musetti 2003: 140 (junior synonym of Phanuromyia Dodd).

Diagnosis. The three genera Phanuromyia, Telenomus and Trissolcus Ashmead cannot be distinguished on the basis of any single, easily recognized morphological character. Rather, they are recognized by the preponderance of evidence from several characters: presence or absence of sculpture on the medial portion of the frons, length of setation between the ommatidia of the compound eyes, shape of the head, number of clavomeres in the female antenna, presence or absence of notauli on the mesoscutum, presence or absence of sculpture on the disk of the mesoscutellum, form of the sternaulus, shape of the first and second metasomal tergites, and sculpture of the second metasomal tergite. To distinguish Phanuromyia, focus should first be placed on the sternaulus. In the large majority of species this is expressed as a line of pits, beginning anteriorly on the mesepisternum near the dorsal apex of the acetabular carina and extending dorsally and posteriorly toward the mesopleural pit. In Telenomus and Trissolcus the sternaulus may have a single irregularly shaped pit, and its course is otherwise represented by fold or crease in the cuticle. Small individuals of Phanuromyia, however, also may have merely a poorly defined line of impression. Supplemental characters to distinguish Phanuromyia are eye setation very short or seemingly absent; frontal depression weakly expressed so that the head appears semiglobose in shape; frontal sculpture highly variable, ranging from smooth to sculptured throughout; female antenna with five clavomeres (defined morphologically, see Johnson 1984); notauli absent; mesoscutellar disk sculpture highly variable; T1 strongly transverse; T2 longer than wide; T2 often with distinctive coriaceous to reticulate microsculpture extending beyond the pits marking the position of the antecostal suture and the longitudinal striae arising between those pits. As Dodd (1914) noted, the ovipositor is often exserted a great distance and is easily seen, but this feature is relevant for only a minority of species.

Within Phanuromyia, we separate the galeata group purely as a practical grouping, and at this point we do not assert its monophyly. The group may be distinguished, first and foremost, by their unusually large body size: most specimens are greater than 2 mm in length. Beyond that, the body is distinctly elongate, T 1 in the female is produced into a horn to house the ovipositor, T 2 is strongly elongate, as often are the following tergites. The group is strictly Neotropical in distribution, extending from the Isthmus of Tehuantepec in the north to Misiones in southern Paraguay.

## Key to assist recognition of Phanuromyia

1 Notauli present........................................................ Trissolcus, Telenomus

- Notauli absent .............................................................................................. 2

2 Sternaulus indicated by a distinct oblique line of foveolae on the mesepisternum most Phanuromyia

- $\quad$ Sternaulus indicated by a shallow fold or by 1 to 2 irregular pits near pro-

3(2) T2 reticulate beyond basal costae............................................Phanuromyia

- T2 smooth beyond basal costae................................................................... 4

4(3) Antennal scrobe absent or weakly impressed, head in lateral profile semiglobose

Phanuromyia

- Antennal scrobe and lateral profile variable ............... Trissolcus, Telenomus


## Phanuromyia comata Nesheim \& Masner, sp. n.

http://zoobank.org/01F7E2C9-511E-4ABF-9826-B206A5D2B20E
http://bioguid.osu.edu/xbiod_concepts/403725
Figures 1-6

Description. Female body length: $2.36 \mathrm{~mm}(\mathrm{n}=1)$.
Median keel on frons: absent. Sculpture of lower frons: with $>6$ transverse rugae medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: distinctly concave. Number of visible terga past T2: 2 or 3. Setation on T2: consisting of thick patches of lateral setae; consisting of widespread scattered pilosity.

Diagnosis. Phanuromyia comata can be recognized by T2 setation consisting of thick patches of lateral setae combined with widespread scattered pilosity dorsally.

Etymology. The name comata is derived from the Latin word for having long hair because this species has diagnostic patches of setae. This name is to be used as a participle.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403725]
Material examined. Holotype, female: BRAZIL: MT, $500 \mathrm{~m}, 12^{\circ} 46 \mathrm{~S}$, $55^{\circ} 30^{\prime} \mathrm{W}$, Vila Vera, X-1973, M. Alvarenga, OSUC 149413 (deposited in CNCI).

Comments. Phanuromyia comata is the only species in the group with widespread pilosity across the entirety of T2, so this character can be used to identify a specimen very quickly.

## Phanuromyia constellata Nesheim, sp. n.

http://zoobank.org/DA85EF19-E1F9-4E63-8164-6437A76CF006
http://bioguid.osu.edu/xbiod_concepts/403720
Figures 7-12

Description. Female body length: $1.43-1.54 \mathrm{~mm}(\mathrm{n}=3)$.


Figures I-6. Phanuromyia comata $q$ (OSUC 149413), I Lateral habitus 2 Dorsal habitus $\mathbf{3}$ Head, mesosoma, lateral view 4 Head, mesosoma, dorsal view 5 Head, mouthparts, anteroventral view 6 T2-T4, dorsal view. Scale bar in millimeters.

Median keel on frons: absent. Sculpture of lower frons: with 3-6 transverse rugae medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with two rows of setiferous punctures converging medially and then diverging ventrally.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: smooth. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.


Figures 7-I 2. Phanuromyia constellata $\odot($ OSUC 322906), 7 Lateral habitus 8 Dorsal habitus 9 Head, mesosoma, lateral view 10 Head, mesosoma, dorsal view II Head, mouthparts, anteroventral view 12 Head, ventral view. Scale bar in millimeters.

T1: swollen in posterior half. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: straight; only slightly concave. Number of visible terga past T2: 4 or 5 . Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia constellate can be recognized by the swirling pattern of setiferous punctures on the frons.

Etymology. The name constellata is derived from the Latin word for constellation because the pattern of punctures on this species' frons is reminiscent of stars in the sky. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403720]
Material examined. Holotype, female: PARAGUAY: Canindeyú Dept., Jejuí-mí, wet grazing floor, MT1, Bosque Mbaracayú Natural Reserve, 29.V-11.VI.1996, Malaise trap, A. C. F. Costa, OSUC 322906 (deposited in OSUC). Paratypes: PARAGUAY: 2 females, OSUC 322905, 322907 (OSUC).

## Phanuromyia corys Nesheim \& Masner, sp. n.

http://zoobank.org/777F2663-8058-4618-8062-B2E0C0E30161
http://bioguid.osu.edu/xbiod_concepts/389325
Figures 13-18

Description. Female body length: 2.80-3.05 mm (n=3).
Median keel on frons: absent. Sculpture of lower frons: with multiple transverse rugae. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with two rows of setiferous punctures converging ventrally.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: straight; only slightly concave. Number of visible terga past T2: 4 or 5. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia corys can be recognized by the two rows of setiferous punctures converging ventrally on the frons.

Etymology. The name corys is derived from a Greek word for helmet because this species has a large head evoking the image of a knight wearing a helmet. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389325]
Material examined. Holotype, female: BRAZIL: RJ, Silva Jardim, VIII-1974, F. M. Oliveira, OSUC 550201 (deposited in CNCI). Paratypes: BRAZIL: 3 females, OSUC 149358-149360 (CNCI).

Comments. This species is recognizable by its large size, only the largest specimens of $P$. odo reach over 2.7 mm in length. Phanuromyia corys may be distinguished from $P$. odo by the converging lines of setiferous punctures on the frons and the straight, transverse apical margin of T2.


Figures I3-I8. Phanuromyia corys $q$ (OSUC 149359), I3 Lateral habitus 14 Dorsal habitus 15 Head, mesosoma, lateral view 16 Head, mesosoma, dorsal view 17 Head, mouthparts, anteroventral view 18 Frons, anteroventral view. Scale bar in millimeters.

## Phanuromyia cranos Nesheim \& Masner, sp. n.

http://zoobank.org/59383F22-EFB5-4638-B7BC-7BBAEB35D567
http://bioguid.osu.edu/xbiod_concepts/389326
Figures 19-24

Description. Female body length: 2.21-2.50 mm (n=20).


Figures 19-24. Phanuromyia cranos $\&$ (OSUC 550034), 19 Lateral habitus 20 Dorsal habitus 21 Head, mesosoma, lateral view 22 Head, mesosoma, dorsal view $\mathbf{2 3}$ Head, mouthparts, anteroventral view 24 Frons, anteroventral view. Scale bar in millimeters.

Median keel on frons: absent. Sculpture of lower frons: with multiple transverse rugae; with irregular rugosity. Shape of mandible: slender. Median tooth of mandible: as large as adjacent teeth. Frons below median ocellus: with 2 perfectly parallel rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.

T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: straight; only slightly concave. Number of visible terga past T2: 2 or 3. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia cranos can be recognized by the 2 perfectly parallel rows of setiferous punctures on the frons.

Etymology. The name cranos is derived from a Greek word for helmet because this species has a large head evoking the image of a knight wearing a helmet. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389326]
Material examined. Holotype, female: ECUADOR: Sucumbíos Prov., Napo River, $270 \mathrm{~m}, 00^{\circ} 30^{\prime} \mathrm{S}, 76^{\circ} 30^{\prime} \mathrm{W}$, Sacha Lodge, 3.IV-13.IV.1994, Malaise trap, P. Hibbs, OSUC 550028 (deposited in CNCI). Paratypes: (21 females) BOLIVIA: 4 females, OSUC 149423, 550038-550040 (CNCI). COSTA RICA: 5 females, OSUC 149421, 164007, 550031-550032, 550035 (CNCI). ECUADOR: 11 females, OSUC 149420, 149422, 164006, 320967, 550027, 550029-550030, 550033-550034, 550036-550037 (CNCI). FRENCH GUIANA: 1 female, OSUC 550111 (CNCI).

Comments. Phanuromyia cranos can be identified most quickly by the distinctive pattern of setiferous punctures on its frons. The setiferous frontal puncture of $P$. odo may at times appear similar, but $P$. odo has the apical margin of T2 distinctly concave.

## Phanuromyia cudo Nesheim \& Masner, sp. n.

http://zoobank.org/9785C5ED-ABDC-405E-B771-999C038B87CC
http://bioguid.osu.edu/xbiod_concepts/389327
Figures 25-30

Description. Female body length: 1.33-1.82 mm (n=20).
Median keel on frons: absent. Sculpture of lower frons: with 3-6 transverse rugae medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: smooth. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: distinctly swollen throughout entire length. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: almost entirely smooth. Posterior margin of T2: straight. Number of visible terga past T2: 3 or 4. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia cudo can be recognized by the swelling of the entire T1 segment.


Figures 25-30. Phanuromyia cudo $\odot$ (OSUC 550006), $\mathbf{2 5}$ Lateral habitus $\mathbf{2 6}$ Dorsal habitus $\mathbf{2 7}$ Head, mesosoma, lateral view $\mathbf{2 8}$ Head, mesosoma, dorsal view $\mathbf{2 9}$ Head, anteroventral view $\mathbf{3 0} \mathrm{T} 1-\mathrm{T} 2$, lateral view. Scale bar in millimeters.

Etymology. The name cudo is derived from the Latin word for a helmet made of raw skin because this species has a large head evoking the image of a knight wearing a helmet. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389327]
Material examined. Holotype, female: COSTA RICA: Heredia Prov., La Selva Biological Station, 100m, X-1992, Malaise trap, P. Hanson, OSUC 549938 (deposited in CNCI). Paratypes: ( 129 females) BELIZE: 1 female, OSUC 550084
(CNCI). BOLIVIA: 10 females, OSUC 149379, 149405-149406, 550016-550019, 550080-550082 (CNCI). BRAZIL: 2 females, OSUC 149400, 550012 (CNCI). COLOMBIA: 1 female, OSUC 149407 (CNCI). COSTA RICA: 37 females, OSUC 149381-149382, 149388, 149390-149391, 149394-149395, 149399, 149408, 549929-549935, 549937, 549956-549957, 549964-549965, 549978-549979, 549983, 549987, 550007, 550010, 550014, 550020-550026, 550079, 575268 (CNCI). ECUADOR: 65 females, OSUC 149387, 149393, 149397, 240600, 549936, 549939-549946, 549948-549955, 549958-549963, 549966-549970, 549973-549977, 549981-549982, 549984-549986, 549988-549996, 549998550006, 550008, 550013, 550113-550115 (CNCI). FRENCH GUIANA: 1 female, OSUC 550099 (CNCI). PANAMA: 7 females, OSUC 149380, 149384, 149389, 149401, 149409, 549971, 549997 (CNCI). PERU: 2 females, OSUC 549980, 550107 (CNCI). TRINIDAD AND TOBAGO: 2 females, OSUC 149383, 550015 (CNCI). VENEZUELA: 1 female, OSUC 149403 (CNCI).

Comments. This species exhibits variation in several characters, but can be reliably diagnosed by the swollen appearance of the entirety of T2.

## Phanuromyia dissidens Nesheim \& Masner, sp. n.

 http://zoobank.org/6F72B99A-A5AA-4F40-AEA0-4197D000A302http://bioguid.osu.edu/xbiod_concepts/403721
Figures 30-36

Description. Female body length: $1.07-2.22 \mathrm{~mm}(\mathrm{n}=4)$.
Median keel on frons: present. Sculpture of lower frons: with multiple transverse rugae. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: evenly covered with setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: costate at sides, smooth medially; entirely costate. Posterior margin of T2: straight; slightly convex. Number of visible terga past T2: 5. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia dissidens can be recognized by the even covering of setiferous punctures on the frons.

Etymology. The name dissidens is derived from the Latin word for differing because this species has an evenly punctured frons, differentiating it from the other species in the group. This name is to be used as a participle.


Figures 3I-36. Phanuromyia dissidens + (OSUC 149412), 3I Lateral habitus 32 Dorsal habitus 33 Head, mesosoma, lateral view $\mathbf{3 4}$ Head, mesosoma, dorsal view $\mathbf{3 5}$ Head, mouthparts, anteroventral view 36 Frons, anteroventral view. Scale bar in millimeters.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403721]
Material examined. Holotype, female: BRAZIL: MT, $500 \mathrm{~m}, 12^{\circ} 46^{\prime} \mathrm{S}, 55^{\circ} 30^{\prime} \mathrm{W}$, Vila Vera, X-1973, M. Alvarenga, OSUC 149412 (deposited in CNCI). Paratypes: (3 females) BOLIVIA: 1 female, OSUC 550077 (CNCI). FRENCH GUIANA: 2 females, OSUC 550105, 550110 (CNCI).

Comments. This species most closely resembles $P$. krossotos. Phanuromyia dissedens may be distinguished by the absence of patches of setae on laterally on T2.

## Phanuromyia galeata Nesheim \& Masner, sp. n.

http://zoobank.org/F0693029-5388-4AE5-A406-067BD87D5179
http://bioguid.osu.edu/xbiod_concepts/386058
Figures 37-42

Description. Female body length: 2.02-2.44 mm (n=15). Male body length: 1.84$2.10 \mathrm{~mm}(\mathrm{n}=5)$.

Median keel on frons: present. Sculpture of lower frons: with multiple transverse rugae. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: without setiferous punctures.

Sculpture on posterior half of mesoscutum: with strong, parallel longitudinal keels. Sculpture of anterior half of mesoscutellum: smooth. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: dark brown to black, contrasting with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: straight; slightly convex. Number of visible terga past T2: 5. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia galeata can be recognized by the median keel on the frons.

Etymology. The name galeata is derived from a Latin word for helmet because this species has a large head evoking the image of a knight wearing a helmet. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=386058]
Material examined. Holotype, female: COSTA RICA: Puntarenas Prov., road to Rincón, 24 km W Pan-American Highway, 200 m, III-1989 - V-1989, Hanson \& Gauld, OSUC 550198 (deposited in CNCI). Paratypes: ( 62 females, 5 males) BELIZE: 1 female, OSUC 550083 (CNCI). BRAZIL: 3 females, OSUC 149315149316, 550188 (CNCI). COLOMBIA: 2 females, OSUC 149320 (CNCI); OSUC 170507 (OSUC). COSTA RICA: 21 females, 3 males, OSUC 149313, 149319, 149321-149324, 149326, 359303, 550087, 550093-550097, 550189-550197, 550199 (CNCI). ECUADOR: 18 females, 2 males, OSUC 149310-149311, 149325, 550170-550184, 550187, 550200 (CNCI). EL SALVADOR: 5 females, OSUC 550088-550092 (CNCI). FRENCH GUIANA: 6 females, OSUC 149317149318, 550102, 550116, 555798, 555801 (CNCI). MEXICO: 1 female, OSUC 320968 (CNCI). PERU: 5 females, OSUC 149312, 149314, 550106, 550185550186 (CNCI).

Comments. This species most closely resembles $P$. galerita, but the two can be easily distinguished from each other by comparing the mandibles: $P$. galeata has a slender mandible with a small median tooth, while $P$. galerita has much broader mandibles and a median tooth as large as the outer teeth.


Figures 37-42. Phanuromyia galeata $\circ+$ (OSUC 555798), 37 Dorsal habitus 38 Lateral habitus 39 Head, mesosoma, dorsal view $\mathbf{4 0}$ Head, mesosoma, lateral view 41 Head, mouthparts, anteroventral view 42 Frons, anteroventral view. Scale bar in millimeters.

## Phanuromyia galerita Nesheim \& Masner, sp. n.

http://zoobank.org/9EE257B5-1447-4290-BB70-9C6026337A3D
http://bioguid.osu.edu/xbiod_concepts/389328
Figures 43-48

Description. Female body length: 2.31-2.55 mm (n=5).
Median keel on frons: absent. Sculpture of lower frons: with irregular rugosity. Shape of mandible: broad. Median tooth of mandible: as large as adjacent teeth. Frons below median ocellus: without setiferous punctures.


Figures 43-48. Phanuromyia galerita $\circ$ (OSUC 550202), 43 Lateral habitus 44 Dorsal habitus 45 Head, mesosoma, lateral view 46 Head, mesosoma, dorsal view 47 Head, mouthparts, anteroventral view 48 Mouthparts, ventral view. Scale bar in millimeters.

Sculpture on posterior half of mesoscutum: with strong, parallel longitudinal keels. Sculpture of anterior half of mesoscutellum: rugose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: dark brown to black, contrasting with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: straight; slightly convex. Number of visible terga past T2: 5. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia galerita can be recognized by the median tooth of the mandible, which is as large as the adjacent teeth.

Etymology. The name galerita is derived from the Latin word for wearing a hood because this species has a large head evoking the image of a hooded figure. This name is to be used as a noun in apposition. This name is to be used as a participle.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389328]
Material examined. Holotype, female: ECUADOR: Sucumbíos Prov., 270m, $00^{\circ} 30^{\prime}$ S, $76^{\circ} 30^{\prime} \mathrm{W}$, Sacha Lodge, 13.VI-23.VI.1994, Malaise trap, P. Hibbs, OSUC 149327 (deposited in CNCI). Paratypes: (4 females) BRAZIL: 1 female, OSUC 149328 (CNCI). ECUADOR: 2 females, OSUC 550202-550203 (CNCI). FRENCH GUIANA: 1 female, OSUC 550101 (CNCI).

Comments. This species most closely resembles $P$. galeata, but the two can be easily distinguished from each other by comparing the mandibles: P. galeata has a slender mandible with a small median tooth, while $P$. galerita has much broader mandibles and a median tooth as large as the outer teeth.

## Phanuromyia hjalmr Nesheim, sp. n.

http://zoobank.org/CDCE8232-4502-4479-9317-5A7DE79295A7
http://bioguid.osu.edu/xbiod_concepts/403730
Figures 49-54

Description. Female body length: 1.19-1.99 mm (n=6).
Median keel on frons: absent. Sculpture of lower frons: with irregular rugosity; with 3-6 transverse rugae medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: rugosepunctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: evenly costate across anterior $1 / 3$ to $1 / 2$, smooth in remaining apical portion. Posterior margin of T2: distinctly concave. Number of visible terga past T2: 2 or 3 . Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia hjalmr can be recognized by the sculpture of T1, which is evenly costate across the anterior $1 / 3$ to $1 / 2$ and smooth in remaining apical portion, combined with the distinctly concave posterior margin of T2.

Etymology. The name hjalmr is derived from the Old Norse word for helmet because this species has a large head evoking the image of a knight wearing a helmet. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403730]


Figures 49-54. Phanuromyia hjalmr $\circ$ (OSUC 550078), 49 Lateral habitus 50 Dorsal habitus 51 Head, mesosoma, lateral view $\mathbf{5 2}$ Head, mesosoma, dorsal view $\mathbf{5 3}$ Head, mouthparts, anteroventral view 54 T1-T2, dorsal view. Scale bar in millimeters.

Material examined. Holotype, female: BOLIVIA: La Paz Dept., Nor Yungas Prov., Coroico, cloud forest, B-03, El Bagante, 1500m, 18.IV.1997, screen sweeping, L. Masner, OSUC 149417 (deposited in CNCI). Paratypes: ( 5 females) COSTA RICA: 1 female, OSUC 550078 (CNCI). ECUADOR: 1 female, OSUC 550050 (CNCI). PARAGUAY: 2 females, OSUC 322901, 324322 (OSUC). VENEZUELA: 1 female, OSUC 149392 (CNCI).

Comments. This species most closely resembles $P$. tonsura, but the two species can be distinguished by their T1 sculpture: $P$. tonsura has the medial portion of T1 smooth from its anterior to posterior margin, while $P$. hjalmr is sculptured across the entire anterior third of T1.

## Phanuromyia krossotos Nesheim, sp. n.

http://zoobank.org/99ACD36B-BD3C-4B7D-BF87-4780C014CFCD
http://bioguid.osu.edu/xbiod_concepts/389330
Figures 55-60

Description. Male body length: $1.46 \mathrm{~mm}(\mathrm{n}=1)$.
Median keel on frons: absent. Sculpture of lower frons: without rugosity. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with setiferous punctures only medially.


Figures 55-60. Phanuromyia krossotos đ (OSUC 550046), 55 Lateral habitus 56 Dorsal habitus 57 Head, mesosoma, lateral view 58 Head, mesosoma, dorsal view 59 Head, mouthparts, anteroventral view $\mathbf{6 0}$ Metasoma, lateral view. Scale bar in millimeters.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: evenly costate across anterior $1 / 3$ to $1 / 2$, smooth in remaining apical portion. Posterior margin of T2: only slightly concave. Number of visible terga past T2: 5. Setation on T2: consisting of thick patches of lateral setae.

Diagnosis. Phanuromyia krossotos can be recognized by the thick lateral patches of setae on T2.

Etymology. The name krossotos is derived from the Greek word for fringed because this species has a distinctive fringe of lateral setae on T2. This name is to be used as an adjective.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389330]
Material examined. Holotype, male: ECUADOR: Sucumbíos Prov., Napo River, $290 \mathrm{~m}, 00^{\circ} 05^{\prime} \mathrm{S}, 76^{\circ} 05^{\prime} \mathrm{W}$, Sacha Lodge, 14.III-24.III.1994, Malaise trap, P. Hibbs, OSUC 550046 (deposited in CNCI).

Comments. This species most closely resembles $P$. dissidens. This species most closely resembles $P$. dissidens. Phanuromyia krossotos may be distinguished by the presence of patches of setae on laterally on T2.

## Phanuromyia odo Nesheim, sp. n.

http://zoobank.org/561818C9-9A51-492A-84C5-910AF4BDDA62
http://bioguid.osu.edu/xbiod_concepts/389324
Figures 61-66

Description. Female body length: $1.51-2.78 \mathrm{~mm}(\mathrm{n}=22)$.
Median keel on frons: absent. Sculpture of lower frons: with 3-6 transverse rugae medially; with irregular rugosity medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: distinctly concave. Number of visible terga past T2: 2 or 3. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia odo can be recognized by the distinctly concave posterior margin of T2 combined with the entirely costate sculpture of T1.


Figures 6I-66. Phanuromyia odo $q$ (OSUC 550248), 61 Lateral habitus 62 Dorsal habitus 63 Head, mesosoma, lateral view 64 Head, mesosoma, dorsal view 65 Head, mouthparts, anteroventral view 66 T2-T6, dorsal view. Scale bar in millimeters.

Etymology. The name odo is derived from the name of the Changeling in the popular television series Star Trek: Deep Space Nine because this species has variable morphology. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389324]
Material examined. Holotype, female: COSTA RICA: Heredia Prov., La Selva Biological Station, 1.V-8.V.1989, Malaise trap, B. V. Brown, OSUC 149335 (deposited in CNCI). Paratypes: (175 females, 2 males) BELIZE: 2 females, OSUC 149352,

550252 (CNCI). BOLIVIA: 6 females, OSUC 149354, 149372, 149375, 550122, 550127-550128 (CNCI). BRAZIL: 9 females, OSUC 149341-149342, 149345, 149378, 550075-550076, 550232, 550238 (CNCI); OSUC 151125 (OSUC). COLOMBIA: 1 female, OSUC 149373 (CNCI). COSTA RICA: 100 females, 1 male, OSUC 149329-149331, 149333-149334, 149336-149337, 149348, 149350, $149353,149355-149357,149362-149364,149367,149369,149377,164001$, 550085-550086, 550098, 550123-550126, 550129-550133, 550135, 550148, 550150, 550157-550158, 550204-550219, 550221-550231, 550233-550237, 550239, 550241-550251, 550253-550268, 550270, 575269-575270 (CNCI); OSUC 575271 (OSUC). ECUADOR: 43 females, 1 male, OSUC 149338-149340, 149343-149344, 149347, 149351, 149361, 149365-149366, 240601, 320966, 550134, 550136-550147, 550149, 550151-550156, 550159-550160, 550162550169, 550220, 550240 (CNCI). EL SALVADOR: 1 female, OSUC 149374 (CNCI). FRENCH GUIANA: 6 females, OSUC 550103-550104, 550108, 555797, 555799-555800 (CNCI). GUATEMALA: 1 female, OSUC 149371 (CNCI). MEXICO: 1 female, OSUC 149346 (CNCI). PANAMA: 1 female, OSUC 149368 (CNCI). PERU: 1 female, OSUC 149376 (CNCI). TRINIDAD AND TOBAGO: 1 female, OSUC 550161 (CNCI). VENEZUELA: 2 females, OSUC 149332, 149349 (CNCI).

Comments. The specimens here referred to as Phanuromyia odo were originally split into two provisional species. This was based upon differences in the relative length of the wings, specifically, whether the wings reached beyond the apex of the metasoma. Figure 67 illustrates the relationship between body size and wing length: larger specimens indeed have relatively shorter wings. However, there is no distinctive gap in the ratio between the two. Therefore, we treat them here as a single species.

## Phanuromyia pauper Nesheim \& Masner, sp. n.

http://zoobank.org/561818C9-9A51-492A-84C5-910AF4BDDA62
http://bioguid.osu.edu/xbiod_concepts/389329
Figures 68-73

Description. Female body length: $1.31-1.62 \mathrm{~mm}(\mathrm{n}=19)$.
Median keel on frons: absent. Sculpture of lower frons: with irregular rugosity medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: medially without costae or foveolae. T2 sculpture: laterally scrobiculate, smooth medially. Sculpture of T1: evenly costate across anterior $1 / 3$ to $1 / 2$, smooth in remaining apical portion. Posterior margin


Figure 67. The specimens identified as Phanuromyia odo were originally split into two separate species, here labeled "small" and "large." The trend line is calculated based on data for all specimens combined. The continuity in both variables and the overlap in specimens divided a priori into small and large categories led to the conclusion that there is insufficient evidence to separate these specimens into two species.
of T2: straight. Number of visible terga past T2: 4 or 5 . Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia pauper can be recognized by the T2 sculpture, which is scrobiculate laterally and smooth medially.

Etymology. The name pauper refers to the lack of longitudinal costae on the base of T2. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=389329]
Material examined. Holotype, female: PERU: Madre de Dios Reg., canopy, $290 \mathrm{~m}, 12^{\circ} 50^{\prime} \mathrm{S}, 69^{\circ} 17^{\prime} \mathrm{W}$, Tambopata National Reserve, III-1983 - IX-1983, fogging, T. L. Erwin, OSUC 149427 (deposited in CNCI). Paratypes: ECUADOR: 18 females, OSUC 149396, 149424-149425, 164002, 549972, 550011, 550062-550067, 550069-550074 (CNCI).

Comments. This species very closely resembles $P$. princeps, although the two species can be distinguished easily by the sculpture of T2: $P$. princeps has a complete scrobiculate angled "belt" while $P$. pauper only has lateral costae.


Figures 68-73. Phanuromyia pauper + (OSUC 550066), 68 Lateral habitus 69 Dorsal habitus 70 Head, mesosoma, lateral view 71 Head, mesosoma, dorsal view $\mathbf{7 2}$ Head, mouthparts, anteroventral view $73 \mathrm{~T} 1-\mathrm{T} 2$, lateral view. Scale bar in millimeters.

## Phanuromyia princeps Nesheim, sp. n.

http://zoobank.org/26939117-0EDA-42F8-9930-2108E2DE1686
http://bioguid.osu.edu/xbiod_concepts/403732
Figures 74-79

Description. Female body length: $1.38-1.48 \mathrm{~mm}(\mathrm{n}=10)$.
Median keel on frons: absent. Sculpture of lower frons: with 3-6 transverse rugae medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.


Figures 74-79. Phanuromyia princeps $\&$ (OSUC 151126), 74 Dorsal habitus 75 Lateral habitus 76 Head, mesosoma, dorsal view $\mathbf{7 7}$ Head, mesosoma, lateral view 78 Head, mouthparts, anteroventral view 79 T1-T3, dorsal view. Scale bar in millimeters.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: ru-gose-punctate. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: medially without costae or foveolae. T2 sculpture: with transverse series of small punctures in shape of incurved chevron. Sculpture of T1: evenly costate across anterior $1 / 3$ to $1 / 2$, smooth in remain-
ing apical portion. Posterior margin of T2: straight; slightly convex. Number of visible terga past T2: 4 or 5 . Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia princeps can be recognized by the T2 sculpture, which consists of a transverse series of small punctures in the shape of an incurved chevron.

Etymology. The name princeps is derived from the prince character in the book The Prince and the Pauper by Mark Twain in reference to its similarity to $P$. pauper. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403732]
Material examined. Holotype, female: BRAZIL: BA, Sapiranga Reserve, sweeping 13, $12^{\circ} 33^{\prime} 27.3^{\prime \prime} S 38^{\circ} 03^{\prime} 05^{\prime \prime} \mathrm{W}$, Mata de São João, 24.VII.2001, sweeping, M. T. Tavares et al., OSUC 150922 (deposited in OSUC). Paratypes: (9 females) BRAZIL: 4 females, OSUC 150923, 151077, 151098, 151126 (OSUC). ECUADOR: 1 female, OSUC 550068 (CNCI). FRENCH GUIANA: 4 females, OSUC 149426, 550100, 550109, 550112 (CNCI).

Comments. This species very closely resembles $P$. pauper, although the two species can be distinguished easily by the sculpture on T2: $P$. princeps has a complete scrobiculate angled "belt," while $P$. pauper only has lateral costae.

## Phanuromyia tonsura Nesheim, sp. n.

http://zoobank.org/90880ED0-8CEA-4475-855D-D42583686F2E
http://bioguid.osu.edu/xbiod_concepts/403728
Figures 80-85

Description. Female body length: $1.32-1.77 \mathrm{~mm}(\mathrm{n}=20)$.
Median keel on frons: absent. Sculpture of lower frons: with irregular rugosity medially. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: uncertain, smooth. Thin median foliaceous lamella on propodeum: absent.

Color of coxae: bright yellow, concolorous with legs.
T1: flat, at most slightly swollen. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: costate at sides, smooth medially. Posterior margin of T2: distinctly concave. Number of visible terga past T2: 2 or 3. Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia tonsura can be recognized by the sculpture of T1, which is costate laterally and smooth medially.


Figures 80-85. Phanuromyia tonsura $+\frac{1}{}$ (OSUC 149418), $\mathbf{8 0}$ Dorsal habitus $8 \mathbf{I}$ Lateral habitus 82 Head, mesosoma, dorsal view $\mathbf{8 3}$ Head, mesosoma, lateral view 84 Head, mouthparts, anteroventral view $85 \mathrm{~T} 1-\mathrm{T} 2$, dorsal view. Scale bar in millimeters.

Etymology. The name tonsura is derived from the Latin word for a shearing and refers to the tonsure hairstyle often worn by monks, because this species has a smooth "bald" area in the middle of T1. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403728]
Material examined. Holotype, female: ECUADOR: Sucumbíos Prov., Napo River, $270 \mathrm{~m}, 0^{\circ} 30^{\prime} \mathrm{S}, 76^{\circ} 30^{\prime} \mathrm{W}$, Sacha Lodge, 10.X-21.X.1994, Malaise trap, P.

Hibbs, OSUC 550269 (deposited in CNCI). Paratypes: (33 females) BRAZIL: 1 female, OSUC 149370 (CNCI). COLOMBIA: 1 female, OSUC 149404 (CNCI). ECUADOR: 26 females, OSUC 149386, 149414-149416, 164005, 240606, 549947, 550041-550045, 550047-550049, 550051-550061 (CNCI). PARAGUAY: 3 females, OSUC 322900, 322902, 322904 (OSUC). PERU: 2 females, OSUC 149402, 149418 (CNCI).

Comments. This species most closely resembles $P$. hjalmr, but the two species can be easily distinguished by their T1 sculpture: $P$. tonsura has the medial portion of T1 smooth from its anterior to posterior margin, while $P$. hjalmr is sculptured across the entire anterior third of T1.

## Phanuromyia tubulifer Nesheim \& Masner, sp. n.

http://zoobank.org/A2D35F8B-369A-466A-9D88-217E0BFE090B
http://bioguid.osu.edu/xbiod_concepts/403723
Figures 86-91

Description. Female body length: 2.24-2.26 mm (n=2).
Median keel on frons: absent. Sculpture of lower frons: with multiple transverse rugae. Shape of mandible: slender. Median tooth of mandible: diminished. Frons below median ocellus: with 2 rows of setiferous punctures.

Sculpture on posterior half of mesoscutum: coriaceous to rugulose, at most with fine irregular longitudinal sculpture. Sculpture of anterior half of mesoscutellum: uncertain, rugose-punctate. Thin median foliaceous lamella on propodeum: present.

Color of coxae: bright yellow, concolorous with legs.
T1: with distinct, tubular horn reaching higher than metascutellum. Anterior margin of T2: with costae or foveolae throughout its width. T2 sculpture: with neither transverse series of small punctures nor scrobiculate lateral areas. Sculpture of T1: entirely costate. Posterior margin of T2: straight; only slightly concave. Number of visible terga past T2: 3 or 4 . Setation on T2: limited to at most 1 row of setae posteriorly and sparse setation laterally.

Diagnosis. Phanuromyia tubulifer can be recognized by the distinct, tubular horn on T1 and the thin foliaceous lamella present medially on the propodeum.

Etymology. The name tubulifer refers to the presence of the diagnostic tubular horn on T1 in this species. This name is to be used as a noun in apposition.

Link to distribution map. [http://hol.osu.edu/map-full.html?id=403723]
Material examined. Holotype, female: GUYANA: Potaro-Siparuni Reg., 100$200 \mathrm{~m}, 04^{\circ} 40^{\prime} 19^{\prime \prime} \mathrm{N}, 58^{\circ} 41^{\prime} 04^{\prime \prime} \mathrm{W}$, Iwokrama Forest Reserve, V-2001 - VI-2001, flight intercept trap, R. Brooks \& Z. Falin, OSUC 149410 (deposited in CNCI). Paratype: BRAZIL: 1 female, OSUC 149411 (CNCI).

Comments. This species is distinct due to its tubular horn, although it is unknown whether the males express this character to any significant degree.


Figures 86-91. Phanuromyia tubulifer + (OSUC 149410), 86 Dorsal habitus 87 Lateral habitus 88 Head, mesosoma, dorsal view 89 Head, mesosoma, lateral view 90 Head, mouthparts, anteroventral view 91 Mesosoma, T1, lateral view. Scale bar in millimeters.

## Key to species of the Phanuromyia galeata group

1 Posterior half to $2 / 3$ of mesoscutum with strong parallel, longitudinal keels (best viewed from behind at 45 -degree angle) (Figs 39, 46); all coxae dark brown to black, contrasting with the otherwise bright yellow legs (Fig. 38) .. 2

- $\quad$ Posterior half of mesoscutum at most with fine, irregular longitudinal sculpture, usually coriaceous to rugulose (Fig. 34); all coxae bright yellow, concolorous with remaining segments of legs (Fig. 1)

2(1) Median keel present on frons; lower frons with multiple transverse rugae; disc of mesoscutellum smooth; mandible slender with median tooth smaller than anterior or posterior tooth (Figs 37-42) (Belize, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Mexico, Peru) .......P. galeata sp. n. $\delta^{\top}$ 早

- No median keel on frons; lower frons irregularly rugose; mesoscutellum rugose punctate at least in anterior half; mandible broad with middle tooth as large as adjacent teeth (Figs 43-48) (Brazil, Ecuador, French Guiana).
P. galerita sp. n. $\mathcal{f}$

3(1) Frons below median ocellus with an irregular pattern of setiferous punctures (Figs 35, 59) 4

- Frons below median ocellus with 2 parallel or subparallel rows of setiferous punctures (Fig. 90)5

4(3) Frons below median ocellus evenly covered with setiferous punctures; no lateral patch of setae on T2; body length 1.0 to 2.3 mm (Figs 31-36) (French Guiana, Bolivia, Brazil)............................................. P. dissidens sp. n. q $^{\pi}$

- Frons below median ocellus with setiferous punctures only medially; lateral patch of $15-20$ setae present on T2; body length 1.5 mm (Figs 55-60) (Ecuador)
P. krossotos sp. n. $\widehat{ }$

5(4) Female T1 with distinct tubular horn; propodeum with foliaceous lamella anterior to T1 horn (Figs 86-91) (Brazil, Guyana) ...... P. tubulifer sp. n. ㅇ

- Female T1 at most moderately swollen (Fig. 38); propodeum without median transverse lamella (Figs 25, 38, 81)
6(5) T 2 with a line of foveae that begins at the anterolateral margin of the sclerite and extends obliquely toward the midline (Fig. 74)7
- Anterior margin of T2 longitudinally costate or with fovea that extend transversely toward the midline (Fig. 37).
7(6) T2 with series of small punctures in the shape of an incurved chevron (Figs 74-79) (Brazil, Ecuador, French Guiana)............. P. princeps sp. n. $\uparrow$
- T2 smooth medially, with scrobiculate lines laterally (Figs 68-73) (Ecuador, Peru)............................................................................ P. pauper sp. n. it
8(6) Basal $2 / 3$ of T 1 with at least a large smooth area medially, sometimes entirely smooth (Fig. 85)
_ Basal $2 / 3$ of T1 longitudinally costate across entire width (Fig. 37)............. 10
9(8) Posterior margin of T2 straight; 3 or 4 terga visible beyond apex of T2 (Figs 25-30) (Belize, Bolivia, Brazil, Costa Rica, Colombia, Ecuador, French Guiana, Panama, Peru, Trinidad and Tobago, Venezuela)....P. cudo sp. n. $q$
- Posterior margin of T2 distinctly concave (Fig. 6); 2 or 3 terga visible beyond apex of T2 (Fig. 80) (Brazil, Colombia, Ecuador, Paraguay, Peru)
P. tonsura sp. n. +

10(8) T2, including laterotergite, with widespread sparse pilosity (Figs 1-6) (Brazil)............................. ...................................... P. comata sp. n. $q$

- T2 at most with 1 row of setae posteriorly and sparse setation laterally, elsewhere glabrous (Fig. 66)

11
11(10) Posterior margin of T2 distinctly concave (Figs 61-66) ..... 12

- Posterior margin of T2 straight or only slightly concave (Fig. 20) ..... 13
12(11) T1 costate throughout its length (Fig. 62) (Belize, Bolivia, Brazil, Colom-bia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Mexico,Panama, Peru, Trinidad and Tobago, Venezuela)
$\qquad$ P. odo sp. n. $\delta^{1}+$
- T1 evenly costate across anterior $1 / 3$ to $1 / 2$, smooth posteriorly (Fig. 54) (Bolivia, Costa Rica, Ecuador, Paraguay, Venezuela) .......P. hjalmr sp. n.
13(12) Metasoma with 2 or 3 terga visible beyond apex of T2; frons below median ocellus with two parallel rows of setiferous punctures (Figs 19-24) (Bolivia, Costa Rica, Ecuador, French Guiana) P. cranos sp. n. $\frac{+}{}$
- Metasoma with 4 or 5 terga visible beyond apex of T2 (Fig. 8); two rows of setiferous punctures below median ocellus not parallel (Fig. 12)14
14(13) Two rows of setiferous punctures below median ocellus converging ventrally; T1 flat at margin with T2; large species, body length 2.8 to 3.0 mm (Figs 13-18) (Brazil)
$\boldsymbol{P}$. corys sp. n. $q$
- Two rows of setiferous punctures below median ocellus converging medially and then diverging ventrally; T1 slightly swollen at margin with T2; body length 1.4 to 1.6 mm (Figs 7-12) (Paraguay) $\qquad$ P. constellata sp. n. +


## Acknowledgements

We thank Luciana Musetti and Sara Hemly for critical logistical support, and Huayan Chen for training on vSysLab and extended-focus imaging.

## References

Dodd AP (1914) Further new genera and species of Australian Proctotrypoidea. Proceedings of the Royal Society of Queensland 26: 91-140. https://doi.org/10.5281/zenodo. 23758
Dodd AP (1916) Australian Hymenoptera Proctotrypoidea. No. 4. Transactions and Proceedings of the Royal Society of South Australia 40: 9-32. https://doi.org/10.5281/zenodo. 23791
Haliday AH (1833) An essay on the classification of the parasitic Hymenoptera of Britain, which correspond with the Ichneumones minuti of Linnaeus. Entomological Magazine 1: 259-276. https://doi.org/10.5281/zenodo. 23723
Johnson NF, Musetti L (2003) Redefinition of the genus Phanuromyia Dodd (Hymenoptera: Scelionidae). Journal of the New York Entomological Society 111: 138-144. https://doi. org/10.1664/0028-7199(2003)111[0138:ROTGPD]2.0.CO;2
Johnson NF (1984) Systematics of Nearctic Telenomus: classification and revisions of the podisi and phymatae species groups (Hymenoptera: Scelionidae). Bulletin of the Ohio Biological Survey 6(3): 1-113. https://doi.org/10.5281/zenodo. 23887

Johnson NF (1991) Revision of Australasian Trissolcus species (Hymenoptera: Scelionidae). Invertebrate Taxonomy 5: 211-239. https://doi.org/10.1071/IT9910211
Johnson NF (1992) Catalog of world Proctotrupoidea excluding Platygastridae. Memoirs of the American Entomological Institute 51: 1-825. https://doi.org/10.5281/zenodo. 23657
Kieffer JJ (1926) Scelionidae. Das Tierreich. Vol. 48. Walter de Gruyter \& Co., Berlin, 885 pp.
Kozlov MA, Kononova SV (1983) [Telenominae of the fauna of the USSR]. Nauka, Leningrad, 336 pp .
Masner L (1976) Revisionary notes and keys to world genera of Scelionidae (Hymenoptera: Proctotrupoidea). Memoirs of the Entomological Society of Canada 97: 1-87. https://doi. org/10.4039/entm10897fv
Mayr E (1942) Systematics and the origin of species, from the viewpoint of a zoologist. Columbia University Press, New York, 334 pp.
Mikó I, Vilhelmsen LB, Johnson NF, Masner L, Pénzes Z (2007) Skeletomusculature of Scelionidae (Hymenoptera: Platygastroidea): head and mesosoma. Zootaxa 1571: 1-78.
Mineo G (2006) European Telenomini: re-descriptions, new taxa, and combinations. Scelionidae (Hymenoptera) 2: 1-48.
Muesebeck CFW, Walkley LM (1956) Type species of the genera and subgenera of parasitic wasps comprising the superfamily Proctotrupoidea (order Hymenoptera). Proceedings of the United States National Museum 105: 319-419. https://doi.org/10.5479/ si.00963801.3359.319
Pélov V (1975) Issidotelenomus, un nouveau genre de la famille Scelionidae (Proctotrupoidea, Hymenoptera). Acta Zoologica Bulgarica 7(3): 89-98. https://doi.org/10.5281/zenodo. 24043
Taekul C, Valerio AA, Austin AD, Klompen H, Johnson NF (2014) Molecular phylogeny of telenomine egg parasitoids (Hymenoptera: Platygastroidea s.l.: Telenominae): evolution of host shifts and implications for classification. Systematic Entomology 39: 24-35. https:// doi.org/10.1111/syen. 12032
Talamas EJ, Masner L, Johnson NF (2011) Revision of the Paridris nephta species group (Hymenoptera, Platygastroidea, Platygastridae). ZooKeys 133: 49-94. https://doi.org/10.3897/ zookeys.133.1613
Veenakumari K, Mohanraj P (2014) Five new species of Phanuromyia Dodd (Hymenoptera: Platygastridae) from India. Entomologists Monthly Magazine 150: 135-147.
Wild AL (2004) Taxonomy and distribution of the Argentine ant, Linepithema humile (Hymenoptera: Formicidae). Annals of the Entomological Society of America 97(6): 1204-1215.

## Appendix I

URI Table matching terms and concepts used in this revision with the Hymenoptera Anatomy Ontology database.

| acetabular carina | http://purl.obolibrary.org/obo/HAO_0000292 |
| :---: | :---: |
| antenna | http://purl.obolibrary.org/obo/HAO_0000101 |
| area | http://purl.obolibrary.org/obo/HAO_0000146 |
| body | http://purl.obolibrary.org/obo/HAO_0000182 |
| body length | http://purl.obolibrary.org/obo/HAO_0002413 |
| carina | http://purl.obolibrary.org/obo/HAO_0000188 |
| coriaceous | http://purl.obolibrary.org/obo/HAO_0002379 |
| costa | http://purl.obolibrary.org/obo/HAO_0000225 |
| cuticle | http://purl.obolibrary.org/obo/HAO_0000240 |
| depression | http://purl.obolibrary.org/obo/HAO_0000241 |
| egg | http://purl.obolibrary.org/obo/HAO_0000286 |
| eye | http://purl.obolibrary.org/obo/HAO_0000217 |
| frons | http://purl.obolibrary.org/obo/HAO_0001044 |
| frontal depression | http://purl.obolibrary.org/obo/HAO_0000911 |
| head | http://purl.obolibrary.org/obo/HAO_0000397 |
| impression | http://purl.obolibrary.org/obo/HAO_0000417 |
| lamella | http://purl.obolibrary.org/obo/HAO_0000188 |
| laterotergite | http://purl.obolibrary.org/obo/HAO_0000493 |
| mandible | http://purl.obolibrary.org/obo/HAO_0000506 |
| margin | http://purl.obolibrary.org/obo/HAO_0000510 |
| median ocellus | http://purl.obolibrary.org/obo/HAO_0000526 |
| mesepisternum | http://purl.obolibrary.org/obo/HAO_0001872 |
| mesopleural pit | http://purl.obolibrary.org/obo/HAO_0001358 |
| mesoscutellum | http://purl.obolibrary.org/obo/HAO_0000574 |
| mesoscutum | http://purl.obolibrary.org/obo/HAO_0001490 |
| mesosoma | http://purl.obolibrary.org/obo/HAO_0000576 |
| metascutellum | http://purl.obolibrary.org/obo/HAO_0000625 |
| metasoma | http://purl.obolibrary.org/obo/HAO_0000626 |
| metasomal tergite | http://purl.obolibrary.org/obo/HAO_0002005 |
| mouthparts | http://purl.obolibrary.org/obo/HAO_0000639 |
| ocellus | http://purl.obolibrary.org/obo/HAO_0000661 |
| ovipositor | http://purl.obolibrary.org/obo/HAO_0000679 |
| pilosity | http://purl.obolibrary.org/obo/HAO_0001990 |
| pit | http://purl.obolibrary.org/obo/HAO_0000241 |
| propodeum | http://purl.obolibrary.org/obo/HAO_0001248 |
| sculpture | http://purl.obolibrary.org/obo/HAO_0000913 |
| segment | http://purl.obolibrary.org/obo/HAO_0000929 |
| sternaulus | http://purl.obolibrary.org/obo/HAO_0001509 |
| suture | http://purl.obolibrary.org/obo/HAO_0000982 |
| tergite | http://purl.obolibrary.org/obo/HAO_0001783 |
| tooth | http://purl.obolibrary.org/obo/HAO_0001019 |
| wing | http://purl.obolibrary.org/obo/HAO_0001089 |

## Supplementary material I

## Occurrences

Authors: Katherine C. Nesheim, Lubomír Masner, Norman F. Johnson
Data type: DarwinCore Archive
Explanation note: Label data for specimens used in study.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

# Phylogenetic analysis of the Lancinae (Gastropoda, Lymnaeidae) with a description of the U.S. federally endangered Banbury Springs lanx 

David C. Campbell', Stephanie A. Clark ${ }^{2.4}$, Charles Lydeard ${ }^{3}$<br>I Department of Natural Sciences, Gardner-Webb University, PO Box 7260, Boiling Springs, NC, 28017, USA 2 Invertebrate Identification, 6535 N Mozart St, Chicago, IL, 60645, USA 3 Department of Biology and Chemistry, Morehead State University, 103 Lappin Hall, Morehead, KY, 40351, USA 4 Invertebrates, Gantz Family Collections Center, Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA

Corresponding author: David C. Campbell (pleuronaia@gmail.com)

Academic editor: E. Neubert | Received 24 November 2016 | Accepted 13 March 2017 | Published 28 March 2017
http://zoobank.org/232968CB-A494-47BF-9653-B764C9132D00
Citation: Campbell SC, Clark SA, Lydeard C (2017) Phylogenetic analysis of the Lancinae (Gastropoda, Lymnaeidae) with a description of the U.S. federally endangered Banbury Springs lanx. ZooKeys 663: 107-132. https://doi. org/10.3897/zookeys.663.11320


#### Abstract

We examined the patelliform snails of the subfamily Lancinae, endemic to northwestern North America, to test whether morphological variation correlated with genetic and anatomical differences. Molecular analyses using cox1, 16 S , calmodulin intron, and 28 S rDNA partial sequences and anatomical data supported recognition of four species in three genera. The relationships of lancines within Lymnaeidae are not yet well-resolved. The federally endangered Banbury Springs lanx is described as a new genus and species, Idaholanx fresti, confirming its distinctiveness and narrow endemicity.


## Keywords

Lanx, Fisherola, Basommatophora, anatomy, molecular

## Introduction

The lancines are relatively large freshwater limpets (up to 20 mm in length), found from the upper Sacramento and Pit Rivers of northern California, north to the Columbia River system in the states of Idaho, Oregon, Washington and Montana in the United States and the province of British Columbia, Canada. Some freshwater limpets in related families have been shown to have high morphological variation within relatively few, widespread species (Walther et al. 2006a, b), but no previous study has analyzed the lancines in detail.

Because of their larger size and color pattern, Tryon (1870) incorrectly suspected that some lancines were mislabeled marine forms. Despite the differences, lancines were generally classified along with other freshwater limpets in Ancylinae until Pilsbry (1925) and H. B. Baker (1925) examined the anatomy and showed that they were lymnaeids. Further studies (Morrison 1955, Walter 1969) have confirmed the lymnaeid anatomy. Although several lymnaeids tend towards few whorls and wide apertures, these are the only truly patelliform members extant in the family. Within the Lancinae, three generic names have been proposed: Lanx Clessin, 1880, Fisherola Hannibal, 1912, and Walkerola Hannibal 1912, but whether they should be recognized as genera, subgenera, or synonyms has varied between authors. Current classification typically recognizes Lanx and Fisherola but treats Walkerola as a subgenus or synonym of Lanx (Burch \& Tottenham, 1980). Nine names (plus one unpublished name cited in the literature) have been proposed for extant species (Table 1). However, there is little agreement in the literature as to whether the variation in shell shape, height, color, and anatomy between populations provide an adequate basis for recognizing all of these taxa (Morrison 1955).

Of particular importance are the questions relating to the status of the Banbury Springs lanx. Banbury Springs lanx was discovered by Terry Frest in 1988 and thought to be a new, undescribed species within the genus Lanx. It is listed as federally endangered in the United States (U.S. Fish and Wildlife Service 1992). Although the small size and different shape distinguish it from other lancines, normal Fisherola occur nearby in the Snake River, raising the possibility that it is just a local ecomorph. However, no populations of Fisherola are known from any other springs (U.S. Fish and Wildlife Service 2006).

The primary objective of this study was to determine the taxonomic status of the United States federally endangered Banbury Springs lanx. We describe it as a new genus and species based on molecular and anatomical data. Secondly, we examine the phylogenetic relationships of the Lancinae using mitochondrial and nuclear gene regions.

## Materials and methods

We sampled populations from throughout the geographic range of Lanx and Fisherola, emphasizing morphologically or geographically distinct populations (Table 2). A few additional lymnaeids were sampled as outgroups. Specimens were preserved in ethanol

Table I. Nominal Recent species names in Lancinae.

| Species name | Type locality | Assignment in present study |
| :--- | :---: | :---: |
| Ancylus altus Tryon, 1865 | Klamath River | Lanx alta (Tryon, 1865) |
| Ancylus crassus Haldeman, 1844 | Columbia drainage | Fisherola nuttallii <br> (Haldeman, 1841) |
| Lanx hannai Walker, 1925 | upper Sacramento River | Lanx patelloides (Lea, 1856) |
| Lanx (Walkerola) klamathensis Hannibal, 1912 | Klamath River | Lanx alta (Tryon, 1865) |
| Ancylus kootaniensis Baird, 1863 [kootenaiensis <br> is an invalid emendation] | Kootenai River <br> (restricted by <br> Morrison 1955) | probably Fisherola nuttallii <br> (Haldeman, 1841) but not <br> directly sampled |
| Fisherola lancides Hannibal, 1912 | Snake River | Fisherola nuttallii <br> (Haldeman, 1841) |
| Ancylus newberryi Lea, 1858 | upper Sacramento <br> (correction by Pilsbry <br> 1925) | Lanx patelloides (Lea, 1856) |
| Ancylus (Velletea) nuttallii Haldeman, 1841 | Columbia drainage | Fisherola nuttallii <br> (Haldeman, 1841) |
| Ancylus patelloides Lea, 1856 | upper Sacramento River | Lanx patelloides (Lea, 1856) <br> Ancylus praeclarus Stimpson ms. cited in Lea, 1867$\quad$ unstated |

in the field. Dissections were carried out using a stereomicroscope fitted with a camera lucida. Typically at least two specimens per population were dissected; in some cases only one specimen was available. DNA extraction used digestion in CTAB overnight at $37^{\circ} \mathrm{C}$, followed by chloroform-isoamyl alcohol separation, isopropanol precipitation, and washing with $70 \%$ ethanol before drying and dissolving in TE (Campbell et al. 2005). PCR amplification was often difficult, so several genes were attempted in an effort to find genes with suitable variation that amplified consistently. ITS failed to amplify. 16S (using the primers from Krebs et al. 2003) amplified for few populations. Coxl (using primers LCO1490 from Folmer et al. 1994 and the external primer from Carpenter and Wheeler 1999) amplified for several but not all samples. The best amplification was obtained for 28 S (primers $2 / 3 \mathrm{~F}$ and 6R from Park and Ó Foighil 2000) and calmodulin intron (primers from Schilthuizen et al. 1999 and new primers ATGAAGTGGATGCTGAYGG and ATTCTGGGAARTCTATYG). However, as observed for other gastropods (Simpson et al. 2005), multiple highly divergent calmodulin intron alleles were obtained, suggesting that multiple copies of calmodulin exist in basommatophorans. The sequence length variation was sufficient to make selection of a single copy straightforward using gel extraction (QIAquick gel extraction kit, Qiagen). The band of about 420 bp (including primers) was selected because it consistently amplified strongly. Because the key variable region in 18 S is in the first part of the gene, we used the $1 \mathrm{~F}-4 \mathrm{R}$ primers (Giribet et al. 1996) to amplify that portion of the gene. PCR cycles used were $95^{\circ} \mathrm{C}, 3 \mathrm{~min} ; 5$ cycles at $92^{\circ} \mathrm{C}$ for $30 \mathrm{sec}, 40^{\circ} \mathrm{C}$ for 30 sec ,
Table 2. Populations sequenced. Species names under "Morphospecies" were assigned based on shell form. Designation is the name assigned based on the present results and used in the trees. A single individual from the Rogue system yielded two distinct calmodulin intron sequences and unique sequences for 28 S and cox 1 .

| Designation | Morphospecies | Locality | Drainage | Accession number |
| :--- | :--- | :---: | :---: | :---: |
| Idaholanx fresti | Banbury lanx | Banbury Springs, Idaho | Snake | $\begin{array}{c}\text { calmodulin HM230326, 28S HM230308, } \\ \text { cox1 HM230356, 16S KT267273 }\end{array}$ |
| Idaholanx fresti | Banbury lanx | Box Canyon Springs, Idaho | Snake | $\begin{array}{c}\text { calmodulin HM230327, 28S HM230309, } \\ \text { cox1 HM230357, 16S KT267273 }\end{array}$ |
| Idaholanx fresti | Banbury lanx | Briggs Spring, Idaho | Snake | 28S HM230310 |
| Idaholanx fresti | Banbury lanx | Thousand Springs, Idaho | Snake | calmodulin HM230328, 28S HM230311 |
| Fisherola nuttallii | F. lancides | off Bancroft Springs, Snake River, Idaho | Snake | $\begin{array}{c}\text { calmodulin HM230330, 28S HM230315, } \\ \text { cox1 HM230359, 16S HM230355 }\end{array}$ |
| Fisherola nuttallii | F. nuttallii | Deschutes River, RM 6.3, Oregon | Columbia | calmodulin HM230329,28S HM230314, 16S KT267274 |
| Fisherola nuttallii | F. nuttallii | $\begin{array}{c}\text { Owyhee River, Whistling Bird Rapids, } \\ \text { Oregon }\end{array}$ | Snake | calmodulin HM230331, 18S HM230306, |
| 28S HM230316, cox1 HM230360 |  |  |  |  |$]$| calmodulin HM230336, 18S HM230307 |
| :--- |


| Designation | Morphospecies | Locality | Drainage | Accession number |
| :--- | :--- | :---: | :---: | :---: |
| Lanx patelloides | L. patelloides | Battle Creek, Sacramento River, <br> California | Sacramento | calmodulin HM230343 |
| Lanx patelloides | L. patelloides | Pit River at CA Hwy 299 bridge, <br> California | Sacramento | calmodulin HM230347 |
| Lanx patelloides | L. patelloides | Sucker Springs lower spring channel, <br> California | Pit | calmodulin HM230348, 28S HM230323 |
| Lanx patelloides | L. species | Lava Creek Lodge, Eastman Lake, Fall <br> River, California | Pit | calmodulin HM230344, HM230349(long), 16S KT267276 |
| Lanx patelloides | L. species | Lost Creek source spring | Pit | calmodulin HM230345 |

$65^{\circ} \mathrm{C}$ for 2 min ; and 40 cycles with at $92^{\circ} \mathrm{C}$ for $30 \mathrm{sec}, \mathrm{x}^{\circ} \mathrm{C}$ for $30 \mathrm{sec}, 65^{\circ} \mathrm{C}$ for 2 min , where x is about $2^{\circ} \mathrm{C}$ below the lower primer annealing temperature; finishing with 10 $\min$ at $72^{\circ} \mathrm{C}$ before cooling to $4^{\circ} \mathrm{C}$. In some cases with weak amplification, nested PCR for calmodulin intron using the Schilthuizen et al. (1999) primers followed by the new primers was used. PCR products were purified using DyeEx 2.0 kits (Qiagen). Sequencing used ABI BigDye 3.1 with cycle sequencing reactions of 4 minutes at $96^{\circ} \mathrm{C}$, followed by 40 cycles with 15 sec at $96^{\circ} \mathrm{C}, 15 \mathrm{sec}$ at about $2^{\circ} \mathrm{C}$ below the lower primer annealing temperature, and 4 min at $65^{\circ} \mathrm{C}$, followed by 10 min at $72^{\circ} \mathrm{C}$ before cooling to $4^{\circ} \mathrm{C}$. Sequences were aligned in BioEdit 7.0.5.3 (Hall 1999). Preliminary alignments made use of CLUSTAL W (Larkin et al. 2007), followed by manual editing to eliminate unnecessary gaps, inconsistent alignment of identical sequences, and other problems. Outgroups were selected based on the availability of 28 S sequence data and at least one of the other included genes. To obtain more complete genetic coverage, three outgroups (Carinifex sp., Polyrhytis emarginata s.l., and Galba modicella s.l.) combined sequences from more than one nominal species, but the species in question are closely related and have sometimes been synonymized.

DNA data were analyzed in PAUP* 4.0a152 (Swofford 1998), TNT (Goloboff et al. 2008) and MrBayes3.2 (Ronquist et al. 2011). Duplicate sequences were eliminated from the phylogenetic analyses. Partition-homogeneity tests ( $\mathrm{P}_{\text {ILD }}$ of Dowton and Austin 2002) were run in PAUP*4.0a152 with 100 replicates of 10 random addition replicates each. This test is sensitive to other factors, such as partition size and evolutionary model, besides data compatibility (Dowton and Austin 2002), but may provide a rough idea of agreement between data sets. Despite the problems of the ILD type of tests, no better alternative has gained wide acceptance. The test requires data for each included taxon and partition, so pairwise comparisons were made between all genes. The only significantly incompatible gene was 16 S data, so it was analyzed separately, but the others were concatenated. Indels were coded as missing data. Parsimony analyses in PAUP* used 500 replicates of TBR swapping, with random taxon addition sequence and holding 10 trees at each addition step. Parsimony bootstrapping used 500 replicates, each replicate being a random-addition heuristic search with 10 random replicates. MrModeltest 2.2 (Nylander 2004) was used to select a maximum likelihood model for the nucleic acid sequences that was then input into MrBayes. Bayesian analyses used 2,000,000 generations and 8 chains, with revmat, shape, pinvar, and statefreq unlinked, and the concatenated sequence had the genes identified as partitions. Duplicate sequences were excluded.

## Abbreviations

FMNH Field Museum of Natural History, Chicago, Illinois, U.S.A.
SAC Invertebrate Identification's invertebrate reference collection, Chicago, Illinois, U.S.A.
DCS Deixis Consultants mollusc reference collection, Seattle, Washington, U.S.A.

Table 3. Outgroup sequences analyzed. Source gives locality for new specimens and literature citation for published sequences. * indicates newly generated sequences.

| Taxon | Gene | Accessions | Sources |
| :---: | :---: | :---: | :---: |
| Acroloxus lacustris (Linnaeus, 1758) | 16S | AY577462 | Jorgensen et al. 2004 |
| Acroloxus lacustris (Linnaeus, 1758) | 28S | DQ328296 | Walther et al. 2006b |
| Acroloxus lacustris (Linnaeus, 1758) | coxl | DQ328271 | Walther et al. 2006b |
| Ancylus fluviatilis Müller, 1774 | 16S | AY577466 | Jorgensen et al. 2004 |
| Ancylus fluviatilis Müller, 1774 | 28 S | DQ328295 | Walther et al. 2006b |
| Ancylus fluviatilis Müller, 1774 | coxl | DQ328270 | Walther et al. 2006b |
| Austropeplea tomentosa (L. Pfeiffer, 1855) | 16 S | EU556238 | Puslednik et al. 2009 |
| Austropeplea tomentosa (L. Pfeiffer, 1855) | 28S | HQ156217 | Holznagel et al. 2010 |
| Austropeplea tomentosa (L. Pfeiffer, 1855) | coxl | AY227365 | Remigio and Hebert 2003 |
| Carinifex newberryi (Lea, 1858) | 28S | *HM230312 | Lava Creek, 1st spring pool N. of Hanna Boathouse, CA |
| Carinifex ponsonbyi Smith, 1876 | 16S | *HM230354 | Hagelstein Park, mid channel E. side center, Klamath River, OR |
| Carinifex ponsonbyi Smith, 1876 | coxl | *HM230358 | Hagelstein Park, mid channel E. side center, Klamath River, OR |
| Dilatata dilatata (Gould, 1841) | 28 S | *HM230313 | Sipsey River near Benevola, Greene Co. AL |
| Dilatata dilatata (Gould, 1841) | coxl | EF012173 | Albrecht et al. 2007 |
| Galba modicella (Say, 1825) | $\operatorname{coxl}$ | KM612000 | Dewaard et al. 2015 |
| Galba obrussa (Say, 1825) | 16S | AF485658 | Remigio 2002 |
| Galba obrussa (Say, 1825) | 28 S | *HM230317 | Sipsey River near Benevola, Greene Co. AL |
| Galba obrussa (Say, 1825) | cam | *HM230332 | Sipsey River near Benevola, Greene Co. AL |
| Lymnaea stagnalis (Linnaeus, 1758) | 16S | AF485661 | Remigio 2002 |
| Lymnaea stagnalis (Linnaeus, 1758) | 28S | AY427490 | Vonnemann et al. 2005 |
| Lymnaea stagnalis (Linnaeus, 1758) | coxl | KT831385 | Gordy et al. 2016 |
| Orientogalba ollula (Gould, 1859) | 16 S | U82067 | Remigio and Blair 1997 |
| Orientogalba ollula (Gould, 1859) | 28 S | AY465065 | Jung et al., unpublished |
| Orientogalba ollula (Gould, 1859) | coxl | KC135900 | Park et al. 2012 |
| Physa acuta (Draparnaud, 1805) | 16 S | JQ390525 | Nolan et al. 2014 |
| Physa acuta (Draparnaud, 1805) | 28 S | DQ256738 | Holznagel et al. 2010 |
| Physa acuta (Draparnaud, 1805) | coxl | JQ390525 | Nolan et al. 2014 |
| Planorbella trivolvis (Say, 1817) | 16S | AY030234 | DeJong et al. 2001 |
| Planorbella trivolvis (Say, 1817) | 28S | AF435688 | Morgan et al. 2002 |
| Planorbella trivolvis (Say, 1817) | coxl | KM612028 | Dewaard et al. 2015 |
| Polyrhytis emarginata (Say, 1821) | 28 S | DQ328299 | Walther et al. 2006b |
| Polyrhytis elodes (Say, 1821) | 16S | AF485652 | Remigio 2002 |
| Polyrhytis exilis (Lea, 1834) | coxl | *HM230364 | Ditch along the Stump Lake access road, Jersey Co., IL |
| Radix auricularia (Linnaeus, 1758) | 16 S | JN794284 | von Oheimb et al. 2011 |
| Radix auricularia (Linnaeus, 1758) | 28S | AY465067 | Jung et al., unpublished |
| Radix auricularia (Linnaeus, 1758) | coxl | KP242340 | Patel et al. 2015 |
| Radix balthica (Linnaeus, 1758) | 16 S | HQ330989 | Feldmeyer et al. 2010 |
| Radix balthica (Linnaeus, 1758) | 28 S | EF417136 | Sonnenberg et al. 2007 |
| Radix balthica (Linnaeus, 1758) | coxl | KP098541 | Feldmeyer et al. 2015 |

## Results

Amplification of 28 S and calmodulin intron were most successful, but representatives of each species (as recognized herein) also amplified for coxl. Within Lancinae, interspecies and intergenus percent variation was lowest for 28 S and highest for coxl. However, the calmodulin intron sequence for lancines was more divergent from Galba obrussa than the maximum variation between lymnaeids for coxl (26-30\% versus $22 \%$ ) (Table 4). Calmodulin sequences for planorbids generated in ongoing study on Vorticifex were apparently homologous based on the beginning and end of the intron sequence, but the middle of the intron was too divergent in sequence and length to obtain a meaningful alignment between the planorbids and lymnaeids. One calmodulin intron paralog of significantly different length was sequenced, but no homology with the chosen paralog was evident (GenBank accession number HM230349).

Several populations yielded identical or nearly identical sequences. These are enumerated in Table 2. No indels were found in coxl within the sampled species, though other Hygrophila do have insertions (pers. obs.). 28 S , 16 S , and calmodulin intron all had several small indels. MrModeltest (Nylander 2004) favored a HKY model for calmodulin intron and GTR $+\mathrm{I}+\mathrm{G}$ for $28 \mathrm{~S}, 16 \mathrm{~S}$, and coxl. Figures $1-2$ show the results of phylogenetic analyses.

Parameters for the trees from these analyses are in Table 5. All Bayesian analyses had a final average standard deviation of split frequencies below $0.6 \%$. Roughly $70 \%$ bootstrap support or $95 \%$ Bayesian posterior probability are thought to reflect significant support, though these empirical estimates are affected by several data and tree characteristics.

As 18 S typically shows little resolution at the species level, it was only sequenced for two species from different lancine genera, and those sequences were identical. Table 6 gives the E10-1 variable region for lymnaeids (present results and published data). The sequences are sufficiently variable to make alignment uncertain. Parsimony analyses using different alignments gave substantially different phylogenetic patterns, so we did

Table 4. Range of percent differences in DNA sequence (raw data, gaps treated as missing).

| Gene | Lymnaeidae | lancine genera | Lanx species | lancine intraspecies |
| :---: | :---: | :---: | :---: | :---: |
| $28 S$ | up to $7.6 \%$ | $1.2-2.8 \%$ | $0.79-1.2 \%$ | $0.00-0.40 \%$ |
| CAM intron | up to $30.1 \%$ | $4.8-8.0 \%$ | $1.3-2.6 \%$ | $0.00-1.87 \%$ |
| $\operatorname{cox} 1$ | up to $21.1 \%$ | $12.9-21.1 \%$ | $7.9-8.6 \%$ | $0.15-1.0 \%$ |
| 16 S | up to $21.3 \%$ | $12.8-16.6$ | no data | $0.00-2.5 \%$ |

Table 5. Tree statistics.

| Gene | Parsimony |  | Bayesian |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | \# trees | length | burnin | maximum ln likelihood | mean ln likelihood |
| 28S, CAM intron, and cox1 | 18 | 1670 | 165000 | -9578.885 | -9602.83 |
| $16 S$ | 2 | 719 | 65000 | -3414.11 | -3427.56 |



## - 0.01 substitutions/site

Figure I. Phylogram of the Bayesian majority-rule consensus tree for 28S, cox1, and calmodulin intron sequence data. Numbers on branches are bootstrap percentages before the slash, then Bayesian posterior probabilities. - indicates a value under $50 \%$ or 0.5 when the other method gave higher values. Taxon names in bold are lancines; starred taxa are Acellinae.
not use them. The alignment in the table is to facilitate comparison and may not reflect actual homology. However, several groups of species have closely similar or identical sequences, supporting a close relationship within these groups.
Table 6. Hypervariable portion of the E10 region of 18 S genes for lymnaeids. * indicates newly generated data. The alignment is meant to facilitate comparison between the different species. Differences between the more divergent sequences are too great for confident homologizing.

| Species | Accession number | Sequence |
| :---: | :---: | :---: |
| Aenigmomphiscola europaea, <br> A. kazakbstanica, Lymnaea stagnalis, Omphiscola glabra, Stagnicola palustris | AY577484, FR797819-FR797829, JN614363, JN614364, HQ659966, JN614368, JN614367 | CCGCG------TGC-GG--GGCGACTCGT-GCGCGGCG |
| Fisherola nuttallii | HM230306* | CCGT-CGC-GCGGGGCGTCAAACCCTCGCCG-GCGGCG |
| Galba cousini | FN598151, JN614345, JN614344 | CCGT-------CGCGGCGCAAGCCGAG-----GCGGCG |
| Galba cubensis | Z83831, JN614326-JN614331, JN614334 | CCGTGTCGTGCCGCGGTGCAAGCCGTGGTCGCGCGGCG |
| Galba humilis | FN182190 | CCGT-------CGCGGCGCAGGCCGAG-----GCGGCG |
| Galba schirazensis | FR772291, JN614335-JN614343 | CCGGC----CATTCATTCACTTGCGTGG----TCGGCG |
| Galba truncatula | Y09019, Z73985, EU152270, EU728668, HQ659965, JN614346-JN614354, FR797815, FR797816 | CCGT-------CCT-TTC----GCGAGG----GCGGTG |
| Galba viator | AF239912 | CCGTGTGCCTCCGTGGTGCAAGCCGTGGTCGCGCGGCG |
| Galba viator | AM412222, AY057088, AY057089, JN614332, JN614333 | CCGTGTGCCTCCGCGGTGCAAGCCGTGGTCGCGCGGCG |
| Lanx alta | HM230307* | CCGT-CGC-GCGGGGCGTCAAACCCTCGCCG-GCGGCG |
| Lymnaea stagnalis | EF489345 | CCG-------------------------------CGGCG |
| Lymnaea stagnalis, Omphiscola glabra, Stagnicola palustris | Y09018, Z73984, AY427525,Y09015, Z73982, JN614365, JN614366, Y09016, Z73983 | CCGCG------TGCCGG--GGCGACTCGT-GCGCGGCG |
| Pectinidens diaphana | EU241865, JF909497, JN614361, JN614362 | CCGC-------CGC-GG--CTCGCGCCGT-G-GCGGCG |
| Pseudosuccinea columella | FN598152, JN614358-JN614360 | CCGT-------CGGTCC--CGCGAGGGGCCG-GCGGTG |
| Pseudosuccinea columella | EU241866 | CCGTT------CGGTCC--CGCGAGGGGCCG-GCGGTG |
| Radix auricularia, Radix peregra | Z73980, Y09017, Z73981, FR797817, FR797818, JN614356, JN614357 | CCGCG------TGCTC---TTCGCGGGGT-GCGCGGTG |
| Radix natalensis | AF192272, EU152269 | CCGCG------TGCTC---CTCACGGGGT-GCGCGGTG |
| Radix natalensis | AF192273 | CCGCG------TGCTC---CTCACGGGGT-GCGTGGTG |


| Species | Accession number | Sequence |
| :--- | :---: | :---: |
| Radix natalensis | AF192274 | CCGCG------TGCTC---CTCCCGGGGT-GCGCGGTG |
| Radix natalensis | JN614355 | CCGCG------TGCTC---CTCGCGGGGT-GCGCGGTG |

Sources: Bargues and Mas-Coma 1997 (Z73980-5); Bargues et al. 1997 (Z83831); Bargues et al. 2007 (AM412222); Bargues et al. 2009 (FN182190); Bargues et al. 2011a (FR772291); Bargues et al. 2011b (FN598151-2); Bargues et al. 2012 (JF909497); Correa et al. 2011 (JN614326-68); Dayrat et al. 2011 (HQ6599656); Duffy et al. 2009 (AF239912, AY057088-9, EU241865-6, EU728668); Jorgensen et al. 2004 (AY577484); Klamath River at Collier Rest Area, California (HM230307*); Klussmann-Kolb et al. 2008 (EF489345) (Note: their analyses excluded variable regions, so perhaps the region was excised from the published sequence rather than originally absent); Marquez, unpublished (Y09015-9); Owyhee River, Whistling Bird Rapids, Oregon (HM230306*); Stothard et al. 2000 (AF192272-4); Vinarski et al. 2011 (FR797815-29) Vonnemann et al. 2005 (AY427525); Walker et al. 2008 (EU152269, EU152270). Only the E10 region is considered above, so there may be differences in other parts of the sequence for ones that are grouped in the table.


Figure 2. Phylogram of the Bayesian majority-rule consensus tree for 16 S sequence data. Numbers on branches are bootstrap percentages before the slash, then Bayesian posterior probabilities. - indicates a value under $50 \%$ or 0.5 when the other method gave higher values. Taxon names in bold are lancines; starred taxa are Acellinae.

## Discussion

In agreement with the anatomical data, molecular data give strong support for placing Lancinae in Lymnaeidae, which favors treating lancines as a subfamily rather than as a separate family. The relationships of lancines to other lymnaeids are not yet well-
resolved. Anatomy (Walter 1969) supports an affinity between Lancinae and the predominantly New World "advanced stagnicoline" group (subfamily Acellinae). Amphipepleinae (Radix, Austropeplea, and Orientogalba) was consistently supported as monophyletic, but the relationships between Amphipepleinae, Lancinae, and the remaining lymnaeids were not well-resolved, probably a function of the limited number of taxa. Sampling of additional lymnaeids, as well as additional genetic data (especially 28S) should greatly improve resolution of the relationships in this diverse and important but taxonomically problematic family.

The Lancinae appear supported as a monophyletic group, relatively divergent from other lymnaeids. Most of the analyses, the 18 S sequence similarity, and several morphological features all support Lancinae. Morphological synapomorphies include the fully patelliform shell, shape of the penial complex and C-shaped to circular columellar muscle (Baker 1925, this work). Patelliform lymnaeids evolved convergently multiple times in the Miocene Paratethys lakes of southeastern Europe (Harzhauser and Mandic 2008), so the molecular data provides a useful test of the morphological similarities. However, the monophyly of Lancinae received low bootstrap support and, in the 16 S analysis, low Bayesian posterior probabilities. Within the Lancinae, the present analyses had Idaholanx more closely related to Fisherola than to Lanx. Some singlegene analyses (not shown) had other patterns of intergeneric relationships in Lancinae. The weak resolution may reflect the limited number of available outgroups with 28 S data. Additionally, variation in the coxl gene may be approaching saturation within Lancinae, as the maximum percent difference between lancines, the maximum difference between any two lymnaeids, and the differences between lymnaeids and other basommatophorans were all about $20 \%$. As a result, convergent effects of multiple mutations in the variable sites probably obscure higher-level relationships in this data set. MacNeil (1939) reported Cretaceous lancines, so the subfamily has had enough time to develop significant genetic variation.

The genetic data consistently support recognition of three major groups within Lancinae. Two correspond to the presently recognized genera Lanx and Fisherola, while the third includes only the Banbury lanx. These results suggest that the Banbury lanx deserves recognition as a distinct genus and species (see description below). Each lancine genus was strongly supported as monophyletic. Genetic variation within Fisherola and Idaholanx was minimal. Within Lanx, there was one clear division and one ambiguous division between populations. The Sacramento-Pit system populations of Lanx (L. patelloides) consistently differed from those from farther west and north. These western and northern Lanx populations include L. alta in the Klamath and Umpqua systems and genetically more variable populations from the Smith and Rogue River systems. The difference between the Smith and Rogue forms and standard $L$. alta was less than the difference between L. alta and L. patelloides (in the case of 28S, only a few bases) but greater than the variation within other drainages. One specimen from the Rogue River system had both the standard L. alta allele and the Smith River allele for calmodulin intron, and the two calmodulin intron alleles obtained for Smith River specimens appear paraphyletic relative to the standard L. alta allele. The
variation within the Rogue and Smith systems therefore appears infraspecific, and the populations are assigned to L. alta. However, the genetic variation may be evolutionarily significant for the conservation of this species. H. B. Baker (1925) and Morrison (1955) noted that the Rogue River population did not exactly match described species from other drainages. Lanx alta, as defined herein, is very plastic in shell shape, so this may not be significant.

The relatively high genetic differences between lancine species contrasts with many other lymnaeids. The present results suggest that only one lancine species is present in each river system, with the exception of Idaholanx fresti in a few springs and Fisherola nuttallii in the main rivers, both in the Columbia-Snake system. The recognition of only two species in Lanx contrasts with most previous classifications. In particular, the widely recognized $L$. subrotunda and $L$. klamathensis are synonymized herein with $L$. alta. Previous tentative synonymization of $L$. hannai with $L$. patelloides and $F$. lancides with $F$. nuttallii are also supported (Morrison 1955, Burch 1982). Although specific populations assigned to $F$. kootaniensis and $L$. newberryi were not sampled in this study, the observed lack of variation within river systems supports previous synonymization with F. nuttallii and L. patelloides, respectively (Pilsbry 1925). Pilsbry (1925) also pointed out that $F$. crassus is an objective synonym of $F$. nuttallii, Haldeman having apparently renamed the same specimen. These synonymies suggest that lancines are relatively variable in shell shape and color pattern, as suspected by Morrison (1955). Similar results from Walther et al. (2006a, b) for the ancylids Ferrissia and Laevapex suggests that lim-pet-shaped Hygrophila have been taxonomically oversplit due to ecomorphic variation. Effects of environmental parameters correlate with shell shape in limpets (Basch 1963, McMahon and Whitehead 1987, Tanaka et al. 2002), and there is also extensive unexplained variation within populations (McMahon 2004). Additionally, limpet shape may be affected by the available substrate (Ridgway et al. 1999). Albrecht et al. (2004) discuss several factors potentially influencing shell shape in freshwater limpets and suggest that waves or currents and predators are the most likely selective pressures. Denny (2000) found that marine intertidal limpets are not optimized to resist wave-produced forces, presumably because the grasping force of a stationary marine limpet typically greatly exceeds observed wave forces. However, the smaller size and thin shells of freshwater limpets and the different environmental parameters for a stream with continual flow versus unpredictably directed waves during tide changes may result in different environmental pressures. Evolutionary pressures and convergent evolution relating to the limpet shape are reviewed in Vermeij (2016), including discussion of the lymnaeids.

The potential for self-fertilization in Hygrophila may account for high genetic divergence. Self-fertilization varies from rare to common in different species (Njiouku et al. 1993, Dillon et al. 2005, Puurtinen et al. 2007). The ultimate population bottleneck of a single individual would produce extreme founder effects and genetic drift, while also producing a genetically uniform founding population, thus accounting for high divergence between taxa and low variation within. Bolotov et al. (2016) found evidence for high divergence due to founder effect in the postglacial invasion of Iceland by lymnaeids. Although the long geologic history of lancines would allow for plenty
of time to accumulate changes, if the modern genera diverged fairly early, the lancines are unusually divergent in coxl protein sequence relative to the other lymnaeids, suggesting additional factors at work. Variation between populations within a river system was quite low. The largest difference between any two alleles within a river system was 9 to 10 bases between calmodulin intron alleles in the Smith and Rogue River populations. Outside of those, there was one individual of $L$. alta from the Klamath River with a single deletion of 6 bases in the calmodulin intron.

The low species diversity of lancines (four species from the entire Pacific Northwest region) contrasts with freshwater caenogastropods such as Juga and Fluminicola in the same river systems, which show high local endemicity within drainages (Hershler et al. 2007, Campbell et al. 2016). The habitat preferences of lancines resemble those of the associated caenogastropods, primarily in cool, flowing, well-oxygenated water, often in springs or spring-influenced areas. The potential for a single hermaphroditic individual to found a new population facilitates dispersal in Hygrophila, in contrast to the gonochoristic caenogastropods. However, unlike many lymnaeids, lancines have a poorly developed lung and are not known to survive out of water for extended periods of time, limiting their potential for dispersal by birds or other overland travel. Dispersal therefore likely occurs primarily within drainages, yet somehow lancines maintain high genetic homogeneity across much larger distances than Juga and Fluminicola, despite apparently similar ecology.

Thus, the present data supports recognition of the Banbury Springs lanx as a distinct genus and species. However, variation within Fisherola and Lanx seems to be largely ecophenotypic, giving a total of only four extant species in the subfamily Lancinae.

## Systematic descriptions

## Family Lymnaeidae Rafinesque, 1815

## Idaholanx Clark, Campbell \& Lydeard, gen. n.

http://zoobank.org/5E7508F1-1AF1-4051-AFD3-E7733DEF094F

Type species. Idaholanx fresti Clark, Campbell \& Lydeard sp. n.
Description. Shell (Figs 3, 5A). Patelliform, 2.0-3.9 mm in height and 4.0-6.7 mm in length and $3.0-5.4 \mathrm{~mm}$ in width. Aperture elliptical. Protoconch smooth, apex positioned posteriorly. Teleoconch sculpture of concentric growth lines. Shell pale to dark reddish brown. Internal columellar muscle scar C-shaped.

Non-genital anatomy. Columnar muscle C-shaped (Fig. 5B), gap on right side, roughly central. Digestive gland, kidney and lung typical of Lymnaeidae and that seen for Lanx and Fisherola (Baker 1925, SAC personal observations). Animal colour dark grey to black.

Genitalia anatomy (Fig. 4): The distinction between the praeputium and penial sheath is not clearly defined, the praeputium and the penial sheath are both about half


Figure 3. Shell, holotype of Idaholanx fresti sp. n.


Figure 4. Reproductive anatomy, holotype of Idaholanx fresti sp. n. AG albumen gland BW body wall NG nidamental gland $\mathbf{O}$ oviduct $\mathbf{O T}$ ovotestis $\mathbf{P}$ penis $\mathbf{P B}$ pyriform body $\mathbf{P G}$ prostate gland $\mathbf{P S}$ penial sheath $\mathbf{S}$ spermatheca $\mathbf{S D}$ spermathecal duct $\mathbf{S V}$ seminal vesicle $\mathbf{U}$ uterus $\mathbf{V}$ vagina $\mathbf{V D}$ vas deferens.
the length of the penial complex. Penis is short and thick. The prostate is elongate and tube like, with the vas deferens entering apically. The uterus is strongly folded, and is surrounded by a large albumen gland. The uterus connects to the proximal part of the oviduct (oviduct I) by a short tubular duct. A roundish nidamental gland joins here. The oviduct widens into the pyriform body which is relatively large, with the anterior portion slightly more swollen than the distal portion. The short oviduct II terminates with a small vagina. The spermatheca is of moderate size and ovate. The spermathecal duct is long and widens at its opening to the vagina.


Figure 5. Comparison of shells and animals of Idaholanx n. gen., Fisherola and Lanx. The shells are oriented with the head of the animal facing right, while the whole animals without shells are dorsal views with the head up. Idaholanx fresti sp. n. A shell B whole animal. Fisherola nuttalli: C shell D whole animal. Lanx patelloides. $\mathbf{E}$ shell $\mathbf{F}$ whole animal. The red arrows indicate the position of the head in $\mathbf{A}, \mathbf{C}$; the position of the gap in the columella muscle in $\mathbf{B}, \mathbf{D}$ and the narrow connection in $\mathbf{F}$. Images not to scale.

Distribution. Idaholanx, as currently recognised, is known from four isolated cold water springs (Thousand, Banbury, Briggs and Box Canyon Springs) that flow into eastern side of an 8 km section of the Snake River, in Gooding County, Idaho.

Remarks. Idaholanx gen. n. differs from Fisherola by having a smaller, taller shell with its apex located towards the middle of the shell and not posteriorly. It differs from Lanx by being smaller and taller and having an open C-shaped columellar muscle and not a closed circular columellar muscle (Fig. 5C-F).

Etymology. A combination of Idaho, the only state the genus is known to occur in and $\operatorname{Lanx}$, the genus it has been historically referred to and which is currently only known from northern California and southern Oregon.

## Idaholanx fresti Clark, Campbell \& Lydeard, sp. n. <br> http://zoobank.org/9B243DB3-ABD2-40CC-B9A0-BC4DD1778971

Type locality. 21-24 ${ }^{\text {th }}$ runs of the lower outflow of Banbury Springs, Gooding County, Idaho, U.S.A. $42^{\circ} 41^{\prime} 20.5^{\prime \prime} \mathrm{N}, 114^{\circ} 49^{\prime} 18^{\prime \prime} \mathrm{W}, 879 \mathrm{~m}, 4$ Sept 2003. Coll: T. Frest \& E. Johannes.

Type material. Holotype Field Museum of Natural History (FMNH) 342894 (dissected), paratypes FMNH 342895, DCS, SAC S.26084; FMNH 342896, DCS, SAC S. 26085 (shell), $13-15^{\text {th }}$ runs of the lower outflow of Banbury Springs, about middle of spring complex along trail with wooden bridges, $42^{\circ} 41^{\prime} 21^{\prime \prime} \mathrm{N}, 114^{\circ} 49^{\prime} 18^{\prime \prime} \mathrm{W}$, 21 Sept 1989; FMNH 342901, lower outflow of Banbury Springs, $42^{\circ} 41^{\prime} 21.8^{\prime \prime} \mathrm{N}$, $114^{\circ} 49^{\prime} 19.4 " W, 11$ Jan 2006; FMNH 342904, SAC S. 23967 (shell), lower outflow of Banbury Springs, $42^{\circ} 41^{\prime} 21^{\prime \prime} \mathrm{N}, 114^{\circ} 49^{\prime} 18^{\prime \prime} \mathrm{W}, 6$ Aug 2006; FMNH 342897 (shells), SAC S. 25699 (shell), lower outflow of Banbury Springs, $42^{\circ} \mathrm{H}^{\prime} 21.8^{\prime \prime} \mathrm{N}$, 114³9'18.5"W, 25 May 2016.

Additional material examined. Idaho. Gooding County. FMNH 342905 (shells), SAC S. 25842 (shell) lower outflow of Box Canyon Spring, about 110 m below diversion dam, $42^{\circ} 42^{\prime} 26.5^{\prime \prime N}, 114^{\circ} 49^{\prime} 02^{\prime \prime} \mathrm{W}, 24$ May 2016; FMNH 342898 (shells) lower outflow of Box Canyon Spring, about 160 m below diversion dam, $42^{\circ} 42^{\prime} 27^{\prime \prime} \mathrm{N}$, $114^{\circ} 49^{\prime} 04^{\prime \prime} \mathrm{W}$, Apr 2016; FMNH 342899 ( 1 dissected), FMNH 342900 (shell) lower outflow of Box Canyon Spring, about 400 m below diversion dam, $42^{\circ} 42^{\prime} 27.5^{\prime \prime} \mathrm{N}$, $114^{\circ} 49^{\prime} 14.5^{\prime \prime W}$ W, 11 Jan 2006; FMNH 342902 ( 1 dissected) outflow of Briggs Spring just below road crossing, $42^{\circ} 40^{\prime} 26.3^{\prime \prime} \mathrm{N}, 114^{\circ} 48^{\prime} 33.4^{\prime \prime} \mathrm{W}, 24$ Jan 2006; FMNH 342906 (shells), SAC S. 25707 (shell) outflow of Briggs Spring about 15 m below diversion dam, $42^{\circ} 40^{\prime} 26.9^{\prime \prime} \mathrm{N}, 114^{\circ} 48^{\prime} 39.2^{\prime \prime} \mathrm{W}, 24$ May 2016; FMNH 342903 ( 1 dissected), outflow of Thousand Springs, $42^{\circ} 44^{\prime} 51.7^{\prime \prime N}, 114^{\circ} 50^{\prime} 42.3^{\prime \prime W}, 24$ Jan 2006.

Description. Shell and anatomical description as for genus. Holotype 2.8 mm in height, 4.8 mm in length and 3.6 mm in width.

Etymology. Named for the late Dr Terrence J. Frest, for his significant contribution to the knowledge of land and freshwater molluscs of North America, especially of the western states and who was also a colleague and friend.

Ecology. This species is found under and on the sides of stones in cold flowing water in the range of $12.2-16.7^{\circ} \mathrm{C}$. It is not known exactly when egg laying occurs or how many eggs are laided at a time. It could be similar to the closely related species


Figure 6. Distribution of Idaholanx fresti. Insets show location of Idaho in the US and of the springs in Idaho.

Fisherola nuttallii (Haldeman, 1841) which occurs in the Snake River and other major tributaries of, as well as the main stem of the Columbia River. Coutant and Becker (1970) observed Fisherola nuttallii laying transparent, suboval gelatinous egg masses containing between 1-12 eggs laid from April to June in the Washington, U.S.A. portion of the Columbia River. They noted that growth rates increased as the availability
of food and temperature increased and that the life span was about a year, with adult mortality increasing rapidly after egg laying and after the temperatures increased above $17.3^{\circ} \mathrm{C}$.

Distribution. Currently known from four small to large isolated spring complexes along an eight kilometer stretch of the Snake River in Gooding County, Idaho (Fig. 6).

Conservation status. Listed as endangered under the U.S. Endangered Species Act of 1973 , under the name Banbury Springs lanx, Lanx sp.

## Acknowledgements

Dave Hopper (U.S. Fish \& Wildlife Service, Boise, Idaho) and Steve Lysne (formerly at the U.S. Fish \& Wildlife Service, Boise now at the College of Western Idaho, Boise, Idaho) helped with collecting specimens, guiding one of us (SAC) to three of the four known locations of Idaholanx and providing access to habitat and ecological data they have collected. Nicolas Hardy, Idaho BLM, Boise, Idaho generated the map. Juna Kurihara did the illustrations of the shell and reproductive anatomy of Idaholanx fresti. Ed Johannes and the late Terry Frest collected specimens and provided copies of their reports. The ABI 3100 automated sequencer was funded by a NSF equipment grant to C. Lydeard, R. Mayden, M. Powell, and P. Harris (DBI-0070351). John Tucker collected the Polyrhytis exilis specimens. Andrew Campbell helped collect the new Hinkleyia caperata specimen. The TNT program is available with the sponsorship of the Willi Hennig Society.

## References

Albrecht C, Kuhn K, Streit B (2007) A molecular phylogeny of Planorboidea (Gastropoda, Pulmonata): insights from enhanced taxon sampling. Zoologica Scripta 36: 27-39. https:// doi.org/10.1111/j.1463-6409.2006.00258.x
Albrecht C, Wilke T, Kuhn K, Streit B (2004) Convergent evolution of shell shape in freshwater limpets: the African genus Burnupia. Zoological Journal of the Linnean Society 140: 577-586. https://doi.org/10.1111/j.1096-3642.2003.00108.x
Baker HB (1925) Anatomy of Lanx, a limpet-like lymnaeid mollusk. Proceedings of the California Academy of Sciences 14: 143-169.
Bargues MD, Artigas P, Dillon RT, Mas-Coma S (2009) Molecular characterization of Lymnaea humilis ( $=$ L. modicella), a major fascioliasis vector in North America, and evaluation of the usefulness of nuclear rDNA and mtDNA markers for Lymnaeidae. Unpublished data on GenBank.
Bargues MD, Artigas P, Khoubbane M, Flores R, Glöer P, Rojas-García R, Ashrafi K, Falkner G, Mas-Coma S (2011a) Lymnaea schirazensis, an overlooked snail distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS ONE 6(9): e24567. http://dx.doi.org/10.1371/journal.pone. 0024567

Bargues MD, Artigas P, Khoubbane M, Mas-Coma S (2011b) DNA sequence characterisation and phylogeography of Lymnaea cousini and related species, vectors of fascioliasis in northern Andean countries, with description of $L$. meridensis n . sp. (Gastropoda: Lymnaeidae). Parasites \& Vectors 4(July): 132. http://dx.doi.org/10.1186/1756-3305-4-132
Bargues MD, Artigas P, Mera y Sierra RL, Pointier JP, Mas-Coma S (2007) Characterisation of Lymnaea cubensis, $L$. viatrix and $L$. neotropica n. sp., the main vectors of Fasciola hepatica in Latin America, by analysis of their ribosomal and mitochondrial DNA. Annals of Tropical Medicine and Parasitology 101(7): 621-641. https://doi.org/10.1179/136485907X229077
Bargues MD, Mangold AJ, Munoz-Antoli C, Pointier JP, Mas-Coma S (1997) SSU rDNA characterization of lymnaeid snails transmitting human fascioliasis in South and Central America. Journal of Parasitology 83(6): 1086-1092. https://doi.org/10.2307/3284367
Bargues MD, Mas-Coma S (1997) Phylogenetic analysis of lymnaeid snails based on 18 S rDNA sequences. Molecular Biology and Evolution 14(5): 569-577. https://doi.org/10.1093/oxfordjournals.molbev.a025794
Bargues MD, Mera y Sierra RL, Artigas P, Mas-Coma S (2012) DNA multigene sequencing of topotypic specimens of the fascioliasis vector Lymnaea diaphana and phylogenetic analysis of the genus Pectinidens (Gastropoda). Memórias do Instituto Oswaldo Cruz 107(1): 111-124. http://dx.doi.org/10.1590/S0074-02762012000100016
Basch PF (1963) Environmentally influenced shell distortion in a fresh-water limpet. Ecology 44(1): 193-194. https://doi.org/10.2307/1933204
Bolotov IN, Aksenova OV, Bespalaya YV, Gofarov MY, Kondakov AV, Paltser IS, Stefansson A, Travina OV, Vinarski MV (2016) Origin of a divergent mtDNA lineage of a freshwater snail species, Radix balthica, in Iceland: cryptic glacial refugia or a postglacial founder event? Hydrobiologia 787(1):73-98. https://doi.org/10.1007/s10750-016-2946-9
Burch JB (1982) Freshwater snails (Mollusca: Gastropoda) of North America. United States Environmental Protection Agency, Cincinnati, vi +294 pp.
Burch JB, Tottenham JL (1980) North American freshwater snails; Species list, ranges and illustrations. Walkerana 1(3): 81-215.
Campbell DC, Clark SA, Johannes EJ, Lydeard C, Frest TJ (2016) Molecular phylogenetics of the freshwater gastropod genus Juga (Cerithioidea: Semisulcospiridae). Biochemical Systematics and Ecology 65: 158-170. https://doi.org/10.1016/j.bse.2016.01.004
Campbell DC, Serb JM, Buhay JE, Roe KJ, Minton RL, Lydeard C (2005) Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. Invertebrate Biology 124(2): 131-164. https://doi.org/10.1111/j.17447410.2005.00015.x

Carpenter JM, Wheeler W (1999) Towards simultaneous analysis of morphological and molecular data in Hymenoptera. Zoologica Scripta 28(1-2): 251-260. https://doi.org/10.1046/ j.1463-6409.1999.00009.x

Clessin S (1882) Die familie der Ancylinen. Systematisches Conchylien-Cabinet 1(6): 1-80, plates 1-9.
Correa AC, Escobar JS, Noya O, Velásquez LE, González-Ramírez C, Hurtrez-Boussès S, Pointier J-P (2011) Morphological and molecular characterization of Neotropic Lymnaeidae
(Gastropoda: Lymnaeoidea), vectors of fasciolosis. Infection, Genetics and Evolution 11(8): 1978-1988. http://dx.doi.org/10.1016/j.meegid.2011.09.003
Coutant CC, Becker CD (1970) Growth of the Columbia River Limpet, Fisherola nuttalli (Haldeman), in normal and reactor-warmed water. BNWL-1537, Pacific Northwest Laboratory, Richland, Washington, 14 pp. https://doi.org/10.2172/4077701
Dayrat B, Conrad M, Balayan S, White TR, Albrecht C, Golding R, Gomes SR, Harasewych MG, De Frias Martins AM (2011) Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. Molecular Phylogenetics and Evolution 59(2): 425-437. http://dx.doi.org/10.1016/j.ympev.2011.02.014
DeJong RJ, Morgan JAT, Paraense WL, Pointier J-P, Amarista M, Ayeh-Kumi PFK, Babiker A, Barbosa CS, Bremond P, Canese AP, de Souza CP, Dominguez C, File S, Gutierrez A, Incani RN, Kawano T, Kazibwe F, Kpikpi J, Lwambo NJS, Mimpfoundi R, Njiokou F, Poda JN, Sene M, Velasquez LE, Yong M, Adema CM, Hofkin BV, Mkoji GM, Loker ES (2001) Evolutionary relationships and biogeography of Biomphalaria (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, Schistosoma mansoni. Molecular Biology and Evolution 18: 2225-2239. https://doi.org/10.1093/ oxfordjournals.molbev.a003769
Denny MW (2000) Limits to optimization: fluid dynamics, adhesive strength and the evolution of shape in limpet shells. Journal of Experimental Biology 203: 2603-2622.
Dewaard JR, Telfer A, Young MR (2015) Barcoding Canada Data Release. Data on GenBank.
Dillon RT Jr, McCullough TE, Earnhardt CE (2005) Estimates of natural allosperm storage capacity and self-fertilization rate in the hermaphroditic freshwater pulmonate snail, Physa acuta. Invertebrate Reproduction and Development 47(2): 111-115. https://doi.org/10.1 080/07924259.2005.9652151
Dowton M, Austin AD (2002) Increased congruence does not necessarily indicate increased phylogenetic accuracy - the behavior of the incongruence length difference test in mixed model analyses. Systematic Biology 51: 19-31. https://doi.org/10.1080/106351502753475853
Duffy T, Kleiman F, Pietrokovsky S, Issia L, Schijman AG, Wisnivesky-Colli C (2009) Realtime PCR strategy for rapid discrimination among main lymnaeid species from Argentina. Acta Tropica 109(1): 1-4. https://doi.org/10.1016/j.actatropica.2008.08.003
Feldmeyer B, Greshake B, Funke E, Ebersberger I, Pfenninger M (2015) Positive selection in development and growth rate regulation genes involved in species divergence of the genus Radix. BMC Evolutionary Biology 15: 164. https://doi.org/10.1186/s12862-015-0434-x
Feldmeyer B, Hoffmeier K, Pfenninger M (2010) The complete mitochondrial genome of Radix balthica (Pulmonata, Basommatophora), obtained by low coverage shot gun next generation sequencing. Molecular Phylogenetics and Evolution 57(3): 1329-1333. http:// dx.doi.org/10.1016/j.ympev.2010.09.012

Folmer O, Hoeh WR, Black MB, Vrijenhoek RL (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.
Giribet G, Carranza S, Baguñà J, Riutort M, Ribera C (1996) First molecular evidence for the existence of a Tardigrada-Arthropoda clade. Molecular Biology and Evolution 13: 76-84. https://doi.org/10.1093/oxfordjournals.molbev.a025573

Goloboff P, Farris J, Nixon K (2008) TNT, a free program for phylogenetic analysis. Cladistics 24: 774-786. https://doi.org/10.1111/j.1096-0031.2008.00217.x
Gordy MA, Kish L, Tarrabain M, Hanington PC (2016) A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada. Parasitology Research 115: 3867-3880. https://doi.org/10.1007/s00436-016-5152-9
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.
Hannibal H (1912) A synopsis of the Recent and Tertiary freshwater Mollusca of the Californian Province, based upon an ontogenetic classification. Proceedings of the Malacological Society of London 10(2): 112-166, plates 5-6.
Harzhauser M, Mandic O (2008) Neogene lake systems of Central and South-Eastern Europe: Faunal diversity, gradients and interrelations. Palaeogeography, Palaeoclimatology, Palaeoecology 260: 417-434. https://doi.org/10.1016/j.palaeo.2007.12.013
Hershler R, Liu H-P, Frest TJ, Johannes EJ (2007) Extensive diversification of pebblesnails (Lithoglyphidae: Fluminicola) in the upper Sacramento River basin, northwestern USA. Zoological Journal of the Linnean Society 149(3): 371-422. https://doi.org/10.1111/ j.1096-3642.2007.00243.x

Holznagel WE, Colgan DJ, Lydeard C (2010) Pulmonate phylogeny based on 28S rRNA gene sequences: A framework for discussing habitat transitions and character transformation. Molecular Phylogenetics and Evolution 57(3): 1017-1025. http://dx.doi.org/10.1016/j. ymper.2010.09.021
Jorgensen A, Kristensen TK, Stothard JR (2004) An investigation of the 'Ancyloplanorbidae’ (Gastropoda, Pulmonata, Hygrophila): preliminary evidence from DNA sequence data. Molecular Phylogenetics and Evolution 32(3): 778-787. https://doi.org/10.1016/j. ympev.2004.02.011
Jung Y, Morgan JAT, Burch JB, Gordon M, Joyce S, Laurson J, Light J, Meyer-Rochov V, Pointier J-P, DeJong RJ, Mkoji GM, Loker ES (Unpublished) A phylogeny of the Basommatophora (Gastropoda: Pulmonata), based on 28 S and actin sequences. Unpublished data on GenBank.
Klussmann-Kolb A, Dinapoli A, Kuhn K, Streit B, Albrecht C (2008) From sea to land and beyond - new insights into the evolution of euthyneuran Gastropoda (Mollusca). BMC Evolutionary Biology 8(57): 1-16. https://doi.org/10.1186/1471-2148-8-57
Krebs RA, Vlasceanu RN, Tevesz MJS (2003) An analysis of diversity in freshwater mussels (Bivalvia: Unionidae) of the Cuyahoga and Rocky River watersheds (Ohio, USA) based on the 16 S rRNA gene. Journal of Great Lakes Research 29(2): 307-316. https://doi. org/10.1016/S0380-1330(03)70436-5
Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948. https://doi.org/10.1093/ bioinformatics/btm404
MacNeil FS (1939) Fresh-water invertebrates and land plants of Cretaceous age from Eureka, Nevada. Journal of Paleontology 13(3): 355-360.
Marquez FJ (Unpublished) Differentiation of Lymnaea subgenus (Galba, Leptolymnaea [sic], Lymnaea s.st., Radix and Stagnicola) (Basommatophora, Lymnaeidae) in base to small
ribosomal DNA helix E10-1 sequence. [A very similar set of sequences appears in Bargues and Mas-Coma 1997]
McMahon RF (2004) A 15-year study of interannual shell-shape variation in a population of freshwater limpets (Pulmonata: Basommatophora: Ancylidae). American Malacological Bulletin 19(1/2): 101-109.
McMahon RF, Whitehead BE (1987). Environmental induction of shell morphometric variation in the European stream limpet, Ancylus fluviatilis (Müller) (Pulmonata: Basommatophora). American Malacological Bulletin 5(1): 105-124.
Meier-Brook C, Bargues MD (2002) Catascopia, a new genus for three Nearctic and one Palaearctic stagnicoline species (Gastropoda: Lymnaeidae). Folia Malacologia 10(2): 83-84. https://doi.org/10.12657/folmal. 010.008
Morgan JA, DeJong RJ, Jung Y, Khallaayoune K, Kock S, Mkoji GM, Loker ES (2002) A phylogeny of planorbid snails, with implications for the evolution of Schistosoma parasites. Molecular Phylogenetics and Evolution 25(3): 477-488. https://doi.org/10.1016/S1055-7903(02)00280-4
Morrison JPE (1955) Notes on the genera Lanx and Fisherola (Pulmonata). The Nautilus 68(3): 79-83.
Njiokou F, Bellec C, Jarne P, Finot L, Delay B (1993) Mating system analysis using protein electrophoresis in the self-fertile hermaphrodite species Bulinus truncatus (Gastropoda: Planorbidae). Journal of Molluscan Studies 59(2): 125-133. https://doi.org/10.1093/ mollus/59.2.125
Nolan JR, Bergthorsson U, Adema CM (2014) Physella acuta: atypical mitochondrial gene order among panpulmonates (Gastropoda). Journal of Molluscan Studies 80: 388-399. https://doi.org/10.1093/mollus/eyu025
Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. https://doi.org/10.1006/mpev.1999.0691
Park D-S, Oh H, Lee M, Kim M, Jung C (2012) Korean Collection for Type Cultures. Data on GenBank.
Park J-K, Ó Foighil D (2000) Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. Molecular Phylogenetics and Evolution 14(1): 75-88.
Patel S, Schell T, Eifert C, Feldmeyer B, Pfenninger M (2015) Characterizing a hybrid zone between a cryptic species pair of freshwater snails. Molecular Ecology 24: 643-655. https:// doi.org/10.1111/mec. 13049
Pilsbry HA (1925) The family Lancinae distinguished from the Ancylidae. Nautilus 38(3): 73-75.
Puslednik L, Ponder WF, Dowton M, Davis AR (2009) Examining the phylogeny of the Australasian Lymnaeidae (Heterobranchia: Pulmonata: Gastropoda) using mitochondrial, nuclear and morphological markers. Molecular Phylogenetics and Evolution 52(3): 643-659. https://doi.org/10.1016/j.ympev.2009.03.033
Puurtinen MK, Knott E, Suonpää S, Nissinen K, Kaitala V (2007) Predominance of outcrossing in Lymnaea stagnalis despite low apparent fitness costs of self-fertilization. Journal of Evolutionary Biology 20: 901-912. https://doi.org/10.1111/j.1420-9101.2007.01312.x

Remigio EA (2002) Molecular phylogenetic relationships in the aquatic snail genus Lymnaea, the intermediate host of the causative agent of fascioliasis: insights from broader taxon sampling. Parasitological Research 88(7): 687-696. https://doi.org/10.1007/s00436-002-0658-8
Remigio EA, Blair D (1997) Molecular systematics of the freshwater snail family Lymnaeidae (Pulmonata: Basommatophora) utilising mitochondrial ribosomal DNA sequences. Journal of Molluscan Studies 63(2): 173-185. https://doi.org/10.1093/mollus/63.2.173
Remigio EA, Hebert PD (2003) Testing the utility of partial COI sequences for phylogenetic estimates of gastropod relationships. Molecular Phylogenetics and Evolution 29(3): 641-647. https://doi.org/10.1016/S1055-7903(03)00140-4
Ridgway TM, Stewart BA, Branch GM (1999) Limited population differentiation in the bearded limpet Patella barbara (Gastropoda: Patellidae) along the coast of South Africa. Journal of the Marine Biological Association of the United Kingdom 79(4): 639-651. https://doi.org/10.1017/S0025315498000800
Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2011) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029
Schilthuizen M, Hoekstra RF, Gittenberger E (1999) Selective maintenance of a rare haplotype in a land snail hybrid zone. Proceedings of the Royal Society of London, Biological Sciences 266(1434): 2181-2185. https://doi.org/10.1007/s00239-004-0232-3
Simpson RJ, Wilding CS, Grahame J (2005) Intron analyses reveal multiple calmodulin copies in Littorina. Journal of Molecular Evolution 60(4): 505-512.
Sonnenberg R, Nolte AW, Tautz D (2007) An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. Frontiers in Zoology 4(6): 12 p . http://dx.doi. org/10.1186/1742-9994-4-6
Stothard JR, Bremond P, Andriamaro L, Loxton NJ, Sellin B, Sellin E, Rollinson D (2000) Molecular characterization of the freshwater snail Lymnaea natalensis (Gastropoda: Lymnaeidae) on Madagascar with an observation of an unusual polymorphism in ribosomal small subunit genes. Journal of Zoology 252(3): 303-315. https://doi. org/10.1111/j.1469-7998.2000.tb00625.x
Swofford DL (1998) PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.
Tanaka MO, Duque-Estrada TEM, Magalhães CA (2002) Dynamics of the acmaeid limpet Collisella subrugosa and vertical distribution of size and abundance along a wave exposure gradient. Journal of Molluscan Studies 68(1): 55-64. https://doi.org/10.1093/mollus/68.1.55
Tryon GW (1870) A Monograph of the Fresh-water Univalve Mollusca of the UnitedStates. Continuation of Prof. S. S. Haldeman's work. Philadelphia, 1-238. https://doi. org/10.5962/bhl.title. 54506
U.S. Fish and Wildlife Service (1992) Endangered and threatened wildlife and plants: determinations of endangered or threatened status for five aquatic snails in South Central Idaho. Federal Register 57: 59244-59256.
U.S. Fish and Wildlife Service (2006) Banbury Springs Lanx (Lanx n. sp.) (undescribed) 5-Year Review: Summary and Evaluation. U.S. Fish and Wildlife Service Snake River Fish and Wildlife Office, Boise, Idaho, ii+30+VII pp.
Vermeij GJ (2016) The limpet form in gastropods: evolution, distribution, and implications for the comparative study of history. Biological Journal of the Linnean Society [Online Early view, not yet assigned to a volume]. https://doi.org/10.1111/bij. 12883
Vinarski MV, Schniebs K, Glöer P, Hundsdoerfer AK (2011) The taxonomic status and phylogenetic relationships of the genus Aenigmomphiscola Kruglov and Starobogatov, 1981 (Gastropoda: Pulmonata: Lymnaeidae). Journal of Natural History 45(33-34): 2049-2068. https://doi.org/10.1080/00222933.2011.574800
Vonnemann V, Schrödl M, Klussmann-Kolb A, Wägele H (2005) Reconstruction of the phylogeny of the Opisthobranchia (Mollusca, Gastropoda) by means of 18 S and 28 S rDNA sequences. Journal of Molluscan Studies 71(2): 113-125. https://doi.org/10.1093/mollus/ eyi014
von Oheimb PV, Albrecht C, Riedel F, Du L, Yang J, Aldridge DC, Bößneck U, Zhang H, Wilke T (2011) Freshwater biogeography and limnological evolution of the Tibetan Plateau - insights from a plateau-wide distributed gastropod taxon (Radix spp.). PLoS ONE 6(10): e26307. https://doi.org/10.1371/journal.pone. 0026307
Walker SM, Makundi AE, Namuba FV, Kassuku AA, Keyyu J, Hoey EM, Prodohl P, Stothard JR, Trudgett A (2008) The distribution of Fasciola hepatica and Fasciola gigantica within southern Tanzania - constraints associated with the intermediate host. Parasitology 135(4): 495-503. https://doi.org/10.1017/s0031182007004076
Walter HJ (1969) Illustrated biomorphology of the 'angulata' lake form of the basommatophoran snail Lymnaea catascopium Say. Malacological Review 2: 1-102.
Walther AC, Taehwan L, Burch JB, Ó Foighil D (2006a) Confirmation that the North American ancylid Ferrissia fragilis (Tryon, 1863) is a cryptic invader of European and East Asian freshwater ecosystems. Journal of Molluscan Studies 72(3): 318-321. https://doi. org/10.1093/mollus/eyl009
Walther AC, Taehwan L, Burch JB, Ó Foighil D (2006b) E Pluribus Unum: A phylogenetic and phylogeographic reassessment of Laevapex (Pulmonata: Ancylidae), a North American genus of freshwater limpets. Molecular Phylogenetics and Evolution 40(2): 501-516. https://doi.org/10.1016/j.ympev.2006.03.019

# Paussus (Scaphipaussus) zhouchaoi sp. n., a new myrmecophilous species from China (Coleoptera, Carabidae, Paussinae, Paussini) 

Cheng-Bin Wang ${ }^{1,2}$<br>I Bin Insect Taxonomy Studio, No.16, Xizhaosi Street, Dongcheng District, Beijing 100061, P. R. China 2 Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, CZ-165 21 Praha 6, Czech Republic<br>Corresponding author: Cheng-Bin Wang (leiodidae@hotmail.com)

Academic editor: A. Casale \| Received 26 December 2016 | Accepted 22 February 2017 | Published 28 March 2017
http://zoobank.org/C957E62E-E76E-4E5B-AA9F-6F17E12FFF07
Citation: Wang C-B (2017) Paussus (Scaphipaussus) zhouchaoi sp. n., a new myrmecophilous species from China (Coleoptera, Carabidae, Paussinae, Paussini). ZooKeys 663: 133-143. https://doi.org/10.3897/zookeys.663.11635


#### Abstract

A new species of flanged bombardier beetles is described from Jiangxi and Sichuan, China, Paussus (Scaphipaussus) zhouchaoi sp. n. (Coleoptera, Carabidae, Paussinae, Paussini). All the type specimens were collected from colonies of the host ant Pheidole sp. (Hymenoptera, Formicidae, Myrmicinae). Important morphological characters of the new species are illustrated by color plates.


## Keywords

Carabidae, China, new species, Paussinae, Paussini, Paussus, Scaphipaussus, taxonomy

## Introduction

The obligate myrmecophilous genus Paussus Linné, 1775 is the most speciose genus of Paussinae (Coleoptera, Carabidae) with members distributed in Afrotropical, Oriental, southwest and southeast Palearctic and Madagascan Regions. Recently, Robertson and Moore (2016) excellently revised Paussus Linné, subdividing and delineating this genus into three series and 10 subgenera:

Paussus I series (Bohemanipaussus Luna de Carvalho, 1982; Bathypaussus Wasmann, 1929; Edaphopaussus Kolbe, 1920);

Paussus II series (Paussus Linné, 1775; Klugipaussus Kolbe, 1927; Scaphipaussus Fowler, 1912; Hylotorus Dalman, 1823; Anapaussus Wasmann, 1929);
Paussus III series (Lineatopaussus Kolbe, 1928; Shuckardipaussus Kolbe, 1938).
However, for the fauna of China, only eleven species were recorded (Luna de Carvalho 1989, Maruyama 2016, Nagel 2003, Robertson and Moore 2016):

Paussus series II: subgenus Paussus Linné, 1775:
P. brancuccii Nagel, 2016 (Guangxi);
P. kjellanderi Luna de Carvalho, 1965 (Jiangsu, Taiwan).

Paussus series II: subgenus Scaphipaussus Fowler 1912:
P. bowringii Westwood, 1850 (Hong Kong);
P. formosus Wasmann, 1912 (Taiwan);
P. hystrix Westwood, 1850 (Hong Kong, Jiangsu, Sichuan);
P. jengi Maruyama, 2016 (Taiwan).

Incertae sedis:
P. elongatus Kanô, 1930 (Taiwan);
P. horikawae Kanô, 1930 (Taiwan);
P. minor Shiraki, 1907 (Taiwan);
P. sauteri Wasmann, 1912 (Taiwan);
P. jousselini Guérin-Méneville, 1836 (Hong Kong, Hunan).

In this paper, a new species from Jiangxi Province and Sichuan Province of China is described and illustrated: Paussus (Scaphipaussus) zhouchaoi sp. n. All the type specimens were collected from colonies of the host ant Pheidole sp. (Hymenoptera, Formicidae, Myrmicinae).

## Material and methods

Specimens were relaxed and softened in a hot saturated solution of potassium hydroxide for three minutes, and then transferred to distilled water to rinse the residual potassium hydroxide off and stop any further bleaching. The softened specimens were moved into glycerin and dissected there to observe morphological details. After examination, the body parts were mounted on a glass slip with Euparal Mounting Medium for future studies. Habitus photograph of Figure 1 was taken using a Canon macro photo lens MP-E 65 mm on a Canon 550D. Observations and measurements were performed using a Zeiss Axio Zoom.V16 motorized stereo zoom microscope (magnification up to $\times 270$ ). Color photographs were taken with a Zeiss AxioCam MRc 5 and the final deep focus images were created with the stacking software Helicon Focus 5.3. Adobe Photoshop CS6 was used for image postprocessing. The morphological terminology follows Nagel (1987) and Robertson and Moore (2016).

The material examined for this study is deposited in the following collections and museums：

BITS Bin Insect Taxonomy Studio，Beijing，China
SNUC Insect Collection of Shanghai Normal University，Shanghai，China
Measurement criteria in millimeters（ mm ）are used as follows：

Body length：axial length from the anterior apex of clypeus to the elytral apex．
Head length：axial length from the anterior apex of clypeus until the constriction of neck．
Head width：maximum width of head（including eyes or temples）．
Eye length：shortest diameter of eye between anterior and posterior margins．
Gena length：shortest distance between antennal insertion and anterior margin of eye．
Pronotal length：axial length of pronotum．
Pronotal width：maximum width of pronotum．
Pronotal anterior part length：axial length from the anterior margin of pronotum to the anterior edge of transverse pronotol cleft．
Pronotal anterior part width：maximum width of pronotal anterior part．
Pronotal posterior part length：length from the level of forefront after transverse pro－ notol cleft to the posterior margin of pronotum．
Pronotal posterior part width：maximum width of pronotal posterior part．
Elytral length：length from the basal border of elytra to its apex along suture．
Elytral width：width across the middle of two elytra combined together．

## Results

Genus Paussus Linné， 1775
Vernacular name：棒角甲属

## Subgenus Scaphipaussus Fowler 1912

Vernacular name：舟棒角甲亚属

## Paussus（Scaphipaussus）zhouchaoi sp．n．

http：／／zoobank．org／B05E8634－5A8D－407F－97FD－FF6BAB547BA5
Vernacular name：周超棒角甲
Figs 1；2A－E；3；4；5；6B－D

Material examined．Holotype：$\widehat{\delta}$ ，CHINA，Sichuan：Chengdu City，Dujiangyan， Zipingpu Town，Lingyanguanyinshan scenic area（灵岩观音山风景区），29．IV．2016， $31.02956 \mathrm{~N}, 103.61651 \mathrm{E}$ ，alt． 1210 m ，ant colony under deadwood bark，leg．Chao


Figure I．Habitus of Paussus（Scaphipaussus）zhouchaoi sp．n．（holotype，ō；dorsal view）．

Zhou \＆Li He（BITS）．Paratypes： 1 ，same data as holotype（BITS）； $1 \delta^{\lambda}$ ，CHINA， Jiangxi：Yichun City，Mingyueshan（明月山）， $27^{\circ} 35^{\prime} 25^{\prime \prime N}$ N， $114^{\circ} 17^{\prime} 02$＂E，alt． 1600 m ， 22．X．2013，Zhong Peng leg．［from a colony of Pheidole sp．］（SNUC）．

Diagnosis. This new species is allocated to the subgenus Scaphipaussus Fowler 1912 according to the groups key of Robertson \& Moore (2016). Within this subgenus the new species is unique with regard to the combination of the following characters: body lustrous, scatteredly and shortly pubescent, seeming hairless to the naked eyes; head vertex distinctly excavated, laterally bordered by auriculate costae; scape longer than wide, cylindrical; fused flagellum elongated subtriangular, with dorsal margin of outer side with five robust teeth and five deep incisions, while ventral margin of that with five weak protrusions and five weak emarginations; pronotum wider than long, width/length $=1.22$, as wide as head, (anterior part width $) /($ posterior part width $)=1.10$, anterior part laterally angulate, posterior part with lateral margins roundly protruded in the apical $3 / 4$ and obliquely substraight in the basal $1 / 4$; elytra not bearing lateral trichome fringes; legs robust; pygidium with posterior dorsal margin distinctly upturned and explanate, marginal trichome fringe dense.

Description. Male holotype. Medium size, body 4.83 mm long. Length (mm) of different body parts: head (0.68) : pronotum (0.91) : pronotal anterior part (0.40) : pronotal posterior part ( 0.43 ) : elytra (3.21) : pronotum-elytra (4.12); width (mm): head (1.13) : pronotum (1.11) : pronotal anterior part (1.11) : pronotal posterior part (1.01) : elytra (1.88). (Pronotum-elytra length) $/($ elytral width $)=2.20$.

Body (Figs 1; 2A-C) oblong and overall appearance lustrous due to lack of punctures or microsculptures on the majority of body parts; unicolor, mostly brown, with elytra lighter and pronotal posterior part darker (the body color is much lighter when the species is alive (Fig. 6C-D) or the specimens are newly collected); integument scatteredly pubescent with short, thin, yellowish setae except pygidium, and the setae on pronotum are distinctly thicker and shorter.

Head (Fig. 3A, C) subglobular, width/length $=1.67$, vertex high and dorsal parts strongly inclined; clypeus indented, depressed at centre and with frontal ridges well demarked; median frontal suture short and dark, not extending to anterior margin of eye; vertex distinctly and broadly excavated, bordered by a raised, auriculate, doublewalled costa at each side; the two costae are separated at their tops by a distinct groove; in addition, one straight and short costa sits in the basal corner of each auriculate costa; eyes reniform, small and less prominent; temples narrow but distinct, slightly projecting laterally beyond eyes in dorsal view; (eye length)/(gena length) $=1.23$; head surface rugosely and contiguously punctate, especially along median frontal suture, while central excavation glabrous and smooth; neck narrow, strongly constricted.

Mouthparts of "closed" type, adjacent to the underside of head. Labrum transverse and rectangular. Palpifer exposed. Maxillary palpomere II large, broad and compressed, almost as wide as long, wider than $2 \times$ width of palpomere III and longer than palpomeres III and IV combined, with mesal margin more or less rounded; palpomere III only slightly wider than palpomere IV; palpomere IV tapering apically and slightly longer than palpomere III. Palpiger exposed. Labial palpomere II with socket for palpomere III positioned along midline; palpomere III narrow, slender, fusiform and slightly compressed, length/width $=3.43$, longer than $2 \times$ length of palpomeres $I$ and


Figure 2. Habitus of Paussus (Scaphipaussus) zhouchaoi sp. n. (A-C holotype, $\widehat{O}^{\top} \mathbf{D}-\mathbf{E}$ paratype,, ) and its host ant ( $\mathbf{F}$ Pheidole sp., minor worker) $\mathbf{A}, \mathbf{D}, \mathbf{F}$ dorsal view $\mathbf{B}, \mathbf{E}$ dorsolateral view $\mathbf{C}$ ventral view.

II combined. Ligula large and broad, with apical margin broadly rounded at middle. Gula with width/length (at narrowest point) $=0.42$.

Antenna (Fig. 4A-D): scape longer than wide, cylindrical, surface rugose, without trichome brush on inner anterior margin. Pedicel vestigial ring-shaped. Fused flagellum elongated subtriangular, 2.59 times as long as wide; dorsal surface with a shallow longitudinal furrow, ending at the level of the most apical incision; ventral surface slightly convex; inner side broadly and weakly undulate; apical side obliquely truncate


Figure 3. Paussus (Scaphipaussus) zhouchaoisp. n. (A, C, E holotype, đ̄ B, D paratype, $\uparrow$ ). A-D heads and pronota $\mathbf{E}$ pygidium $\mathbf{A}-\mathbf{B}$ oblique anterodorsal view $\mathbf{C}-\mathbf{E}$ dorsal view.


Figure 4. Antenna of Paussus (Scaphipaussus) zhouchaoi sp. n. (holotype, $J^{\top}$ ). A dorsal view, in ethanol solution $\mathbf{B}$ dorsal view $\mathbf{C}$ dorsoposterior view $\mathbf{D}$ posterior view.
and rounded at tip; both apical side and inner side (especially along apical half) marginate and the marginal band rugosely punctate; outer side longitudinally excavated from outer basal angle to short distance before apex; dorsal margin of outer side with five robust teeth and 5 deep incisions; ventral margin of outer side with five weak protrusions and five weak emarginations; inner basal corner roundly protruded; outer basal angle large, prominent and extended, without trichome; basal side between insertion and outer basal angle simply emarginate without traces of indentations.

Pronotum (Fig. 3A, C) wider than long, width/length $=1.22$, as wide as head, widest at lateral angulations of anterior part; transversally cleft, anterior part almost as long as posterior part, (anterior part width) $/($ posterior part width $)=1.10$; anterior part moderately raised, strongly divided by a longitudinal groove, weakly edged behind and laterally angulate; posterior part with lateral margins roundly protruded in the apical $3 / 4$ and obliquely substraight in the basal $1 / 4$, medially with a deep, wide and longitudinal furrow towards scutellum; transverse furrow with trichome-bearing glandular openings at the furthest lateral ends; trichomes dark yellow, not much contrasting with the brown pronotum, (distance between trichomes) $/($ trichome width $)=3.38$; median excavation glabrous and smooth, both sides of excavation equally rugose.

Scutellum ligulate, wider than long, surface densely punctate.
Elytra oblong, length/width = 1.71; humeri hardly demarcated; surface smooth and lustrous, without punctures, merely sculptured with micropores; areas along suture devoid of pubescence. Metathoracic wings fully developed.

Legs robust, with smooth surfaces. Tibiae compressed; protibiae straight, mesotibiae slightly sinuate, metatibiae almost straight; metatibiae broader than meso- and protibiae; tibial spurs absent; pubescence denser on apical part of all tibiae. Tarsi with tarsomeres I-IV subequal in length, with apical margins entire, dorsally straight or inconspicuously emarginate; all tarsomeres without adhesive pads but loosely setose on lateral parts of ventral surfaces.

Stridulatory organ present: scraper as a curved row of transverse spines on abdominal ventrite I, and file present at inner base of metafemur.

Pygidium (Fig. 3E) with disc shining, only sparsely covered with microtrichiae; posterior dorsal margin distinctly upturned and explanate; marginal trichome fringe dense.

Aedeagus as shown in Figs. 5A-D: median lobe elongate, slender and arcuate, apex distinctly emarginate; subbasal articulation tubercle well developed; parameres slender, apical parts narrow, apex rounded and devoid of setae; strut present, embedded in membraneous tissue in the basal part of median lobe.

Female paratype (Figs 2D-E; 3B, D). Sexual dimorphism weak, no distinct differences between shape of eyes, shape of costae at head vertex, shape and microstructure of fused flagellum, curvature of metatibiae. The abdomen of the female paratype, including female genitalia, was poured into wash basin when the present author working in a drunken state.

Host ant. All the type specimens were collected from colonies of the host ant Pheidole sp. (Hymenoptera, Formicidae, Myrmicinae). The male holotype and the female paratype from Sichuan Province were collected from a Pheidole colony under


Figure 5. Aedeagus of Paussus (Scaphipaussus) zhouchaoi sp. n. (holotype, ${ }^{\top}$ ). A dorsal view B left lateral view $\mathbf{C}$ ventral view $\mathbf{D}$ right lateral view.


Figure 6. Field observations (Sichuan: Lingyanguanyinshan scenic area) of Paussus (Scaphipaussus) zhouchaoi sp. n. (Photoed by Li He) A biotope B the rotten deadwood inhabited by $P$. (S.) zhouchaoi sp. n. and its host ants (Pheidole sp.) C $P$. (S.) zhouchaoi sp. n. on the rotten deadwood D $P$. (S.) zhouchaoi sp. n. on the palm of the collector Li He .
deadwood bark. Two Pheidole sp. minor workers (Fig. 2F) glued to a card and pinned together with the holotype.

Field observations. Biotope in Lingyanguanyinshan scenic area (Sichuan) as shown in Fig. 6A-B.

Etymology. The specific epithet is dedicated to Mr. Chao Zhou (Chengdu, Sichuan, China), one of the collectors of this new species and a good amateur obsessing with beetles.

Distribution. China (Jiangxi, Sichuan).
Remarks. Maruyama (2016) partially revised the Paussus hystrix group (to which unequivocally the new species belongs) and described 19 species from East Asia and

Southeast Asia. Comparing with these species, Paussus (Scaphipaussus) zhouchaoi sp. n. seem to be hairless to the naked eye and it also can be distinguished by the combination of the characters in the above paragraph of Diagnosis.

## Acknowledgements

I would like to express my sincere gratitude to Chao Zhou (Chengdu, Sichuan, China) and Li He (Chengdu, Sichuan, China) for loaning the invaluable specimens of the new species. I am very grateful to Xiao-Bin Song (Shanghai, China) for his generosity in giving me the priority to describe this new species when I knew he also possess one male specimen. I also quite appreciate Michael Staab (University of Freiburg, Freiburg, Germany) for identifying the host ant. I am indebted to Jan Růžička (Czech University of Life Sciences, Prague, Czech Republic) for offering me the opportunity to continue my taxonomic research in Europe. I am obliged to Hong-Zhang Zhou (Institute of Zoology, Chinese Academy of Sciences, Beijing, China) for his supervision during my PhD time. Special thanks to Bin Liu, an insect-obsessed eccentric on Cerambycidae, the founder and investor of Bin Insect Taxonomy Studio (Beijing, China), for his years of assistance and company. My appreciations to Wen-I Chou (Taitung, Taiwan, China), Liang He (Institute of Zoology, Chinese Academy of Sciences, Beijing, China), Liang Lü (Institute of Zoology, Chinese Academy of Sciences, Beijing, China), Hao Xu (the Bureau of Forest Resource Management of Mt. Simianshan, Chongqing, China), Qiao-Zhi Yang (Beijing, China) for their continued support of my studies. I am grateful to Andrea Di Giulio (University "Roma Tre", Roma, Italy) and James A. Robertson (University of Arizona, Tucson, USA) who provided constructive comments on previous versions of the manuscript. This study was supported by the Bin Insect Taxonomy Studio (No. 2016).

## References

Luna de Carvalho E (1989) Essai monographique des Coléoptères Protopaussines et Paussines. Memórias do Instituto de Investigação Científica Tropical, 2a serie, 70(1987): 1-1028.
Maruyama M (2016) Revision of the hystrix Westwood, 1850 group of the genus Paussus Linné, 1775 (Coleoptera: Carabidae: Paussinae) I. Descriptions of nineteen new species. Japanese Journal of Systematic Entomology 22(1): 55-86.
Nagel P (1987) Arealsystemanalyse afrikanischer Fühlerkäfer (Coleoptera, Carabidae, Paussinae). Ein Beitrag sur Rekonstruktion der Landschaftsgenese. Erdwissenschaftliche Forschung Band XXI. Franz Steiner, Stuttgart, 233 pp.
Nagel P (2003) Subfamily Paussinae Latreille, 1807. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. Volume 1: Archostemata - Myxophaga - Adephaga. Apollo Books, Stenstrup, 208-211.
Robertson JA, Moore W (2016) Phylogeny of Paussus L. (Carabidae: Paussinae): unravelling morphological convergence associated with myrmecophilous life histories. Systematic Entomology. https://doi.org/10.1111/syen. 12205

# A new species of genus Chorebus Haliday (Hymenoptera, Alysiinae) parasitising Hexomyza caraganae Gu (Diptera, Agromyzidae) from NW China 

Tao Li', Cornelis van Achterberg ${ }^{2,3}$<br>I General Station of Forest Pest Management, State Forestry Administration, Shenyang 110034, P. R. China 2 Shaanxi Key Laboratory for Animal Conservation / Key Laboratory of Resource Biology and Biotechnology in Western China, College of Life Sciences, Northwest University, 229 North Taibai Road, Xi'an, Shaanxi 710069, China 3 Department of Terrestrial Zoology, Naturalis Biodiversity Center, Postbus 9517, 2300 RA Leiden, The Netherlands

Corresponding author: Tao Li (litao200105@163.com)

Academic editor: M. Sharkey | Received 19 January 2017 | Accepted 2 March 2017 | Published 28 March 2017
http://zoobank.org/C2955CE8-F790-4418-8740-F7DD98826459
Citation: Li T, van Achterberg C (2017) A new species of genus Chorebus Haliday (Hymenoptera, Alysiinae) parasitising Hexomyza caraganae Gu (Diptera, Agromyzidae) from NW China. ZooKeys 663: 145-155. https://doi. org/10.3897/zookeys.663.11874


#### Abstract

Chorebus (Stiphrocera) hexomyzae sp. n. (Hymenoptera, Braconidae, Alysiinae, Dacnusini) is described and illustrated. It was reared from twig galls of Hexomyza caraganae Gu (Diptera, Agromyzidae) on Caragana korshinskii Kom. f. (Fabaceae) in Ningxia and Inner Mongolia (NW China). A partial key to related or similar Chorebus species is provided.


## Keywords

Alysiinae, biology, Caragana korshinskii, Chorebus, Dacnusini, Hexomyza caraganae, host, new species, parasitoid, twig gall

## Introduction

The subfamily Alysiinae (Hymenoptera, Braconidae) is a large and common subfamily containing $2,440+$ valid species worldwide (Yu et al. 2016). The subfamily is characterized by having mandibles with 3 or 4 more or less outwardly curved ("exodont") teeth (Fig. 7; Shaw and Huddleston 1991; van Achterberg 1993; Belokobylskij and Kostromina 2011). Rarely, the mandibles have up to 5-7 teeth or lobes, or possess only 1-2 teeth; in all cases the mandibles, if they are closed, do not touch each other. Traditionally, the Alysiinae are divided into two tribes: Alysiini and Dacnusini. The tribe Alysiini contains 76 valid genera and nearly double the number of species compared to the Dacnusini with only 31 valid genera (Yu et al. 2016). The presence (Alysiini) or absence (Dacnusini) of vein r-m of the fore wing is the main morphological difference between the two tribes, with wingless or brachypterous specimens included in the Alysiini. In general, Alysiini are koinobiont endoparasitoids of larval cyclorrhaphous Diptera in moist substrates like dung, carcasses or other decaying organic matter (Wharton 1984; Shaw and Huddleston 1991). In contrast, Dacnusini are koinobiont endoparasitoids of larval cyclorrhaphous Diptera mining in leaves or stems (Yu et al. 2016). The new species of Chorebus Haliday, 1833, (Alysiinae, Dacnusini) belongs to a large cosmopolitan genus with 460 valid species (Yu et al. 2016), but most species are described from the northern hemisphere. The new species is peculiar because of its association with a dipterous twig-galler. To date, it is only the second known reliable host association of a Chorebus sp. with a twig-galler. Up to now, only C. gedanensis (Ratzeburg, 1852) was reared multiple times from the poplar twig gall fly, Hexomyza schineri (Giraud, 1861) in Europe (Nixon 1937, 1944; Griffiths 1967; Georgiev 2004). The Nearctic Chorebus agromyzae (Gahan, 1913) is recorded from the same host on willow, but this is based solely on a reference by Fulmek (1968) which is most likely incorrect considering the host association in the original description by Gahan (1913), viz., Cerodontha (Butomomyza) angulata (Loew, 1869), a species leaf mining Carex spp. The two references concerning galls of Cynipidae by Rudow (1918) are obviously erroneous as hymenopterous larvae are not parasitized by Alysiinae. For the recognition of the subfamily Alysiinae, see van Achterberg $(1976,1990,1993)$ and for additional references see Yu et al. (2016).

## Materials and methods

Twig galls of Caragana korshinskii Kom. f. (Fabaceae) were collected in Ningxia and Inner Mongolia (NW China), and placed in a large nylon cage at room temperature in the laboratory. Distilled water was sprayed over the galls twice a week to prevent desiccation and the emerged insects were collected daily. The galls were induced by Hexomyza caraganae Gu (Diptera: Agromyzidae); the inducer was kindly identified by Dr. Guang-Chun Liu (Shenyang University, Shenyang).

For the morphological terminology used in this paper, see van Achterberg (1993) and Harris (1979). The descriptions, measurements and figures were made using a Leica M205A microscope with a Leica Microsystem DFC550 digital camera. Photographs were combined using Leica Application Suite (Version 4.5.0).

The holotype and some paratypes are deposited at the Department of Life Sciences, Northwest University, Xi'an (NWUX), China. Most paratypes and hosts are deposited in the Insect Museum of the General Station of Forest Pest Management (GSFPM), State Forestry Administration, Shenyang, China. Some paratypes are deposited at the Naturalis Biodiversity Center (RMNH), Leiden.

## Results

## Key to Palaearctic species reared from Hexomyza Enderlein and similar species

$1 \quad$ First metasomal tergite 1.1-1.3 times as long as its apical width and its apical half distinctly widened posteriorly (Fig. 5; but subparallel-sided in C. singularis); hind coxa evenly setose dorsally (Figs 1, 6, 11); vein r of fore wing distinctly longer than width of pterostigma and strongly oblique (Fig. 2); vein 3-CU1 of fore wing 3.0 times longer than vein CU1b 2

- $\quad$ First tergite 1.8-2.5 times as long as its apical width and its apical half nearly parallel-sided; basal half of hind coxa with dorsal tuft of dense setae; vein $r$ of fore wing slightly shorter than width of pterostigma and moderately oblique; vein 3-CU1 of fore wing 1.5 times longer than vein CU1b; [marginal cell of fore wing slender, 3.0-3.5 times longer than its maximum width] 5
2 Two apical segments of maxillary palp nearly as long as height of head; hind coxa yellow; precoxal sulcus almost smooth; apical half of first metasomal tergite subparallel-sided; [mesoscutum punctulate and almost entirely setose]... C. singularis (Tobias, 1962)
- Two apical segments of maxillary palp 0.2-0.3 times as long as height of head; hind coxa black; precoxal sulcus at least distinctly narrowly crenulate; apical half of first tergite distinctly widened posteriorly (Fig. 5)3

3 Palpi and hind femur dark brown; mandibles largely blackish; marginal cell of fore wing stout, 2.5 times longer than its maximum width (Fig. 2); mandible distinctly narrowed apically because of subbasally situated lower tooth (Figs 12-19); precoxal sulcus narrowly crenulate (Figs 1, 4); middle lobe of mesoscutum smooth, except some punctures (Fig. 4) ... C. hexomyzae sp. n.

- Palpi and hind femur yellow; mandibles largely reddish brown; marginal cell of fore wing slender, 4-5 times longer than its maximum width; mandible subparallel-sided or slightly widened apically and lower tooth subapically situated; precoxal sulcus broadly crenulate; middle lobe of mesoscutum superficially rugose; [pronotum laterally and mesopleuron partly granulate]... 4

4 Second－fourth segments of hind tarsus yellow，contrasting with its blackish telotarsus；lateral lobes of mesoscutum partly with short setae medially；first metasomal tergite without median carina．．．．．．．．C．coxator（Thomson，1895）
－$\quad$ Second－fourth segments of hind tarsus and its telotarsus dark brown；lateral lobes of mesoscutum glabrous except for some long setae laterally；first tergite usually with median carina

C．nydia（Nixon，1937）
5 Temple behind base of mandible rectangular，protruding，and nearly as wide as base of mandible；first metasomal tergite 2．2－2．5 times as long as its apical width $\qquad$ C．gedanensis（Ratzeburg，1852）
－$\quad$ Temple behind base of mandible evenly curved and much narrower than base of mandible；first tergite 1．8－2．2 times as long as its apical width 6
6 Temple in dorsal view 1.4 times as long as eye；ovipositor sheath slightly pro－ jecting beyond apex of metasoma，its blackish part 0.7 times as long as hind basitarsus；occiput less densely setose

C．ares（Nixon，1944）
－Temple in dorsal view nearly as long as eye；part of ovipositor sheath project－ ing beyond apex of metasoma approx．as long as second segment of hind tarsus，its blackish part 0.9 times as long as hind basitarsus；occiput more densely setose．

C．senilis（Nees，1812）

## Taxonomy

## Chorebus（Stiphrocera）hexomyzae sp．n． <br> http：／／zoobank．org／0025B40D－6DD5－4DB6－8897－51F5D4AC378E <br> Figures 1－21

Type material．Holotype，$Q_{\text {，（NWUX）＂NW China：Ningxia Hui Autonomous Re－}}$ gion，Shizuishan，Dawukou（N 39 $06^{\prime}$ ，E $106^{\circ} 20^{\prime}, 1140 \mathrm{~m}$ ），26．v． $2015^{\prime \prime}$ and reared from Hexomyza caraganae Gu in twig galls on Caragana korshinskii Kom．f．Paratypes

 id．，25．v．2015； $5 q$ q $10 \delta^{\top} \delta^{\lambda}$ ，id．，27．v．2015；1q，id．，25．vi．2015； $3 q q 1 \delta^{\top}$ ，NW China： Inner Mongolia Autonomous Region，Hangjinqi（N 39²4＇，E 108²4＇， 1460 m ），




 17ずす，id．，1－14．vi．2016．

Diagnosis．Antenna with 27－34 segments；eye in dorsal view 1．1－1．2 times as long as temple；temple medium－sized and rounded ventrally，moderately densely se－ tose with medium－sized setae and hardly protruding behind base of mandible（Figs 4， $12,14,16,19$ ）；third segment（including annellus） 1.4 times as long fourth segment；


Figure I. Chorebus (Stiphrocera) hexomyzae sp. n., female, paratype, habitus lateral.
mandible with four teeth, middle tooth ( $=\mathrm{t} 2$ ) wide triangular, acute, much longer than both lateral teeth, with an extra protuberance on ventral side of middle tooth and ventral $(=\mathrm{t} 3$ ) tooth rather close to base of mandible resulting in apically narrowed mandible (Figs 10, 12-19); notauli nearly complete and largely smooth (Fig. 4); lateral lobes of mesoscutum largely glabrous; length of vein $r$ of fore wing almost equal to width of pterostigma (Fig. 2); vein CU1b short of fore wing short and first subdiscal cell closed and robust; vein 3-SR+SR1 rather short and regularly bent, resulting in a robust marginal cell (Fig. 2); first tergite slightly longer than its apical width, evenly convex and longitudinal rugae not obscured by setosity, dorsope small, and dorsal carinae united and connected with median carina (Fig. 5); second tergite smooth and posterior half sparsely setose (Fig. 11); setose part of ovipositor sheath 0.05 times as long as fore wing and 0.2 times as long as hind tibia (Fig. 1).


Figures 2-II. Chorebus (Stiphrocera) hexomyzae sp. n., female, holotype, but 2 of paratype. $\mathbf{2}$ Wings $\mathbf{3}$ Mesosoma lateral $\mathbf{4}$ Mesosoma dorsal $\mathbf{5}$ Propodeum and first metasomal tergite dorsal $\mathbf{6}$ Hind leg lateral 7 Head anterior 8 Head dorsal 9 Head antero-dorsal IO Mandible, full view on middle tooth II Metasoma dorsal.

Description. Holotype, $\uparrow$, length of body 3.9 mm , of fore wing 3.5 mm .
Head. Transverse and shiny in dorsal view, slightly widened posteriorly (Fig. 9), width of head 1.9 times its lateral length, in anterior view subcircular (Fig. 7), and 1.1 times wider than mesoscutum; antenna 0.7 times as long as fore wing and with 30 segments, short setose but apically with few long bristles, length of third segment (including annellus) 1.4 times as long as fourth segment, length of third, fourth and penultimate segments $2.6,1.8$ and 1.5 times their width, respectively (Figs 1, 3); length of maxillary palp 0.8 times height of head; eye in dorsal view 1.1 times as long as temple (Fig. 8); eye in lateral view 1.4 times higher than wide; frons convex laterally, remotely punctulate and setose, and slightly depressed behind antennal sockets and with shallow groove in front of anterior ocellus (Fig. 9); vertex rather convex and with long setae (Fig. 8); OOL:diameter of ocellus:POL= 14:7:8; face 1.4 times wider than high, rather evenly convex, with long setae and largely smooth, sparsely punctulate and with satin sheen; clypeus largely smooth, convex and transverse, depressed and slightly concave medio-ventrally (Fig. 7); malar space absent; mandible with four teeth, middle tooth ( $=\mathrm{t} 2$ ) wide triangular, acute, much longer than both lateral teeth, with an extra protuberance on ventral side of middle tooth (similar to t3) and ventral ( $=\mathrm{t} 3$ ) tooth rather close to base of mandible resulting in apically narrowed mandible (Figs 10, 12-19); medial length of mandible nearly equal its maximum width and mandible ventro-basally with large flat part nearly as wide as dorsal part of mandible (Figs 12-15, 19).

Mesosoma. Length of mesosoma 1.5 times its height; pronope wide, elliptical and large; side of pronotum largely smooth, sparsely setose and finely punctulate, only posterior half of oblique groove coarsely crenulate and some crenulae anteriorly (Fig. 3); mesoscutum without lateral carina in front of tegula, but with lateral groove (Fig. 4); tegula square and large; epicnemial area crenulate; precoxal sulcus narrow and finely crenulate, its posterior third absent (Fig. 3), remainder of mesopleuron smooth; pleural sulcus smooth; episternal scrobe medium-sized, oval and connected to pleural sulcus; metapleuron largely smooth dorsally and rugulose ventrally, without specialised central area, setae directing postero-ventrally, but setae of dorsal groove directing dorsally (Fig. 3); notauli nearly complete, smooth except some fine crenulae anteriorly and posteriorly separated from long and narrow linear medio-posterior depression (Fig. 4); lateral lobes of mesoscutum largely glabrous and with satin sheen, remainder of mesoscutum largely setose; scutellar sulcus deep and wide, with 3 long carinae and 2 short ones, sulcus 4 times wider than its median length; scutellum smooth and moderately convex, superficially impressed medio-posteriorly; axilla densely setose; metanotum with long setae and with regular and complete coarse median carina (Figs 4, 5); surface of propodeum rugose, medially with some coarse transverse rugae, with open setosity leaving sculpture well visible, but postero-laterally rather densely setose, anteriorly with short and rather weak median carina and areola absent (Fig. 5).

Wings (Fig. 2). Fore wing: r:2-SR:3-SR+SR1 = 10:14:49; 1-SR+M slightly sinuate; SR1 evenly bent (Fig. 2); r approx. equal width of pterostigma and oblique; cu-a postfurcal; 1-CU1:2-CU1 = 3:11; 3-CU1 much longer than short CU1b; m-cu antefurcal,


Figures I2-19. Chorebus (Stiphrocera) hexomyzae sp. n., female, holotype. I2-19. Mandible at different angles; t1, t2, t3 = upper, middle and lower tooth, respectively; t4 = additional tooth on ventral side of middle tooth.
nearly straight and distinctly converging to $1-\mathrm{M}$ posteriorly; first subdiscal cell 2.1 times as long as wide; $\mathrm{M}+\mathrm{CU} 1$ largely unsclerotised. Hind wing: $\mathrm{M}+\mathrm{CU}: 1-\mathrm{M}: 1 \mathrm{r}-\mathrm{m}=$ 30:14:13; m-cu absent; cu-a straight.

Legs. Hind coxa largely smooth, without dense dorso-basal tuft of setae but with long whitish setae (Fig. 6); tarsal claws medium-sized, almost as long as arolium and with few bristles (Fig. 6); length of femur, tibia and basitarsus of hind leg 3.6, 9.0 and 4.4 times their width, respectively; length of hind tibial spurs 0.35 and 0.40 times their basitarsus; hind basitarsus slightly widened submedially (Fig. 6).

Metasoma. Length of first tergite 1.1 times its apical width, its dorsal carinae united at basal quarter and connected with median carina, medially evenly convex and rather


Figures 20, 2 I. 20 Twig gall of Hexomyza caraganae Gu, with emergence hole of Chorebus (Stiphrocera) hexomyzae sp. n. 2 I Adult of $C$. (S.) hexomyzae emerging from puparium of $H$. caraganae Gu .
regular and coarse longitudinal rugae distinctly visible despite long setosity (Fig. 5); dorsope small and round, laterope obsolescent; second tergite smooth and medio-anteriorly glabrous, remainder sparsely setose; setose part of ovipositor sheath 0.05 times as long as fore wing (total visible sheath 0.08 times), narrowed apically and 0.2 times as long as hind tibia (Fig. 1).

Colour. Black (including mandible); palpi, legs (but coxa and dorsally femora black), pterostigma and veins dark brown; wing membrane subhyaline.

Male. Similar to female. Antenna with 3-4 segments more than in female and slightly slenderer.

Variations. Length of body of $Q 3.0-3.9 \mathrm{~mm}$, and of fore wing $2.7-3.5 \mathrm{~mm}$; length of body of ふ $3.3-3.9 \mathrm{~mm}$, and of fore wing $2.9-3.7 \mathrm{~mm}$; antenna of $q$ with 25(1), 26(1), 27(5), 28(12), 29(19), 30(10), 31(7) segments; antenna of ${ }^{\top}$ with 30(2), 31(6), 32(9), 33(16), 34(12), 35(1), 36(1) segments; first metasomal tergite $1.0-1.1$ times longer than its apical width; setose part of ovipositor sheath $0.04-0.05$ times as long as fore wing; setae of second tergite as subposterior row or also laterally present; lateral lobes of mesoscutum nearly completely glabrous or anterior third setose and remainder glabrous; femora and tibiae dark brown with blackish streaks or yellowish brown; palpi dark brown or yellowish brown; mandible black or dark brown.

Biology. Larval endoparasitoid of Hexomyza caraganae Gu, 1991 (Diptera: Agromyzidae) in twig galls on Caragana korshinskii Kom. (Fabaceae).

Distribution. Palaearctic China (Inner Mongolia, Ningxia).
Remarks. The new species belongs to the subgenus Stiphrocera Foerster, 1863, because it has smooth hind coxa without a dorsal tuft, and runs in the key to Far East Russian species by Tobias (1998) to Chorebus coxator (Thomson, 1895) and C. singularis (Tobias, 1962). The new species is easily separated by its dark palpi, mandible and legs. In addition, the shape of the mandible and the mesosomal setosity are different as indicated in the key. The new species is very different from the only other named species reared from Hexomyza twig galls, the European C. gedanensis (Ratzeburg, 1852), because of the elongate first metasomal tergite and shorter vein $r$ of the fore wing of the latter. Two similar species occurring in the East Palaearctic region (C. ares (Nixon, 1944) and C. senilis (Nees, 1812)) are included in the key for comparison.

Etymology. The specific name is derived from the host's generic name: Hexomyza Enderlein, 1936.

## Acknowledgements

The authors are deeply grateful to Dr. Michael Sharkey (Department of Entomology, University of Kentucky, USA) and one anonymous referee for valuable comments and suggestions. We are also indebted to Dr. Guang-Chun Liu (Shenyang University, Shenyang, China) for identifying the host. This research was supported by the "Twelfth Five-year" National Science and Technology Support Program of China (No. 2012BAD19B0701) and the National Natural Science Foundation of China (NSFC, No. 31070585; NSFC, No. 31501887).

## References

Belokobylskij SA, Kostromina TS (2011) Two late-spring braconid genera of the family Alysiinae (Hymenoptera: Braconidae) new for the fauna of Russia. Zoosystematica Rossica 20(1): 85-95.
Fulmek L (1968) Parasitinsekten der Insektengallen Europas. Beiträge zur Entomologie 18(7/8): 719-952.
Gahan AB (1913) New Ichneumonoidea parasitic on leaf-mining Diptera. Canadian Entomologist 45: 145-154. https://doi.org/10.4039/Ent45145-5
Georgiev G (2004) Chorebus gedanensis (Hymenoptera: Braconidae), a new parasitoid of the poplar twiggall fly, Hexomyza schineri (Diptera: Agromyzidae) in Bulgaria. Acta Zoologica Bulgarica 56(1): 115-118.
Griffiths GCD (1967) The Alysiinae (Hym. Braconidae) parasites of the Agromyzidae (Diptera) IV. The parasites of Hexomyza Enderlein, Melanagromyza Hendel, Ophiomyia Braschnikov and Napomyza Westwood. Beiträge zur Entomologie 17(5/8): 653-696.
Harris R (1979) A glossary of surface sculpturing. Occasional Papers in Entomology of the California Department of Food and Agriculture 28: 1-31.
Nixon GEJ (1937) A revision of the European Dacnusini (Hym., Braconidae, Dacnusinae). Entomologist's Monthly Magazine 80: 88-108.
Nixon GEJ (1944) British species of Dacnusa (Braconidae). Transactions of the Society for British Entomology 4: 1-88.
Rudow F (1918) Braconiden und ihre Wirte. Entomologische Zeitschrift 32: 4, 7-8, 11-12, 15-16.
Shaw MR, Huddleston T (1991) Classification and biology of Braconid wasps (Hymenoptera: Braconidae). Handbooks for the Identification of British Insects 7(11): 1-126.
Tobias VI (1998) Alysiinae (Dacnusini) and Opiinae. In: Ler PA (Ed.) 'Key to the insects of Russian Far East. Vol. 4. Neuropteroidea, Mecoptera, Hymenoptera. Pt 3. Dal'nauka, Vladivostok, 299-411, 558-655.
van Achterberg C (1976) A preliminary key to the subfamilies of the Braconidae (Hymenoptera). Tijdschrift voor Entomologie 119: 33-78.
van Achterberg C (1990) Illustrated key to the subfamilies of the Holarctic Braconidae (Hymenoptera: Ichneumonoidea). Zoologische Mededelingen Leiden 64(1): 1-20.
van Achterberg C (1993) Illustrated key to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). Zoologische Verhandelingen Leiden 283: 1-189.
Wharton RA (1984) Biology of the Alysiini (Hymenoptera: Braconidae), parasitoids of cyclorrhaphous Diptera. Texas Agricultural Experimental Station. Technical Monograph 11: 1-39.
Yu DS, van Achterberg C, Horstmann K (2016). Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. www.taxapad.com, Nepean, Ontario, Canada.

