RESEARCH ARTICLE



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

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Abstract

The first morphological observation of a euryalid brittle star, *Asteronyx loveni*, using non-destructive Xray micro-computed tomography (μ 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 μ 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 μ 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 (μ CT) is a non-destructive imaging technique using X-ray. This method allows rapid creation of three dimensional (3D) morphological and anatomical images at μ 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 μ 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 2012; Faulwetter et al. 2013; Kohtsuka 2014; Sentoku et al. 2015; Landschoff and Griffiths 2015).

In the Ophiurida and Ophintegrida, μ 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 μ 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 μ 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 μ CT scanning.

Materials and methods

Sample preparations

Applying μ 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 μ 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 μ 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.

µCT observation and 3D reconstructions

A ScanXmate B100TSS110 μ 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. 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 2D 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

Catalog Number	Locality	Water Depth (m)	Date
NSMT E-6986	East China Sea, southwestern Japan, 26°56.30'N, 127°37.00'E	648	June 1, 2011
NSMT E-5638	Off Miyako, northeastern Japan, 39°20.19'N, 142°51.39'E; -39°19.22'N, 142°49.17'E	1709-1737	November 6, 2007

Table 1. Sampling information of the two examined specimens of Asteronyx loveni.

Table 2. Scanning parameters of µCT for the observations of *Asteronyx loveni*.

Observed specimen	Source voltage (kV)	Source current (µA)	Exposure time for 1 frame (sec)	Total number of frames	Total time for scanning (min)	Detector size (pixel)	Resolution (µm)
Entire body	80	155	1.0	1,500	25	1,024 × 1,012	15.440
Basal part of an arm	100	100	1.2	1,200	16	1,024 × 1,012	13.759
Isolated vertebral arm ossicle	75	43	0.4	1,200	50	1,024 × 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 μ 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 4th 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 μ 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 1. Microscopic (**A**, **D**, **G**), μ CT volume rendered (**B**) and μ CT surface rendered (**C**, **E**, **F**, **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 **D**, **E** whole animal, aboral view **F** mouth frame, virtually dissecting aboral view, peristomial plates are colored green **G**, **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 (**A**, **C**, **E**) and μ CT surface rendered (**B**, **D**, **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 **E** oral disc, oral view. An arrow in **E** indicates the orientation of observation of **F**; **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 1st vertebrae and an oral plate **C** disc and basal part of an arm, vertical section of central disc **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 (**A**), μ CT volume rendered (**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 **D** Inter-vertebral muscles are colored purple in **D** Black arrow heads in **B** and **D** indicate identical vertebra, and ST and AS labels also indicates the identical characters in **A**, **B** and **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 2D 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, granule-shaped (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 (**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 **B** vertical longitudinal section, adradial side of the arm **C**, **D** horizontal longitudinal section, aboral view **C** is set on more aboral side than **D** horizontal longitudinal section, or al view **E–G** transverse vertical sections from basal (**E**) to distal (**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 (**B–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 μ 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 extend to the distal side and dead-ended inside the vertebra (Fig. 6K–O).



Figure 6. SEM (**A**), μCT surface rendering (**B**, **C**) and 2D section (**D**–**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 *Asteronyx loveni* were successfully observed by μ 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 μ 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, µCT 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 μ CT scanning. This study has shown that μ CT is a powerful tool for species- to order-level taxonomy in Ophiuroidea as Ziegler (2012) suggested. Considering that specimens remain non-dissected following μ 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 μ CT images in this study (ca. 4.5–15.5 μ 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 3D 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 μ 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 μ 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.

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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 μ 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 μ 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 μ CT surface rendering images of the isolated vertebral ossicles of *Asteronyx loveni* (NSMT E-5638).
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RESEARCH ARTICLE



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

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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* **sp. 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* **sp. 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

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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 haemuli* 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) (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

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using forceps and then stored in 80% ethanol. *Anilocra haemuli* from *H. flavolineatum* Desmarest, 1823, (family Haemulidae) (St. John, USVI, n = 4, 2011; n = 2, 2012; n = 1, 2013; Guana Island, BVI, n = 1, 2012; n = 2, 2013; St. Thomas, USVI, 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, 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 µl Thermo Scientific DreamTaq PCR master mix (2x) (2x DreamTag buffer, 0.4 mM of each dNTP, and 4 mM MgCl2), 1.25 µl of each primer, 1 µl DNA, and 9 µl of PCR-grade nuclease free water (Thermo Scientific, Vilnius, Lithuania). Total volume per reaction was $25 \,\mu$ l, and PCR reactions were conducted using a ProFlex[™] PCR thermal cycler (Applied Biosystems by Life Technologies). Reactions were amplified under the following PCR conditions: Stage 1, 94°C for 5min, Stage 2, 36 cycles of 94°C for 30s, 47°C for 50s, 72°C for 2min, and Stage 3, 72°C for 10min. 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 Gen-Bank 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 Bunkley-Williams 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 (\mathcal{Q}) 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 104–109nt (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* pareopods 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 fla-volineatum* (USNM 184796); allotype (male, TL, W unknown) (USNM 184797); Para-types (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: \bigcirc (32, 13, AMNH_IZC 250203; 32, 14, AMNH_IZC 250204) Mosquito Island, BVI; \bigcirc (30, 10) West End Enrique Reef, La Parguera, Puerto Rico, 30 Nov 1976; \bigcirc (30,12, AMNH_IZC 250205) San Cristobal Reef, La Parguera, Puerto Rico, 30 Nov 1976; \bigcirc (30, 11, AMNH_IZC 250206; 29,14) Mingo Cay, St. John, USVI, 4 Mar 1977; \bigcirc (32, 13; 34, 14, AMNH_IZC 250207) Lameshur Bay, St. John, USVI, 2 Mar 1977; \bigcirc (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: \bigcirc (30, 11; 31, 12) Cinnamon Bay, St. John, Jun 2011; \bigcirc (28, 12) White Bay, Guana Island, BVI; Jul 2011; \bigcirc (damaged; 25, 9) St. Thomas, USVI, Jun 2012; \bigcirc (25, 10) White Bay, Guana Island, BVI, Jul 2012; \bigcirc (26, 11) Jumbee Bay, St. John, USVI, Jul 2013; \bigcirc (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 2 0.8 times as long as article 1; article 3 0.9 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 3 1.6 times as long as article 2; article 4 1.2 times as long as wide, 1.5 times as long as article 3; article 5 1.3 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 I. Anilocra haemuli female (29 mm) A–D Anilocra haemuli female (23 mm) E–I: A dorsal view
B lateral view C dorsal view of cephalon D ventral view of cephalon. E dorsal pleotelson F pereopod 7
G pereopod 2 H pereopod 1 I pereopod 6.



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 **C** article 3 of maxilliped **D** maxillule **E** mandible **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 basis 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 **C** dorsal view of cephalon **D** ventral view of cephalon **E** pereopod 7 **F** dorsal pleotelson **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 **C** mandible **D** maxilliped **E** article 3 of maxilliped **F** article 3 of mandibular palp **G** maxillule **H–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

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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. *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 percopods, 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. 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 ♀ (38, 17, AMNH_IZC 250209), Lameshur Bay, St. John, 18°18'59"N, 64°43'25"W, US Virgin Islands, 2 Mar 1977, coll. EH and LB Williams.

Paratype. Ovigerous \bigcirc 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: \bigcirc (33, 13, AMNH_IZC 250211; 24, 9) San Cristobal Reef, La Parguera, Puerto Rico 28–29 Jan 1977; \bigcirc (35, 15, AMNH_IZC 250212; 32, 13 AMNH_IZC 250213) Lameshur Bay, St. John, USVI, 2 Mar 1977; \bigcirc (39, 16, AMNH_IZC_250214) Buck Island, St. Thomas, USVI, 5 Mar 1977; \bigcirc (34, 15, AMNH_IZC 250215; 25, 10 AMNH_IZC 25016) Laurel Reef, La Parguera, Puerto Rico, 18 May 1977; \bigcirc (30, 12) Ensenada Honda, Vieques, Puerto Rico, 20 Dec 1983. Collected by PC Sikkel and ER Brill: \bigcirc (27, 10; 30, 13; 26, 10; 31, 12; 29, 12; 29, 12; damaged) TS (11,6) White Bay, Guana Island, 18°28'0"N, 64°33'59"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 2 1.5 times as long as article 1; article 3 0.9 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 **B** lateral view **C** dorsal view of cephalon **D** pleotelson **E** ventral view of cephalon **F** pereopod 1 **G** pereopod 2 **H** pereopod 6 **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 **C** article 3 of maxilliped **D** maxillule **E** mandible **F** maxilliped **G-K** pleopods 1–5 respectively.

cle terminating in 1 short simple seta. *Antenna* comprised of 9–10 articles, peduncle article 3 1.5 times as long as article 2; article 4 1.3 times as long as wide, 1.1 times as long as article 3; article 5 1.6 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. *Maxillula* nesial 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** percopod 1 **C** dorsal pleotelson **D** percopod 7 **E** dorsal view of cephalon **F** ventral view of cephalon **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; dactylus 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 **C** mandible **D** maxilliped **E** article 3 of maxilliped **F** article 3 of mandibular palp **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. haemuli*. 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

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. holocanthi 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.

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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

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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.
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RESEARCH ARTICLE



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

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Abstract

Five species of *Pireneitega* spiders from China are surveyed, of which three are new to science: *P. huashanensis* Zhao & Li, **sp. n.** ($\mathcal{J} Q$), *P. lushuiensis* Zhao & Li, **sp. n.** ($\mathcal{J} Q$), *P. siyankouensis* Zhao & Li, **sp. n.** ($\mathcal{J} Q$). Two 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: A = epigynal atrium; ALE = anterior lateral eye; 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; CF = cymbial furrow; CO = conductor; d = dorsal; E = embolus; EB = embolic base; ET = epigynal teeth; FD = fertilization ducts; Fe = femur; H = epigynal hood; MA = median apophysis; Mt = metatarsus; p = prolateral; PA = patellar apophysis; Pa = patella; PLE = posterior lateral eye; PME = posterior median eye; PME-PLE = distance between PME and PLE; PME-PME = distance between PME and PME; R = receptacle; r = retrolateral; RTA = retrolateral tibial apophysis; ST = subtegulum; T = tegulum; Ta = tarsus; TC = tip of conductor; 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.

Species	GenBank accession number	Sequence length	Collection localities (all in China)
P. huashanensis sp. n.	KY593329	1194bp	Shaanxi Prov.: Huayin Prefecture: Mt. Huashan
P. liansui	KY593330	1194bp	Hunnan Prov.: Daoxian Co.
P. lushuiensis sp. n.	KY593327	1194bp	Yunnan Prov.: Lushui Co.
P. triglochinata	KY593328	1194bp	Sichuan Prov.: Mt. Emei
P. xiyankouensis sp. n.	KY593331	1194bp	Guangxi Prov.: Yizhou City

Table 1. Voucher specimen information.

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 huashanensis Zhao & Li, sp. n.

http://zoobank.org/A75AC3BA-9598-4DEA-B235-1485879B4EFB Figs 1–2, 11

Type material. Holotype 3° : China: *Shaanxi*: Huayin Prefecture: Mt. Huashan, Duyukou Village, 34°31'42"N, 110°07'22"E, 530 m, 30.IX.2013, Y. Li and J. Liu. **Paratypes:** 1 3° , same data as holotype; $4^{\circ}1^{\circ}3^{\circ}$, same area, $34^{\circ}32'46$ "N, 110°07'06"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 1. Palp of *Pireneitega huashanensis* sp. n., male holotype. A Prolateral view **B** Ventral view **C** Retrolateral view. Scale bar: equal for **A**, **B**, **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 **C** Male habitus, dorsal view **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D**, **E**.

	Fe	Pt	Ti	Mt	Ta
Ι	3d 2p 1r	_	1p 3-3v	1p 3-3v	_
II	3d 1p 2r	1d 1p 1r	2p 3-3v	3p 3-3v	_
III	3d 2p	1p 1r	2p 2r 3-3v	2d 5p 3r 3-3v	1p 1r
IV	3d 1r	1p 1r	2p 2r 3-3v	4p 4r 3-3v	2p 2r

Spination in male:

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 (*Bd*), median part running anteriorly (*Md*), 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.

	Fe	Pt	Ti	Mt	Ta
Ι	3d 2p 1r	-	1p 3-3v	1p 3-3v	_
II	3d 2p 2r	1d 1p	2p 3-3v	2p 3-3v	_
III	3d 2p 2r	2d 1p 1r	2d 2p 2r 3-3v	2d 4p 2r 3-3v	1p 1r
IV	3d 1p 2r	1d 1p 1r	2d 2p 2r 3-3v	3d 2p 2r 3-3v	2p 2r

Spination in female:

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 (♀). Holotype ♀ from Hunan, Daoxian County, 25°31'N, 111°36'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 (\bigcirc).

Material examined. 3913, China: *Hunan*: Daoxian County: Dongzhou Village, 25°31'45"N, 111°36'17"E, 168 m, 5.XI.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; AME-AME 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 **D**, **E**.

	Fe	Pt	Ti	Mt	Ta
Ι	2d 2p	_	3-3v	1r 3-3v	—
II	3d 2p 2r	_	2p 3-3v	3p 3-3v	_
III	3d 2p 1r	1p 1r	2p 2r 3-3v	5p 5r 3-3v	1p 1r
IV	3d 2p 1r	1p 1r	2p 2r 3-3v	1d 4p 5r 3-3v	1p 1r

Spination in male:

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 3: China: *Yunnan*: Nujiang Lisu Autonomous Prefecture, Lushui County, Pianma Town, 25°59'52"N, 98°37'53"E, 2257 m, 28.VI.2016, Y. Li, M. Xu & M. Hu. **Paratypes:** $8 \oplus 5 3$, same data as holotype; $3 \oplus 2 3$, Nujiang Lisu Autonomous Prefecture, Lushui County, 25°59'38"N, 98°39'42"E, 2337 m, 29.VI.2016, Y. Li, M. Xu & M. Hu; 7 \oplus , Baoshan Prefecture, Tengchong City, Mangbang Town, Changlinggan Village, 24°58'07"N, 98°36'54"E, 2032 m, 23.VI.2013, Z. Zhao & J. Liu; $2 \oplus 1 3$, Baoshan Prefecture, Tengchong City, Mt. Gaoligong National Park, 24°49'44"N, 98°46'03"E, 2177 m, 21–22.VI.2013, Z. Zhao and J. Liu; $10 \oplus$, Baoshan Prefecture, Tengchong City, Mingguang Town, Xinjie, Yunyan Temple, 25°29'19"N, 98°32'35"E, 1797 m, 28.XI.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 (*vs* 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.

	Fe	Pt	Ti	Mt	Ta
Ι	2d 4p 1r	_	1p 3-3v	3-3v	_
II	3d 1p 1r	_	1r 3-3v	1r 3-3v	_
III	3d 2p 1r	1p 1r	2p 2r 3-3v	5p 5r 3-3v	1p
IV	3d 2p	1d 1p 1r	1d 2p 3-3v	5p 5r 3-3v	1p 1r

Spination in male:

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 **D** Female habitus, dorsal view **E** Female habitus, ventral view. Scale bars: equal for **D**, **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.

	Fe	Pt	Ti	Mt	Ta
Ι	3d 2p	_	3-3v	3-3v	_
II	3d 2p 2r	—	2p 3-3v	2p 3-3v	—
III	3d 2p 2r	1r	2p 2r 3-3v	5p 5r 3-3v	1r
IV	3d 1p 2r	1r	2p 2r 3-3v	5p 5r 3-3v	1p 1r

Spination in female:

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 (♀ only, male mismatched). Holotype ♀: China: Sichuan: Mt. Emei. Types lost (originally at Jilin University).

Coelotes triglochinatus: Song et al. 1999: 388, f. 225W–X, 227J, 228K (♀ only, male mismatched).

Pireneitega triglochinata: Wang & Jäger 2007: 48 (transfer from Coelotes).

Material examined. China: *Sichuan*: 2♂, Mt. Emei, Yuanhong Cave, 29°34'08"N, 103°24'32"E, 858 m, 29.IX.2016, Z. Zhao & X. Zhang; 2♀5♂, Mt. Emei, 29°34'11"N, 103°25'36"E, 834 m, 29.IX.2016, Z. Zhao & X. Zhang.

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 a bifurcate tip of the septum (*vs* broad 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.

	Fe	Pt	Ti	Mt	Ta
Ι	2d 2p 1r	_	3-3v	3-3v	_
II	3d 2p 2r	_	2p 3-3v	2p 2r 3-3v	_
III	3d 2p 2r	1p 1r	1d 2p 2r 3-3v	5p 5r 3-3v	_
IV	3d 2p 1r	1d 1p 1r	2p 2r 3-3v	5p 5r 3-3v	1r

Spination in male:

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 **D**, **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.

	Fe	Pt	Ti	Mt	Ta
Ι	1d 1p 1r	_	1p 3-3v	4-4v	_
II	3d 2p 2r	_	2p 3-3v	2p 3-3v	_
III	3d 2p 2r	1p 1r	2p 2r 3-3v	5p 5r 3-3v	1r
IV	3d 2p 1r	1r	2p 2r 3-3v	5p 5r 3-3v	1p 1r

Spination in female:

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 d: China: *Guangxi*: Hechi Prefecture: Yizhou City: Xiyankou Village, Mt. Baihu, Xiannvyan, 24°29'17"N, 108°34'02"E, 110 m, 11.XII.2012, Z. Chen & Z. Zhao. **Paratypes:** 2° , same data as holotype; 1° , Hechi Prefecture: Donglan County: Sanshi Town: Gongping Village, unnamed cave, 24°21'44"N, 107°23'11"E, 383 m, 11.II.2015, Y. Li & Z. Chen; 1♀, Hechi Prefecture: Donglan County: Bala Village, unnamed cave, 24°26'37"N, 107°20'50"E, 385 m, 18.III.2015, Y. Li & Z. Chen; 2913, Hechi Prefecture: Nandan County: Chengguan Town, unnamed cave, 25°02'11"N, 107°25'00"E, 559 m, 2.II.2015, Y. Li & Z. Chen; $1 \oplus 1_{0}^{2}$, Chongzuo Prefecture: Daxin County: Fulong Town: Pingliang Village, Banzhongtun, Shuiniu Cave, 22°57'55"N, 107°28'12"E, 248 m, 24.XII.2012, Z. Chen & Z. Zhao; 3 $\bigcirc 2$ \land , Baise Prefecture: Debao County: Yandong Town: Yandong Village, Chuanshan Cave, 23°10'00"N, 106°40'01"E, 596 m, 20.XII.2012, Z. Chen & Z. Zhao; 19, Chongzuo Prefecture: Pingxiang City: Liancheng County, Baiyu Cave, 22°07'44"N, 106°45'55"E, 326 m, 28.XII.2012, Z. Chen & Z. Zhao; 1♀, Chongzuo Prefecture: Tiandeng County: Dukang Town: Bakong Village, Yuanliutun, entrance to unnamed cave, 23°06'45"N, 107°04'33"E, 457 m, 26.XII.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 (*vs* 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 (*vs* 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) (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 **D**, **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
Ι	2d 2p 2r	-	1p 3-3v	2p 3-3v	-
II	2d 3p 2r	1p	1p 3-3v	1p 1r 3-3v	-
III	2d 2p 2r	2d 1p 1r	1d 2p 2r 3-3v	3d 2p 4r 3-3v	1r
IV	2d 2p 1r	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	Ti	Mt	Ta
Ι	3d 2p 1r	-	3-3v	3-3v	-
II	3d 2p 2r	1p	2p 3-3v	2p 1r 3-3v	-
III	3d 2p 3r	2d 1p 1r	1d 2p 2r 3-3v	4p 5r 3-3v	1r
IV	3d 2p 1r	1p 1r	2p 2r 3-3v	2d 2p 3r 3-3v	-

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

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Figure 11. Collection localities of five *Pireneitega* species from China. 1 *P. huashanensis* sp. n. 2 *P. liansui* 3 *P. lushuiensis* sp. n. 4 *P. triglochinata* 5 *P. xiyankouensis* sp. n.

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RESEARCH ARTICLE



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

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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, Qingryllus, 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 1. 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% 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 \mathcal{J} : CHINA: Sichuan, Chongzhou, Jiguanshan, Shaoyaogou, 29.V.2016, leg. Fuming Shi. Paratypes: $1\mathcal{J}$, $1\mathcal{Q}$: 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 4th joint, slightly shorter than 5th joint, 5th joint widened apically, apical margin transversal and arc-shaped; last joint of labial palpus slightly longer than 2th 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 4B) long and straight, near the end not swollen, apex acute.

Colouration. Body black brown to black. Part of 4th and 5th 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 **B** ventral view **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

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RESEARCH ARTICLE



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

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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. constellata* Nesheim, **sp. n.** (Paraguay), *P. corys* Nesheim & Masner, **sp. n.** (Brazil), *P. constellata* Nesheim, **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, French Guiana), *P. galeata* Nesheim, **sp. n.** (Belize, Brazil, Colombia, Costa Rica, Ecuador, French Guiana), *P. galeata* Nesheim, **sp. n.** (Bolivia, Costa Rica, Ecuador, Peru), *P. galeatia* 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, 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, *Teleno-mus* 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, 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, T1 in the female is produced into a horn to house the ovipositor, T2 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 for	veolae on the mesepister-
	num	most Phanuromyia
_	Sternaulus indicated by a shallow fold or by 1 to 2	2 irregular pits near pro-
	mesothoracic suture	
3(2)	T2 reticulate beyond basal costae	Phanuromyia
_	T2 smooth beyond basal costae	

4(3)	Antennal scrobe absent or weakly impressed, head in	n lateral profile semiglo-
	bose	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 mm (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: rugose-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, 500m, 12°46'S, 55°30'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 mm (n=3).



Figures 1–6. *Phanuromyia comata* \bigcirc (OSUC 149413), **I** Lateral habitus **2** Dorsal habitus **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 7–12. *Phanuromyia constellata* \bigcirc (OSUC 322906), **7** Lateral habitus **8** Dorsal habitus **9** Head, mesosoma, lateral view **10** Head, mesosoma, dorsal view **11** 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' from 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*: PARA-GUAY: 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: rugose-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 13–18. *Phanuromyia corys* ♀ (OSUC 149359), 13 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* \bigcirc (OSUC 550034), 19 Lateral habitus 20 Dorsal habitus 21 Head, mesosoma, lateral view 22 Head, mesosoma, dorsal view 23 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: rugose-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, 270m, 00°30'S, 76°30'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* ♀ (OSUC 550006), **25** Lateral habitus **26** Dorsal habitus **27** Head, mesosoma, lateral view **28** Head, mesosoma, dorsal view **29** Head, anteroventral view **30** T1–T2, 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, 549988–550006, 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-AEA0-4197D000A302 http://bioguid.osu.edu/xbiod_concepts/403721 Figures 30–36

Description. Female body length: 1.07–2.22 mm (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: rugose-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 31–36. *Phanuromyia dissidens* ♀ (OSUC 149412), 31 Lateral habitus 32 Dorsal habitus 33 Head, mesosoma, lateral view 34 Head, mesosoma, dorsal view 35 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, 500m, 12°46'S, 55°30'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 mm (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 149315–149316, 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 149317–149318, 550102, 550116, 555798, 555801 (CNCI). **MEXICO:** 1 female, OSUC 320968 (CNCI). **PERU:** 5 females, OSUC 149312, 149314, 550106, 550185–550186 (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* \bigcirc (OSUC 555798), 37 Dorsal habitus 38 Lateral habitus 39 Head, mesosoma, dorsal view 40 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* \bigcirc (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°30'S, 76°30'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: rugose-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: 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* \bigcirc (OSUC 550078), 49 Lateral habitus 50 Dorsal habitus 51 Head, mesosoma, lateral view 52 Head, mesosoma, dorsal view 53 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 mm (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* 3° (OSUC 550046), **55** Lateral habitus **56** Dorsal habitus **57** Head, mesosoma, lateral view **58** Head, mesosoma, dorsal view **59** Head, mouthparts, anteroventral view **60** 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: rugose-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, 290m, 00°05'S, 76°05'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 mm (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: rugose-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 61–66. *Phanuromyia odo* \bigcirc (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). CO-LOMBIA: 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, 550162-550169, 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). MEXI-CO: 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 mm (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: rugose-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, 290m, 12°50'S, 69°17'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* \bigcirc (OSUC 550066), **68** Lateral habitus **69** Dorsal habitus **70** Head, mesosoma, lateral view **71** Head, mesosoma, dorsal view **72** Head, mouthparts, anteroventral view **73** T1–T2, 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 mm (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* \bigcirc (OSUC 151126), **74** Dorsal habitus **75** Lateral habitus **76** Head, mesosoma, dorsal view **77** 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: rugose-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°33'27.3"S 38°03'05"W, Mata de São João, 24.VII.2001, sweeping, M. T. Tavares et al., OSUC 150922 (deposited in OSUC). *Paratypes*: (9 females) BRA-ZIL: 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 mm (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* \bigcirc (OSUC 149418), **80** Dorsal habitus **81** Lateral habitus **82** Head, mesosoma, dorsal view **83** Head, mesosoma, lateral view **84** Head, mouthparts, anteroventral view **85** T1–T2, 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, 270m, 00°30'S, 76°30'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 m, 04°40'19"N, 58°41'04"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

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) <i>P. galeata</i> sp. n. \mathcal{E}°
_	No median keel on frons; lower frons irregularly rugose; mesoscutellum ru- gose punctate at least in anterior half; mandible broad with middle tooth as large as adjacent teeth (Figs 43–48) (Brazil, Ecuador, French Guiana)
3(1)	Frons below median ocellus with an irregular pattern of setiferous punctures (Figs 35, 59)
_	Frons below median ocellus with 2 parallel or subparallel rows of setiferous punctures (Fig. 90)
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)
_	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)
5(4)	Female T1 with distinct tubular horn; propodeum with foliaceous lamella anterior to T1 horn (Figs 86–91) (Brazil, Guyana) <i>P. tubulifer</i> sp. n. \bigcirc
_	Female T1 at most moderately swollen (Fig. 38); propodeum without me- dian transverse lamella (Figs 25, 38, 81)
6(5)	T2 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) <i>P. princeps</i> sp. n. \bigcirc
_	T2 smooth medially, with scrobiculate lines laterally (Figs 68–73) (Ecuador, Peru)
8(6)	Basal ² / ₃ of T1 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 12 straight; 3 or 4 terga visible beyond apex of 12 (Figs 25–30) (Belize, Bolivia, Brazil, Costa Rica, Colombia, Ecuador, French
_	Guiana, Panama, Peru, I rinidad and Tobago, Venezuela) <i>P. cudo</i> sp. n. \Im Posterior margin of T2 distinctly concave (Fig. 6); 2 or 3 terga visible beyond apex of T2 (Fig. 80) (Brazil, Colombia, Ecuador, Paraguay, Peru)
10(8)	T2, including laterotergite, with widespread sparse pilosity (Figs 1–6) (Brazil).
_	T2 at most with 1 row of setae posteriorly and sparse setation laterally, else- where glabrous (Fig. 66)

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) <i>P. odo</i> sp. n. 👌
_	T1 evenly costate across anterior 1/3 to 1/2, smooth posteriorly (Fig. 54)
	(Bolivia, Costa Rica, Ecuador, Paraguay, Venezuela) <i>P. bjalmr</i> 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. Q
_	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)
_	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) <i>P. constellata</i> sp. n. Q

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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.

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RESEARCH ARTICLE



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

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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*, 16S, calmodulin intron, and 28S 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
Species name	Type locality	Assignment in present study
Ancylus altus Tryon, 1865	Klamath River	Lanx alta (Tryon, 1865)
Ancylus crassus Haldeman, 1844	Columbia drainage	<i>Fisherola nuttallii</i> (Haldeman, 1841)
Lanx hannai Walker, 1925	upper Sacramento River	Lanx patelloides (Lea, 1856)
Lanx (Walkerola) klamathensis Hannibal, 1912	Klamath River	Lanx alta (Tryon, 1865)
<i>Ancylus kootaniensis</i> Baird, 1863 [<i>kootenaiensis</i> is an invalid emendation]	Kootenai River (restricted by Morrison 1955)	probably <i>Fisherola nuttallii</i> (Haldeman, 1841) but not directly sampled
<i>Fisherola lancides</i> Hannibal, 1912	Snake River	<i>Fisherola nuttallii</i> (Haldeman, 1841)
Ancylus newberryi Lea, 1858	upper Sacramento (correction by Pilsbry 1925)	Lanx patelloides (Lea, 1856)
Ancylus (Velletea) nuttallii Haldeman, 1841	Columbia drainage	<i>Fisherola nuttallii</i> (Haldeman,1841)
Ancylus patelloides Lea, 1856	upper Sacramento River	Lanx patelloides (Lea, 1856)
Ancylus praeclarus Stimpson ms. cited in Lea, 1867	unstated	not validly proposed; Lea stated that <i>newberryi</i> differs from it in several ways but never directly said anything about <i>praeclarus</i>
Ancylus subrotundatus Tryon, 1865	Umpqua River	Lanx alta (Tryon, 1865)

Table 1. Nominal Recent species names in Lancinae.

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°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. Cox1 (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 28S (primers 2/3F 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 18S is in the first part of the gene, we used the 1F-4R primers (Giribet et al. 1996) to amplify that portion of the gene. PCR cycles used were 95°C, 3 min; 5 cycles at 92°C for 30 sec, 40°C for 30 sec,

				4 4 4
Designation	Morphospecies	Locality	Drainage	Accession number
Idaholanx fresti	Banbury lanx	Banbury Springs, Idaho	Snake	calmodulin HM230326, 285 HM230308, <i>cax1</i> HM230356, 16S KT267273
Idaholanx fresti	Banbury lanx	Box Canyon Springs, Idaho	Snake	calmodulin HM230327, 285 HM230309, <i>cox1</i> HM230357, 16S KT267273
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	calmodulin HM230330, 28S HM230315, <i>cox1</i> HM230359, 16S HM230355
Fisherola nuttallii	F. nuttallii	Deschutes River, RM 6.3, Oregon	Columbia	calmodulin HM230329, 28S HM230314, 16S KT267274
Fisherola nuttallii	F. nuttallii	Owyhee River, Whistling Bird Rapids, Oregon	Snake	calmodulin HM230331, 18S HM230306, 28S HM230316, <i>cox1</i> HM230360
Lanx alta	L. alta	Klamath River at Collier Rest Area, California	Klamath	calmodulin HM230336, 18S HM230307
Lanx alta	L. klamathensis	Barclay Spring, Hagelstein Park, Upper Klamath Lake, Oregon	Klamath	calmodulin HM230335, 28S HM230319
Lanx alta	L. klamathensis	Link River at Hwy bridge, Klamath Falls, Oregon	Klamath	calmodulin HM230337
Lanx alta	L. species	Smith River National Recreation Area, California	Smith	calmodulin HM230341, 28S HM230321
Lanx alta	L. species	Smith River National Recreation Area, California	Smith	calmodulin HM230342
Lanx alta	L. species cf. L. alta	Rogue River at Gold Nugget Recreation area (BLM), Oregon	Rogue	calmodulin HM230338, HM230340 (identical sequence from two specimens)
Lanx alta	L. species cf. L. alta	Rogue River at Gold Nugget Recreation area (BLM), Oregon	Rogue	calmodulin HM230339, 28S HM230320, <i>cox1</i> HM230362
Lanx alta	L. subrotundata	Amacher City Park, Roseburg, Umpqua River, Oregon	Umpqua	calmodulin HM230334, 28S HM230318, <i>cox1</i> HM230361
Lanx patelloides	L. hannai	McCloud River S. of Ah-Di-Na Camp Ground, California	Sacramento	calmodulin HM230346, 28S HM230322, <i>cox1</i> HM230363

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 28S and coxI.

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

65°C for 2 min; and 40 cycles with at 92°C for 30 sec, x°C for 30 sec, 65°C for 2 min, where x is about 2°C below the lower primer annealing temperature; finishing with 10 min at 72°C before cooling to 4°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°C, followed by 40 cycles with 15 sec at 96°C, 15 sec at about 2°C below the lower primer annealing temperature, and 4 min at 65°C, followed by 10 min at 72°C before cooling to 4°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 28S 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 (P_{IID} 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 16S 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.

Taxon	Gene	Accessions	Sources
Acroloxus lacustris (Linnaeus, 1758)	16S	AY577462	Jorgensen et al. 2004
Acroloxus lacustris (Linnaeus, 1758)	28S	DO328296	Walther et al. 2006b
Acroloxus lacustris (Linnaeus, 1758)	cox1	DQ328271	Walther et al. 2006b
Ancylus fluviatilis Müller, 1774	165	AY577466	Jorgensen et al. 2004
Ancylus fluviatilis Müller, 1774	28S	DO328295	Walther et al. 2006b
Ancylus fluviatilis Müller, 1774	cox1	DQ328270	Walther et al. 2006b
Austropeplea tomentosa (L. Pfeiffer, 1855)	16S	EU556238	Puslednik et al. 2009
Austropeplea tomentosa (L. Pfeiffer, 1855)	285	HQ156217	Holznagel et al. 2010
Austropeplea tomentosa (L. Pfeiffer, 1855)	cox1	AY227365	Remigio and Hebert 2003
Carinifex newberryi (Lea, 1858)	285	*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	cox1	*HM230358	Hagelstein Park, mid channel E. side center, Klamath River, OR
Dilatata dilatata (Gould, 1841)	28S	*HM230313	Sipsey River near Benevola, Greene Co. AL
Dilatata dilatata (Gould, 1841)	cox1	EF012173	Albrecht et al. 2007
Galba modicella (Say, 1825)	cox1	KM612000	Dewaard et al. 2015
Galba obrussa (Say, 1825)	16S	AF485658	Remigio 2002
Galba obrussa (Say, 1825)	285	*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)	cox1	KT831385	Gordy et al. 2016
Orientogalba ollula (Gould, 1859)	165	U82067	Remigio and Blair 1997
Orientogalba ollula (Gould, 1859)	28S	AY465065	Jung et al., unpublished
Orientogalba ollula (Gould, 1859)	cox1	KC135900	Park et al. 2012
Physa acuta (Draparnaud, 1805)	16S	JQ390525	Nolan et al. 2014
Physa acuta (Draparnaud, 1805)	28S	DQ256738	Holznagel et al. 2010
Physa acuta (Draparnaud, 1805)	cox1	JQ390525	Nolan et al. 2014
Planorbella trivolvis (Say, 1817)	16S	AY030234	DeJong et al. 2001
Planorbella trivolvis (Say, 1817)	285	AF435688	Morgan et al. 2002
Planorbella trivolvis (Say, 1817)	cox1	KM612028	Dewaard et al. 2015
Polyrhytis emarginata (Say, 1821)	28S	DQ328299	Walther et al. 2006b
Polyrhytis elodes (Say, 1821)	16S	AF485652	Remigio 2002
Polyrhytis exilis (Lea, 1834)	cox1	*HM230364	Ditch along the Stump Lake access road, Jersey Co., IL
Radix auricularia (Linnaeus, 1758)	165	JN794284	von Oheimb et al. 2011
Radix auricularia (Linnaeus, 1758)	285	AY465067	Jung et al., unpublished
Radix auricularia (Linnaeus, 1758)	cox1	KP242340	Patel et al. 2015
Radix balthica (Linnaeus, 1758)	16S	HQ330989	Feldmeyer et al. 2010
Radix balthica (Linnaeus, 1758)	28S	EF417136	Sonnenberg et al. 2007
Radix halthica (Linnaeus, 1758)	cor 1	KP098541	Feldmever et al. 2015

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

Results

Amplification of 28S and calmodulin intron were most successful, but representatives of each species (as recognized herein) also amplified for *cox1*. Within Lancinae, interspecies and intergenus percent variation was lowest for 28S and highest for *cox1*. However, the calmodulin intron sequence for lancines was more divergent from *Galba obrussa* than the maximum variation between lymnaeids for *cox1* (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 *cox1* within the sampled species, though other Hygrophila do have insertions (pers. obs.). 28S, 16S, and calmodulin intron all had several small indels. MrModeltest (Nylander 2004) favored a HKY model for calmodulin intron and GTR+I+G for 28S, 16S, and *cox1*. 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 18S 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

Gene	Lymnaeidae	lancine genera	Lanx species	lancine intraspecies
285	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%
cox1	up to 21.1%	12.9-21.1%	7.9-8.6%	0.15-1.0%
165	up to 21.3%	12.8–16.6	no data	0.00-2.5%

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

Table 5. Tree statistics.

Come	Pars	imony		Bayesian	
Gene	# trees	length	burnin	maximum ln likelihood	mean In likelihood
28S, CAM intron, and <i>cox1</i>	18	1670	165000	-9578.885	-9602.83
16S	2	719	65000	-3414.11	-3427.56



0.01 substitutions/site

Figure 1. 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.

of the E10 region of 18S genes for lymnaeids. * indicates newly generated data. The alignment is meant to facilitate comparison	fferences between the more divergent sequences are too great for confident homologizing.
Table 6. Hypervariable portion of the E10 region of 1	between the different species. Differences between the n

Species	Accession number	Sequence
Aenigmomphiscola europaea, A. kazakhstanica, Lymnaea stagnalis, Omphiscola glabra, Stagnicola palustris	AY577484, FR797819-FR797829, JN614363, JN614364, HQ659966, JN614368, JN614367	CCGCGIGC-GGGGCGACTCGT-GCGCGGCG
Fisherola nuttallii	HM230306*	CCGT-CGC-GCGGGGCGTCAAACCCTCGCCG-GCGGCG
Galba cousini	FN598151, JN614345, JN614344	CCGTCGCGGCGCGCAAGCCGAGGCGGCG
Galba cubensis	Z83831, JN614326-JN614331, JN614334	CCGTGTCGTGCCGCGGTGCAAGCCGTGGTCGCGCGCG
Galba humilis	FN182190	CCGTCGCGGCGCGCGGGCGAGGCGGCG
Galba schirazensis	FR772291, JN614335-JN614343	CCGGCCATTCATTCACTTGCGTGGTCGGCG
Galba truncatula	Y09019, Z73985, EU152270, EU728668, HQ659965, JN614346-JN614354, FR797815, FR797816	CCGTCCT-TTCGCGAGGGCGGTG
Galba viator	AF239912	CCGTGTGCCTCCGTGGTGCAAGCCGTGGTCGCGCGCG
Galba viator	AM412222, AY057088, AY057089, JN614332, JN614333	CCGTGTGTCCTCCGCGGTGCAAGCCGTGGTCGCGCGCG
Lanx alta	HM230307*	CCGT-CGC-GCGGGGCGTCAAACCCTCGCCG-GCGGCG
Lymnaea stagnalis	EF489345	CCGCGGCG
Lymnaea stagnalis, Omphiscola glabra, Stagnicola palustris	Y09018, Z73984, AY427525,Y09015, Z73982, JN614365, JN614366, Y09016, Z73983	CCGCGTGCCGGGGCGACTCGT-GCGCGCG
Pectinidens diaphana	EU241865, JF909497, JN614361, JN614362	CCGCCGC-GGCTCGCGCCGT-G-GCGGCG
Pseudosuccinea columella	FN598152, JN614358-JN614360	CCGTCGGTCCCGCGAGGGGCCG-GCGGTG
Pseudosuccinea columella	EU241866	CCGTTCGGTCCCGCGAGGGGCCG-GCGGTG
Radix auricularia, Radix peregra	Z73980, Y09017, Z73981, FR797817, FR797818, JN614356, JN614357	CCGCGTGCTCTTCGCGGGGT-GCGCGGTG
Radix natalensis	AF192272, EU152269	CCGCGTGCTCCTCACGGGGT-GCGCGGTG
Radix natalensis	AF192273	CCGCGTGCTCCTCACGGGGT-GCGTGGTG

Species	Accession number	Sequence
Radix natalensis	AF192274	CCGCGTGCTCCTCCCGGGGT-GCGCGGTG
Radix natalensis	JN614355	CCGCGIGCTCCTCGCGGGGT-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 (AF192272-4); Vinarski et al. 2011 (FR797815-29) Vonnemann et al. 2005 (AY427525); Walker et al. 2008 (EU152269, EU152270). Only the E10 region is 6); 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 al. 2011a (FR772291); Bargues et al. 2011b (FN598151-2); Bargues et al. 2012 (JF909497); Correa et al. 2011 (JN614326-68); Dayrat et al. 2011 (HQ659965sequence rather than originally absent); Marquez, unpublished (Y09015-9); Owyhee River, Whistling Bird Rapids, Oregon (HM230306*); Stothard et al. 2000 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 16S 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 wellresolved. 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 18S 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 16S 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 28S data. Additionally, variation in the cox1 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 limpet-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 *cox1* 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 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 **O** oviduct **OT** ovotestis **P** penis **PB** pyriform body **PG** prostate gland **PS** penial sheath **S** spermatheca **SD** spermathecal duct **SV** seminal vesicle **U** uterus **V** vagina **VD** 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*. **E** shell **F** whole animal. The red arrows indicate the position of the head in **A**, **C**; the position of the gap in the columella muscle in **B**, **D** and the narrow connection in **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 *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.

http://zoobank.org/9B243DB3-ABD2-40CC-B9A0-BC4DD1778971

Type locality. 21–24th runs of the lower outflow of Banbury Springs, Gooding County, Idaho, U.S.A. 42°41'20.5"N, 114°49'18"W, 879m, 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), 13–15th runs of the lower outflow of Banbury Springs, about middle of spring complex along trail with wooden bridges, 42°41'21"N, 114°49'18"W, 21 Sept 1989; FMNH 342901, lower outflow of Banbury Springs, 42°41'21.8"N, 114°49'19.4"W, 11 Jan 2006; FMNH 342904, SAC S.23967 (shell), lower outflow of Banbury Springs, 42°41'21"N, 114°49'18"W, 6 Aug 2006; FMNH 342897 (shells), SAC S.25699 (shell), lower outflow of Banbury Springs, 42°41'21.8"N, 114°49'18.5"W, 25 May 2016.

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

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RESEARCH ARTICLE



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

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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);

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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 65mm 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, ChinaSNUC 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 pronotol 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: ♂, CHINA, Sichuan: Chengdu City, Dujiangyan, Zipingpu Town, Lingyanguanyinshan scenic area (灵岩观音山风景区), 29.IV.2016, 31.02956N, 103.61651E, alt. 1210 m, ant colony under deadwood bark, leg. Chao



Figure 1. Habitus of *Paussus (Scaphipaussus) zhouchaoi* sp. n. (holotype, d; dorsal view).

Zhou & Li He (BITS). **Paratypes:** 1[♀], same data as holotype (BITS); 1♂, CHINA, Jiangxi: Yichun City, Mingyueshan (明月山), 27°35′25″N, 114°17′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, double-walled 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 project-ing 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× 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× length of palpomeres I and



Figure 2. Habitus of *Paussus (Scaphipaussus) zhouchaoi* sp. n. (**A–C** holotype, \mathcal{T} **D–E** paratype, \mathcal{D}) and its host ant (**F** *Pheidole* sp., minor worker) **A**, **D**, **F** dorsal view **B**, **E** dorsolateral view **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) zhouchaoi* sp. n. (**A, C, E** holotype, \bigcirc **B, D** paratype, \bigcirc). **A–D** heads and pronota **E** pygidium **A–B** oblique anterodorsal view **C–E** dorsal view.



Figure 4. Antenna of *Paussus (Scaphipaussus) zhouchaoi* sp. n. (holotype, \Im). **A** dorsal view, in ethanol solution **B** dorsal view **C** dorsoposterior view **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, \mathcal{J}). **A** dorsal view **B** left lateral view **C** ventral view **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.

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RESEARCH ARTICLE



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

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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 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 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]... 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; 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) Second-fourth segments of hind tarsus and its telotarsus dark brown; lateral lobes of mesoscutum glabrous except for some long setae laterally; first tergite 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 C. gedanensis (Ratzeburg, 1852) Temple behind base of mandible evenly curved and much narrower than base Temple in dorsal view 1.4 times as long as eye; ovipositor sheath slightly pro-6 jecting beyond apex of metasoma, its blackish part 0.7 times as long as hind Temple in dorsal view nearly as long as eye; part of ovipositor sheath projecting 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

Taxonomy

Chorebus (Stiphrocera) hexomyzae sp. n.

http://zoobank.org/0025B40D-6DD5-4DB6-8897-51F5D4AC378E Figures 1–21

Type material. Holotype, \bigcirc , (NWUX) "NW **China: Ningxia** Hui Autonomous Region, Shizuishan, Dawukou (N 39°06', E 106°20', 1140 m), 26.v.2015" and reared from *Hexomyza caraganae* Gu in twig galls on *Caragana korshinskii* Kom. f. Paratypes (88♀♀ 79♂♂): (GSFPM, NWUX, RMNH): 1♀, same data as holotype; 6♀♀ 3♂♂, id., but 17.v.2015; 5♀♀ 5♂♂, id., 23.v.2015; 3♀♀ 8♂♂, id., 24.v.2015; 4♀♀ 2♂♂, id., 25.v.2015; 5♀♀ 10♂♂, id., 27.v.2015; 1♀, id., 25.vi.2016; 3♀♀ 1♂, NW **China: Inner Mongolia** Autonomous Region, Hangjinqi (N 39°45', E 108°44', 1460 m), 22.v.2016; 4♀♀ 6♂♂, id., 23.v.2016; 1♂, id., 24.v.2016; 1♂, id., 26.v.2016; 2♀♀ 1♂, id., 27.v.2016; 1♀ 2♂♂, id., 28.v.2016; 2♀♀ 1♂, id., 30.v.2016; 2♀♀ 2♂♂, id., 31.v.2016; 1♀ 3♂♂, id., 2.vi.2016; 1♀, id., 3.vi.2016; 4♀♀ 4♂♂, id., 6.vi.2016; 1♀, id., 7.vi.2016; 1♀, id., 8.vi.2016; 2♂♂, id., 12.vi.2016; 15♀♀ 10♂♂, Inner Mongolia Autonomous Region, Dalate (N 40°17', E 109°54', 1020 m), 23–30.v.2016; 26♀♀ 17♂♂, 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 setose 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;

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Figure 1. Chorebus (Stiphrocera) hexomyzae sp. n., female, paratype, habitus lateral.

mandible with four teeth, middle tooth (= t2) wide triangular, acute, much longer than both lateral teeth, with an extra protuberance on ventral side of middle tooth and ventral (= t3) 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 2–11. Chorebus (Stiphrocera) hexomyzae sp. n., female, holotype, but 2 of paratype. 2 Wings
3 Mesosoma lateral 4 Mesosoma dorsal 5 Propodeum and first metasomal tergite dorsal 6 Hind leg lateral
7 Head anterior 8 Head dorsal 9 Head antero-dorsal 10 Mandible, full view on middle tooth 11 Metasoma dorsal.

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); eve 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 (= t2) wide triangular, acute, much longer than both lateral teeth, with an extra protuberance on ventral side of middle tooth (similar to t3) and ventral (= t3) 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 post-furcal; 1-CU1:2-CU1 = 3:11; 3-CU1 much longer than short CU1b; m-cu antefurcal,



Figures 12–19. *Chorebus (Stiphrocera) hexomyzae* sp. n., female, holotype. **12–19.** 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-M posteriorly; first subdiscal cell 2.1 times as long as wide; M+CU1 largely unsclerotised. Hind wing: M+CU:1-M:1r-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, 21.20 Twig gall of *Hexomyza caraganae* Gu, with emergence hole of *Chorebus* (*Stiphrocera*) *hexomyzae* sp. n. **21** 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 \bigcirc 3.0–3.9 mm, and of fore wing 2.7–3.5 mm; length of body of \bigcirc 3.3–3.9 mm, and of fore wing 2.9–3.7 mm; antenna of \bigcirc with 25(1), 26(1), 27(5), 28(12), 29(19), 30(10), 31(7) segments; antenna of \bigcirc 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.

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