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DATA PAPER

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The Bryozoa collection of the Italian National Antarctic Museum, with an updated checklist from Terra Nova Bay, Ross Sea

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Abstract

This study provides taxonomic and distributional data of bryozoan species from the Ross Sea area, mainly around Terra Nova Bay, based on specimens curated at the Italian National Antarctic Museum (MNA, Section of Genoa). Bryozoan specimens were collected at 75 different sampling stations in the Ross Sea and in the Magellan Strait, in a bathymetric range of 18–711 meters, during 13 expeditions of the Italian National Antarctic Research Program (PNRA) conducted between 1988 and 2014. A total of 282 MNA vouchers corresponding to 311 specimens and 127 morphospecies have been identified and included in the present dataset. 62% of the species were already reported for the Terra Nova Bay area, where most of the Italian samples come from, with a 35% of samples representing new records classified at the specific level, and 3% classified at the genus level. These new additions increase to 124 the total number of species known to occur in Terra Nova Bay. Four 3D-models of Antarctic bryozoans from the Ross Sea are also presented and will be released for research and educational purposes on the Museum website.

Keywords

Antarctica, Bryozoa, checklist, MNA, new records, outreach, Ross Sea, Terra Nova Bay, 3D models

Introduction

In the last 30 years, several Italian expeditions have been conducted in the Ross Sea leading to the publication of contributions on different taxonomic groups. Among the different phyla, Bryozoa was extensively studied until 2000 with papers reporting data on bryozoan species obtained during the first Italian Antarctic campaigns in Terra Nova Bay (i.e., 1988–1995) (Di Geronimo and Rosso 1990; Rosso 1990, 1991, 1992a, 1994; Rosso and Sanfilippo 2000).

From 2000 onwards, new Antarctic campaigns were annually conducted and the number of new samples acquired by the Italian National Antarctic Museum (MNA, section of Genoa) progressively increased. However, no new taxonomic characterization was carried out on this material until now. Few samples from the Magellan Strait, collected during the VI Italian National Antarctic Program (PNRA) expedition in 1991, are also included here. The present study provides distributional data and taxonomic identification, at the lowest possible level, of bryozoans collected during 13 scientific expeditions of the PNRA in the Ross Sea and the Magellan Strait. These data are combined with previous literature checklists for the area with the aim of providing an updated checklist for Terra Nova Bay and distributional data for all the available vouchers. All bryozoan specimens reported in this paper are stored at the MNA and at the museum of the IPOP in Catania (Figure 1). This dataset is the sixth MNA contribution to the Antarctic Biodiversity Portal, the thematic Antarctic node for both the Ocean Biogeographic Information System (AntOBIS) and the Global Biodiversity Information Facility (ANTABIF) (http://www.biodiversity.aq). Previous contributions on Mollusca, Tanaidacea, Fungi, Ophiuroidea and Porifera were respectively published in Ghiglione et al. (2013), Piazza et al. (2014), Selbmann et al. (2015), Cecchetto et al. (2017) and Ghiglione et al. (2018).

Project description

Project title: Antarctic Bryozoa in the collection of the Italian National Antarctic Museum (MNA)

Curator and Promoter: Stefano Schiaparelli

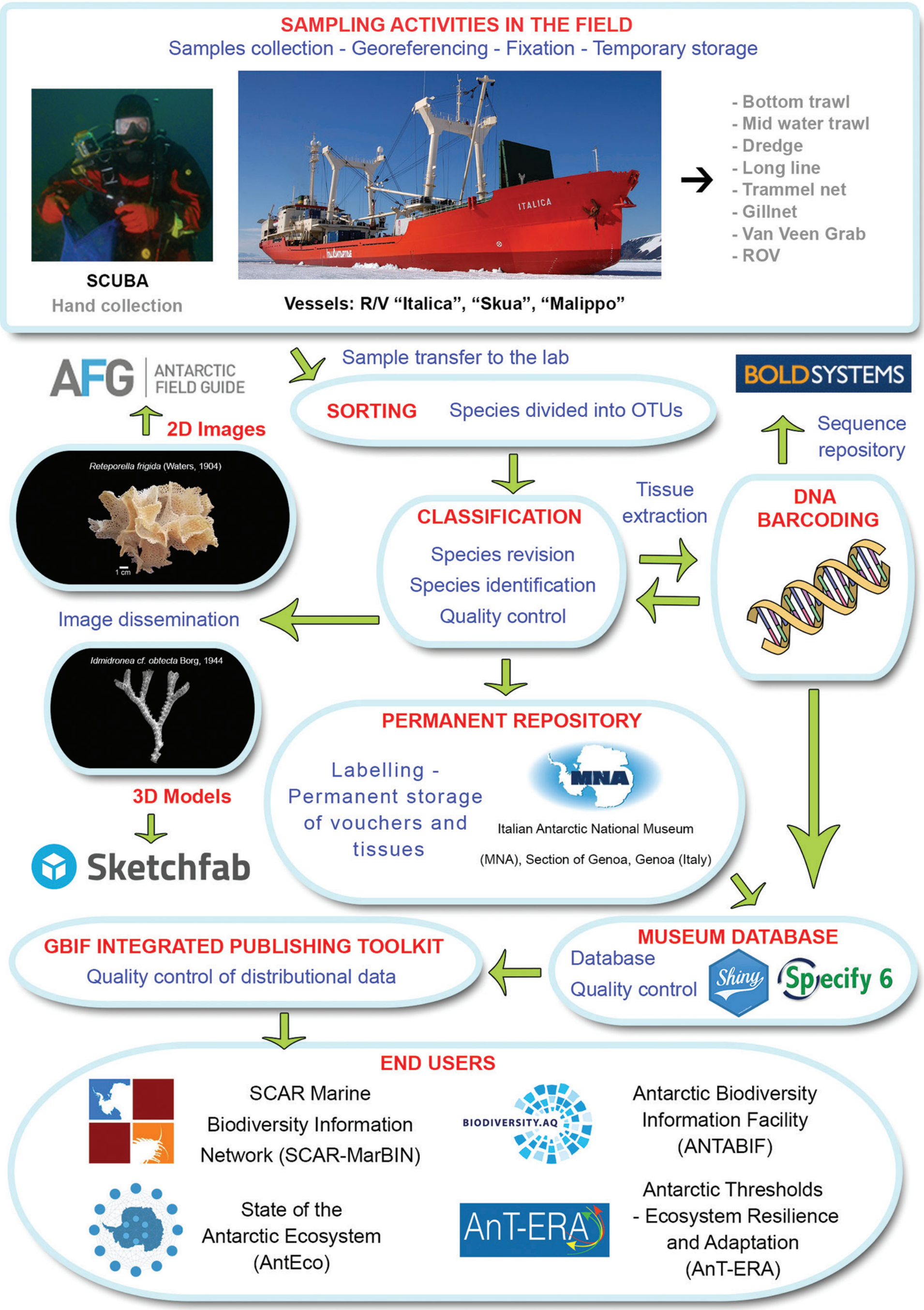

Figure 1. Flowchart depicting major stages in dataset development and publishing.

Personnel: Matteo Cecchetto, Chiara Lombardi, Simonepietro Canese, Silvia Cocito, Piotr Kuklinski, Claudio Mazzoli, Stefano Schiaparelli

Funding: The Bryozoa specimens were collected during 13 different Antarctic expeditions conducted from 1988 to 2014 and 18 research projects funded by the PNRA listed below (with the project name or category under the PNRA program in italic, followed by the project code, the expedition number and the corresponding years):

– Necton e risorse da pesca 2.1.4.6, III expedition (1987/1988)
– Oceanografia & Benthos 2.1.4.3, III expedition (1987/1988)
– Benthos 3.2.1.2.5, V expedition (1989/1990)
– Oceanografia geologica 3.2.1.4, V expedition (1989/1990)
– Benthos, Magellan strait 3.7.8, VI expedition (1990/1991)
– Ecologia e biogeochimica dell'Oceano Meridionale - ROSSMIZE 2d.2, XI expedition (1995/1996)
– Ecologia e biogeochimica dell'Oceano Meridionale 2b.3, XIII expedition (1997/1998)
– Struttura e dinamica delle cenosi marine di Baia Terra Nova 2b.3.1, XIV expedition (1998/1999)
– Processi genetici e significato paleoclimatico e paleoceanografico dei CARBONati marini biogenici in ANTartide - CARBONANT 4.7, XVII expedition (2001/2002)
– Molecole e geni di organismi marini antarctici in funzione evolutiva, adattativa e applicativa 1.1, XVIII (2002/2003)
– The costal ecosystem of Victoria Land coast: distribution and structure along the latitudinal gradient 2002/8.6, XIX expedition (2003/2004)
– L'ecosistema costiero di Baia Terra Nova - Latitudinal Gradient Project 2006/08.01, XXV expedition (2009/2010)
– Barcoding of Antarctic Marine Biodiversity - BAMBi 2010/A1.10, XXVII expedition (2011/2012)
– Vulnerabilità dei pesci polari al cambiamento climatico: ciclo vitale, habitats e relazione con il ghiaccio marino in Pleuragramma antarcticum 2010/A1.11, XXVIII expedition (2012/2013)
– Barcoding of Antarctic Marine Biodiversity - BAMBi 2010/A1.10, XXVIII expedition (2012/2013)
– Diversità genetica spazio temporale di endoparassiti delle regioni polari: uno studio per la valutazione dell'impatto dei cambiamenti globali sulle reti trofiche marine 2009/A1.09, XXVIII expedition (2012/2013)
– Integrità dell'ecosistema marino antarctico come presupposto per lo studio dell'interazione parassita-ospite: un approccio genetico, molecolare ed immunologico 2013/AZ1.09, XXIX expedition (2013/2014)
– Barcoding of Antarctic Marine Biodiversity - BAMBi 2010/A1.10, XXIX expedition (2013/2014)

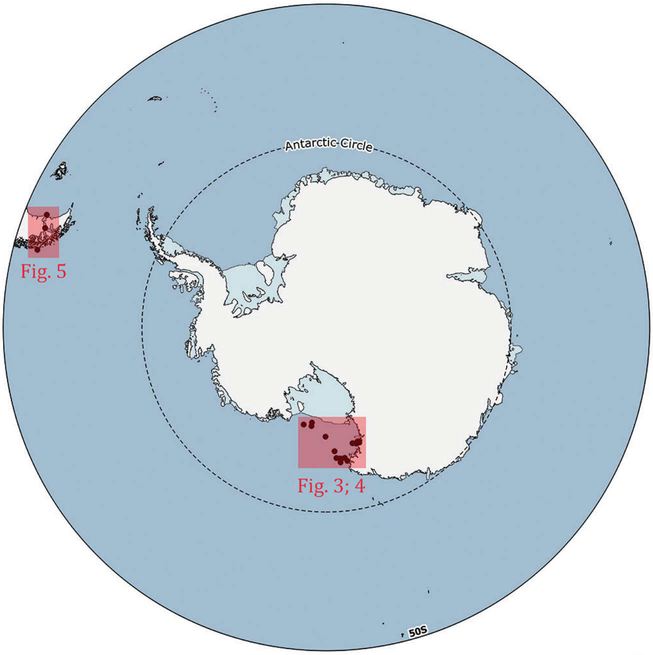

Figure 2. General map of Antarctica with the study areas highlighted (red boxes). Detailed maps of the sampling areas are provided in Figs 3–5.

Study area: Bryozoa specimens were collected in the Ross Sea sector of the Southern Ocean (Figs 2–4) and in the Magellan Strait (Figs 2, 5).

Design: Data were assembled by revising all the distributional records of the specimens stored in the MNA collections (section of Genoa, Italy) and in the museum of the IPOP in Catania.

Materials and methods

Sampling

The distributional data of Bryozoa, here illustrated, came from different research expeditions, conducted between 1988 and 2014, and include 75 sampling stations, located between 18 and 711 metres of depth (Figs 2–5). Sampling was performed by using a variety of methods and gears such as dredges (Charcot dredge, Naturalist dredge, Triangular dredge, and Picard dredge), Van Veen grabs of different volumes and, for opportunistic sampling, fishing long lines, mid-water trawls (that accidentally touched the bottom due to a failure of the winches providing additional material), trammel nets and other fishing nets. In addition, during the XXV PNRA expedition, some samples

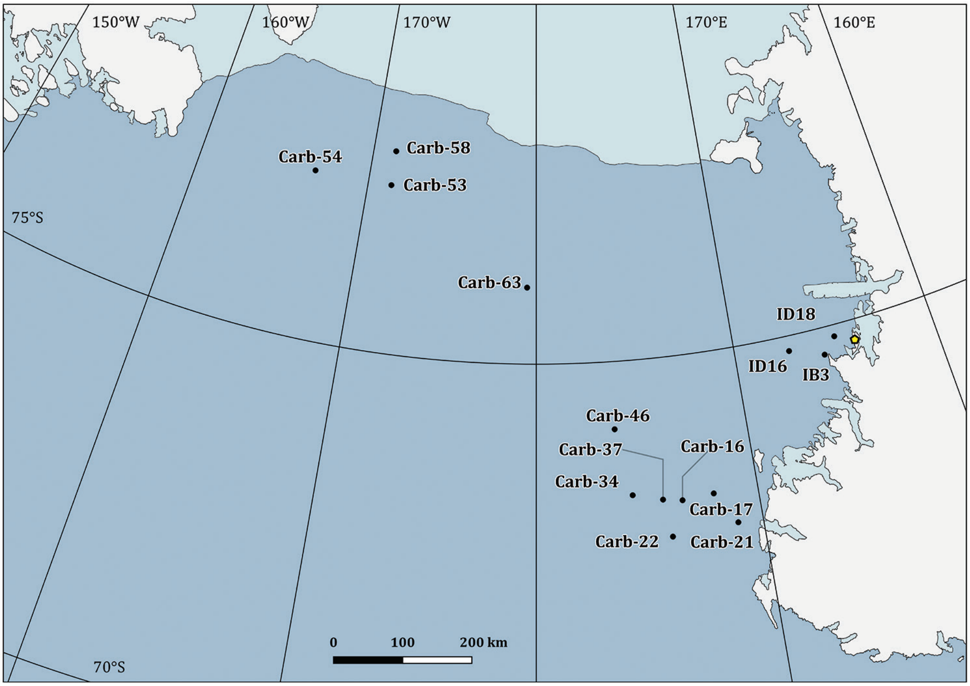

Figure 3. Sampling stations map in the Ross Sea area, Antarctica. The yellow pentagon indicates the location of the research station "Mario Zucchelli" in Terra Nova Bay. A detailed map of the sampling area for Terra Nova Bay is provided in Figure 4.

were hand-collected by SCUBA diving by one of the authors (SS) (Figs 6, 7). Images of bryozoan colonies were also obtained through ROV video transects performed during the XXIX expedition, and subsequently identified at least to the genus level (Figure 7). Station coordinates and sampling events were recorded during sampling activities based on various GPS systems. The data flowchart (Figure 1) illustrates the sampling, sorting and storing procedures for specimens as well as data and image availability.

Quality control

Once at the MNA, all specimens were classified at the lowest possible taxonomic level; only those that were classified at least to genus level were included in the present dataset. Several researchers contributed in classifying the specimens, and the last taxonomic revision of the MNA bryozoan collection was conducted in the past two years by Chiara Lombardi, Silvia Cocito, and Piotr Kuklinski. During all phases of sorting, classification and storage of samples at the MNA, quality control and data cleaning have been undertaken at various stages in order to produce high quality data, and make consistent cross-references between the database and samples' labels. The MNA (www.mna.it)

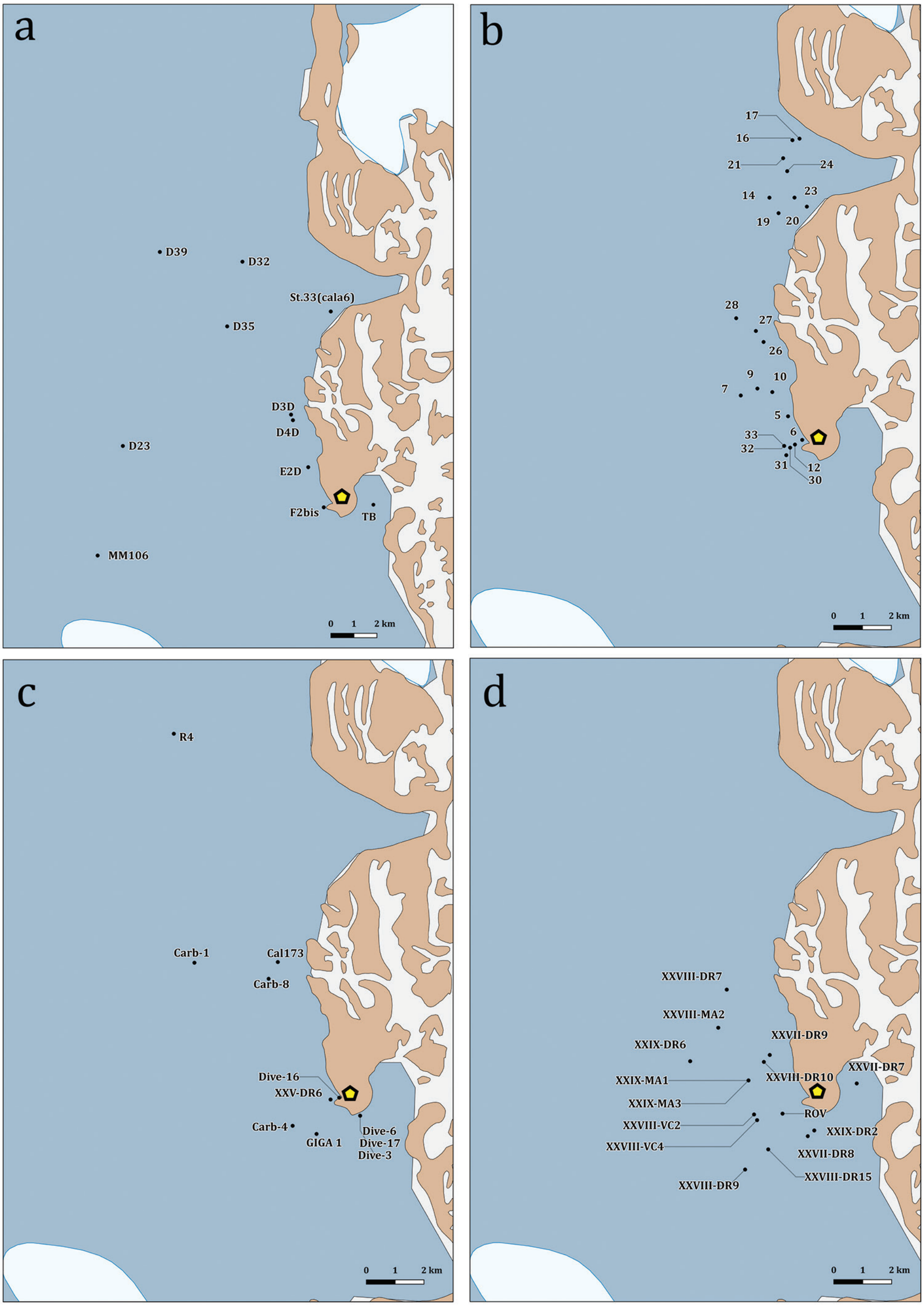

Figure 4. Detailed map of the sampling stations of the PNRA expeditions III, V and XI (**a**); XVIII (**b**); XIII, XIX, XVII and XXV (**c**); XXVII, XXVIII and XXIX (**d**). The yellow pentagon indicates the location of the research station “Mario Zucchelli”.

Figure 5. Detailed map of the sampling stations of the sixth PNRA expedition (Magellan Strait).

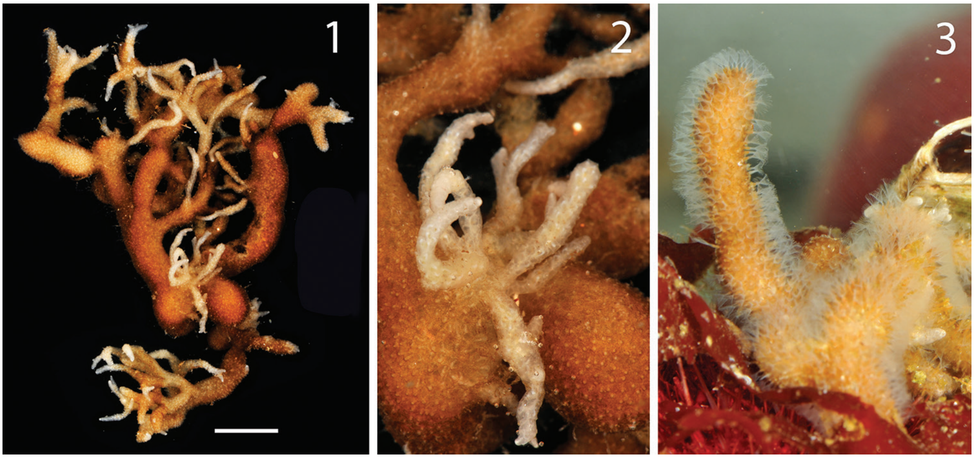

Figure 6. *Alcyonidium* sp. colony (MNA 2733) collected by SCUBA diving. Entire colony (**1**) and details (**2**) of the specimens after fixation in ethanol. Detail of the colony depicted in aquarium immediately after the collection (**3**).

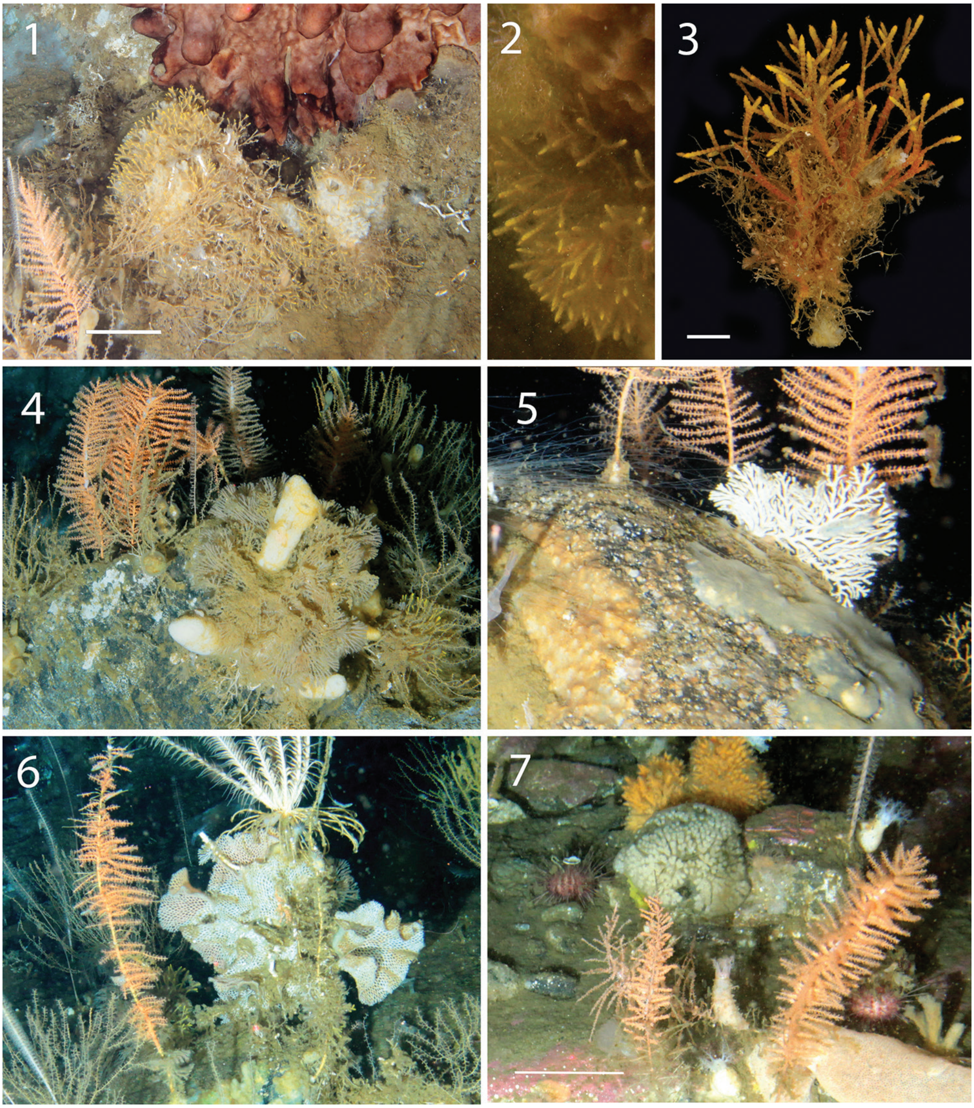

Figure 7. Images of bryozoan colonies in the field obtained through ROV video transect and SCUBA diving. Bushy-colony of *Klugella buski* Hastings, 1943 (NR) living in association with other bryozoans, hydroids and sponge (**1**), a detail of the yellow tips of the colony in the field (**2**), and the whole fresh colony after the collection (**3**); the last two corresponding to the same voucher (MNA 2872) collected by SCUBA diving; colony of the cyclostome *Hornera* enclosing a sponge (**4**); fan-shaped colony of the cyclostome *Hornera* sp. growing on a boulder (**5**); colony of the cheilostome *Reteporella* sp. living in association with other bryozoans, hydroids, polychaetes (**6**); rounded greyish colony of the cyclostome *Fasciculipora ramosa* D'Orbigny 1839 growing on the sea bottom (**7**).

uses the R-Shiny web application (https://steu.shinyapps.io/MNA-generale/) to manage and show its collections, and a Microsoft SQL database (Specify 6) to link all the data (photos, glass slides, etc.) to the physical samples in the collection. Of a total of 282 MNA vouchers, 29 samples were collected and studied before the MNA was established in 1999, and they were stored at the museum of the Istituto Policattedra di Oceanologia e Paleoecologia (IPOP) in Catania. All other samples are permanently curated at the MNA and available for study to the scientific community.

Results

Taxonomic coverage

The present dataset reports distributional data on bryozoan specimens belonging to the classes Stenolaemata and Gymnolaemata collected in the Ross Sea and in Magellan Strait. It includes a total of 311 specimens corresponding to 282 MNA vouchers and belonging to 127 morphospecies. Out of these, 100 were classified to species level and 27 to genus level, representing three orders and 34 families (Figs 8, 9).

Considering only the records collected in the Terra Nova Bay area, 41 species (62.12%) were already known for the area, whereas 25 are new records, out of which 23 (34.85%) are classified at species level and two (3.03%) at the genus level. These new records are annotated by 'NR' immediately after the species name in the Checklist. By merging previously published records (i.e., Di Geronimo and Rosso 1990, Rosso 1990, Rosso 1991, Rosso 1992a, Rosso 1994, Rosso and Sanfilippo 2000) with the new ones, the number of bryozoan morphospecies occurring in the Terra Nova Bay area increases to 124 (Fig. 10 and Suppl. material 1: Table 1). From this regional checklist we have excluded identifications of specimens lacking key morphological characters, so as to avoid future misidentifications (e.g., *Larvapora* cf. *mawsoni*, *Fenestrulina* sp.). Therefore, the total number of species reported in the area may increase in the future.

The MNA collection includes one bryozoan holotype: *Melicerita digeronimoi* (voucher: MNA 1054). This species, deposited at the museum of the IPOP in Catania (catalogue number IPOP.B1.8.8.1991) in 1991, was published by Rosso in 1992 (Rosso 1992b), i.e., eight years before the establishment of the MNA. To keep track of this voucher, an MNA catalogue number (MNA 1054) has also been assigned, although the sample is curated by the IPOP.

The updated list includes two classes, Stenolaemata, with the order Cyclostomatida (15 morphospecies), and Gymnolaemata, with the orders Ctenostomatida (one morphospecies) and Cheilostomatida (108 morphospecies). The majority of the species are characterized by well-calcified skeletons (88 species), 35 species with slightly calcified skeletons, and only one soft-bodied species (*Alcyonidium* sp., Ctenostomatida). Concerning colony growth habits (i.e., encrusting, erect flexible, erect rigid) (Hageman et al. 1998), erect rigid forms are the commonest (46 species), followed by encrusting (44 species) and erect flexible (32 species) ones.

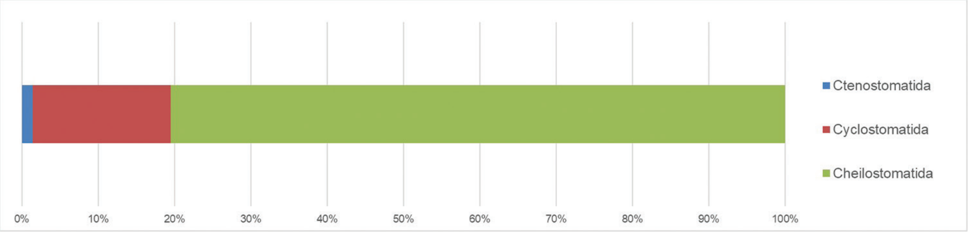

Figure 8. Taxonomic coverage (reported in percentage of specimens per order) of the MNA Bryozoa collection. Cheilostomatida cover the vast majority of the collection (~80% of the collection specimens), followed by Cyclostomatida (~18%) and Ctenostomatida (~2%).

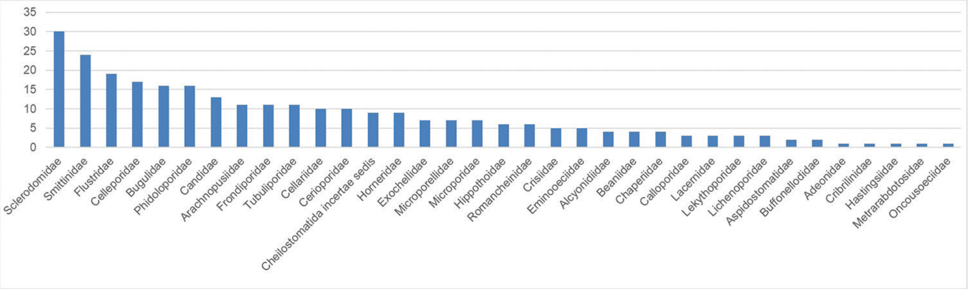

Figure 9. Histogram depicting the number of specimens per family of the MNA Bryozoa collection.

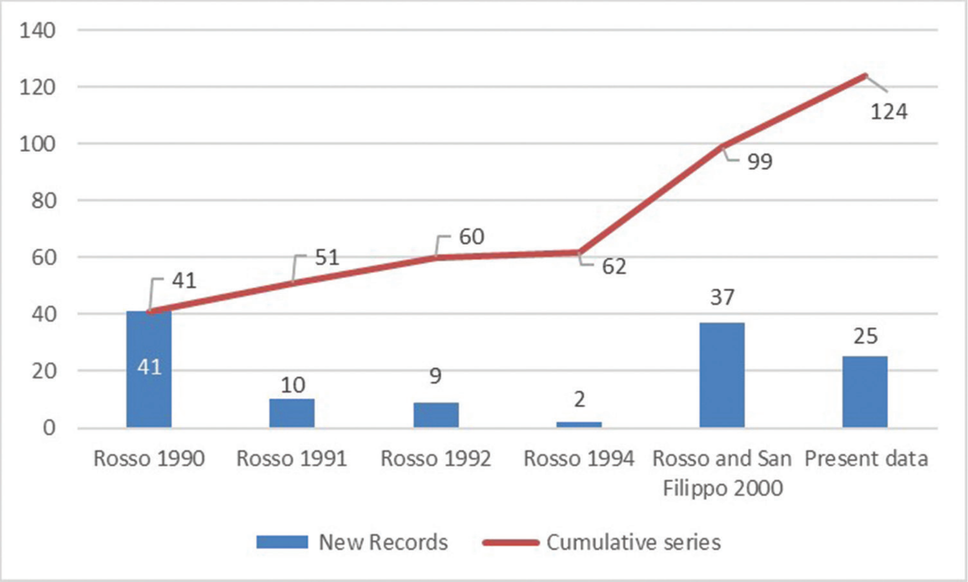

Figure 10. Histogram depicting the number of new records (blue bars) and their cumulative series (red line) reported by literature and this publication for the Terra Nova Bay area.

Old bryozoan names and synonyms were cross-checked in Bryozoa.net (Indexes to bryozoan taxa http://www.bryozoa.net/indexes.html) and WoRMS (World Register of Marine Species; http://www.marinespecies.org; last accessed 20 April 2018). Whenever necessary, taxonomic updates were applied to our checklist. The updated and detailed checklist is reported below.

Taxonomic update for Cheilostomatida species previously reported in the literature

Adelascopora jeqolqa Moyano, 1989 is now *Adelascopora secunda* Hayward & Thorpe, 1988; *Clithriellum inclusum* (Rogick, 1956), originally classified by Rosso (1990), is now named *Polirhabdotos inclusum* (Waters, 1904) according to the original classification of Hayward and Thorpe; *Cellaria vitrimuralis* (Rogick, 1956) (reported by Rosso 1990) is now *Cellaria diversa*, *Celleporella antarctica* Moyano & Gordon, 1980 (reported in Rosso 1990) is classified as *Antarctothoa antarctica* whereas *Chaperia simplicissima* Kluge, 1914 is named *Exallozoon simplicissimum* as stated in Bock and Gordon (2013). Regarding the Family Flustridae, *Flustra drygalskii* Kluge, 1914 (reported by Rosso 1990) is now classified as *Klugeflustra drygalskii* (Bock & Gordon, 2013) (Rosso 1994, Hayward 1995), *Flustra angusta* Kluge, 1914 (reported by Rosso 1990, 1992a, 1994) is *Isosecuriflustra angusta* (Bock & Gordon, 2013) (Hayward 1995), and *Flustra tenuis* Kluge, 1914 (reported by Rosso 1994) is *Isosecuriflustra tenuis* (Bock & Gordon, 2013) (Hayward 1995). *Hippellozoon gelidum* Moyano, 1966 (reported by Rosso 1992) is named *Reteporella gelida* (Waters, 1904) (Hayward 1995, Bock and Gordon 2013). *Mawsonia membranacea* Livingstone, 1928 (reported by Rosso 1990) is classified as *Swanomia membranacea* (Thornely, 1924); *Porella antarctica* Powell, 1967 (reported by Rosso 1991) is *Aimulosia antarctica* and the genus *Sertella* is now *Reteporella* (Hayward 1995). Among Smittinidae, *Smittina gelida* (as reported by Rosso 1990) is classified as *S. directa* (Waters, 1904) by Hayward (1995), whereas *S. oblongata* (as reported by Rosso 1990), reported as conspecific with *S. antarctica* (Waters, 1904) (Hayward 1995) is classified as *S. antarctica* according to recent taxonomic literature (Bock and Gordon 2013, Kuklinski 2016).

Taxonomic update for Cyclostomatida species previously reported in literature

Defrancia sarsi (Borg, 1944), reported for Terra Nova Bay in 2000 (Rosso and Sanfilippo 2000), is here classified as *Apsendesia sarsi* following Bock and Gordon (2013); *Idmidronea magna* Androsova, 1968 (as reported by Rosso 1990) is synonym of *Idmidronea obtecta* Borg, 1944. *Tubulipora tubigera* Busk, 1866 (reported by Rosso 1991, Rosso and Sanfilippo 2000) is classified as *Supercytis tubigera* as stated in Bock and Gordon (2013). Lastly, although the record *Bearta* sp. has been reported in the literature (Rosso 1990), it is certainly a misspelling and should be referred to *Beania* sp.

Taxonomic ranks

Kingdom: Animalia
Phylum: Bryozoa
Class: Stenolaemata
Order: Cyclostomatida
Families: Cerioporidae, Crisiidae, Frondiporidae, Hastingsiidae, Horneridae, Lichenoporidae, Oncousoeciidae, Tubuliporidae
Genera: *Bicrisia*, *Crisia*, *Disporella*, *Fasciculipora*, *Hastingsia*, *Hornera*, *Idmidronea*, *Neofungella*, *Oncousoecia*, *Tubulipora*
Species: *Bicrisia edwardsiana* (NR), *Bicrisia* sp., *Crisia* sp., *Disporella humilis*, *Disporella* sp. (NR), *Fasciculipora ramosa*, *Hastingsia irregularis*, *Hornera* cf. *smitti*, *Hornera smitti* (NR), *Hornera* sp., *Idmidronea* cf. *antarctica*, *Idmidronea* cf. *obtecta*, *Idmidronea obtecta*, *Idmidronea* sp., *Neofungella claviformis*, *Oncousoecia* sp., *Tubulipora* sp.

Class: Gymnolaemata
Orders: Cheilostomatida, Ctenostomatida
Families: Adeonidae, Alcyonidiidae, Arachnopusiidae, Aspidostomatidae, Beaniidae, Buffonellodidae, Bugulidae, Calloporidae, Candidae, Cellariidae, Celleporidae, Chaperiidae, Cribrilinidae, Eminooeciidae, Exochellidae, Flustridae, Hippothoidae, Lacernidae, Lekythoporidae, Metrarabdotosidae, Microporellidae, Microporidae, Phidoloporidae, Romancheinidae, Sclerodomidae, Smittinidae
Genera: *Adelascopora*, *Adeonella*, *Aimulosia*, *Alcyonidium*, *Amastigia*, *Amphiblestrum*, *Andreella*, *Antarcticaetos*, *Arachnopusia*, *Austroflustra*, *Beania*, *Bostrychopora*, *Buffonellodes*, *Caberea*, *Camptoplites*, *Carbasea*, *Cellaria*, *Cellarinella*, *Cellarinelloides*, *Celleporella*, *Dakariella*, *Dendroperistoma*, *Ellisina*, *Eminooecia*, *Escharella*, *Exallozoon*, *Exochella*, *Favosthimosia*, *Fenestrulina*, *Flustra*, *Himantozoum*, *Hippothoa*, *Isoschizoporella*, *Isosecuriflustra*, *Klugeflustra*, *Klugella*, *Kymella*, *Lageneschara*, *Larvapora*, *Melicerita*, *Micropora*, *Nematoflustra*, *Notoplites*, *Orthoporidra*, *Osthimosia*, *Pemmatoporella*, *Plesiothoa*, *Polirhabdotos*, *Reteporella*, *Smittina*, *Stephanollona*, *Swanomia*, *Systenopora*, *Thrypticocirrus*, *Toretocheilum*, *Tricellaria*
Species: *Adelascopora secunda*, *Adeonella* sp., *Aimulosia antarctica*, *Alcyonidium australe*, *Alcyonidium* sp. (NR), *Amastigia crassimarginata* (NR), *Amphiblestrum inermis*, *Andreella* sp., *Antarcticaetos bubeccata*, *Arachnopusia* cf. *aviculifera*, *Arachnopusia decipiens*, *Arachnopusia latiavicularis*, *Arachnopusia monoceros*, *Arachnopusia* sp., *Austroflustra vulgaris*, *Beania challengeri* (NR), *Beania erecta*, *Beania* sp., *Bostrychopora dentata*, *Buffonellodes umbonata*, *Caberea darwinii*, *Camptoplites angustus* (NR), *Camptoplites bicornis*, *Camptoplites latus* (NR), *Camptoplites* sp., *Camptoplites tricornis*, *Carbasea curva*, *Carbasea ovoidea*, *Cellaria aurorae*, *Cellaria* cf. *aurorae*, *Cellarinella* cf. *latilaminata*, *Cellarinella* cf. *nutti*, *Cellarinella edita* (NR), *Cellarinella latilaminata*, *Cellarinella margueritae*, *Cellarinella njegovanae*, *Cellarinella nutti*, *Cellarinella rogickae*, *Cellarinella* sp., *Cellarinella watersi* (NR), *Cellarinelloides crassus*, *Celleporella* sp., *Dakariella concinna*, *Dendroperistoma pro-*

jecta, *Ellisina antarctica*, *Eminooecia carsonae* (NR), *Escharella watersi*, *Exallozoon simplicissimum*, *Exochella avicularis*, *Exochella hymanae*, *Exochella longirostris*, *Exochella* sp., *Favosthimosia milleporoides*, *Fenestrulina parvipora*, *Fenestrulina* sp., *Flustra anguloavicularis* (NR), *Himantozoum (Himantozoum) antarcticum*, *Hippothoa flagellum*, *Isoschizoporella secunda* (NR), *Isoschizoporella similis*, *Isoschizoporella* sp., *Isosecuriflustra angusta*, *Isosecuriflustra* sp., *Klugeflustra antarctica* (NR), *Klugeflustra drygalskii*, *Klugeflustra vanhoeffeni*, *Klugella buski* (NR), *Kymella polaris*, *Lageneschara lyrulata*, *Larvapora* cf. *mawsoni*, *Larvapora* sp., *Melicerita digeronimoi*, *Melicerita obliqua*, *Micropora brevissima*, *Micropora* sp., *Nematoflustra flagellata*, *Notoplites antarcticus* (NR), *Notoplites drygalskii*, *Notoplites* sp., *Notoplites tenuis*, *Orthoporidra compacta* (NR), *Orthoporidra* sp., *Osthimosia bicornis*, *Osthimosia cf. clavata*, *Osthimosia cf. curtioscula*, *Osthimosia clavata* (NR), *Osthimosia mariae* (NR), *Osthimosia* sp., *Pemmatoporella marginata*, *Plesiothoa calculosa*, *Polirhabdotos inclusum*, *Reteporella antarctica* (NR), *Reteporella frigida*, *Reteporella longichila* (NR), *Reteporella parva* (NR), *Reteporella* sp., *Smittina anecdota* (NR), *Smittina antarctica*, *Smittina directa*, *Smittina pileata* (NR), *Smittina rogickae*, *Smittina* sp., *Stephanollona longispinata*, *Swanomia belgica*, *Swanomia membranacea*, *Systenopora contracta* (NR), *Thrypticocirrus contortuplicata*, *Thrypticocirrus phylactelloides*, *Toretocheilum turbinatum*, *Tricellaria* sp.

Spatial coverage of dataset

General geographic description:

Ross Sea, Antarctica (Figs 2–4) and the Magellan Strait (Figs 2, 5)

Coordinates:

PNRA III expedition: -74.64833 and -74.84833; 164.92167 and 165.61167
PNRA V Exp: -74.69672 and -74.73528; 164.13183 and 164.47500
PNRA VI Exp: -52.51000 and -52.87167; -68.05500 and -74.97500
PNRA XI Exp: -74.69280; 164.60000
PNRA XIII Exp: -74.68850; 164.15833
PNRA XIV Exp: -74.74375; 164.14667
PNRA XVII Exp: -72.65917 and -77.65133; -166.79183 and 176.25783
PNRA XVIII Exp: -74.69557 and -74.79013; 164.03790 and 164.14782
PNRA XIX Exp: -74.82167 and 164.19167
PNRA XXV Exp: -74.69027 and -74.69768; 164.10255 and 164.13108
PNRA XXVII Exp: -74.68562 and -74.71337; 164.05915 and 164.14903
PNRA XXIX Exp: -74.68677 and -74.71828; 164.12278 and 164.24206

Temporal coverage of dataset

PNRA III Exp: 5 January – 28 January 1988
PNRA V Exp: 24 December 1989 – 3 January 1990
PNRA VI Exp: 24 February – 3 March 1991
PNRA XI Exp: 22 October 1995 - 2 February 1996
PNRA XIII Exp: 21 February 1998
PNRA XIV Exp: 25 January 1999
PNRA XVII Exp: 4 – 29 January 2002
PNRA XVIII Exp: 31 January – 18 February 2003
PNRA XIX Exp: 20 February 2004
PNRA XXV Exp: 10 December 2009 – 11 January 2010
PNRA XXVII Exp: 28 January - 3 February 2012
PNRA XXIX Exp: 16 January – 1 February 2014

Description of selected species

Parent collection identifier: Italian National Antarctic Museum (MNA, section of Genoa, Italy)

Collection name: Bryozoa collection of the Italian National Antarctic Museum (MNA)

Specimen preservation method: Part of the old collection was initially fixed in 4% formalin and then transferred in 70% ethanol. Samples collected from 2002 onwards were directly fixed in ethanol (99%) for molecular studies or air-dried. Bryozoan MNA vouchers are now preserved in 90% ethanol (~38% of the entire collection) or dried (~62%).

Database virtual collection of vouchers and 3D-models: 3D-models of four Antarctic bryozoans (Figs 11–15) were obtained from four specimens through micro-CT imaging performed at the Department of Geosciences (University of Padua) by CM. Acquisitions were performed using a bench-top Skyscan 1172 micro-CT system (Bruker), equipped with a Hamamatsu 100/250 microfocus X-ray source and a Hamamatsu C9300 11 megapixel camera (with a pixel size of 8.68 μm) filtered by a 0.5 mm Aluminium foil. Projection images were acquired with 70 kV source voltage, 141 μA current, 540 ms exposure time, 2×2 binning mode, 0.25° rotation step over 360°, averaged over 12 frames and in vertical random movement mode to minimise noise, providing an image pixel size of about 9 μm. Two connected scans were necessary to comprise the whole sample height. The run time for each sample was about 400 minutes. Post-acquisition reconstruction was performed using the NRecon (Bruker microCT) software package, starting from raw projection images, and applying thermal correction, misalignment compensation, ring artefact reduction and beam hardening correction. Segmentation was then performed with CT Analyser (Bruker microCT) software package, using a 3D-adaptive thresholding procedure (mean of minimum and maximum value) within spherical kernels of radius 8 pixels, starting from a predetermined pre-thresholding value. Resulting images were saved as monochrome (1 bit) bitmaps and imported in the CTVox (Bruker microCT) software package to perform 3D-rendering and animations. The model will be available on the MNA web site (www.mna.it) and on Sketchfab (https://sketchfab.com/MNA).

Hastingsia irregularis Borg, 1944 (MNA 10490, Figure 11) belongs to the Cyclostomatida, the only extant order of the class Stenolaemata, whose species are widely spread from all over the world, including the Antarctic Region. The scarce knowledge of cyclostome species for the Ross Sea, compared to cheilostomes, is attributable to the complex taxonomy of the group, which has been studied partly by Borg (1944) and Androsova (1968) but not for the Ross Sea or continent as a whole. The scan shows the structure of an *H. irregularis* colony, which develops well-calcified 3D-architectures, offering a space-resource to other species. *H. irregularis* forms erect colonies composed of 'fascicles' of long narrow zooids (ridge-like structures) becoming autozooids at their distal ends. The long and narrow zooids have small-scattered pseudopores and their skeletal walls are characterized by grooves between adjacent zooids and distinct growth ridges. Gonozooids (i.e., reproductive zooids) develops between colony branches (see the median branch developing a gonozooid on the top, Figure 11).

This model clearly provides an example of how the cyclostome colony acts as a resource for another bryozoan genus, *Arachnopusia* (Cheilostomatida, shown in detail in Figure 12). The genus *Arachnopusia* includes encrusting species, usually occurring in shallow shelf seas and only growing on biogenic carbonates (Hayward 1995). The species illustrated in the model (MNA 10491) grows on the reverse (dorsal) side of *H. irregularis* branches, where the surface is smoother compared to the rough substrate created by the tubular processes on the other side. Thus, this encruster might take advantage of the erect habit of the cyclostome (i.e., accessing the food on the water column) without interfering with its activities such as feeding and growing, which mainly occur on the other side.

Idmidronea cf. *obtecta* Borg, 1944 (MNA 9890, Figure 13) is a cyclostome forming large, erect, well-calcified colonies, with cylindrical branches. These branches are dichotomous, not very regularly spaced, with an oval transverse section, subcircular or rounded trapezoidal. Although bifurcations are not very regular on *I. obtecta* colonies, the whole zoarial shape is 3D-structured. The species represents another example of bioconstructional bryozoan, thus offering substrate and space for other organisms to live and settle. Interestingly, the model shows on reverse sides of branches the presence of arcuate growth lines. The presence of "growth check lines", especially among Antarctic erect cheilostomes, is very common, indicating a transitional phase between growth and stop in the colony, usually during the winter period (Winston, 1983). These bryozoans represent good bioindicators and key-species for experimental studies thanks to their "growth lines", which can be easily measured, allowing the quantification of the growth of the entire colony, often related to variations in environmental conditions detectable via stable isotope analyses (i.e., food availability, salinity, temperature and pCO_2 variations) (Barnes 2015).

Figure 11. Video of the 3D-model of *Hastingsia irregularis* Borg, 1944 (MNA 10490) and *Arachnopusia* sp. (MNA 10491). Height and width of the *H. irregularis* colony are respectively ~5.1 mm and ~6.2 mm.

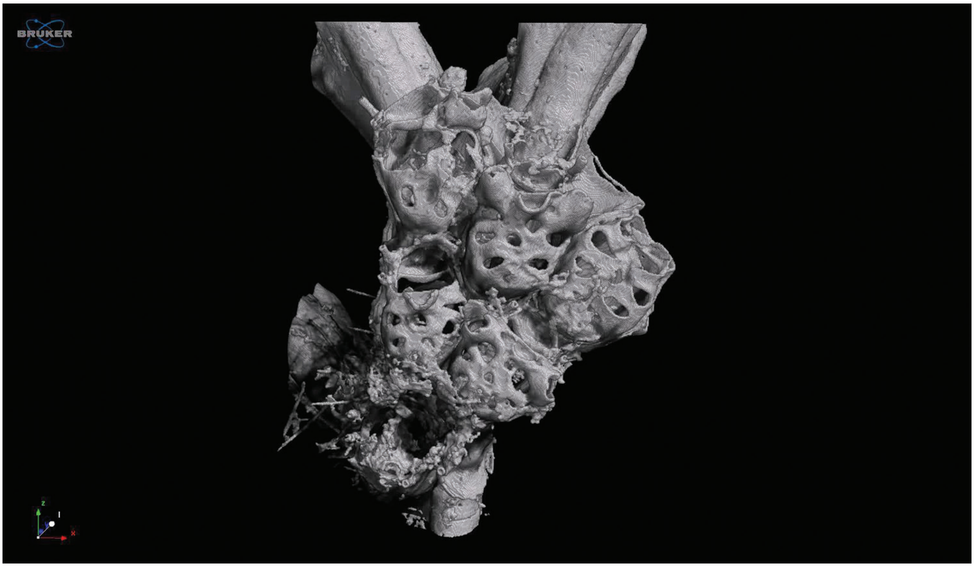

Figure 12. Close-up video of the 3D-model of *Arachnopusia* sp. (MNA 10491) shown in Figure 11. The width of the colony is 1.2 mm.

Eminoecia carsonae (Rogick, 1957) (NR) (MNA 8408, Figure 14) is an erect calcitic cheilostome bryozoan, originating from an encrusting base. This species develops erect branches, bifurcating dichotomously and shaping 3D colonies. Different characteristic morphological features of the autozooids, such as thick crenulated edges, tuberculate and coarse frontal walls, two pairs of pores at the proximal and distal end of the zooid and

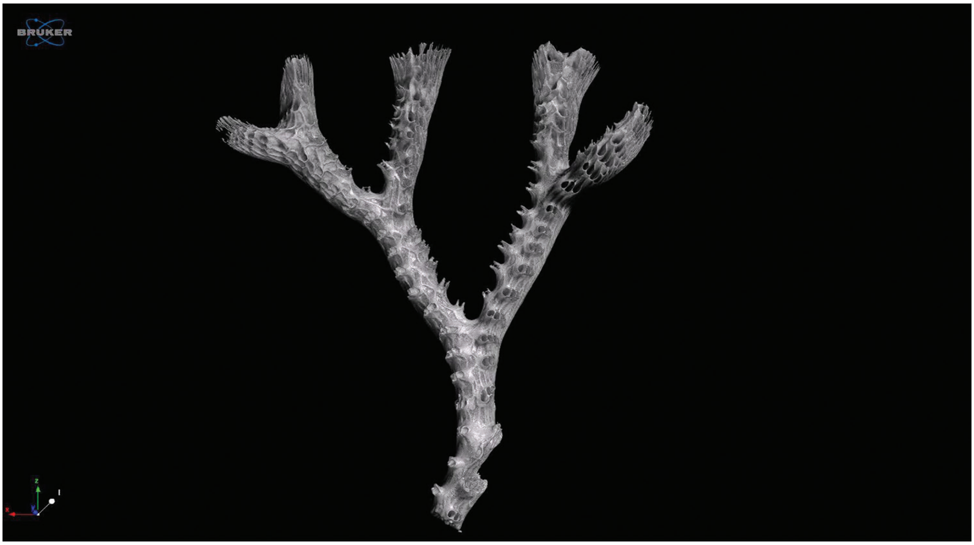

Figure 13. Video of the 3D-model of *Idmidronea* cf. *obtecta* Borg, 1944 (MNA 9890). Height and width of the colony are respectively ~20.4 mm and ~17.5 mm.

Figure 14. Video of the 3D-model of *Eminooecia carsonae* (Rogick, 1957) (MNA 8408). Height and width of the colony are respectively ~21.7 mm and ~1.8 mm.

frontal suboral avicularia, organized in bands on the colony branches, are illustrated in Figure 14. Being a bioconstructional bryozoan, this species offers space and advantages to other species to live and settle, thus it has a key role in promoting the biodiversity. Described as an endemic Antarctic species, *E. carsonae* is widespread in the Ross Sea (Hayward 1995; Moyano 2005) but it is here recorded for the first time for Terra Nova Bay.

Figure 15. Video of the 3D-model of *Smittina directa* (Waters, 1904) (MNA 9883). Height and width of the colony are respectively ~17.2 mm and ~16.9 mm.

Smittina directa (Waters, 1904) (MNA 9883, Figure 15), an endemic Antarctic species, represents another example of an erect calcitic cheilostome, developing slender, cylindrical colonies originating from an encrusting base. Its autozooids, budding simultaneously, are organized in whorls of four or five, with frontal orifices opening around the whole branch (Figure 15). The primary orifice has an anvil-shaped lyrula (e.g., median tooth) with a straight edge projecting corners and a peristome, and its distal third is in a continuum with the calcification of the next autozooid (Hayward 1995). *Smittina directa* is subject to marked secondary calcification processes, which lead, in later ontogeny, to the thickening of the frontal walls, thus some of its morphological features are hardly recognizable. In addition to the autozooids, the branch represented in Figure 15 bears kenozooids (e.g., zooids without muscles and primary orifice), whose main function is to provide structural strength to the colony.

Datasets

Dataset metadata:

This dataset contains data on the phylum Bryozoa, represented by two classes and three orders in the Ross Sea, with focus on Terra Nova Bay, and the Magellan Strait. The present dataset has been formatted in order to fulfil the Darwin Core standard protocol required by the OBIS scheme (http://www.iobis.org/manual/lifewatchqc/) and according to the SCAR-MarBIN Data Toolkit (available at http://www.scarmarbin.be/documents/SM-FATv1.zip). The dataset was uploaded and integrated with the

ANTOBIS database (the geospatial component of SCAR-MarBIN). Two studies have been based on this dataset: Rosso and Sanfilippo 1991; Rosso 1992b.

The Darwin Core elements included in the dataset are: ID, Institution code (i.e., the name of the institution where the samples are curated), basis of record, occurrence ID, catalogue number (i.e., MNA catalogue number), individual count, preparation (preservation method and more info about the sample, e.g., ETOH, dry, glass slides, etc.), event ID (i.e., original sampling station code), sampling protocol (sampling gear), event date, year, month, day, verbatim event date, field number (sampling station code as showed in the maps), event remarks (i.e., expedition), maximum depth meters, decimal latitude, decimal longitude, locality, taxon ID, scientific name ID, scientific name, kingdom, phylum, class, order, family, genus, subgenus, specific epithet, infraspecific epithet, scientific name authorship, taxon remarks. Some of the sampling stations are dredge stations, which have two sets of coordinates: the starting and ending points. In such cases, the coordinates reported in the dataset refer to the starting point of the dredge station.

Object name: Bryozoa collection of the Italian National Antarctic Museum (MNA) – Data
Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 1.0
Distribution: https://doi.org/10.15468/u08az1
Language: English
Metadata language: English
License of use: This dataset [Bryozoa collection of the Italian National Antarctic Museum (MNA) - Data] is made available under the Creative Commons Attribution License (CC-BY) 4.0: http://www.creativecommons.org/licenses/by/4.0/legalcode
Date of metadata creation: 25 May 2018
Hierarchy level: Dataset

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Supplementary material 1

Terra Nova Bay check-list of Bryozoa species

Authors: Matteo Cecchetto, Chiara Lombardi, Simonepietro Canese, Silvia Cocito, Piotr Kuklinski, Claudio Mazzoli, Stefano Schiaparelli

Data type: (measurement/occurrence/multimedia/etc.)

Link: https://doi.org/10.3897/zookeys.812.26964.suppl1

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RESEARCH ARTICLE

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Analysis of mitochondrial genomes resolves the phylogenetic position of Chinese freshwater mussels (Bivalvia, Unionidae)

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Abstract

The Yangtze River basin is one of the most species-rich regions for freshwater mussels on Earth, but is gravely threatened by anthropogenic activities. However, conservation planning and management of mussel species has been hindered by a number of taxonomic uncertainties. In order to clarify the taxonomic status and phylogenetic position of these species, mitochondrial genomes of four species (*Acuticosta chinensis*, *Schistodesmus lampreyanus*, *Cuneopsis heudei* and *Cuneopsis capitatus*) were generated and analyzed along with data from 43 other mitogenomes. The complete F-type mitogenomes of *A. chinensis*, *S. lampreyanus*, *C. heudei,* and *C. capitatus* are 15652 bp, 15855 bp, 15892 bp, and 15844 bp, respectively, and all four F-type mitogenomes have the same pattern of gene arrangement. ML and BI trees based on the mitogenome dataset are completely congruent, and indicate that the included Unionidae belong to three subfamilies with high bootstrap and posterior probabilities, i.e., Unioninae (*Aculamprotula*, *Cuneopsis*, *Nodularia*, and *Schistodesmus*), Anodontinae (*Cristaria*, *Arconaia*, *Acuticosta*, *Lanceolaria*, *Anemina*, and *Sinoanodonta*), and Gonideinae (*Ptychorhynchus*, *Solenaia*, *Lamprotula*, and *Sinohyriopsis*). Results also indicate that *A. chinensis* has affinities with *Arconaia lanceolata* and *Lanceolaria grayii* and is a member of the subfamily Anodontinae.

Keywords
China, classification, freshwater, F-type mitogenome, mussel

Introduction

The freshwater mussel family Unionidae is the most species-rich family within the order Unionida, including more than 620 species representing 142 genera (Graf and Cummings 2007; Bogan 2008). The Unionidae is widely distributed, and its members are found on all continents, with the exception for Antarctica (Graf and Cummings 2007; Bogan 2008; Lopes-Lima et al. 2017a). Unfortunately, freshwater mussels are one of the most threatened animal groups in the world, due to habitat destruction, commercial exploitation, and water pollution (Lydeard et al. 2004; Vaughn et al. 2010; Lopes-Lima et al. 2014; Wu et al. 2017a).

Well-supported phylogenetic hypotheses for the Unionidae are crucial for understanding the evolutionary history and biogeography of its genera (e.g., Roe 2013; Graf et al. 2015), for formulating reliable classifications (e.g., Campbell et al. 2005), and for developing conservation priorities (Lopes-Lima et al. 2017b, 2018). Advances in developing improved phylogenetic hypotheses for the Unionidae have occurred in the past several decades (Davis 1984; Lydeard et al. 1996; Nagel and Badino 2001; Hoeh et al. 2001, 2002; Giribet and Wheeler 2002; Graf 2002; Campbell et al. 2005; Zanatta and Murphy 2006; Graf and Cummings 2007; Campbell and Lydeard 2012a, b; Froufe et al. 2014; Prié and Puillandre 2014; Graf et al. 2015; Pfeiffer and Graf 2015). Most of these studies have focused on North American, Australian, and European taxa, although more recently, African (Whelan et al. 2011; Graf 2013; Elderkin et al. 2016) and Asian (Huang et al. 2002; Zhou et al. 2007; Huang et al. 2013; Bolotov et al. 2017a, b) taxa have been included, and a global phylogenetic framework of the Unionidae has recently been established (Bolotov et al. 2017a; Lopes-Lima et al. 2017a). Despite these advances, the incorporation of Asian taxa into unionid phylogenetic hypotheses, particularly those from China has lagged.

The middle and lower reaches of the Yangtze River are a diversity hotspot for unionids in East Asia (Graf and Cummings 2007; He and Zhuang 2013; Zieritz et al. 2017), and this region may harbor as many as 15 unionid genera (Wu et al. 2000; Shu et al. 2009; Wu et al. 2017a). As with North American freshwater mussels, much of the early descriptive work on Chinese taxa occurred during the latter part of the 19th Century (Heude 1875, 1877a, b, 1878, 1879, 1880a, b, 1881, 1883, 1885). Pierre Marie Heude was a Jesuit priest who collected freshwater and terrestrial mollusks in China. During a ten-year period between 1882 and 1902, Heude described close to 600 species including 140 freshwater mussel species (Johnson 1973). However, the validity and classification of many of these species were called in to question by Simpson (1900, 1914) and Haas (1969). Simpson (1900, 1914) presented a modified classification based on anatomical information such as marsupium size and shape, larval type and umbo sculpture in addition to conchological characters. Simpson condensed the number of Chinese freshwater mussels down to 85 species in 14 genera and placed them into two subfamilies, the Unioninae and the Hyriinae. Haas (1969) further revised the classification of the Unionidae and reduced the number of Chinese unionids to 56 species and subspecies in 20 genera, and placed them into four subfamilies: Unioninae, Quadrulinae, Anodontinae and Lampsilinae. After 1949, Chinese malacologists (e.g., Lin 1962; Tchang et al. 1965a, b; Liu et al. 1964, 1979, 1980, 1982; Wu et al. 2000) conducted a substantial amount of work on the classification of the Unionidae, and placed Chinese species into either the Unioninae or Anodontinae, based on the presence or absence of hinge teeth. In the 1990s, malacologists began to refocus their attention on the soft anatomy and changes to the classification, based on the shape of the glochidia and type of marsupium were made (Wei and Fu 1994; Wu et al.1999a, b; Shu et al. 2012). Despite these advances, the higher-level taxonomy of Chinese unionids was not updated, and only the subfamilies Unioninae and Anodontinae remained in the revised system.

At the beginning of this century, Chinese researchers investigated the molecular systematics of the Unionidae and made great progress revising the earlier classifications (Huang et al. 2002; Wang et al. 2013; Ouyang et al. 2011, 2015; Huang et al. 2013, 2015, 2018; Song et al. 2016; Zhou et al. 2007, 2016a, b; Wu et al. 2016, 2017b). However, there continued to be many discrepancies regarding the classification of genera (Table 1). Most recently, Lopes-Lima et al. (2017a) constructed a phylogenetic framework for the worldwide Unionidae; however, it only contained 17 Chinese freshwater mussel species. Wu et al. (2018b) generated a phylogeny based on portions of the mitochondrial COI and ND1genes that included 34 Chinese unionids. While the resultant trees from these studies resolved a number of relationships, branch support values at certain nodes were low, and the placements of some genera (*Sinohyriopsis* and *Lepidodesma*) were not clarified.

The purpose of this study was to clarify the taxonomic status and phylogenetic position of Chinese Unionidae using the DNA sequences of mitochondrial genomes to infer phylogenetic relationships. Phylogenetic hypotheses based on the analysis of mitochondrial genomes of unionids are becoming more common (Walker et al. 2006; Huang et al. 2013, 2018; Burzyński et al. 2017). In the Unionoida, Mytiloida, and Veneroida, an unusual mode of mitochondrial DNA transmission termed Doubly Uniparental Inheritance (DUI) occurs, in which two distinct, tissue-specific and gender-associated mitogenomes (i.e., F-type and M-type) (Breton et al. 2007) are present. For the remainder of this paper, all references to mitogenomes refer to the F-type mitogenome.

In this study, we sequenced and described the complete mitogenomes of four Chinese unionids: *Acuticosta chinensis* (Lea, 1868), *Schistodesmus lampreyanus* (Baird & Adams, 1867), *Cuneopsis heudei* (Heude, 1874), and *Cuneopsis capitatus* (Heude, 1874), with the aim of combining these new genome sequences with existing mitochondrial genomes to develop a phylogenetic framework for the Chinese Unionidae. In addition, we were particularly interested in determining the taxonomic position of the genus *Acuticosta*. This genus was erected by Simpson (1900) and *Acuticosta chinensis* (Lea, 1868) was used as the type species. The genus *Acuticosta* has been placed in a number of unionid subfamilies including the Hyriinae (Simpson, 1900), Unioninae (Liu 1979), Acuticostinae (Prozorova et al. 2005), and Unioninae (Huang et al. 2002, Graf and Cummings 2007, Zhou et al. 2007, Ouyang et al. 2011, 2015), and most recently, the Anodontinae (Wu et al. 2018b).

Table 1. Chinese freshwater mussels (Unionidae) systematic taxonomy history. Shaded genera indicate classification disputes.

Genus	Liu et al. 1979	Huang et al. 2002	Zhou et al. 2007	Ouyang et al. 2011	Huang et al. 2013	Ouyang et al. 2015	Wu et al. 2018b	This study
Aculamprotula Wu et al., 1999	–	–	Unioninae	Unioninae	Unioninae	Unioninae	Unioninae	Unioninae
Sinanodonta Modell, 1944	Anodontinae	Anodontinae	Anodontinae	Anodontinae	–	Anodontinae	Anodontinae	Anodontinae
Cristaria Schumacher, 1817	Anodontinae	Anodontinae	Anodontinae	–	Anodontinae		Anodontinae	Anodontinae
Cuneopsis Simpson, 1900	Unioninae	Unioninae	Unioninae	Unioninae	–	Unioninae	Unioninae	Unioninae
Schistodesmus Simpson, 1900	Unioninae	Unioninae	Unioninae	Unioninae	–	Unioninae	Unioninae	Unioninae
Nodularia Conrad, 1853	Unioninae	Unioninae	Unioninae	Unioninae	–	Unioninae	Unioninae	Unioninae
Anemina Haas, 1969	Anodontinae	Anodontinae	–	Anodontinae	–	Anodontinae	Anodontinae	Anodontinae
Acuticosta Simpson, 1900	Unioninae	Unioninae	Unioninae	Unioninae	–	Unioninae	Anodontinae	Anodontinae
Arconaia Conrad, 1865	Unioninae	Unioninae	Unioninae	–	–	–	Anodontinae	Anodontinae
Lamprotula Simpson, 1900	Unioninae	Ambleminae	Ambleminae	Ambleminae	–	Ambleminae	Gonideinae	Gonideinae
Lanceolaria Conrad, 1853	Unioninae	Unioninae	Unioninae	Unioninae	–	Unioninae	Anodontinae	Anodontinae
Lepidodesma Simpson, 1896	Anodontinae	Unioninae	–	–	–	–	**Incertae sedis**	**Incertae sedis**
Ptychorhynchus Simpson, 1900	–	Ambleminae	–	–	–	–	Gonideinae	Gonideinae
Solenaia Conrad, 1869	Anodontinae	Ambleminae	–	Ambleminae	Gonideinae	Ambleminae	Gonideinae	Gonideinae
Sinohyriopsis Starobogatov, 1970	Unioninae	Ambleminae	Ambleminae	Ambleminae	–	Ambleminae	**Incertae sedis**	Gonideinae

Materials and methods

Taxon sampling, mitochondrial genome sequencing, and assembly

Samples of four species were collected from Poyang Lake (28°47.84'N; 116°2.03'E) in Jiangxi Province, China (Figure 1), and specimens were preserved and vouchers deposited in the Biological Museum of Nanchang University. Information for primers used for PCR amplification of F-type mitogenomes can be found in Table 2. Complete mitogenomes were sequenced and annotated according to our previous study (Wu et al. 2016).

Dataset construction

We downloaded all published unionid mitogenomes from GenBank (as of March 2018), and combined them with the four mitogenomes generated in this study for a total of 41 unionid mitogenomes (22 Chinese taxa). In addition, we included additional genomes, also downloaded from GenBank, from the Margaritiferidae (four species), Iridinidae (one species), and Hyriidae (one species) as out-groups for the phylogenetic analysis (Table 3).

Figure 1. Shells of the unionids species in this study. **A** *Acuticosta chinensis* (Lea, 1868) **B** *Schistodesmus lampreyanus* (Baird & Adams, 1867) **C** *Cuneopsis heudei* (Heude, 1874) **D** *Cuneopsis capitatus* (Heude, 1874). Scale bar: 4 cm. Photogaphs R-W Wu.

Alignments, partitioning strategies, and phylogenetic analyses

Nucleotide sequences of 12 mitochondrial protein-coding genes (we excluded *atp8*) and 2 rRNA genes were concatenated for construction of the phylogenetic trees. Nucleotide sequences of protein coding genes (PCG) were translated to amino acid sequences using MEGA 5.0 (Tamura et al. 2011), and genes were aligned based on the amino acid sequence (PNGs), or nucleotide sequence (rRNA) using the MUSCLE program (Edgar 2004) with default settings. Alignments of sequences were manually checked and areas of ambiguous alignment were excluded. Finally, 12 PCGs and the 2 rRNA genes were concatenated (11862 bp) using SequenceMatrix (Vaidya et al. 2011). The dataset was then partitioned according to codon position of each PCG and each rRNA gene for phylogenetic analysis. Prior to phylogenetic analysis, a partition homogeneity test was carried out in PAUP* version 4.0b10 (Swofford 2003) to determine rate heterogeneity among genes and codon positions. The partition homogeneity test indicated there was no significant difference in signals ($P > 0.05$).

PartitionFinder v1.1.1 (Lanfear et al. 2012) was used to select optimal substitution models for the 2 rRNA genes and each codon position of the 12 PCG. Bayesian analyses were undertaken in MrBayes Version 2.01 (Ronquist et al. 2012), four chains were run simultaneously for 1 million generations, and trees were sampled every 1000

Table 2. Primers used for PCR amplification of female *Acuticosta chinensis*, *Schistodesmus lampreyanus*, *Cuneopsis heudei,* and *Cuneopsis capitatus* mitochondrial genomes.

Fragment	Primer name	Primer sequence (5' to 3')	Length
COI (universal primer)	LCO1490	GGTCAACAAATCATAAAGATATTGG	~700 bp
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
16S (universal primer)	16SarL	CGCCTGTTTATCAAAAACAT	~500 bp
	16SbrH	CCGGTCTGAACTCAGATCACGT	
ND1 (universal primer)	Leu-uurF	TGGCAGAAAAGTGCATCAGATTAAAGC	~1000 bp
	LoGlyR	CCTGCTTGGAAGGCAAGTGTACT	
COI→ND1 (*A. chinensis*)	ZGCNH	TTGGGACTGGCTGGAC	~500 bp
	ZGCNR	TTACTAGGAGCTATTCGAGC	
	2ZGCNH	GAGTCTTGGGGTTTATTGT	~1400 bp
	2ZGCNR	AGTAGAAAGACCAAAACCG	
	3ZGCNH	CAGTTCGGTGTTATCTTCAT	~3400 bp
	3ZGCNR	TGGCTAGTAGTGATTCTTGC	
ND1→16S (*A. chinensis*)	ZGN1H	CGAAGCCTGACAATGTCTA	~4500bp
	ZGN1R	TATCGAAAGTTGGGTTTGC	
16S→COI (*A. chinensis*)	ZG1CH	CTAGTGTTGCCTTTCACTG	~5200 bp
	ZG1CR	AGACAAGGGAGGATAAACC	
COI→ND1 (*S. lampreyanus*)	SXCNH	CTGGTTGGACGGTGTATC	~3200 bp
	SXCNR	ATAGCCATCCCAGTAGCC	
	2SXCNH	GTTATACTCTTCCGATCATCCT	~2100 bp
	2SXCNR	AACCAGCACAGAACTCAATA	
ND1→16S (*S. lampreyanus*)	SXN1H	GAGATGGTTTGAGCTATGG	~4500 bp
	SXN1R	CGATGTTGGCTTAAGGATA	
16S→COI (*S. lampreyanus*)	SX1CH	TTCCTAGTCTTGCCATTCA	~3600 bp
	SX1CR	GCAGGCACAAGTAATCAAA	
COI→ND1 (*C. heudei*)	YTCNH	TCTGGTGATGCCAATAATGA	~6200 bp
	YTCNR	TCCCCTCCTTTATAGTTTCA	
ND1→16S (*C. heudei*)	YTN1H	TGTCTCTGCGAGGATTACT	~1300 bp
	YTN1R	ACATAAGTGCAACCGCTAT	
	2YTN1H	TTCTGCCACCTTGCTTCA	~3300 bp
	2YTN1R	GGCTGACTCATACGAACCAT	
16S→COI (*C. heudei*)	YT1CH	TTACTGGTTCCAAGATTGC	~5600 bp
	YT1CR	AATCAAACCAGGAGATCGT	
COI→ND1 (*C. capitatus*)	JSCNH	GTTGCTGAGCGTATTCCTT	~5300 bp
	JSCNR	CTTTGACTTTGCAGAGGGA	
ND1→16S (*C. capitatus*)	JSN1H	GTATTTGGAGTTGGATGATC	~4700 bp
	JSN1R	GAATGGCAAGACTAGGAATA	
16S→COI (*C. capitatus*)	JS1CH	TATTCCTAGTCTTGCCATTC	~5000 bp
	JS1CR	CAATAATCTTCCAGGTTGAC	

generations, with a burn-in of 25%. Stationarity was considered to be reached when the average standard deviation of split frequencies was less than 0.01.

The gene and codon site-based partitioned ML analysis was performed in RAxML implemented in raxmlGUI v.1.3 (Stamatakis 2014), using the GTRGAMMAI model of nucleotide substitution with the search strategy set for rapid bootstrapping. ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017) implemented

Table 3. F-type mitochondrial genomes used in this study.

Taxon	GenBank accession number	Reference
UNIONIDAE		
Ambleminae		
Quadrula quadrula (Rafinesque, 1820)	FJ809750	Breton et al. 2009
Venustaconcha ellipsiformis (Conrad, 1836)	FJ809753	Breton et al. 2009
Potamilus alatus (Say, 1817)	KU559011	Wen et al. 2017
Leptodea leptodon (Rafinesque, 1820)	NC_028522	Feng et al. 2016
Toxolasma parvum (Barnes, 1823)	HM856639	Breton et al. 2011
Lampsilis ornata (Conrad, 1835)	NC_005335	Serb and Lydeard 2003
Gonideinae		
Pronodularia japanensis (Lea, 1859)	AB055625	Unpublished
Lamprotula leaii (Griffith & Pidgeon, 1833)	NC_023346	Chen et al. 2012
Ptychorhynchus pfisteri (Heude, 1874)	KY067440	Zhou et al. 2016a
Potomida littoralis (Cuvier, 1798)	NC_030073	Froufe et al. 2016
Solenaia oleivora (Heude, 1877)	NC_022701	Huang et al. 2015
Solenaia carinatus(Heude, 1877)	NC_023250	Huang et al. 2013
Sinohyriopsis schlegelii (Martens, 1861)	HQ641406	Unpublished
Sinohyriopsis cumingii (Lea, 1852)	NC_011763	Unpublished
Anodontinae		
Acuticosta chinensis (Lea, 1868)	MH919390	This study
*Arconaia lanceolat*a (Lea, 1856)	KJ144818	Wang et al. 2014
Lanceolaria grayana (Lea, 1834)	NC_026686	Unpublished
Pyganodon grandis (Say, 1829)	FJ809754	Breton et al. 2009
Utterbackia peninsularis Bogan & Hoeh, 1995	HM856636	Breton et al. 2011
Utterbackia imbecillis (Say, 1829)	HM856637	Breton et al. 2011
Lasmigona compressa (Lea, 1829)	NC_015481	Breton et al. 2011
Anodonta anatina (Linnaeus, 1758)	NC_022803	Soroka et al. 2015
Sinanodonta woodiana (Lea, 1834)	HQ283346	Soroka et al. 2010
Sinanodonta lucida (Heude, 1877)	KF667529	Song et al. 2016
Anemina arcaeformis (Heude, 1877)	KF667530	An et al. 2016
Anemina euscaphys (Heude, 1879)	NC_026792	Xue et al.2016
Cristaria plicata (Leach, 1814)	KM233451	Wang et al. 2016
Unioninae		
Lepidodesma languilati (Heude, 1874)*	NC_029491	Zhou et al. 2016b
Schistodesmus lampreyanus (Baird & Adams, 1867)	MH919388	This study
Cuneopsis pisciculus (Heude, 1874)	NC_026306	Han et al. 2016
Cuneopsis heudei (Heude, 1874)	MH919389	This study
Cuneopsis capitatus (Heude, 1874)	MH919387	This study
Nodularia douglasiae (Griffith & Pidgeon, 1833)	NC_026111	Unpublished
Unio delphinus Spengler, 1793	KT326917	Fonseca et al. 2017
Unio pictorum (Linnaeus, 1758)	NC_015310	Soroka et al. 2010
Unio crassus Retzius, 1788	KY290446	Burzyński et al. 2017
Unio tumidus Retzius, 1788	KY021076	Soroka et al. 2018
Aculamprotula tortuosa (Lea, 1865)	NC_021404	Wang et al. 2013
Aculamprotula scripta (Heude, 1875)	MF991456	Wu et al. 2017b
Aculamprotula coreana (Martens, 1886)	NC_026035	Lee et al. 2016
Aculamprotula tientsinensis (Crosse & Debeaux, 1863)	NC_029210	Wu et al. 2016

Taxon	GenBank accession number	Reference
MARGARITIFERIDAE		
Gibbosula rochechouartii (Heude, 1875)	KX378172	Huang et al. 2018
Margaritifera falcata (Gould, 1850)	NC_015476	Breton et al. 2011
Cumberlandia monodonta (Say, 1829)	NC_034846	Guerra et al. 2017
Margaritifera dahurica (Middendorff, 1850)	NC_023942	Yang et al. 2015
HYRIIDAE		
Echyridella menziesii(Dieffenbach, 1843)	NC_034845	Guerra et al. 2017
IRIDINIDAE		
Mutela dubia (Gmelin, 1791)	NC_034844	Guerra et al. 2017

(*) indicates this species is incertae sedis

in IQ-TREE was used to choose the appropriate models, which additionally considers the FreeRate heterogeneity model (+R). IQ-TREE (Nguyen et al. 2015) was also used for ML tree reconstruction, and 1000 ultrafast bootstrap replicates were run to estimate branch support (Minh et al. 2013). The optimal substitution models for each partition by PartitionFinder and ModelFinder are shown in Suppl. material 1: Tables S1, S2.

Results

General features of the mitochondrial genomes

The lengths of the complete mitogenomes of *Acuticosta chinensis*, *Schistodesmus lampreyanus*, *Cuneopsis heudei*, and *Cuneopsis capitatus* were 15652bp, 15855bp, 15892bp and 15844bp, respectively. The newly sequenced four mitogenomes all contained 13 protein-coding genes, two rRNA genes, 22 tRNAs, and one female specific gene (FORF). All four F-type mitogenomes had the same pattern of gene arrangement. Among the 38 mitochondrial genes, 11 genes were encoded on the heavy chain, and the remaining 27 genes were encoded on the light chain (Figure 2).

The nucleotide composition of the *Acuticosta chinensis*, *Schistodesmus lampreyanus*, *Cuneopsis heudei* and *Cuneopsis capitatus* had obvious A+T bias (*A. chinensis*: 65.73%; *S. lampreyanus*: 64.54%; *C. heudei*: 62.45%; *C. capitatus*: 63.69%). In the base composition analysis for the four species, the A+T skews were negative, and the G+C skew were positive, indicating that the bases composition ratios of the four mitogenomes were T biased to A, and G biased to C. In invertebrate mitochondria, there are three conventional start codons: ATG, ATA and ATT, and three alternative start codons: ATC, TTG, and GTG (Wolstenholme 1992). The mitochondrial genomes of *A. chinensis* and *C. capitatus* had eleven protein coding genes which used the conventional start codons, and the remaining two used alternative start codons. *S. lampreyanus* and *C. heudei* had 12 PCG which used the common start codons, and one used the alternative start codon (Table 4).

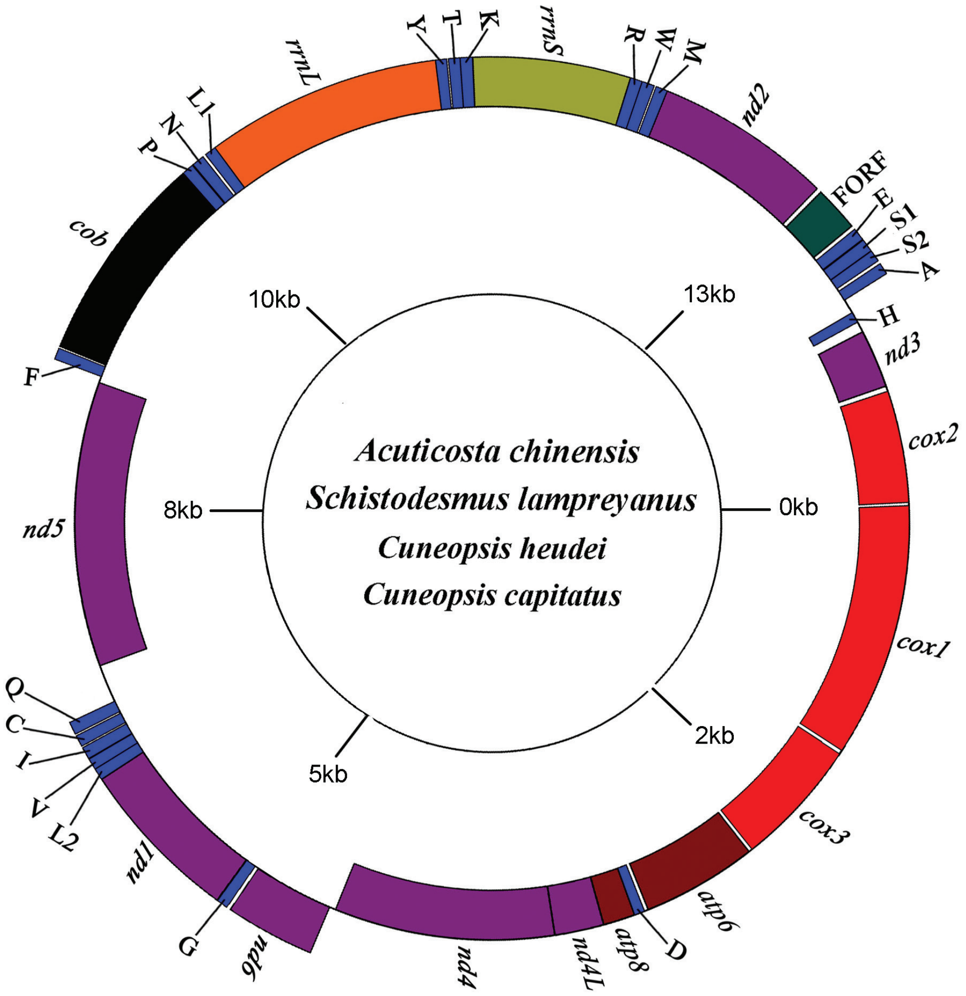

Figure 2. The gene arrangement of the F-type mitochondrial genome of *Acuticosta chinensis*, *Schistodesmus lampreyanus*, *Cuneopsis heudei*, and *Cuneopsis capitatus*.

The overlapping of neighboring genes is common in freshwater mussel mitochondria. There were three overlaps of neighboring genes in the mitochondrial genome of *Acuticosta chinensis* and *Schistodesmus lampreyanus*, and two in *Cuneopsis heudei*. The position of the largest gene overlap (8 bp) was between ND4 and ND4L. The mitochondrial genome of *Cuneopsis capitatus* only had one overlapping region between tRNA^{Met} and ND2. There were 29 non-coding regions (NCRs) in *A. chinensis*, *C. heudei*, and *C. capitatus*, and 27 NCRs in *S. lampreyanus*. The longest NCRs of the *A. chinensis*, *S. lampreyanus*, *C. heudei*, and *C. capitatus* were 224 bp, 349 bp, 216 bp, and 323 bp, respectively; all were located between ND5 and tRNA^{Gln} (Table 4).

Table 4. Structural characteristics of F-type mitochondrial genomes of *Acuticosta chinensis*, *Schistodesmus lampreyanus*, *Cuneopsis heudei*, and *Cuneopsis capitatus*. For each protein coding genes, start and stop codons and anticodons are presented in parentheses. Gene lengths are in bp.

	A. chinensis	*S. lampreyanus*	*C. heudei*	*C. capitatus*
Total size (bp)	15652	15855	15892	15844
AT%	65.73	64.54	62.45	63.69
CG%	34.27	35.46	37.55	36.31
AT skew	-0.18	-0.19	-0.21	-0.18
GC skew	0.28	0.33	0.33	0.32
No. of NCR	29	27	29	29
No. of overlapping genes	3	3	2	1
Size range of gene overlap	1 to 8	1 to 8	1 to 8	1
cox1	1539 (TTG/TAG)	1578 (ATA/TAG)	1566 (TTG/TAA)	1542 (TTG/TAG)
tRNA-Asp (D)	63 (GTC)	64 (GTC)	64 (GTC)	64 (GTC)
cox3	780 (ATG/TAA)	780 (ATG/TAA)	780 (ATG/TAA)	780 (ATG/TAG)
atp6	702 (ATG/TAA)	702 (ATG/TAG)	702 (ATG/TAG)	702 (ATG/TAG)
atp8	189 (ATG/TAA)	192 (ATG/TAA)	192 (ATG/TAG)	192 (ATG/TAG)
nd4L	297 (GTG/TAG)	279 (ATG/TAA)	255 (ATG/TAG)	255 (ATG/TAG)
nd4	1347 (ATT/TAA)	1347 (ATT/TAA)	1347 (ATT/TAA)	1329 (ATA/TAA)
nd6	489 (ATT/TAG)	486 (ATC/TAA)	507 (ATA/TAA)	507 (ATA/TAA)
tRNA-Gly (G)	62 (TCC)	63 (TCC)	63 (TCC))	63 (TCC))
nd1	900 (ATA/TAA)	900 (ATA/TAG)	900 (ATA/TAG)	900 (ATA/TAA)
tRNA-Leu (L2)	64 (TAA)	64 (TAA)	63(TAA)	64 (TAA)
tRNA-Val (V)	64 (TAC)	63 (TAC)	63 (TAC)	64 (TAC)
tRNA-Ile (I)	64 (GAT)	67 (GAT)	64 (GAT)	64 (GAT)
tRNA-Cys (C)	64 (GCA)	62 (GCA)	64 (GCA)	61 (GCA)
tRNA-Gln (Q)	69 (TTG)	70 (TTG)	69 (TTG)	69 (TTG)
nd5	1728 (ATA/TAA)	1713 (ATA/TAA)	1794 (ATA/TAA)	1734 (ATG/TAA)
tRNA-Phe (F)	66 (GAA)	65 (GAA)	65 (GAA)	64 (GAA)
Cob	1137 (ATA/TAA)	1146 (ATT/TAA)	1149 (ATA/TAA)	1020 (ATC/TAA)
tRNA-Pro (P)	64 (TGG)	66 (TGG)	64 (TGG)	64 (TGG)
tRNA-Asn (N)	65 (GTT)	66 (GTT)	68 (GTT)	65 (GTT)
tRNA-Leu (L1)	66 (TAG)	64 (TAG)	63 (TAG)	64 (TAG)
rrnL	1285	1304	1302	1297
tRNA-Tyr (Y)	60 (GTA)	61 (GTA)	63 (GTA)	63 (GTA)
tRNA-Thr (T)	61 (TGT)	66 (TGT)	64 (TGT)	63 (TGT)
tRNA-Lys (K)	68 (TTT)	70 (TTT)	70 (TTT)	70 (TTT)
rrnS	853	857	859	853
tRNA-Arg (R)	66 (TCG)	67 (TCG)	65 (TCG)	65 (TCG)
tRNA-Trp (W)	65 (TCA)	64(TCA)	63 (TCA)	62 (TCA)
tRNA-Met (M)	65 (CAT)	65 (CAT)	65 (CAT)	65 (CAT)
nd2	966 (ATG/TAA)	966 (ATG/TAA)	966 (ATG/TAA)	966 (ATG/TAA)
tRNA-Glu (E)	63 (TTC)	72 (TTC)	68 (TTC)	68 (TTC)
tRNA-Ser (S2)	68 (AGA)	73 (AGA)	68 (TCT)	68 (TCT)
tRNA-Ser (S1)	64 (TGA)	64 (TGA)	64 (CGA)	64 (CGA)
tRNA-Ala (A)	67 (TGC)	65 (TGC)	66 (TGC)	64 (TGC)
tRNA-His (H)	65 (GTG)	69 (GTG)	69 (GTG)	67 (GTG)
nd3	357 (ATG/TAG)	357 (ATG/TAG)	357 (ATG/TAA)	357 (ATG/TAG)
cox2	681 (ATG/TAA)	681 (ATG/TAG)	681 (ATG/TAA)	681 (ATG/TAG)

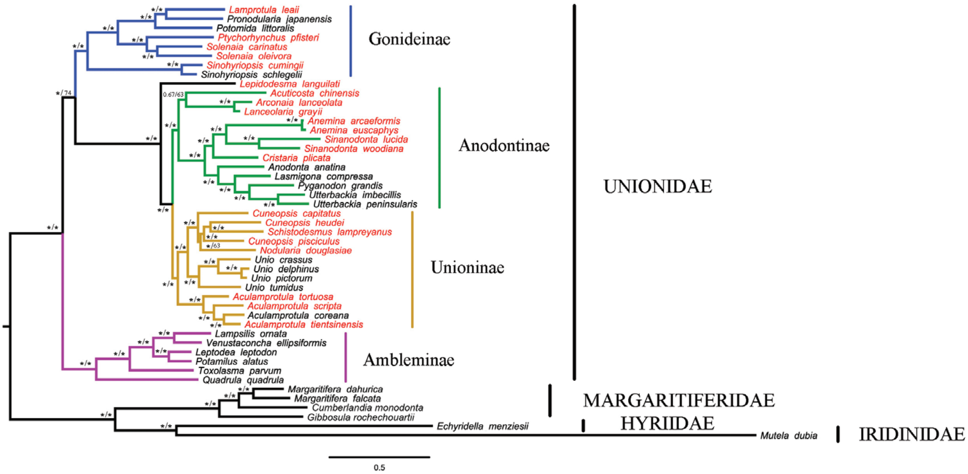

Figure 3. Phylogenetic trees of freshwater mussels obtained by Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of 12 mitochondrial protein-coding gene sequences (except *atp8*) and two rRNA combined dataset. Support values above the branches are posterior probabilities and bootstrap support. (*) indicates 100 percent bootstrap support and posterior probabilities. Red font indicates Chinese species.

All four mitochondria contained 22 tRNAs, including two serine tRNAs and two leucine tRNAs. The histidine tRNA and aspartate tRNA were located in the heavy chain, whereas the remaining 20 tRNAs were encoded by the light chain. The length of tRNAs differed slightly in each species (Table 4). The tRNA anticodons were the same in all species with the exception of two serine tRNAs. The anticodons of the two serines tRNAs of *A. chinensis* and *S. lampreyanus* were AGA and TGA, while those of *C. heudei* and *C. capitatus* were TCT and CGA (Table 4).

Phylogenetic analyses

ML and BI trees have completely congruent topologies and in general are well supported by high bootstrap and posterior probability values at almost all nodes (Figure 3). The mitogenomic dataset supports the monophyly of four Unionidae subfamilies (i.e., Unioninae, Anodontinae, Ambleminae, and Gonideinae) by both ML and BI methods. Phylogenetic analyses reveal the following relationships: (((Unioninae + Anodontinae) + Gonideinae) + Ambleminae) within the Unionidae.

Our phylogenetic analyses indicate that except for *Lepidodesma languilati* (Heude, 1874), the 21 Chinese species belong to the following three subfamilies: Unioninae (*Aculamprotula*, *Cuneopsis*, *Nodularia* and *Schistodesmus*), Anodontinae (*Cristaria*, *Arconaia*, *Acuticosta*, *Lanceolaria*, *Anemina* and *Sinoanodonta*), and Gonideinae (*Ptychorhynchus*, *Solenaia*, *Lamprotula*, *Sinohyriopsis*). Our results support the placement of *Acuticosta chinensis* in the Anodontinae, but *Leidodesma languilati* is not placed as a member of any subfamily, but instead is the well-supported sister taxon to the monophyletic group formed by the Unioninae and Anodontinae.

Discussion

Phylogenetic relationships of subfamilies in the Unionidae

In this study, we provide a novel phylogenetic hypothesis for relationships between subfamilies in the Unionidae (Figure 4). Other phylogenetic analyses of the Unionidae have been based on selected gene regions. For example, Lopes-Lima et al. (2017a) proposed the phylogenetic relationship of the subfamily based on COI and 28S as follows: (Anodontinae + Unioninae) + (Rectidentinae + (Ambleminae + Gonideninae)). Bolotov et al. (2017a) proposed relationships based on three loci (COI, 16S and 28S), and adding more taxa: ((Anodontinae + Unioninae) + (Ambleminae + Gonideninae)) + (Rectidentinae + Pseudodontinae). Prior investigations into subfamily relationships in the Unionidae, based on complete mitochondrial genomes, seem to be consistent with these earlier studies, (Anodontinae + Unioninae) + (Ambleminae + Gonideninae) (Huang et al. 2013; Burzyński et al. 2017; Huang et al. 2018; Wu et al. 2016, 2017b). The current study is based on the mitochondrial genome sequences for the largest number of unionid species (41). By increasing the number of taxa and the amount of DNA sequences, we obtain a unique set of phylogenetic relationships: ((Anodontinae + Unioninae) + Gonideninae) + Ambleminae). Our phylogeny differs from other studies based on mitochondrial genome sequences in that the Ambleminae is the basal subfamily as opposed to the sister Gonideninae.

Bolotov et al. (2017a) proposed that the most recent common ancestor (MRCA) of the Anodontinae, Unioninae, Ambleminae, and Gonideninae likely originated in East Asia (Probability 65.8%). Under this scenario the MRCA of Anodontinae + Unioninae arose in East Asia during the Cretaceous period, whereas the MRCA of Ambleminae + Gonideninae was continuously distributed in East Asia and North America. The ancestor of the Ambleminae was most likely to originate in North America. The diversification of each subfamily occurred in the late Cretaceous (Bolotov et al. 2017a). The results of phylogenetic analyses in the current study have different evolutionary implications. Our results indicate that the Ambleminae is basal to the other three subfamilies, and its origin is therefore earlier than the other three subfamilies. Globally, eight subfamilies (Anodontinae, Unioninae, Pseudodontinae, Gonideinae, Ambleminae, Rectidentinae, Parreysiinae, and Modellnaiinae) are recognized in the Unionidae (Bolotov et al. 2017a; Lopes-Lima et al. 2017a; Whelan et al. 2011). The lack of mitochondrial genomes for Rectidentinae, Parreysiinae, Modellnaiinae, and Pseudodontinae, precluded their incorporation into this study. However, we believe that the fully resolved phylogenetic tree, with high branch support in the present study, serves as a framework for further studies on the Unionidae, Future phylogenetic analyses based on complete mitochondrial genome sequences of representatives of all the subfamilies in the Unionidae will ultimately produce well-supported phylogenetic hypotheses for the Unionidae.

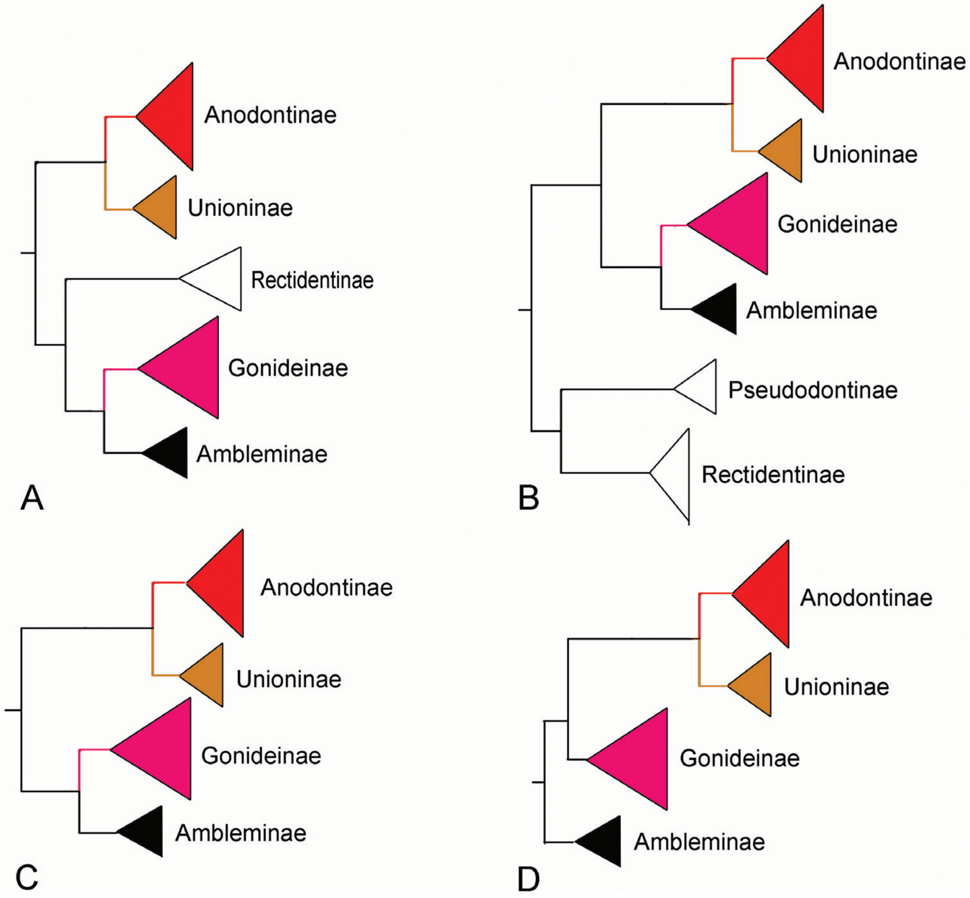

Figure 4. Hypotheses of phylogenetic relationships among subfamilies of the Unionidae form this and other studies. **A** Lopes-Lima et al. (2017a) **B** Bolotov et al. (2017a) **C** Huang et al. 2013; Burzyński et al. 2017; Huang et al. 2018; Wu et al. 2016, 2017b **D** This study.

Phylogeny and taxonomy of Chinese taxa

The classification of the Chinese unionid genera has been in a state of flux, different studies having placed the same genus in different subfamilies. For example, based on the presence or absence of the glochidial hooks and the type of marsupium, Wu et al. (1999a) divided the genus *Lamproula sensu lato* Simpson, 1900 into *Lamprotula sensu stricto* and *Aculamprotula* Wu, Liang, Wang & Ouyang, 1999. This distinction was later confirmed by molecular data (Zhou et al. 2007; Pfeiffer and Graf 2013; Wu et al. 2018b), but the classification of *Lamprotula* has also been disputed. Our results do not support the taxonomy of Huang et al. (2002), Zhou et al. (2007) and Ouyang et al. (2011; 2015) that placed *Lamprotula* sensu stricto in the Ambleminae. Our phylogenetic analyses instead confirm the results of Pfeiffer and Graf (2013), Lopes-Lima et al.

(2017a), Bolotov et al. (2017a; b) and Wu et al. (2018b) that *Lamprotula* is a member of the Gonideninae. The classification of the genus *Sinohyriopsis* has also been unstable. The shape of the glochidia of *Sinohyriopsis cumingii* (Lea, 1852) is semi-elliptical and unhooked, and resembles the typical morphology of glochidia in the Gonideninae (Wu et al. 2018a). But the marsupium of *S. cumingii* is restricted to the outer two demibranchs of the gills (ectobranchous), whereas in other species in the Gonideninae (*Lamprotula leaii* (Griffith & Pidgeon, 1833) *Solenaia carinatus* (Heude, 1877) and *Solenaia oleivora* (Heude, 1877)) the marsupium includes all four demibranchs (tetragenous) (Wu et al. 2018a). Therefore, based on anatomical features alone, the classification of the *Sinohyriopsis* in the Gonideninae has always been in doubt. Prior phylogenetic analyses based on one or two mitochondrial molecular markers (Huang et al. 2002; Zhou et al. 2007; Ouyang et al. 2011; 2015) placed *Sinohyriopsis* in the Ambleminae, However, our results indicate that *Sinohyriopsis* should be placed in the Gonideninae, confirming the conclusions of Lopes-Lima et al. (2017a) and Bolotov et al. (2017a, b). The placement of *Aculamprotula* has not been as controversial and our results place it in the Unioninae.

The genus *Lepidodesma* Simpson, 1896 is endemic to China and *Lepidodesma languilati* (Heude, 1874) is the type species. The juvenile of this species is thin and fragile, and the adult shell is robust. In addition, adults lack pseudocardinal teeth, but possess lateral teeth and the glochidia are triangular and have hooks. The breeding period is from February to August, and the type of marsupium is ectobranchous (Wu et al. 2018a). These characteristics are similar to species in the subfamily Unioninae and Anodontinae. Other characters, such as the size of the glochidia, which is large, and the tripartite water tubes (Wu et al. 2018a), indicate an affinity with the subfamily Anodontinae. The classification of *Lepidodesma* has alternated between these two subfamilies with some (Simpson 1900, Huang et al. 2002, Graf and Cummings 2007, Zhou et al. 2016) placing it in the Unioninae, and others (Haas 1969, Liu et al. 1979, Prozorova et al. 2005) in the Anodontinae. The results of our study indicate a novel result in which *L. languilati* is place in neither of these subfamilies, but is sister to a clade that includes both the Unioninae and Anodontinae. The robust branch support values indicate that *L. languilati* is not a member of either subfamily, but is instead a member of another, as yet unrecognized clade or perhaps is the remnant of a once larger more diverse group. Owing to the lack of available mitochondrial genomes for representatives of the Rectidentinae, Parreysiinae, and Pseudodontinae, our study did not include these subfamilies, and we recognize that their inclusion could produce a different set of relationships.

Due to the emphasis on the morphological characteristics of the shell, malacologists have consistently supported including both *Arconaia* and *Lanceolaria* in the Unioninae (Haas 1969; Liu 1979; Graf and Cummings 2007). The shells of *Arconaia* and *Lanceolaria* are thick and have distinct hinge teeth, and the morphology of the glochidia (triangular; hooked) and type of marsupium (ectobranchous) are similar to species of the subfamily Unioninae and Anodontinae (Wu et al. 2018a). The phylogenetic relationships inferred by different molecular markers, seem to confirm the phylogenetic position of these genera in the Unioninae (Huang et al. 2002; Zhou et al. 2007; Ouyang et al. 2015). However, the above-mentioned phylogenetic analyses included a limited number of taxa, and several key nodes in the phylogeny had low branch support. The results of the current study support the placement of *Arconaia* and *Lanceolaria* in the Anodontinae, confirming the results of Lopes-Lima et al. (2017a) and Wu et al. (2018b).

The genus *Acuticosta* was erected by Simpson and *Acuticosta chinensis* (Lea, 1868) was designated as the type species. Based on the marsupium, anatomy, larvae type and umbo sculpture, Simpson (1900) placed this genus in the Hyriinae. Subsequently, Chinese malacologists (Liu et al. 1979) re-classified the genus as a member of the Unioninae based on the presence or absence of hinge teeth. Prozorova et al. (2005) in a review of the bivalves in the Yangtze River drainage, placed the genus in Acuticostinae, although Graf and Cummings (2007) still maintained *Acuticosta* in the Unioninae. Molecular genetic analyses of a variety of markers by Huang et al. (2002), Zhou et al. (2007), and Ouyang et al. (2011; 2015) all indicated that *A. chinensis* was a member of the Unioninae. However, the limited taxon sampling and low branch support values in molecular phylogenetic analyses have allowed questions concerning the true affinities of *Acuticosta* to persist (Pfeiffer and Graf 2013; Huang et al. 2013; Lopes-Lima et al. 2017). Recently, Wu et al. (2018b) indicated that *A. chinensis* is a member of the Anodontinae based on mitochondrial DNA sequences of two genes. The current analysis of mitochondrial genomes provides further support for the placement of *Acuticosta* in the Anodontinae and indicates affinity of *Acuticosta* to the genera *Arconaia* and *Lanceolaria*.

Endangered status and conservation implications

China is a vast territory with a huge number of lakes and rivers. As a result, it is one of the most species-rich regions in the world (Zieritiz et al. 2017; Cai et al. 2018). However, in recent decades, freshwater mussels in China have declined drastically, and species diversity has been seriously threatened. At present, 40 species of Chinese unionids are included in the 2018 IUCN Red List, although 32 of these are categorized as data deficient or least concern. In addition, nearly half of the species included had not been evaluated. At present, advancing urbanization in the Yangtze River Basin, increasingly threatens the habitat of freshwater mussels, and conservation and management efforts targeting freshwater taxa are urgently needed.

Understanding of the phylogenetic diversity of freshwater mussels has important significance for determining the priority conservation strategies of species (Lopes-Lima et al. 2017b, 2018). This study provides support for the classification of a number of Chinese species, and lays the foundation for the future development of a more comprehensive phylogenetic based classification for freshwater unionids in China. Accurate taxonomic placement of rare and understudied species is central to many aspects of conservation as important biological characteristics (e.g., habitat preferences, reproductive traits) can be inferred from closely related taxa. Future research on Chinese unionids should focus on species delimitation and classification. In addition, more research is needed on understanding the basic ecology of Chinese mussels including species distributions, habitat preferences, and host fish identification.

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Supplementary material 1

Supplementary Tables S1, S2

Authors: Rui-Wen Wu, Xiong-Jun Liu, Sa Wang, Kevin J. Roe, Shan Ouyang, Xiao-Ping Wu

Data type: molecular data

Explanation note: Table S1: Partitioning strategies from PartitionFinder for mt genome dataset; Table S2: Partitioning strategies from ModelFinder for mt genome dataset.

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RESEARCH ARTICLE

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A new species and range extensions for three other species of pebblesnails (Lithoglyphidae, *Fluminicola*) from the upper Klamath basin, California–Oregon

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Abstract

This is the fifth in a recent series of papers on the poorly known western North American pebblesnail genus *Fluminicola* (Caenogastropoda, Lithoglyphidae). Herein we clarify the taxonomic status of the currently undescribed pebblesnail fauna in the upper Klamath River drainage (UKL) based on morphologic evidence, and mitochondrial DNA sequence data from 58 UKL collection localities. We describe one new species (*F. klamathensis*) from eight UKL localities which is differentiated by mtDNA sequences and unique penial morphology, and document range extensions to the UKL for three species from closely proximal drainages (*F. fresti*, *F. modoci*, *F. multifarius*). *Fluminicola fresti* was found at a single locality along the western edge of upper Klamath Lake. *Fluminicola modoci* and *F. multifarius* are widely distributed in the UKL; both species exhibit marked morphologic variation yet are relatively little differentiated genetically in this basin.

Keywords

Caenogastropoda, freshwater, mitochondrial DNA, morphology, Pacific Northwest, systematics, Truncatelloidea

Introduction

This is the fifth in a recent series of papers on the freshwater pebblesnails (Lithoglyphidae: *Fluminicola*) of the Pacific Northwest, USA. The previous contributions in this series, which treated the faunas in the Rogue–Umpqua (Hershler et al. 2017), upper Sacramento River (Hershler et al. 2007), and Snake River watersheds (Hershler and Liu 2012; Liu et al. 2013), increased the number of *Fluminicola* species from 9 to 27 and also documented large range extensions for *F. coloradoense* Morrison, 1940 and *F. multifarius* Hershler, Liu, Frest & Johannes, 2007.

The pebblesnails in the upper Klamath River drainage (UKL), California–Oregon, have been little studied historically and are currently unassigned to species (Hershler and Frest 1996). In various contract reports documenting their extensive field surveys of UKL freshwater mollusks, Frest and Johannes (1998, 2000, 2004, 2005, and other references cited therein) recognized 24 purportedly undescribed, narrowly ranging pebblesnail species from this watershed (based largely on shells and body pigmentation) and gave them provisional scientific (e.g., "*Fluminicola* n. sp. 1") and colloquial names. Although the UKL pebblesnails have subsequently become a focus of conservation attention–e.g, four of the putative novelties recognized by Frest and Johannes were incorporated into the Northwest Forest Plan as "survey and management species" (USDA and USDI 1994; also see Frest and Johannes 1999) and three of these were petitioned (unsuccessfully) for addition to the Federal List of Threatened and Endangered Species (Curry et al. 2008; USFWS 2012)–there have been no recently published studies of this fauna aside from a molecular phylogenetic analysis which delineated a close relationship between specimens from the Link River (the outlet of Upper Klamath lake) and *F. modoci* Hannibal, 1912 from the Goose Lake basin (Hershler et al. 2007). Herein we utilize both molecular (mitochondrial DNA sequences) and morphological data to delimit the UKL pebblesnail species. The former has proved very useful in previous taxonomic studies of pebblesnails, enabling delineation of both morphologically cryptic, and morphologically variable species (Hershler et al. 2007, 2017; Liu et al. 2013).

Methods

For this project we sequenced specimens from 58 UKL localities that were sampled in August 2012 and May and September 2013. Collections were made at three localities (Harriman Springs, Wood River south spring source, spring brook below Schoolhouse Meadow) on more than one occasion in an effort to increase sample sizes. Specimens were collected by hand or with a small sieve and preserved in 90% (non-denatured) ethanol in the field. Portions of several samples were relaxed with menthol crystals, fixed in dilute formalin, and preserved in 70% ethanol for anatomical study. Vouchers were deposited in the Smithsonian Institution's National Museum of Natural History (USNM) collection.

Some of the collections contained multiple shell morphotypes which were sorted and analyzed separately, yielding a total of 80 samples (UKL12–UKL91). Cytochrome c oxidase subunit I (COI) and cytochrome B (cytB) sequences were obtained from 283 and 259 UKL specimens, respectively. Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin 1992); each specimen was analyzed for mtDNA individually. LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify a 709 base pair (bp) fragment of COI; cytB427F (5'TGA GGK GCN ACT GTT ATT ACT AA3') and cytB1049R (5'GTG AAA ACT TGS CCR ATT TGC TC3') were used to amplify a 644 bp fragment of the cytB gene. The cytB427F and cytB1049R primers were designed based on conserved regions of cytB in an alignment using previously published sequences from *Oncomelania hupensis* (Gredler) (NC13073) and *Potamopyrgus antipodarum* (Gray) (GQ996433). Amplification conditions and sequencing of amplified polymerase chain reaction product methods were those of Liu et al. (2013). Sequences were determined for both strands and then edited and aligned using SEQUENCHER™ version 5.4.1 (Gene Codes Corporation, Ann Arbor, MI). In order to generate easily readable topologies, one example of each unique UKL haplotype was used in the phylogenetic analyses, which were performed separately for the COI and cytB datasets. The analyses of the COI dataset also included the previously published UKL haplotypes (from a single collection locality), and sequences from 14 regional *Fluminicola* species and representatives of two other North American lithogyphid genera (*Somatogyrus*, *Taylorconcha*). Trees were rooted with *Pristinicola hemphilli* (Pilsbry) (Hydrobiidae). The cytB dataset also included sequences from 13 *Fluminicola* species (a cytB sequence is not available for *F. gustafsoni* Hershler & Liu). Given that cytB sequences are not available for other North American lithoglyphid genera, basally positioned *F. virens* was used to root the trees based on this dataset (Hershler et al. 2007; Hershler and Liu 2012). Sample codes, locality and voucher details, and GenBank accession numbers for the sequences used in the molecular phylogenetic analyses are in Suppl. material 1.

Genetic distances were calculated using MEGA7 (Kumar et al. 2016), with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of missing data. MRMODELTEST v. 2.3 (Nylander 2004) selected the GTR + I + G model parameters as the best fit for both the COI and cytB datasets (using the Akaike Information Criterion). Phylogenetic analyses were performed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods; trees were also generated using a distance method. The MP and ML analyses were performed using PAUP* v. 4.0b10 (Swofford 2003) and the BI analyses were conducted using MRBAYES v. 3.2.6 (Ronquist et al. 2012). The MP analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 100 random additions. Nodal support was evaluated by 10,000 bootstrap replicates. The ML analyses were performed using the GTR + I + G model. The optimized parameter values for COI were base frequencies of A = 0.3089, T = 0.3856, C = 0.1684, G = 0.1371; shape of gamma distribution = 1.1801; proportion of invariant sites = 0.5691. The optimized parameter values for cytB were base frequencies of A = 0.3146, T = 0.3671, C = 0.1917, G = 0.1268; shape of gamma distribution = 0.7706; proportion of invariant sites = 0.3818. A GTR distance-based neighbor-joining (NJ) tree was used as the initial topology for branch-swapping. Nodal support was evaluated by 1,000 bootstrap pseudo-replicates. For the BI analyses Metropolis-coupled Markov chain Monte Carlo simulations were run with four chains (using the model selected by MRMODELTEST) for 5,000,000 generations. Markov chains were sampled at intervals of 100 generations to obtain 50,000 sample points. We used the default settings for the priors on topologies and the GTR + I + G model parameters. At the end of the analyses, the average standard deviations of split frequencies were 0.005763 (COI dataset) and 0.004997 (cytB dataset) and the potential scale reduction factor (PSRF) was 1, indicating that the runs had reached convergence. The sampled trees with branch lengths were used to generate 50% majority rule consensus trees, with the first 25% of the samples removed to ensure that the chain sampled a stationary portion. For the distance analyses, HKY distances were used to generate neighbor-joining (NJ) trees (Saitou and Nei 1987).

We also studied pertinent specimens in the USNM collection, including UKL material collected by Frest and Johannes that was acquired during the planning stage of this project. The total number of shell whorls (WH) was counted for each specimen; and the height and width of the entire shell (SH, SW), body whorl (HBW, WBW), and aperture (AH, AW) were measured from camera lucida outline drawings (Hershler 1989). Photographs of alcohol preserved specimens (that had been anaesthetized with menthol crystals prior to fixation) were taken using a Coolpix 990 mounted on an Olympus SZX12 dissecting microscope. Other methods of morphological study were routine (Hershler et al. 2007). Shell descriptive statistics were generated using SYSTAT FOR WINDOWS 11.01 (SSI 2004).

Results

Ninety-four (94) COI and 96 cytB haplotypes were detected in the analyzed UKL specimens (Suppl. materials 2, 3, respectively). The molecular phylogenetic and distance analyses of the two datasets generated closely similar trees in which the UKL haplotypes were resolved into four clades. Three of the clades were strongly supported (>95% bootstrap or posterior probability) in all analyses, while the fourth (clade A) was strongly supported only in the cytB BI analysis. The BI topology based on the COI sequences is shown in Figure 1 and the geographic distributions of the four clades are shown in Figure 2. Pairs of the lineages were sympatric at eight localities and three of the lineages co-occurred at one site (Wood River south headsprings).

Clade C is composed of pebblesnails from eight UKL localities. This lineage is well differentiated genetically from currently recognized *Fluminicola* species (7.6–17.2% for COI, 6.4–20.3% for cytB) and is further distinguished by unique penial morphology; we describe it as a new species below. The phylogenetic relationships of this new species were not well resolved.

Clades A, B and D contain both UKL pebblesnails and currently recognized species from other regional drainages (Figs 1, 2). Clade A contains *F. multifarius* and a large number of UKL populations varying in shell size and shape (Fig. 3). Although most of

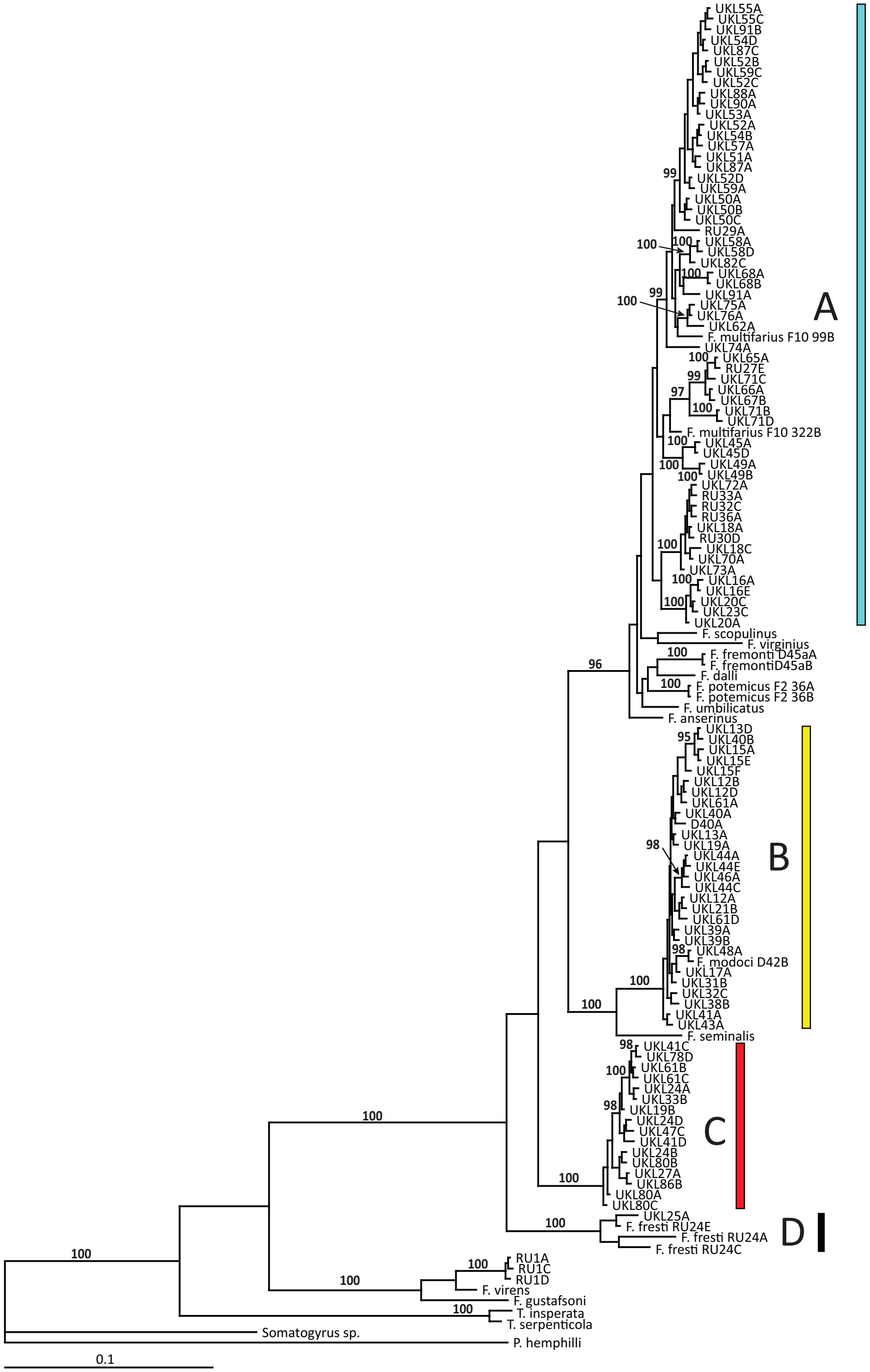

Figure 1. Bayesian tree based on the COI dataset. The four clades (**A–D**) containing UKL haplotypes are color coded as in Figure 2. Posterior probabilities for nodes are shown when ≥ 95%. Specimen codes are from Suppl. materials 1–3.

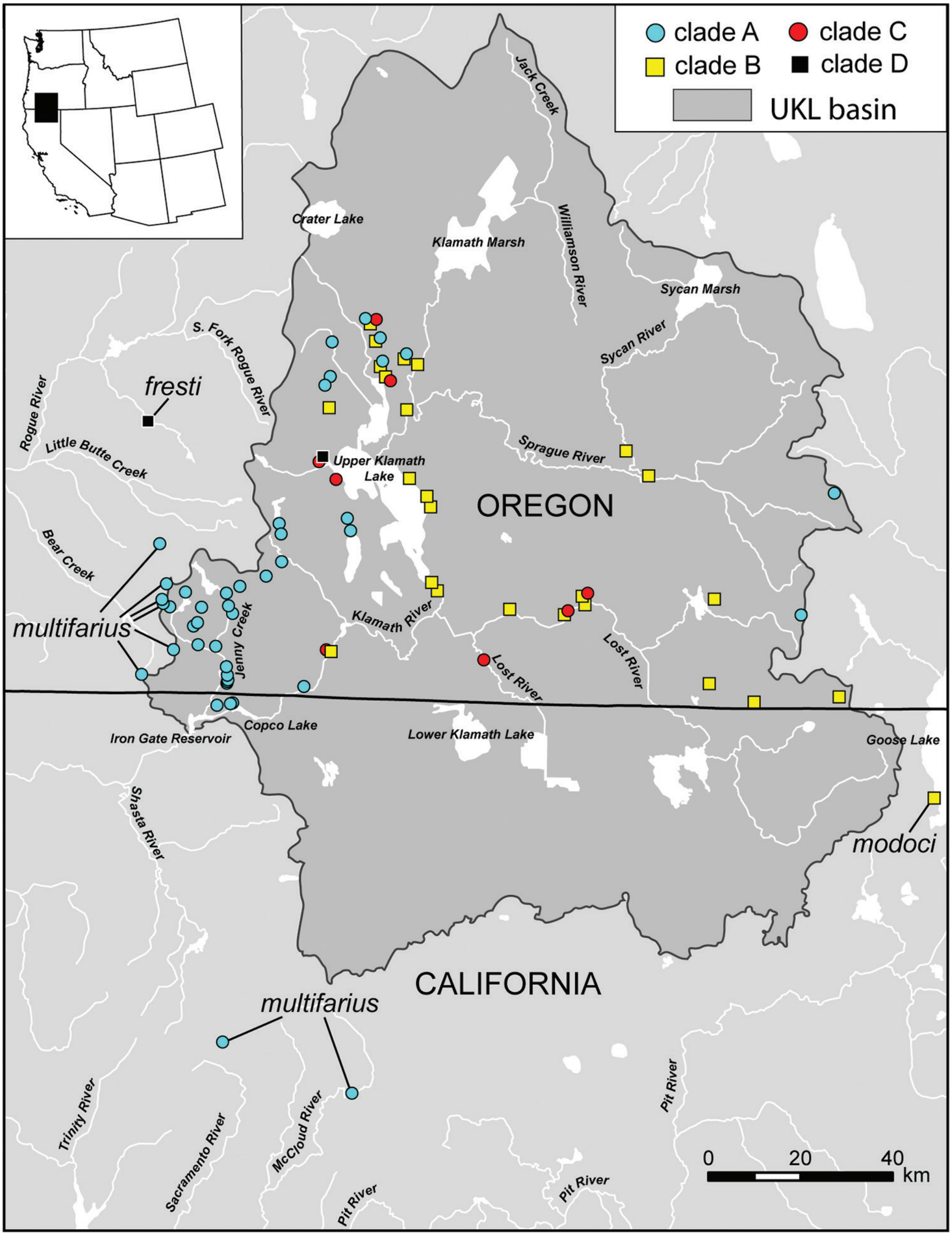

Figure 2. Map of southwest Oregon and northwest California showing the distribution of mtDNA clades **A–D** with color codes matching those in Figure 1.

the UKL pebblesnails in this clade have subglobose to narrowly conical shells conforming to *F. multifarius*, populations in the Jenny Creek drainage often contain additional forms that fall outside of the range of variation previously reported for this species (e.g., Fig. 3F, shell neritiform; Fig. 3H, shell having a distinct swelling on the inner aper-

Figure 3. Shells of UKL *F. multifarius* **A** USNM 1144951 **B** USNM 1144587 **C** USNM 1190091 **D, E** USNM 1020970 **F, G** USNM 1145066 **H** USNM 1144903 **I** USNM 1190128 **J** USNM 1190104 **K** USNM 1144463. Scale bar: 1.0 mm.

tural lip). Clade B contains *F. modoci* and UKL pebblesnails that well conform to this species aside from having a much larger range in shell size among populations (Fig. 4A–G). Clade D is composed of *F. fresti* Hershler, Liu & Hubbart, 2017 and pebblesnails from Harriman Springs that closely resemble this species in their shells (Fig. 4H).

The sequence divergence between the UKL pebblesnails and the extra-limital species in clades A, B, and D is <4% for both genes (Table 1), which is relatively small compared

Table 1. Sequence divergence within and between the extra-limital and UKL components of clades A, B, and D. Values are mean ± standard deviation.

	COI		cytB	
Clade A	UKL	*F. multifarius*	UKL	*F. multifarius*
UKL	2.2 ± 0.3%		2.9 ± 0.4	
F. multifarius	2.3 ± 0.3%	2.0 ± 0.4%	3.3 ± 0.5	3.1 ± 0.5
Clade B	UKL	*F. modoci* (D42)	UKL	*F. modoci* (D42)
UKL	0.4 ± 0.1%		0.4 ± 0.1%	
F. modoci (D42)	1.0 ± 0.3%	–	1.2 ± 0.5%	–
Clade D	UKL25A	*F. fresti*	UKL	*F. fresti*
UKL25A	–		–	
F. fresti	3.8 ± 0.7%	2.2 ± 0.3%	3.9 ± 0.6%	2.2 ± 0.3%

UKL25A consists of a single sequenced specimen.

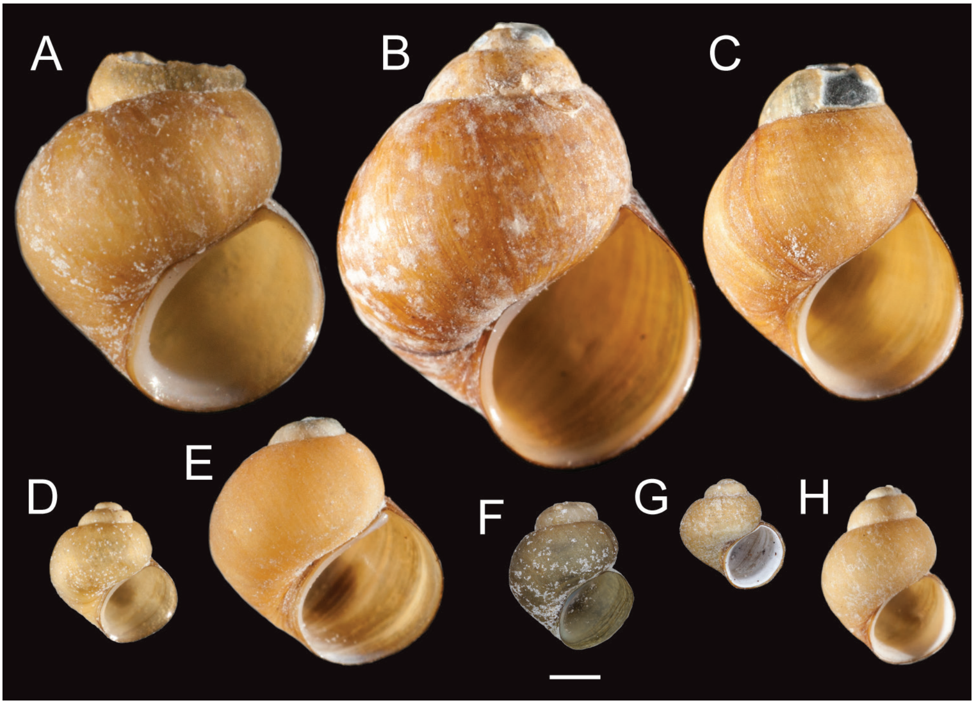

Figure 4. Shells of UKL *F. modoci* (**A–G**) and *F. fresti* (**H**) **A** USNM 1144454 **B** USNM 1144520 **C** USNM 1144942 **D** USNM 1144407 **E** USNM 1144966 **F** USNM 1190096 **G** USNM 1144565 **H** USNM 1144900. Scale bar: 1.0 mm.

to that among currently recognized *Fluminicola* species, which ranges from 1.7–18.7% for COI and 1.4–25.7% for cytB, with 93% (301/325) and 96% (288/300) of the pairwise comparisons >4%, respectively. The morphologically diverse UKL pebblesnails in both clades A and B are also relatively little differentiated genetically (clade A, mean sequence divergence 2.2% for COI and 2.9% for cytB; clade B, 0.4% for both genes). (Note that clade B was shallowly structured in all the trees while clade A was somewhat structured only in the BI topologies.) We also found that the pronounced phenotypic variation within populations (belonging to clade A) in the Jenny Creek drainage is not accompanied by substantial genetic divergence. For example, specimens having neritiform, simple conical, and conical shells with a swelling on the inner apertural lip that were collected in sympatry at the Fall Creek locality (UKL 50–52) differ by only 0.6–0.7% for COI, and 0%–0.5% for cytB. Based on this body of evidence we recognize clades A, B and D as single species corresponding to *F. multifarius*, *F. modoci*, and *F. fresti*.

Taxonomic treatments

Family Lithoglyphidae Troschel, 1857
Genus *Fluminicola* Carpenter, 1864

Fluminicola klamathensis Liu & Hershler, sp. n.

http://zoobank.org/CC65E345-A320-4B5D-A267-EAF676F7B950
Figures 5, 6, 7, 8A

Types. Holotype, USNM 1144499 (a cleaned shell), Lost River at Stukel Bridge, Klamath County, Oregon, 42.0825N, 121.6617W, 10/5/1997, Terrence J. Frest and Edward J. Johannes. Paratypes, USNM 1468970 (a large series of dry shells and alcohol-preserved specimens), from same lot.

Referred material. OREGON. *Klamath County*: USNM 1207966, Lost River at Stukel Bridge, USNM 1144894, USNM 1469075, USNM 1469082, USNM 1469090, Wood River, south spring source (42.7372N, 121.9775W), USNM 1469072, USNM 1469077, USNM 1469078, USNM 1469080, Tecumseh Spring (42.6424N, 121.9432W), USNM 1144346, USNM 1190088, Camporee Spring (42.4308N, 122.0614W), USNM 883517, USNM 1144348, USNM 1190089, USNM 1207965, USNM 1225874, Harriman Spring, outflow of main spring (42.4673N, 122.1009W), USNM 1469076, USNM 1469086, Big Springs at Bonanza (42.1982N, 121.4004W), USNM 1469074, USNM 1469088, Lost River, below Harpold Dam (42.1702N, 121.4530W).

Diagnosis. A large *Fluminicola* (shell height, 6.5–8.4 mm) with a subglobose to ovate-conic shell often having an eroded spire. *Fluminicola klamathensis* is readily distinguished by its penis, which does not gently taper along its length as in other congeners, but instead abruptly narrows distally and has a well demarcated, short filament. This new species is further differentiated from closely similar and frequently sympatric *F. modoci* in its generally darker colored shell periostracum, broad central cusps on the central and lateral radula teeth, very short outer wing of the lateral teeth, small number of cusps on the inner and outer marginal teeth, light pigment on the dorsal surface of the penis, large core of internal dark pigment in the distal section of the penis, and the fairly large seminal receptacle.

Description. Shell (Fig. 5A–F) subglobose to ovate-conic, spire often eroded in large part, whorls (in specimens having a complete spire), 4.25–4.5. Teleoconch whorls

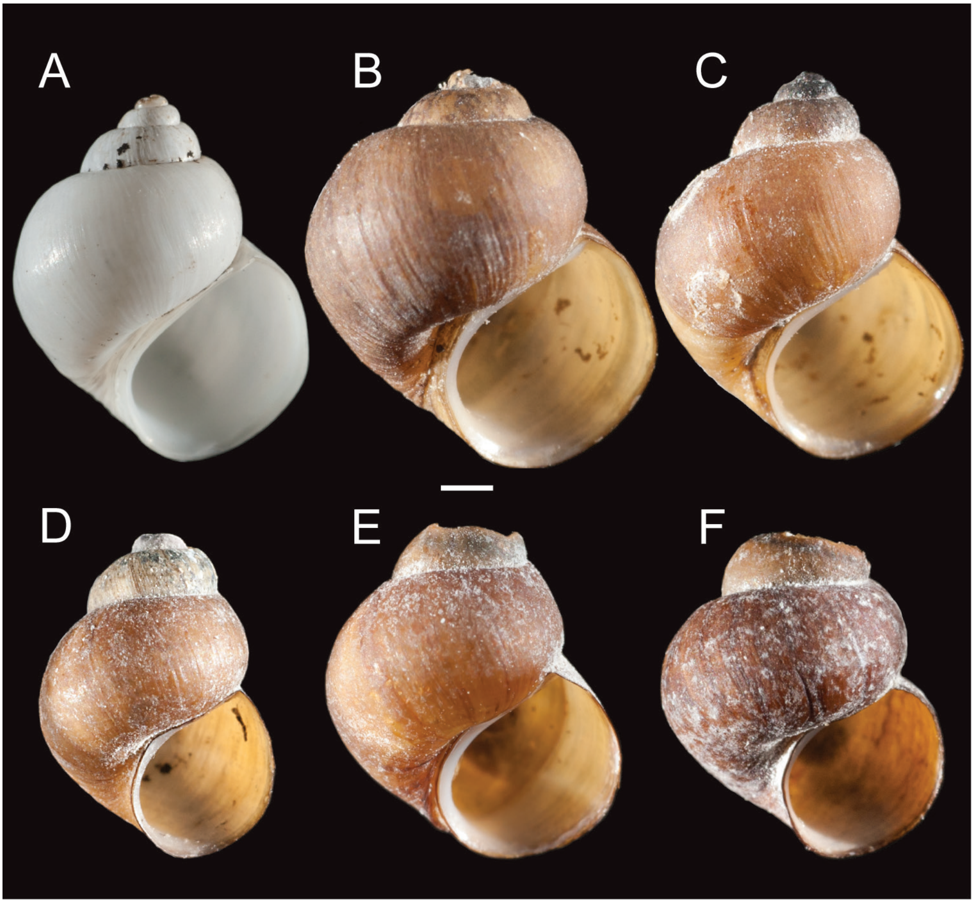

Figure 5. Shells of *F. klamathensis*, sp. n. **A** Holotype, USNM 144499 **B, C** USNM 1469076 **D** USNM 1469078 **E** USNM 1144346 **F** USNM 1144894. Scale bar: 1.0 mm.

low to medium convex, sometimes weakly shouldered. Aperture pyriform; inner lip complete, variably thickened, broadly adnate to parietal wall; columellar shelf narrow or extending over much of umbilical region. Outer lip thin, prosocline. Umbilicus absent or a narrow slit, umbilical region sometimes excavated. Shell white, periostracum brown, fairly thick, sometimes covered with black deposits. Shell measurements and whorl count data are summarized in Table 2.

Operculum (Fig. 6A–C) as for genus; muscle attachment margin little thickened on inner side; rim sometimes present on inner side near outer edge (Fig. 6B). Radula (Fig. 6D–H) as for genus; dorsal edge of central teeth concave, lateral cusps 2–5, hoe-shaped; basal cusp 1–5. Lateral teeth having 2–4 cusps on inner side and 3–5 cusps on outer side; outer wing slightly longer than length of cutting edge. Inner marginal teeth with 11–19 cusps, outer marginal teeth with 11–22 cusps. Radula data are from USNM 144346, USNM 1468970.

Table 2. Shell parameters for *F. klamathensis*. Measurements are in mm.

	WH	SH	SW	HBW	WBW	AH	AW
			Holotype, USNM 11444996				
	4.50	7.07	5.43	5.78	4.32	3.93	3.43
			Paratypes, USNM 1468970 (*N* = 5)				
Mean	4.40	6.68	5.53	5.53	4.27	3.94	3.38
S.D.	0.14	0.52	0.48	0.36	0.30	0.38	0.25
Range	4.25–4.50	6.10–7.12	4.93–6.10	5.12–5.81	3.93–4.58	3.51–4.39	3.06–3.68

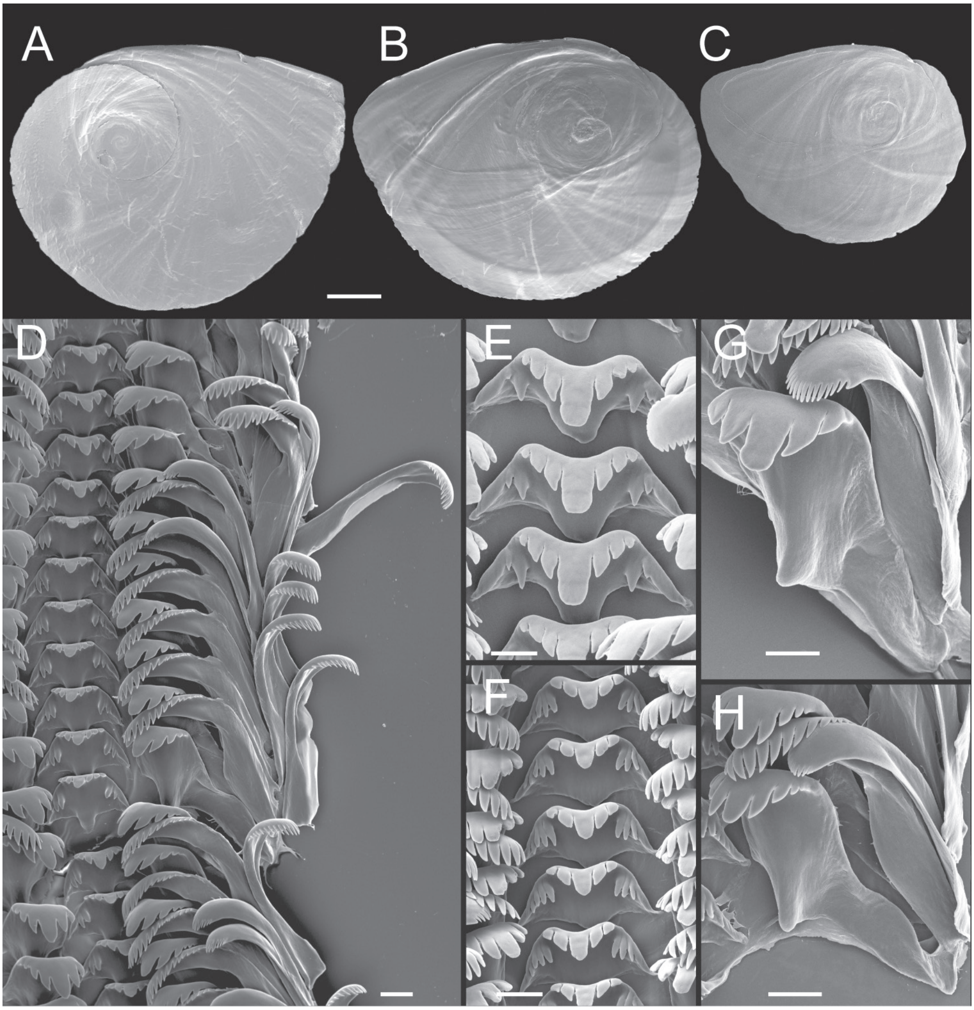

Figure 6. Opercula and radula, *F. klamathensis*, sp. n. **A, B** Opercula (outer, inner sides), USNM 1468970 **C** Operculum (inner side), USNM 144348 **D** Portion of radular ribbon, USNM 1468970 **E, F** Central teeth, USNM 144346, USNM 1144348 **G, H** Lateral teeth, USNM 1144346, USNM 1144348. Scale bars: 500 µm (**A–C**); 20 µm (**D–H**).

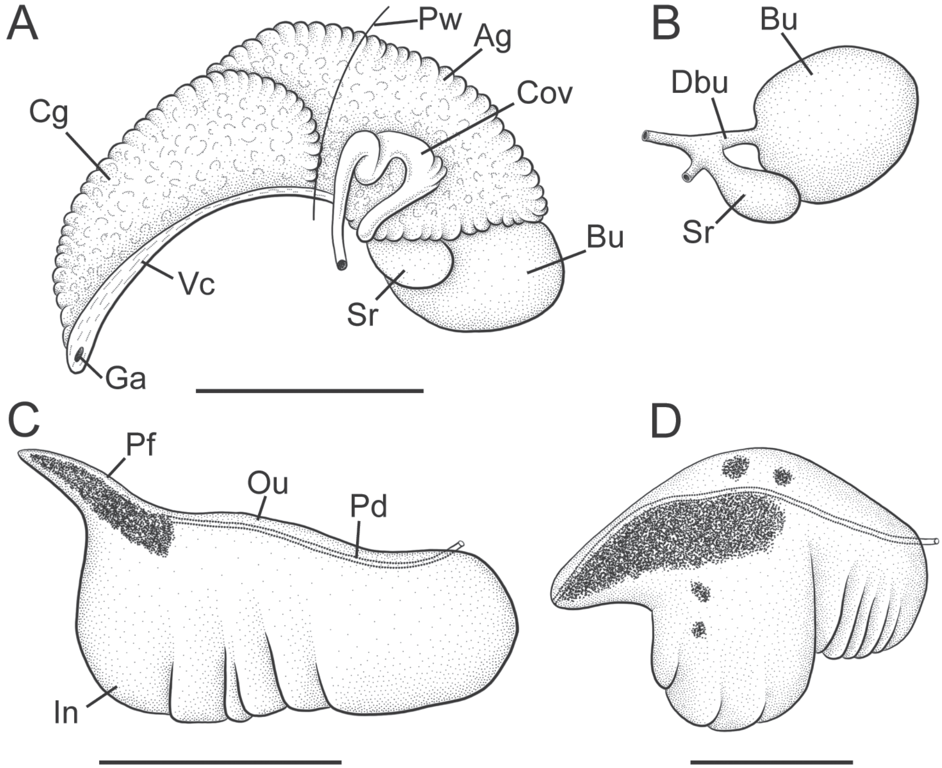

Figure 7. Reproductive anatomy, *F. klamathensis* sp. n. **A** Female glandular oviduct and associated structures (viewed from left side), USNM 1225874 **B** Bursa copulatrix and seminal receptacle, USNM 1225874 **C, D** Penis (dorsal surface), USNM 1225874, USNM 1469090. **Ag** albumen gland **Bu** bursa copulatrix **Cg** capsule gland **Cov** coiled oviduct **Dsr** seminal receptacle duct **Ga** genital aperture **In** inner edge of penis **Ou** outer edge of penis **Pd** penial duct **Pf** penial filament **Pw** posterior wall of pallial cavity **Sr** seminal receptacle **Vc** ventral channel of capsule gland. Scale bars: 1.0 mm (**A–C**); 500 µm (**D**).

Snout, cephalic tentacles grey or black, pigment light around eyespots; pallial roof, visceral coil usually light brown; foot variably pigmented dorsally, sole pale. Ctenidial filaments 33–36 (*N* = 5), broadly triangular. Glandular oviduct and associated structures shown in Figure 7A, B. Coiled oviduct circular, anterior arm kinked, posterior arm sometimes having small accessory pouches containing sperm. Bursa copulatrix large, ovate or globular, partly overlapped by albumen gland. Bursal duct narrow, much shorter than bursa. Seminal receptacle medium-sized, pouch-like, partly overlapped by albumen gland. Albumen gland having small pallial component. Capsule gland slightly shorter than albumen gland. Genital aperture a small, terminal pore. Penis (Figs 7C, D, 8A) large, base rectangular, often having a distinct, lobe-like swelling along inner edge distally (Fig. 7D); distal end of penis blunt, with short, narrow, filament. Distal section of penis having dense core of internal black pigment; dorsal surface pale or lightly pigmented. Penial duct near inner edge, narrow, nearly straight.

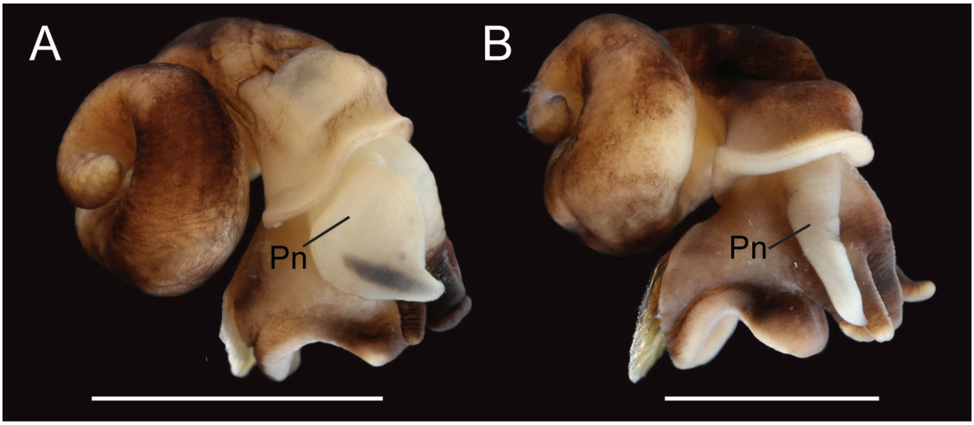

Figure 8. Photographs of ethanol preserved, relaxed specimens of *F. klamathensis* sp. n. (**A**) and *F. modoci* (**B**) from the Wood River south headspring, showing the differences in penial morphology **A** USNM 1469072 **B** USNM 1144736. **Pn** penis. Scale bar: 200 µm.

Etymology. The species name is an adjectival geographic epithet referring to the distribution of this pebblesnail in the (upper) Klamath River basin.

Distribution. Large, spring-influenced habitats in the UKL.

Remarks. We selected the Lost River at Stukel Bridge as the type locality because the pebblesnails in this population do not have apically eroded shells. However, we did not have suitably relaxed material from this locality for anatomical study and thus used specimens from Harriman Springs for this purpose.

The shells of *F. klamathensis* and *F. modoci* can be difficult to distinguish although the former usually has darker, thicker periostracum, and tends to be larger when found in sympatry. As noted above, these two species are most readily differentiated by the shape and pigmentation of the penis (Fig. 8).

Sixteen (16) COI and 13 cytB haplotypes were detected in *F. klamathensis* (Suppl. materials 2, 3, respectively).

The "Tall pebblesnail" (also referred to as *Fluminicola* n. sp. 2) that was recognized by Frest and Johannes in their UKL contract reports (also see Frest and Johannes 1999) and subsequently included in the Northwest Forest Plan as a Survey and Manage species, corresponds to *F. klamathensis*.

Fluminicola fresti Hershler, Liu, Frest & Hubbart, 2017

Figure 4H

Fluminicola fresti–Hershler et al. 2017: 10–14, figs 4E–G, 5 (Diversion from Big Butte Springs through Butte Falls Hatchery, just south of Butte Falls–Fish Lake Road (Jackson County 321) and 0.16 km west of Butte Falls–Prospect Road (Jackson County 922), Jackson County, Oregon, 42.5389N, 122.5551W).

Distribution. North Fork Umpqua River drainage and Rogue River basin north of Little Butte Creek, Oregon (Hershler et al. 2017).

Referred material. OREGON. *Klamath County*. USNM 1144900, USNM 1469091, Harriman Spring, outflow of main spring (42.4673N, 122.1009W),

Remarks. The Harriman Springs pebblesnails have small (shell height, about 4.0 mm), narrowly conical shells with convex whorls that well conform to *F. fresti*; they also closely resemble this species in details of radula morphology. Radula: central teeth with 3–4 lateral cusps, 1 basal cusp; lateral teeth with 3–4 cusps on outer side, 2 cusps on inner side; inner marginal teeth with 23–28 cusps; outer marginal teeth with 28–36 cusps(USNM 1144900).

We sequenced only a single specimen of *F. fresti* from Harriman Springs, which was collected during our first visit to this locality. Our subsequent collections from this site that were preserved in 90% ethanol for mtDNA analysis did not contain this species although a few specimens were found in one of the subsamples that had been prepared for anatomical study.

This new record extends the range of *F. fresti* about 26 km eastward from the Rogue River headwaters.

Fluminicola modoci Hannibal, 1912

Figures 4, 8B

Fluminicola modoci–Hannibal 1912: 187, pl. 8: fig. 30 (in part; Fletchers Spring, south end, Goose Lake, California).

Distribution. Several springs in the Goose Lake basin, California–Oregon (Hershler and Frest 1996; Hershler 1999; Hershler et al. 2007).

Referred material. OREGON. *Klamath County*. USNM 1190095, USNM 1207964, Wood River, south spring source (42.7372N, 121.9775W), USNM 1144333, USNM 1190094, Tecumseh Spring (42.6424N, 121.9432W), USNM 874187, USNM 874935, USNM 1144336, USNM 1144337, USNM 1144942, USNM 1190086, Barkley Spring (42.3822N, 121.8111W), USNM 1190102, Klamath River, east of Boyle Power Station (42.0934N, 122.0964W), USNM 1190087, Ouxy Spring (42.3989N, 121.8235W), USNM 1144390, USNM 1144923, USNM 1190100, Brown Spring (42.4951N, 121.2956W), USNM 1190093, Spring, Klamath Fish Hatchery (42.6519N, 121.9479W), USNM 1190096, Reservation Spring (42.7023N, 121.9629W), USNM 1144407, USNM 1190098, Spring, Williamson River campground (42.6584N, 121.8499W), USNM 1190097, Spring Creek, headspring (42.6690N, 121.8860W), USNM 1144411, USNM 1190090, Crystal Spring (42.5736N, 122.0823W), USNM 1144966, USNM 1190121, Big Springs at Bonanza (42.1982N, 121.4004W), USNM 1144418, USNM 1185800, Casebeer Spring, ouflow at Gerber Dam Road (42.2056N, 121.0592W), USNM 1144454, USNM 1190123, Duncan Spring, north complex (42.0416N, 121.0689W), USNM 1190123, Lost River at Big Springs City Park, Bonanza (42.1976N, 121.4002W), USNM 114469, USNM 1190124, Gwinn Spring Creek at Gwinn Spring Creek Road (42.0063N, 120.9545W), USNM 1020714, USNM 1144673, USNM 1144925, USNM 1154376, Link River under US97/OR140 bridge (42.2185N, 121.7892W). USNM 1190137, Link River, above Link River dam (42.2341N, 121.8036W), USNM 1144494, USNM 1144986, USNM 1207967, Lost River, Stevenson County Park (42.1831N, 121.5994W), USNM 1190139, Lost River, below Harpold Dam (42.1702N, 121.4530W), USNM 1144503, USNM 1190101, Sprague River at Beatty Gap (42.4476N, 121.2377W), USNM 1144515, USNM 1190099, Williamson River, Klamath County park (42.5705N, 121.8791W), USNM 1144520, USNM 1190140, Upper Klamath Lake, south of Modoc Point (42.4373N, 121.8672W). *Lake County*: USNM 1144565, USNM 1185798, Spring southeast of Slash Spring, Yocum Valley (42.0174N, 120.7316W).

Remarks. The UKL *F. modoci* range from 2.6–8.4 mm in shell height and include specimens with eroded spires that closely resemble the type material for this species (e.g., Fig. 4A) as well as individuals with fully intact, ovate-conic to trochoidal shells. Radula: central teeth with 2–6 lateral cusps, 1–4 basal cusps; lateral teeth with 3–7 cusps on outer side, 2–5 cusps on inner side; inner marginal teeth with 13–30 cusps; outer marginal teeth with 20–43 cusps (USNM 1144337, USNM 1144418, USNM 1144925).

The new records extend the range of *F. modoci* about 130 km westward from the northwestern portion of the Goose Lake basin.

Twenty-seven (27) COI and 31 cytB haplotypes were detected in the UKL specimens of *F. modoci* (Suppl. materials 2, 3, respectively).

The "Klamath pebblesnail" (also referred to as *Fluminicola* n. sp. 1) that was recognized by Frest and Johannes in their UKL contract reports (also see Frest and Johannes 1999) and subsequently included in the Northwest Forest Plan as a Survey and Manage species, may correspond to *F. modoci*.

Fluminicola multifarius Hershler, Liu, Frest & Johannes, 2007

Figures 3, 9

Fluminicola multifarius–Hershler et al. 2007: 415, 417, 419, figs 7M, 24, 25 (Big Springs (source) at Big Springs City Park northwest of the city of Mount Shasta, south of Spring Hill, Siskiyou County, California ([UTM zone 10] 556400 E, 4575265 N, 1092 m).

Distribution. Sacramento River headwater region, upper reaches of the McCloud and Rogue River drainages, California–Oregon (Hershler et al. 2007; Hershler et al. 2017).

Referred material. CALIFORNIA. *Siskiyou County*: USNM 1207974, Spring on Close Butte (41.9884N, 122.3229W), USNM 1190109, Spring northwest of Copco Reservoir (41.9873N, 122.3275W), USNM 1145066, USNM 1190108, Fall Creek above Copco Road bridge (41.9834N, 122.3623W). OREGON. *Jackson County*: USNM 1144324, USNM 1144325, USNM 1144326, USNM 1144898, USNM 1145117, USNM 1190128, USNM 1243229, USNM 1254453, Fredenburg Spring

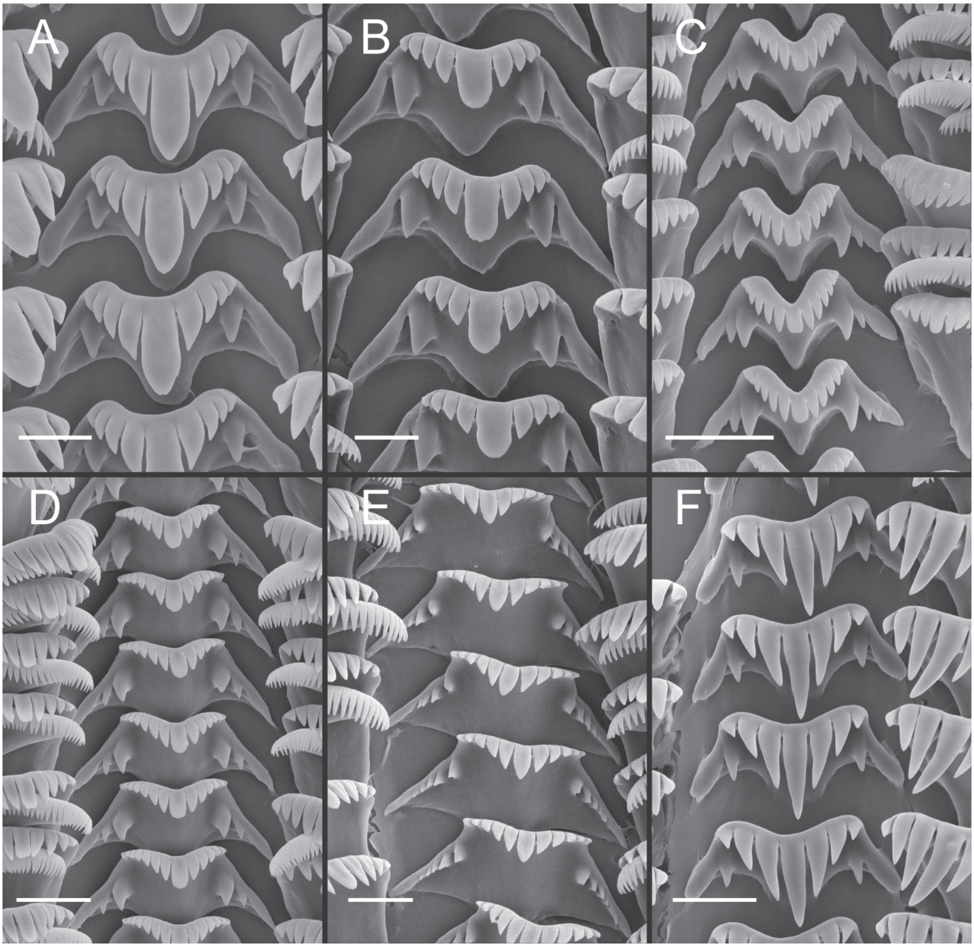

Figure 9. Variation in central radular teeth, UKL *F. multifarius* **A, C, E** USNM 1207968 **B** USNM 144951 **D** USNM 1144368 **F** Central teeth, USNM 1207970. Scale bar: 10 µm.

(42.1669N, 122.3268W), USNM 1207971, Spring Creek north of Schoolhouse Meadow (42.0357N, 122.3397W), USNM 1144903, USNM 1190105, USNM 1207969, Spring brook below Schoolhouse Meadow (42.0288N, 122.3374W), USNM 1144342, USNM 1144943, USNM 1144365, USNM 1144366, USNM 1144484, USNM 1190114, Keene Creek, east of bridge on Mill Creek Road (42.1046N, 122.4136W), USNM 1144368, USNM 1144718, USNM 1144946, USNM 1190103, Rattlesnake Spring (42.0625N, 122.3389W), USNM 1190104, Shoat Spring, source (42.0466N, 122.3360W), USNM 1207968, Shoat Springs, outflow near source (42.0456N, 122.3367W), USNM 1207970, Spring channel above Schoolhouse Meadow, adjacent to cabin ruins (42.0327N, 122.3379W), USNM 1144536, USNM 1144537, USNM 1144941, USNM 1144942, USNM 1190118, Spring north of Hyatt Reservoir (42.2064N, 122.4498W), USNM 1144540, USNM 1144541, USNM 1144907, USNM 1144993, USNM 1190131, Nameless Spring, outflow (42.2183N, 122.3087W), USNM 1190129, Bluejay Spring (42.1810N, 122.3368W), USNM

1144587, USNM 1190115, Spring, Chinquapin Mountain (42.1409N, 122.4268W), USNM 1190116, Spring along Beaver Creek Road (42.1467N, 122.4165W), USNM 1190117, Spring, Craine Prairie (42.1754N, 122.4086W), USNM 1190111, Spring north of Soda Spring (42.1025N, 122.3684W), USNM 1190119, Spring, west side of Burnt Creek Road (42.1761N, 122.4911W), USNM 1144655, USNM 1145079, USNM 1190130, Jenny Creek Spring (42.2034N, 122.3443W). *Klamath County*: USNM 1469081, USNM 1469089, Wood River, south spring source (42.7372N, 121.9775W), USNM 1190138, Spring west of Klamath River (42.0257N, 122.1351W), USNM 1144392, USNM 1144951, USNM 1190127, Tiger Lily Spring (42.6156N, 122.0935W), USNM 1144393, USNM 1190092, Four Mile Spring (42.6331N, 122.0778W), USNM 1469079, Spring, Klamath Fish Hatchery (42.6519N, 121.9479W), USNM 1469083, Reservation Spring (42.7023N, 121.9629W), USNM 1469084, Spring Creek, headspring (42.6690N, 121.8860W), USNM 1144414, USNM 1144965, USNM 1190091, Short Creek, headspring (42.7000N, 122.0776W), USNM 1144416, USNM 1207975, Rainbow Springs (42.3239N, 122.2040W), USNM 1190134, Cold Creek, south of Lake of the Woods (42.3434N, 122.2083W), USNM 1144463, USNM 1190133, Spring along western edge of Buck Lake (42.2670N, 122.1995W), USNM 1190132, Johnson Creek (42.2401N, 122.2399W), USNM 1144468, USNM 1190136, Spring (northernmost), Denny Creek (42.3552N, 122.0286W), USNM 1144887, USNM 1144984, USNM 1190135, Spring (southernmost), Denny Creek (42.3324N, 122.0221W). *Lake County*: USNM 1190125, Spring, Holmes Meadow (42.1761N, 120.8350W), USNM 1144564, USNM 1185799, Blonde Spring (42.4149N, 120.7467W).

Remarks. The UKL *F. multifarius* range from 2.1–5.1 mm in shell height and, as noted above, vary considerably in shell shape and appearance of the inner apertural lip between and sometimes within populations. There is also considerable variation in the number of cusps on the radular teeth; and the size and shape of the cusps and indentation of the dorsal edge of the central teeth (Fig. 9). Radula: central teeth with 2–6 lateral cusps, 1–6 basal cusps; lateral teeth with 3–7 cusps on outer side; 2–6 cusps on inner side; inner marginal teeth with 16–35 cusps; outer marginal teeth with 22–40 cusps (USNM 1144326, USNM 1144342, USNM 1144368, USNM 1144463, USNM 1144540, USNM 1144564, USNM 1144588, USNM 1144951, USNM 1145079, USNM 1207968, USNM 1207970, USNM 1207974).

Fifty-one (51) COI and 52 cytB haplotypes were detected in the UKL specimens of *F. multifarius* (Suppl. materials 2, 3, respectively).

The "Fredenburg pebblesnail" (also referred to as *Fluminicla* n. sp. 17) and "Klamath Rim pebblesnail" (also referred to as *Fluminicola* n. sp. 3), which were recognized by Frest and Johannes in their UKL contract reports (also see Frest and Johannes 1999) and subsequently included in the Northwest Forest Plan as Survey and Manage species, correspond to *F. multifarius*.

The new records detailed herein extend the range of *F. multifarius* about 150 km eastward from the Rogue River headwaters. Populations of this species are distributed in close proximity (ca 1 km) across the divide between the Rogue River and UKL basins (springs in Sampson Creek and Burnt Creek drainages, respectively).

Discussion

Our findings, based on both morphologic and genetic (mtDNA sequences) evidence, have shown that contrary to previous assertions in the grey literature (Frest and Johannes 1998, 2000, 2004, 2005), the UKL does not have a large, highly endemic fauna of undescribed pebblesnails, but instead contains only four species, one of which is new and three of which were previously described from other regional drainages. Although we only surveyed 58 of the >200 UKL pebblesnail localities reported by Frest and Johannes, we sampled at least one locality for each of the putatively new species that they recognized, and we sampled numerous localities in the Jenny Creek watershed where much of the phenotypic diversity of UKL pebblesnails is concentrated. Thus, we are confident that we have well delineated the taxonomic diversity of UKL pebblesnails. Additional studies will be needed to further delineate the distributions of the four species in the UKL and to determine whether these pebblesnails range into the lower reach of the large Klamath River watershed.

Our study also provides evidence of striking morphologic variation in *F. modoci* and *F. multifarius* similar to what has been observed in various marine caenogastropod lineages (e.g., *Littorina*; Reid 1996). Further investigations utilizing rapidly evolving nuclear markers such as microsatellites should provide additional insight into the apparent decoupling of morphologic and genetic variation in these two species and, together with ecological studies, help tease apart the underlying mechanisms for the sympatric occurrence of multiple *F. multifarius* morphotypes at various localities in the Jenny Creek area.

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Supplementary material 1

Table S1

Authors: Hsiu-Ping Liu, Robert Hershler

Data type: specimen data

Explanation note: Sample codes, USNM voucher numbers (for newly sequenced specimens), locality details, and GenBank accession numbers for COI and cytB sequences.

Link: https://doi.org/10.3897/zookeys.812.29205.suppl1

Supplementary material 2

Table S2

Authors: Hsiu-Ping Liu, Robert Hershler

Data type: molecular data

Explanation note: Distribution of COI haplotypes. Exemplars used in the phylogenetic analyses are in parentheses.

Link: https://doi.org/10.3897/zookeys.812.29205.suppl2

Supplementary material 3

Table S3

Authors: Hsiu-Ping Liu, Robert Hershler

Data type: molecular data

Explanation note: Distribution of cytB haplotypes. Exemplars used in the phylogenetic analyses are in parentheses.

Link: https://doi.org/10.3897/zookeys.812.29205.suppl3

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doi: 10.3897/zookeys.812.29764
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RESEARCH ARTICLE

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Discovery of a new species of the genus *Stygepactophanes* from a groundwater-fed spring in southern France (Crustacea, Copepoda, Harpacticoida, Canthocamptidae)

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Abstract

A new species of the genus *Stygepactophanes* Moeschler & Rouch, 1984 (Copepoda, Harpacticoida, Canthocamptidae) is established to accommodate a small canthocamptid population collected from a spring system in the "Parc du Mercantour", Var catchment, southern France. The population analysed in the present study is defined by a set of morphological characters of the female, namely a very large maxilliped, a rudimentary mandibular palp, P1 with 3-segmented exopod and 2-segmented endopod, a falcate terminal claw of the P1 endopod, dorsal seta of caudal rami inserted on the inner margin, and anal operculum not overreaching the insertion of the caudal rami, thus supporting its assignment into the genus *Stygepactophanes*. The new species *Stygepactophanes occitanus* shows marked differences with the nominotypical species of the genus that was originally described by monotypy with the species

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† Deceased

Stygepactophanes jurassicus Moeschler & Rouch, 1984. The main diagnostic traits of *S. jurassicus* are the absence of the P5 and a falcate outer terminal claw of P1 endopod. *Stygepactophanes jurassicus* also shows a reduced armature of the antennal exopod, bearing one seta, 1-segmented P2–P4 endopods, a reduced armature of P2–P4 exopodal segments 3 (3,4,4 armature elements, respectively), P6 bearing only one long seta, a rounded short and smooth anal operculum. Conversely the female of *S. occitanus* Galassi & Fiers, **sp. n.** has a well-developed P5, with rudimentary intercoxal sclerite, together with a falcate outer terminal claw of P1 endopod, antennal exopod bearing two elements, P4 endopod 1-segmented versus 2-segmented in P2–P3, P2–P4 exopodal segment 3 with five armature elements, P6 with three setae of different lengths, rounded anal operculum, bearing 3–4 strong spinules.

According to our present knowledge, *S. occitanus* Galassi & Fiers, **sp. n.** is assigned to the genus *Stygepactophanes* as the most conservative solution, waiting for the male to be discovered. The genus *Stygepactophanes* represents a distinct lineage within the harpacticoid family Canthocamptidae that colonised southern European groundwater, the genus being known only from the saturated karst in Switzerland and a fissured saturated aquifer in southern France. Both species of the genus are stygobites and narrow endemics, the nominotypical species being known from the type locality Source de la Doux in Délemont (Switzerland), and *S. occitanus* Galassi & Fiers, **sp. n.** described herein from a spring system of the Var catchment (France).

Keywords

Groundwater, stygobite, systematics, taxonomy, Var catchment

Introduction

The “Parc du Mercantour” in southern France and the “Parco Naturale Alpi Marittime” in north-western Italy have promoted the development of an inventory of biological resources, including poorly known species belonging to different domains (ATBI program: All Taxa Biodiversity Inventory) and coming from different ecosystems, with special attention on the groundwater habitats. The project was also supported by the “European Distributed Institute of Taxonomy” (EDIT) (Dole-Olivier et al. 2015). The Mercantour massif has long been recognized as a European hotspot of biodiversity for both fauna and flora (Ozenda and Borel 2006, Giudicelli and Derrien 2009, Deharveng et al. 2015, Villemant et al. 2015). Its uniqueness is related to its location in Europe, where three (Alpine, Mediterranean and Continental) out of nine biogeographical regions coexist (European Environmental Agency 2018). This area is of great biogeographical interest also because of its role as a refugia during the Last Glacial Maximum, hosting a high number of narrow endemics (Biancheri and Claudin 2002, Dole-Olivier et al. 2015).

The Mercantour National Park (1465 km^2) is situated at the south-western end of the Alpine arc. The landscape is highly diversified and defined by a complex geology (Comité de Bassin 1995a, b, c). Three major geological units are present: a central crystalline massif (granite, gneiss), external and intensively folded sedimentary formations of Secondary and Tertiary ages, and intra-Alpine thrust sheets coming from Italy and covering the subalpine zone. Groundwater is mainly represented by aquifers in fissured consolidated rocks (Comité de Bassin 1995a, b, c; Cornu et al. 2013).

In two spring mouths belonging to the same spring system out of the 27 sampled in the studied area, a small population of an unknown canthocamptid harpacticoid was discovered. The new species shows morphological affinities with *Stygepactophanes jurassicus* Moeschler & Rouch, 1984, the only species at present known for the genus. Detailed morphological analyses, and a direct comparison with the type-material of *S. jurassicus*, the type species of the genus, supported the establishment of the second species of the genus, *S. occitanus* sp. n.

Materials and methods

Sampling was carried out according to the PASCALIS protocol, which was designed to assess groundwater biodiversity at regional scale (Malard et al. 2002). A stratified random sampling was adopted in spring-summer 2009 and in summer 2010 for the Var catchment, where 6 sampling sites were selected. Springs were sampled by using three techniques in order to maximize the sampling effort. A drift net was used to collect organisms flushed out from the aquifer by drift (Rouch et al. 1968), a Surber sample was taken to collect organisms at the surface of spring sediments and in the aquatic vegetation (Surber 1936), and a Bou-Rouch pump (Bou and Rouch 1967) was used to collect organisms at depth from the interstices of spring sediments (when present). The drift net (150 µm mesh size) was positioned at the spring outlet for eight to twelve hours (Rouch 1980). Once animals in the drift had been collected, the Surber sample was taken by moving cobbles upstream of the Surber net (150 µm mesh size) in order to dislodge animals at the surface of the spring bed sediments. Finally, sampling at depth into the spring bed sediments was carried out whenever the sediment thickness was > 30 cm. A mobile pipe was inserted into the spring bed sediments (maximum depth 50 cm below the bed surface) and 5–10 L of interstitial water and fine particles were extracted with a Bou-Rouch pump. Whatever the sampling method used, samples were elutriated, filtered through a 200-µm mesh net in the field and immediately fixed with 95% alcohol.

Only two spring mouths belonging to the Var catchment, in the Entraunes municipality, France (sites 30 and 31 in Dole-Olivier et al. (2015): Table 1, page 532), revealed the presence of two adult females of the new species collected in the drift, and three copepodids with the Surber sampling technique at the spring mouths.

Observations and drawings were made with a phase contrast Leitz Diaplan light microscope SFZ28, equipped with a drawing tube (standard magnification 1.25×, terminal lens 18×). Morphological details were also analysed with the aid of a Leica DM 2500 interferential microscope.

The type material of *Stygepactopanes jurassicus* was also analysed. The specimens were mounted in glycerine with modelling clay dots under the cover glass; the slides were re-sealed with polyurethane varnish in the course of the present study.

Abbreviations used: Aesth: aesthetasc; P1–P6: legs 1 to 6. Armature presentation in Tables 1, 2: Roman numerals referring to spines, Arabic numerals to setae; armament position indicated as x.x.x referring to outer.distal.inner elements.

Taxonomy

Order Harpacticoida Sars, 1903
Family Canthocamptidae Brady, 1880
Genus *Stygepactophanes* Moeschler & Rouch, 1984
Type species *Stygepactophanes jurassicus* Moeschler & Rouch, 1984
Other species *Stygepactophanes occitanus* Galassi & Fiers, sp. n.

Stygepactophanes occitanus Galassi & Fiers, sp. n.

http://zoobank.org/DA7D1261-22C6-4A77-B3CB-2063518C43DF
Figures 1–5

Material examined. ***Holotype*** here designated. Adult ♀ completely dissected and mounted in polyvinyl lactophenol on one slide, coll. M.-J. Dole-Olivier and Dominique Martin, 21 July 2009, deposited at the Muséum national d'Histoire naturelle de Paris. ***Paratypes.*** 1 ♀, same data as holotype, preserved in alcohol, coll. M.-J. Dole-Olivier and Dominique Martin, 9/08/2010. ***Additional material:*** 3 ♀ copepodids collected at the Sanguinière spring system, from a spring mouth located at 2199 m above sea level.

Etymology. The specific epithet refers to the region Occitania, derived from the Medieval Latin Occitania (from where the new species was collected, a region now encompassing the French administrative region Languedoc-Roussillon-Midi-Pyrénées which is located on part of the traditional Occitania and includes Roussillon).

Type locality. Sanguinière spring system, Var Department, Mercantour National Park, France, Var river catchment at Entraunes municipality at 2040 m above sea level; coordinates 44.25226354N, 6.77111744E.

Diagnosis. *Stygepactophanes occitanus* Galassi and Fiers, sp. n. has a well-developed P5, with rudimentary intercoxal sclerite, together with a falcate outer terminal claw of P1 endopod, antennal exopod bearing two elements, P4 endopod 1-segmented versus 2-segmented in P2–P3, P2–P4 exopodal segment 3 with five armature elements, P6 with three setae of different lengths, rounded anal operculum, bearing 3–4 strong spinules.

Description of the female. Body (Fig. 1A, B) slender and cylindrical in dorsal view, with urosome slightly narrower than prosome. Body length of holotype 425 µm, female paratype 410 µm. Podoplean flexure indistinct; prosome and urosome of same length. Integument without pits and very feeble sclerotization. Integumental windows absent; female genital and first abdominal somites completely fused forming genital double-somite; double-somite short, length/width ratio: 0.45; genital field located near anterior margin of genital somite and extended far beyond proximal half of somite. Genital complex expanded caudally to end of second third of ventral surface (Fig. 2A), with rather large reniform orifices of receptacles and long copulatory duct; the latter with wide funnel and copulatory pore; slit shaped pore present on both sides of copulatory pore.

Body ornamentation: integument of cephalothorax and urosome unornamented in the paratype, with short lateral row of spinules on left side of P4-bearing somite in the holotype (Fig. 2C); P5-bearing somite either completely smooth (holotype) or with short row of spinules on left side (paratype) (Fig. 1A, B); integument of genital double-somite either unornamented (holotype) or with short posterodorsal row of spinules (paratype; Fig. 1A); posterolateral and posteroventral margins ornamented with slender spinules, short medially (Fig. 1B), absent medioventrally (Fig. 2A); posterior margins of urosomites IV and V lateroventrally and posteroventrally with narrow spinules, interrupted mid-ventrally on urosomite IV, continuous row of spinules on urosomite V (Fig. 2A). Posterodorsal hyaline frills of body somites narrow and straight, plain. Anal somite unornamented along posterodorsal and posterolateral margins; posteroventral margin with two sets of spinules medially; outer ones minute, inner ones long and slender; anal sinus smooth, anal orifice with few slender hairs (Fig. 2A). Anal somite as long as preceding one with convex anal operculum bearing four (holotype: Fig. 2D) or three (paratype: Fig. 1A) coarse spinules along distal margin; distal margin of operculum not extending beyond anal sinus.

Caudal rami (Fig. 1A–C): conical and truncate, in both dorsal and lateral view, slightly divergent; length/width ratio: 2.75 (holotype) and 2.80 (paratype). Anterolateral accessory seta (I) minute, inserted on proximal third of caudal ramus, anterolateral seta (II) inserted on distal third of caudal ramus, accompanied by two or three long and slender spinules at insertion (Figs 1C, 2A), ca. 1.5 times longer than anterolateral accessory seta; posterolateral seta (III) slender and unornamented, outer terminal seta (IV) 1.5 times longer than ramus, sparsely serrate; inner terminal seta (V) (Fig. 2B) as long as the whole body, rather slender, sparsely serrate along outer margin (Fig. 2B); both setae IV and V without breaking plane; terminal accessory seta (VI) short, less than 1/3 length of caudal ramus; dorsal seta (VII) located at more than half of caudal ramus, near inner margin, articulating on a single basal section.

Rostrum (Figs 1A, 3A): triangular with tongue-shaped apex and apparently completely fused to cephalothorax; apex reaching just the first antennule segment; integument smooth; sensilla pair present, located subapically.

Antennule (Fig. 3A, B): rather short, backwards bent, reaching halfway along cephalothorax at most; 7-segmented, without particular integument ornamentation; armature (from proximal to distal segment): 1–8-5–2+Aesth-1–3-9+Aesth. Aesthetasc on segment IV rather wide, leaf shaped (but wrinkled in both specimens), overreaching segment VII, and fused at base with accompanying seta (acrothek); aesthetasc on segment VII slender and tongue-shaped, fused at base with terminal seta (acrothek).

Antenna (Fig. 3C): with allobasis and 1-segmented exopod; syncoxa robust, unornamented; allobasis cylindrical, 1.75 times longer than wide with two smooth setae and some slender spinules along abexopodal margin; terminal endopodal segment armed with nine elements: three lateral ones (two spines, one seta) and six distal ones (one spine, five setae); both outer distal ones fused at base; armature elements partially squamous and serrate; outer margin with two clusters of spinules; exopod located at proximal fourth of allobasis, 1-segmented and well developed, bearing one lateral and one terminal delicately serrate setae.

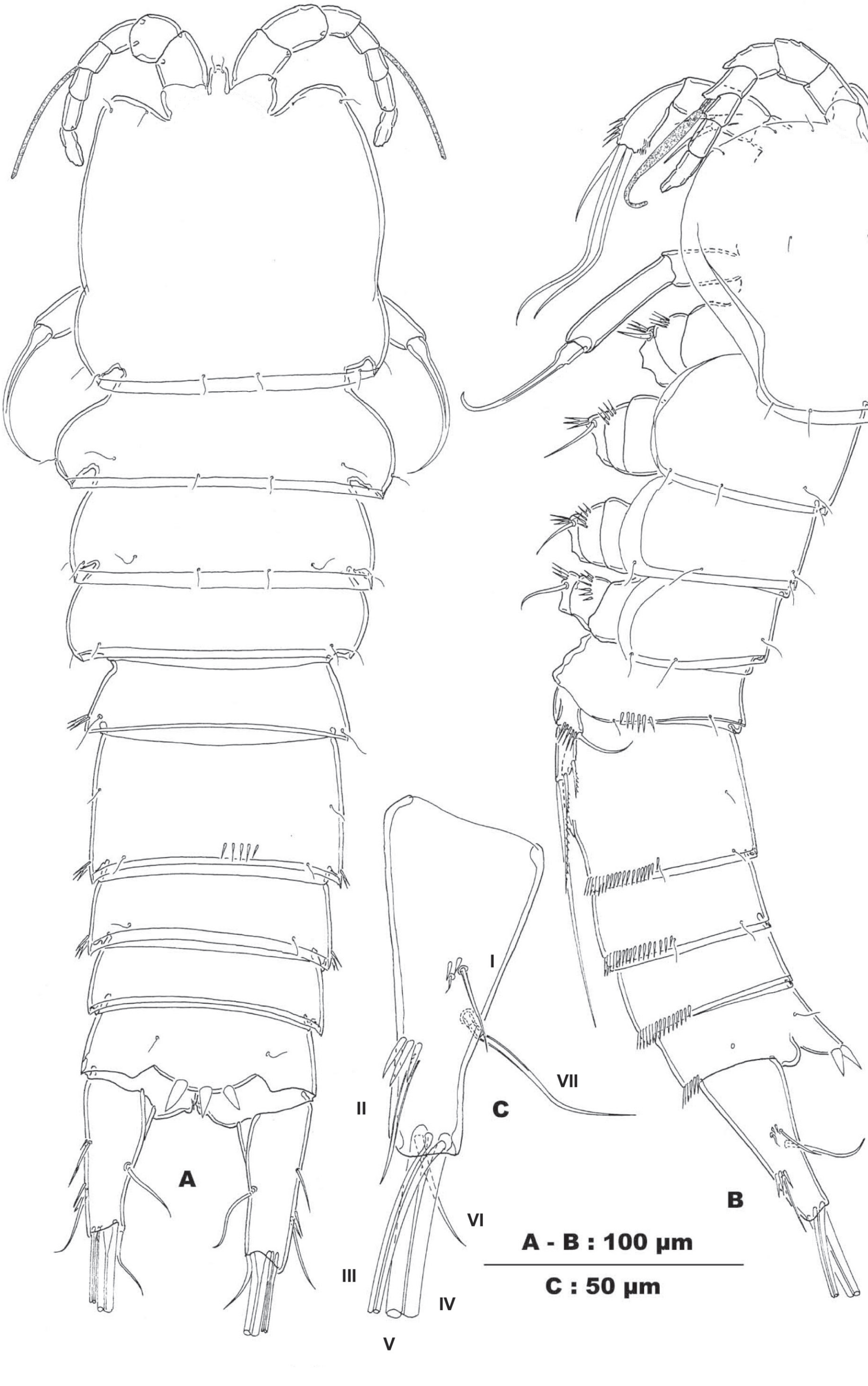

Figure 1. *Stygepactophanes occitanus* sp. n. (female paratype) **A** Habitus, dorsal view **B** Habitus, lateral view **C** Left caudal ramus, outer lateral view, enlarged.

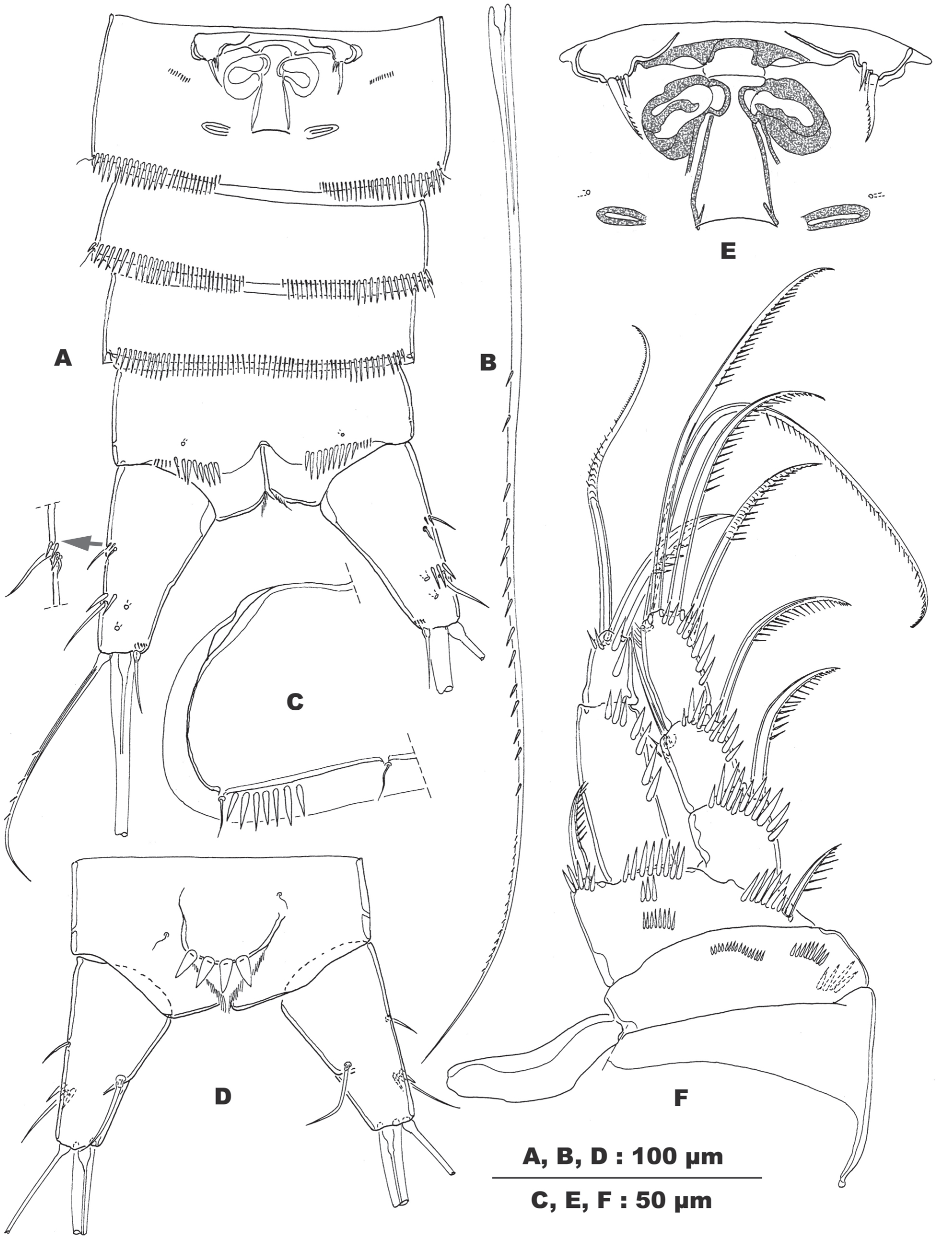

Figure 2. *Stygepactophanes occitanus* sp. n. (female paratype) **A** Urosome, ventral view (arrow indicates anterolateral setae, enlarged) **B** Inner terminal seta (V) of caudal rami **C** Posteroventral edge of P4-bearing somite **D** Anal somite and caudal rami, dorsal view **E** P6 and genital complex, enlarged **F** P1, frontal.

Mandible (Fig. 3D): rather slender, coxal gnathobase slender, without ornamentation; palp rudimentary bearing two short slender setae; cutting edge consisting of two strong bi-dentate oral and four-five multi-dentate aboral teeth.

Labrum (Fig. 3E): crescent with a subapical transverse row of spinules and an apical transverse row of setules; both edges with a short row of strong spinules.

Labium (Fig. 3F): with an oblique row of strong spinules on each side, free distal margin with setules; paragnaths armed distally with several clusters of fragile hair-like and slender spinulose elements.

Maxillule (Fig. 3G, H): well developed arthrite incorporated into praecoxa, with seven strong curved uni- or multi-serrate armature elements inserted on free distal margin, two long lateral setae and two anterior surface setae. Basis cylindrical with a total of seven elements: four naked setae on outer margin, three apical elements, one of which strong and falcate.

Maxilla (Fig. 3I): syncoxa with 2 well-developed endites that are not defined at their bases, distal endite with three setae, one serrate and two slender, bare setae; proximal endite with two setae, one bare and one serrate. Allobasis drawn out into strong unipinnate claw, rather slender, medial structure armed with widely spaced slender spinules, accompanied by one serrate and one smooth setae; endopod rudimentary, represented by two smooth setae.

Maxilliped (Figs 3J, 5A): subchelate; syncoxa short, rather quadrangular, ornamented with short rows of spinules, but lacking armature elements; basis very long (length/width ratio: 3.4) with a short row of spinules at the middle of anterior and posterior surfaces; endopod 1-segmented, extended in a long sparsely ornamented claw.

P1 (Figs 2F, 5B): prehensile; well-developed praecoxa, coxa, basis and wide intercoxal sclerite; 3-segmented exopod and 2-segmented endopod; exopod and endopod subequal in length; endopodal segment 1 quite overreaching exopodal segments 1 and 2; praecoxa and intercoxal sclerite spineless; coxa with short rows of small spinules on frontal and caudal surfaces; basis with a row of coarse spinules near articulation of each ramus, on frontal side, and near insertion of inner seta; outer seta on basis short, robust, sparsely pinnate; inner seta, bent outwards, reaching halfway along endopod 1, spiniform, serrate along outer margin only; exopod segments with coarse spinules near distal outer corner, slender spinules near insertion of inner armature elements on second and third segments; endopod with spinules along outer margin of first segment and near insertion of armature elements on second segment; armature elements of exopod serrate along outer margin only; outer terminal seta on endopodal segment 2 robust, claw-shaped, serrate midway of outer margin; inner seta geniculate, slightly serrate; armature formula as in Table 1.

P2–P4 (Figs 4A, B, D; 5C): well-developed praecoxa, coxa, intercoxal sclerite and basis; P2–P3 with 3-segmented exopods and 2-segmented endopods, P4 with 1-segmented endopod; praecoxa and intercoxal sclerite spineless, coxa and basis with short rows of spinules on anterior surface, and spineless on caudal surface; exopodal segments with coarse spinules on outer distal edge and naked inner margin except for two hair-like elements on second segment of P2 and P3; P2–P3 endopod 1 quadrate,

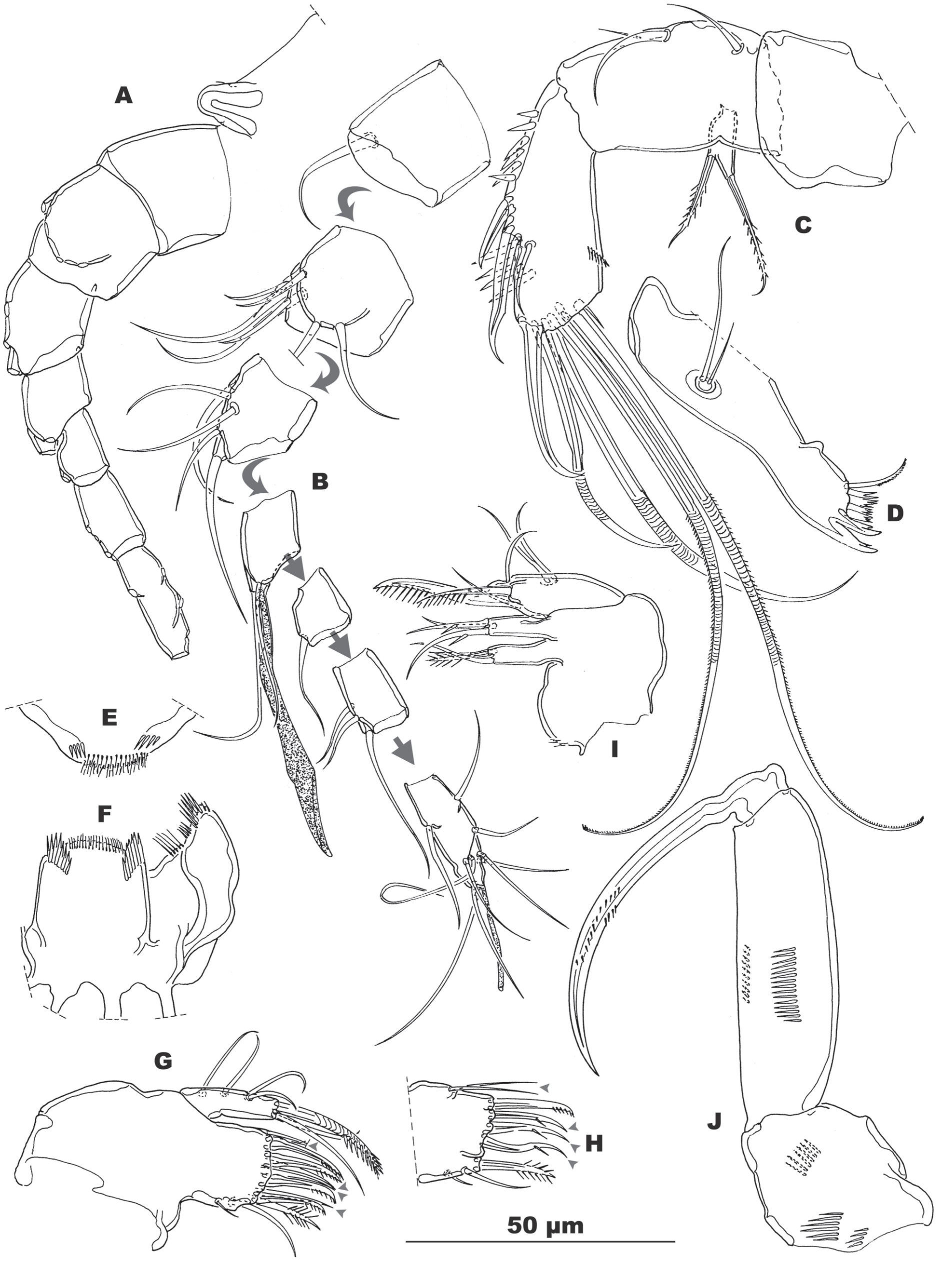

Figure 3. *Stygepactophanes occitanus* sp. n. (female paratype) **A** Contour of rostrum and antennule, dorsal view **B** Antennule, exploded, armament distribution **C** Antenna **D** Mandible **E** Labium **F** Labrum **G** Maxillule, frontal view (arrows indicating elements on caudal face, see H) **H** Maxillular arthrite, caudal view (arrows indicating elements not discernable in frontal view) **I** Maxilla, frontal view **J** Maxilliped, frontal view.

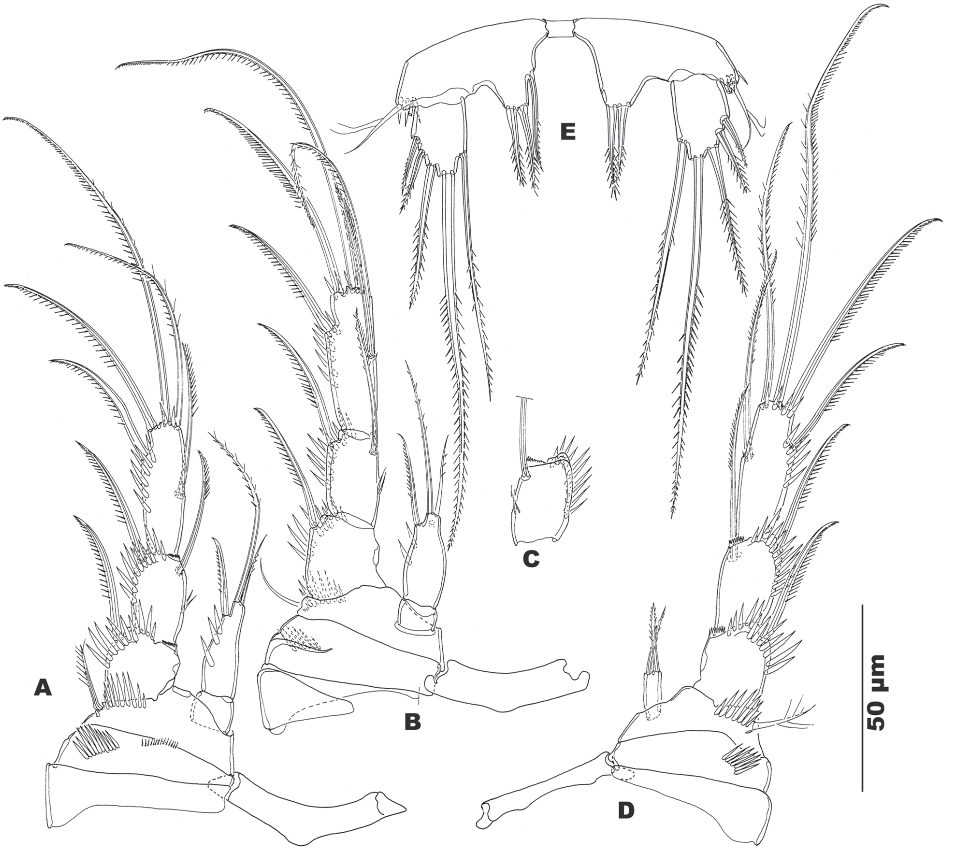

Figure 4. *Stygepactophanes occitanus* sp. n. (female paratype) **A** P2, frontal view **B** P3, caudal view **C** P3, exopod 2, opposite side, frontal view **D** P4, frontal view **E** P5, caudal view.

unarmed, and spineless; P2–P3 endopodal segment 2 ca. three times longer than wide, with two or three coarse spinules along outer margin; inner margin bare; P4 endopod small, rectangular, 2.5 times longer than wide, not reaching the middle of exopodal segment 1, and unornamented; armature formula of P1–P4 exopods and endopods as in Table 1.

P5 (Figs 4E, 5D): baseoendopod and exopod not fused; baseoendopod with short inner lobe, not reaching the middle of exopod, and with short spiniform setae; left lobe with two apical and one medial setae, right lobe with two apical setae; intercoxal sclerite present, spineless; basipodal outer seta slender and short, sparsely pinnate, accompanied by cluster of spinules at insertion; exopod semi-rectangular, 1.5 times longer than wide, with five robust sparsely serrate setae: three outer, one apical, one medial; apical and medial setae the longest.

P6 (Fig. 2A, E): remnants fused, symmetrical, bearing three small setae; medial and middle setae minute and smooth, outer seta four times longer than the inner ones, robust and serrate along medial margin; legs fused medially forming a genital operculum.

Male. Unknown.

Table 1. Female armature of P1–P4 of *Stygepactophanes occitanus* sp. n. (female only).

	basis outer element	basis inner element	exopod	endopod
P1	+	+	I.0-I.1-II.2.0	0.0-I.1.0
P2	+	–	I.0-I.1-II.2.1	0.0-I.2.0
P3	+	–	I.0-I.1-II.2.1	0.0-I.2.0
P4	+	–	I.0-I.1-II.2.1	0.II.0

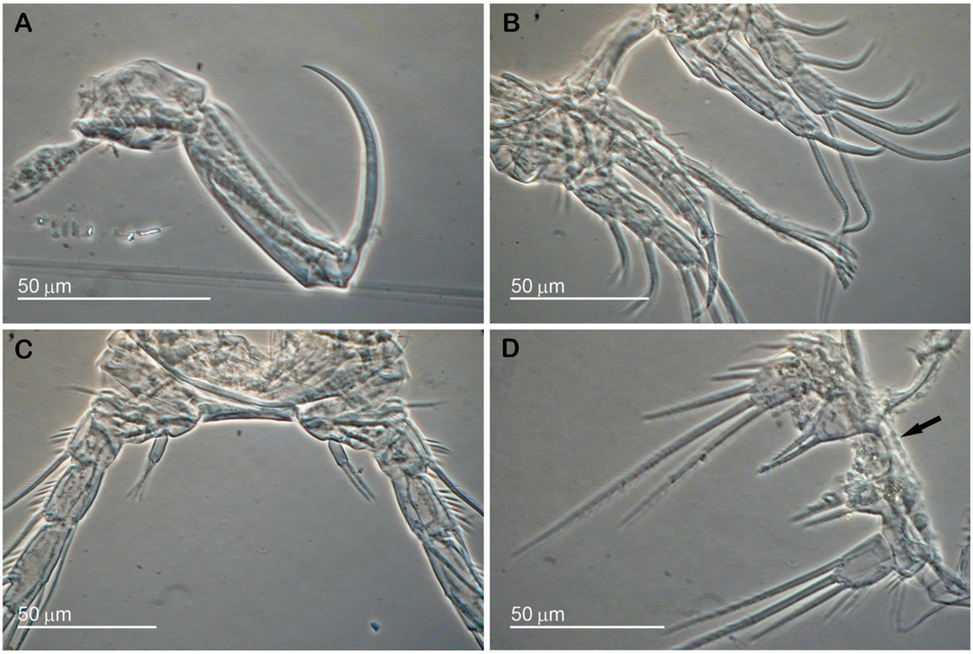

Figure 5. *Stygepactophanes occitanus* sp. n. (female paratype) Optical microscopy micrographs. **A** Maxilliped **B** P1 **C** P4 **D** P5.

Genus *Stygepactophanes* Moeschler & Rouch, 1984

Stygepactophanes jurassicus Moeschler & Rouch, 1984
Figures 6, 7

Material examined. ♀ labeled as "holotype" collected from "source de la Doux à Delémont" (Jura, Switzerland), 1 ♂ from "Galerie de la captage de Champ-du-Moulin", Gorges de l'Areuse (Neufchâtel, Switzerland) without type indication; each specimen dissected with the parts mounted in glycerine. Material deposited at the Department of Arthropodology and Entomology of the Museum of Natural History of Geneva (Switzerland). The type material consists of a slide with the dissected female holotype and a slide with a dissected male; the latter without status indication and labeled to be obtained in the "Galerie de la captage de Champ-du-Moulin". The mounts are of poor quality and many appendages appear to be absent or lost. The other specimens mentioned by Moeschler and Rouch (1984) (i.e., 2 ♂ – including 1 ♂ paratype and 1 copepodid) are missing. They seem absent in the Rouch collection and hosted at the Muséum national d'Histoire Naturelle de Paris and are certainly not present in the Genève Museum (F Fiers, pers. obs.). Fortunately, the original description by Moeschler and Rouch (1984) is detailed. The present contribution is a slight emendation of the original description, focussing on the finer morphological details, and aimed at analysing the status of the male specimen kept in Genève.

Supplementary description. Female. Urosome (Fig. 6A) without P5, urosomite I unornamented; genital double-somite short, length/width ratio: 0.73, with small receptacle orifices and wide, bell-shaped copulatory funnel and wide copulatory pore. Posterodorsal and posterolateral margins smooth; posteroventral margin with six sets of spinules of different lengths; hyaline frill absent; urosomites IV and V ornamented with six groups of spinules on posteroventral margins. Anal somite as long as preceding one, with smooth free margin of anal operculum; anal sinus not covered by operculum, smooth except for few hairs along anal orifice. Posterodorsal and posterolateral margins smooth, posteroventral margin with spinules, either long or short.

Caudal rami (Fig. 6A, C): cylindrical, only slightly enlarged at proximal part and truncate at distal part, anterolateral accessory seta (I) absent; anterolateral seta (II) inserted on distal third of caudal ramus, with some minute spinules at insertion; posterolateral seta (III) broken, accompanied by two long spinules at insertion; outer and inner terminal setae (IV–V) fused at base, both sparsely serrate and without breaking planes (Fig. 6A, B); basal part of inner terminal seta slightly inflated with narrow hyaline outer and inner membranes (outer membrane arrowed in Fig. 6A); terminal accessory seta (VI) ca. as long as half caudal rami; posteroventral margins of caudal rami with three long spinules; dorsal seta (VII) inserted at second third of caudal rami, near inner margin, articulated on basal part, and accompanied by one or two long spinules at insertion.

Antenna: with short coxa, half as long as wide, unornamented; spinules on abexopodal margin long, reaching distal fourth of allobasis; exopod with one seta, sparsely serrate along one side; endopod with distal margin bearing four elements (one spine and three setae).

P1–P4 armature as in Table 2. P3 (Fig. 7B, C): praecoxa, coxa and intercoxal sclerite well developed, unarmed and unornamented; basis with outer seta and cluster of spinules near articulation with exopod; outer elements of exopod pectinate; frontal surface of exopodal segment 3 with large subapical cuticular pore (Fig. 7C); outer element on endopod spiniform, inner one setiform.

P5 absent.

P6 (Fig. 6A): reduced, represented by a single (smooth?) long seta, and confluent midventrally forming a caudally expanded convex plate covering anterior part of genital field.

Male. Urosome (Fig. 6C): urosomite I without P5, unornamented (Fig. 7G); urosomites II–V ornamented (urosomites III–V as in female, and urosomite II ornamented as urosomites III–V); outer terminal and inner terminal setae (IV–V) of caudal rami fused at base; seta V not inflated and lacking hyaline membranes; dorsal seta (VII) inserted near or on inner margin of caudal rami.

Table 2. Female and male armature of P1–P4 of *Stygepactophanes jurassicus* Moeschler & Rouch, 1984 (* possible presence of two outer spines on the second segment, but likely attributable to an anomaly). Armature of female P1, P2, and P4, and male P2 taken from Moeschler and Rouch (1984).

	basis outer element	basis inner element	exopod	endopod
P1 female	–	1	I.0-I.0-II.1.1 or I.0-I.0-II.1.0	0.0-I.1.0 or 0.0-I.0.0
P1 male	–	1	I.0-I.0*-II.1.1 or I.0-I.0*-II.1.0	0.0-I.1.0
P2 female and male	–	–	I.0-I.1-I.2.0	I.1.0
P3 female	+	–	I.0-I.1-I.2.1	I.1.0
P3 male	+	–	I.0-I.1-I.2.1	0.0-modified
P4 female and male	–	–	I.0-I.0-I.2.1	I.1.0

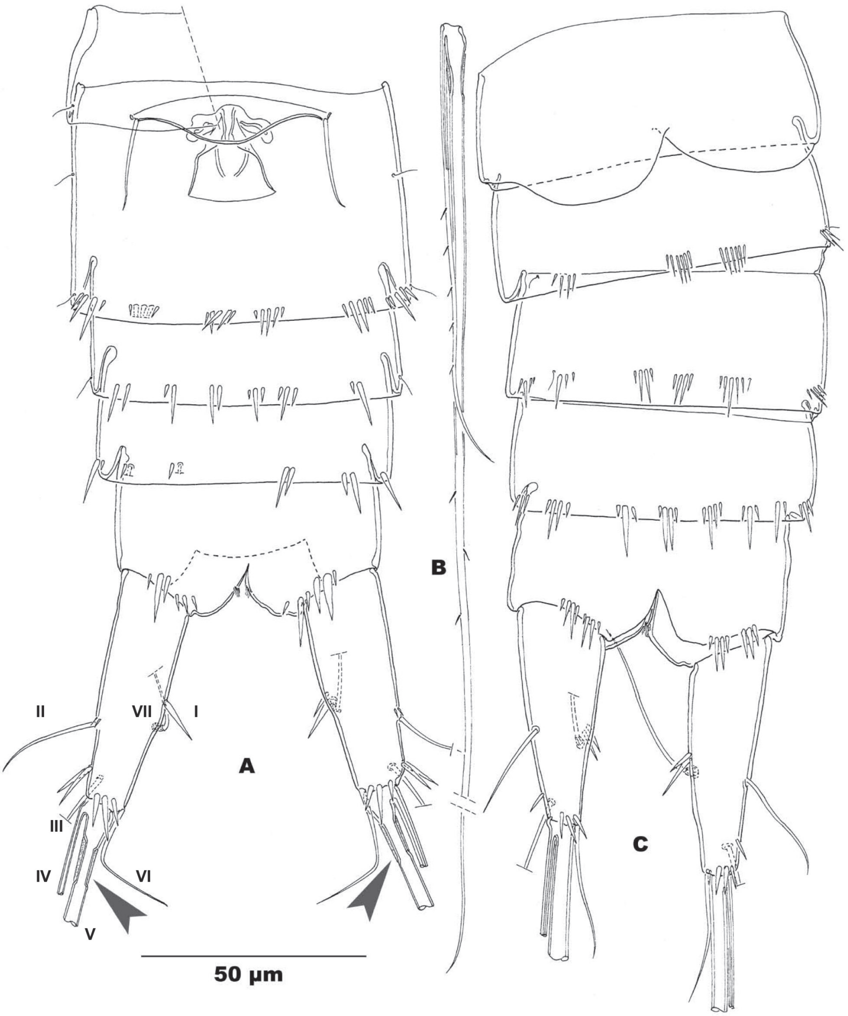

Figure 6. *Stygepactophanes jurassicus* Moeschler & Rouch, 1984. **A** Female urosome, ventral view (P5-bearing somite with P5 absent, left side, right side broken) **B** Female inner terminal seta (V), ventral view **C** Male urosome, ventral (**A, B** female holotype **C** male paratype).

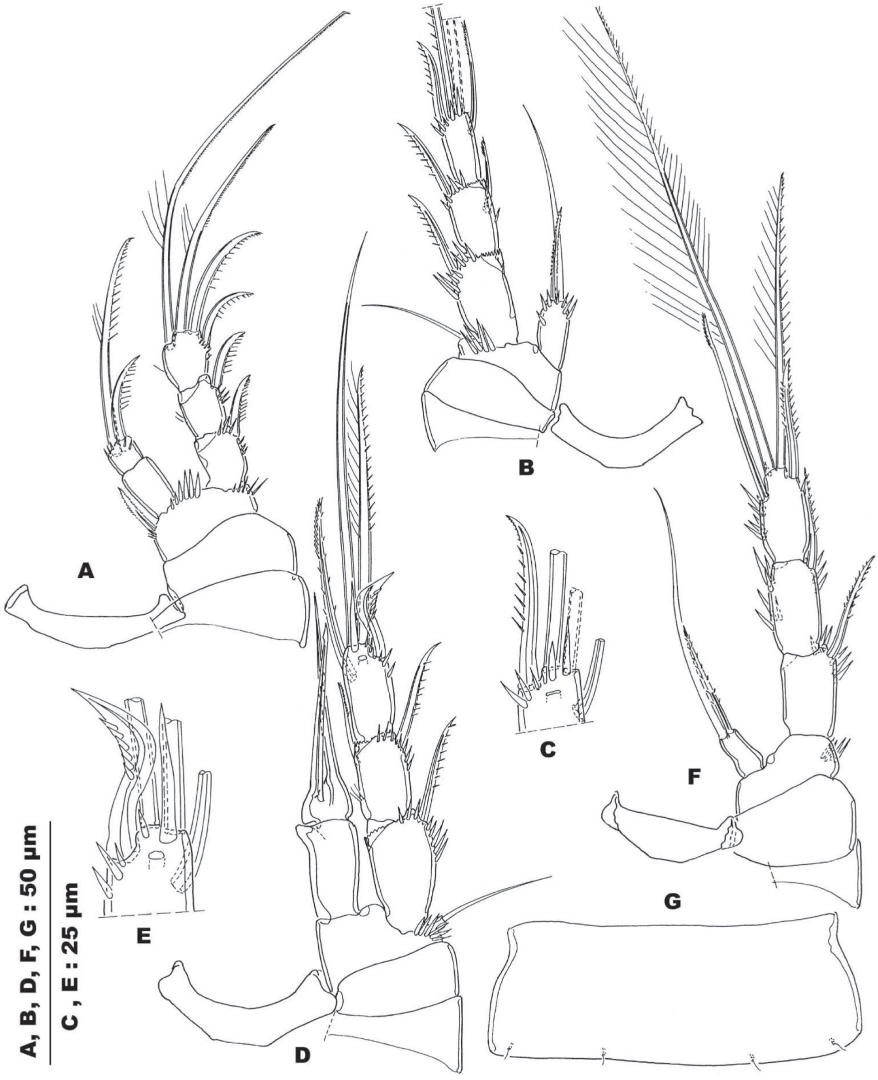

Figure 7. *Stygepactophanes jurassicus* Moeschler & Rouch, 1984. **A** P1, frontal **B** P3, frontal **C** P3, distal end of exopodal segment 3, enlarged **D** P3, frontal **E** P3, distal end of exopodal segment 3, enlarged **F** P4, caudal view **G** P5-bearing somite with P5 absent, ventral view (**B, C** female holotype **A, D–G**: male paratype).

P1 (Fig. 7A): praecoxa, coxa and intercoxal sclerite unarmed and unornamented; intercoxal sclerite narrow and wide; medial margin of exopodal segments 2 and 3 with sparse hairy ornament; outer margins of exopodal segments with few spinules; armature elements of inner margin and inner distal margin delicately serrate outwardly, plumose midway inwardly; endopodal segment 1 without spinule ornament, endopodal segment 2 with spinules along distal margin; outer terminal element on endopodal segment 2 claw-shaped (falcate), serrate along outer margin; inner element robust with spinular appearance, outwardly serrate, plumose midway inwardly, and at least twice as long as outer element; left and right legs identical.

P3 (Fig. 7D, E) with well-developed praecoxa, coxa and intercoxal sclerite, all unarmed and unornamented; basis as in female; medial armature element on exopodal segment 3, segment more robust than in female; outer spine on exopodal segment 3 robust, claw-shaped and strongly serrate in middle of outer margin; apical margin with long median spinule and wide subapical pore on frontal surface (Fig. 7E); endopod 2-segmented; proximal segment twice as long as wide, with medial distal corner forming a truncate expansion; distal segment globular and extended into two equally long sharp apophyses overreaching exopod.

P4 (Fig. 7F) with well-developed praecoxa and coxa; intercoxal sclerite unarmed and unornamented; basis without outer seta and with short row of spinules near outer margin; medial element on exopodal segment 3 with distal inner margin pectinate; endopod 1-segmented, twice as long as wide, with outer terminal element spiniform and inner one setiform.

P5 absent.

P6 (Fig. 6C) represented as a caudally symmetrical bilobate plate, without setae and completely smooth.

Moeschler and Rouch (1984) reported the aberrant nature of the exopodal armature of P1 in the male specimen collected at the "Captage de Champ-du-Moulin". They provided an illustration (Moeschler and Rouch (1984): fig. 7b, page 968) of a leg with two spines on exopodal segment 2, and only three armature elements on its terminal segment. The opposite leg was mentioned as being armed in the same way as described for the female holotype with one outer spine on exopodal segment 2 and four elements on the terminal segment.

Re-examination of the slide kept at Genève labeled: "Galerie de la Captage de Champ-du-Moulin, Gorges de l'Areuse (NE); 17.11.1981" revealed, however, that both legs are identical, and resemble the female P1 as illustrated in Moeschler and Rouch (1984): fig. 5a, page 965. This observation confirms that the male paratype deposited at Genève must have been mislabeled during processing of the slides.

Key to the species of the genus *Stygepactophanes* (based on females only)

1 P1 endopodal segment 1 ca. 2 times longer than endopodal segment 2, slightly overreaching exopodal segment 1; P2–P4 endopods 1-segmented, P5 absent, caudal rami cylindrical and long (length/width ratio: 3.3–3.5), anal operculum rounded and smooth .... ***Stygepactophanes jurassicus* Moeschler & Rouch, 1984**

– P1 endopodal segment 1 ca. 3 times longer than endopodal segment 2, quite overreaching exopodal segment 2; P2–P3 endopods 2-segmented, P4 endopod 1-segmented, P5 well developed, with rudimentary intercoxal sclerite, caudal rami subconical and long (length/width ratio: 2.54), anal operculum with strong spinules .... ***Stygepactophanes occitanus* sp. n.**

Remarks

Stygepactophanes occitanus sp. n. does not fit the diagnosis of any defined genus in the keys available to date (Lang 1948, Borutzky 1952, Dussart and Defaye 1995, 2001, Boxshall and Halsey 2004) and led us to the genus *Epactophanes* Mrázek, 1893 using the identification keys of Wells (2007). In more detail, *S. occitanus* sp. n. could be placed among the genera unified in Borutzky's (1952) subfamily Epactophaninae Borutzky, 1952 currently including *Epactophanes* and *Epactophanoides* Borutzky, 1966 (and eventually *Ceuthonectes* Chappuis, 1924, see Dussart and Defaye 2001, and below).

There are several indications that *S. occitanus* sp. n. is an obligate groundwater species (as well as *S. jurassicus*). The fine integument almost completely devoid of ornamentation, the body transparency and the absence of eye pigmentation, the large and wide antennule main aesthetasc and the reduced appendages are relevant stygomorphic traits. Moreover, the presence of the new species in the outflow of two spring mouths fed by the same aquifer, and its low abundance (no other specimens were found in additional samplings (M-J Dole-Olivier and D Martin, pers. comm.) are supplementary arguments to support this contention.

Obligate groundwater canthocamptids such as members of *Stygepactophanes*, *Lessinocamptus* Stoch, 1997, *Spelaeocamptus* Chappuis, 1933, and *Paramorariopsis* Brancelj, 1991, among others, are known to have a limited distribution, and in most cases are narrow endemics (Galassi 1997, Fiers and Moldovan 2008, Galassi et al. 2009, Brancelj 2009, 2011, Di Lorenzo et al. 2018). Their affinities with the epigean members of the family often remain obscure. Apparently they represent local derived strays displaying adaptations, mostly in terms of reduction and/or characters' losses. These reductions and/or characters' losses are frequently found in obligate groundwater species of other harpacticoid families, such as Ectinosomatidae, Ameiridae, Rotundiclipeidae, and Leptopontiidae and even in some stygobiotic cyclopoid genera (Galassi et al. 1999a, b, Galassi and De Laurentiis 2004a, b).

As far as the stygobiotic Canthocamptidae are concerned, although their roots have different origins in the evolutionary history of the family, they share remarkable similarities such as simplified body shape, delicate integument, long and widened main antennule aesthetasc, short P1 endopods with prominent falcate terminal claw (even more conspicuous in *Stygepactophanes* than in *Lessinocamptus* and *Elaphoidella* Chappuis, 1929), and reduction in mouthparts and swimming legs, likely as a result of adaptive convergence by means of heterochrony (Galassi et al. 1999a, b, Galassi et al. 2009). The very long maxilliped shared by *S. jurassicus* and *S. occitanus* sp. n. may either be considered an autapomorphy of the genus, or the result of adaptation to a similar trophic niche (adaptive trait?). Actually long maxillipeds are present also in members of other harpacticoid families (Galassi and De Laurentiis 2004b), as in the Parastenocarididae (e.g., *Simplicaris lethaea* Galassi and De Laurentiis, 2004, and in *Parastenocaris andreji* Brancelj, 2000) suggesting also that this character may have appeared more than once in the evolutionary history of the Harpacticoida.

Relationships between *Stygepactophanes* and the *Epactophanes-Epactophanoides* lineage, as might be assumed, cannot be substantiated as they do not share the particular caudally displaced female genital complex, as found in *Stygepactophanes* and the topology of the dorsal seta on caudal rami inserted near or on the inner margin of caudal rami (considered herein an autapomorphy of the *Stygepactophanes* lineage).

Stygepactophanes jurassicus and *S. occitanus* sp. n. share similar habitus, long maxilliped, P1 with falcate outer apical element, P1 endopodal segment 1 from 2 to 3.5 times longer than endopodal segment 2, topology and development of the genital field in the female extending at least to the proximal half of the genital double-somite, and the inner position of the dorsal seta of the caudal rami.

Nevertheless, the females are clearly distinguishable on the basis of the following characters: antennal exopod with one seta in *S. jurassicus* versus two in *S. occitanus* sp. n.; mandibular palp 1-segmented in *S. jurassicus* but absent in *S. occitanus* sp. n., where only two remnant setae are present, 1-segmented P2–P3 endopods in *S. jurassicus* versus 2-segmented in *S. occitanus* sp. n.; P2 exopodal segment 3 with three elements in *S. jurassicus* versus five elements in *S. occitanus* sp. n., P3–P4 exopodal segment 3 with four elements in *S. jurassicus* versus five elements in *S. occitanus* sp. n.; P5 absent in *S. jurassicus* but present and well developed in *S. occitanus* sp. n.; P6 with one outer long seta in *S. jurassicus* versus three setae, the outer the longest in *S. occitanus* sp. n.; anal operculum rounded and smooth in *S. jurassicus* versus ornamented by strong spinules in *S. occitanus* sp. n. The affinities of *S. occitanus* sp. n. and *S. jurassicus* are indisputable, and the main difference relies on the primitive character states shown by *S. occitanus* sp. n.; namely the well-developed P5, the 2-segmented P2–P3 endopods, and a higher number of armature elements of the exopodal segment 3 of P2–P4.

To our present knowledge, and on the basis of the missing information about the male of *S. occitanus* sp. n., the assignment of the new species to the genus *Stygepactophanes* is the most conservative solution. Pending the discovery of the male, an emended diagnosis is provided based on females only.

Emended diagnosis of the genus *Stygepactophanes*

Canthocamptidae. Small canthocamptid with cylindrical body without clear demarcation between prosome and urosome; integument without pits and very feeble sclerotization. Eyeless. Integumental windows absent; female genital and first abdominal somites completely fused forming a genital double-somite; genital field located near anterior margin of genital somite and developed at least as far as the middle length of the same somite. Body ornamentation: integument of cephalothorax and urosome unornamented. Posterodorsal frills of body somites narrow and straight, plain; anal somite unornamented along posterodorsal and posterolateral margins. P5-bearing somite either completely smooth or with short row of spinules; integument of genital double-somite either unornamented or with short posterodorsal row of spinules; posterolateral and posteroventral margins ornamented with slender spinules, short medially, absent medioventrally. Operculum not protruding beyond the insertion of caudal rami, rounded and smooth or bearing strong spinules. Caudal rami cylindrical or conical, elongated, bearing seven setae or missing the anterolateral accessory seta (I); dorsal seta inserted near or on inner margin of caudal ramus. Rostrum small, not defined at base; antennule 7-segmented with main aesthetasc on segment IV very large; antenna allobasis with two abexopodal setae and 1-segmented exopod bearing one or two setae; mandibular palp rudimentary, represented by a small segment bearing one short seta, or represented by two setae only; maxillule and maxilla reduced, the latter with 2 endites; maxilliped very long, clearly discernible in dorsal view with a long and thin basis and falcate endopod; P1–P4 with 3-segmented exopods; P1–P3 with 1- or 2-segmented endopods, P1 with 2-segmented endopod, falcate and bearing a serrate claw; endopodal segment 1 always shorter than endopodal segment 2; P4 with 1-segmented endopod; P5 absent or present and well developed; in the latter case, intercoxal sclerite present.

Discussion

Moeschler and Rouch (1984) faced problems in allocating *S. jurassicus* among the canthocamptids, and resurrected Borutzky's (1952) family subdivision in subfamilies. Fortunately, they have had the chance to collect and analyse the male. *Stygepactophanes* was assumed to be related either to the Morariinae Borutzky, 1952 or to the Epactophaninae Borutzky, 1952, with a certain bias towards the latter, an opinion that has not been challenged so far. Borutzky's (1952) subfamilies have not elicited much support and have been generally ignored (Wells 2007), with a few exceptions (Dussart and Defaye 1995, 2001), and currently partially re-introduced by Walter and Boxshall (2018). The validity of these canthocamptid subfamilies is still debated and in need of revision.

That the subfamily Epactophaninae with the only genera *Epactophanes* Mrázek, 1893 and *Epactophanoides* Borutzky, 1966 may constitute a natural group has some ground based on the unique male P3 endopod and the morphology of the female genital complex. The proposal that *Stygepactophanes*, *Ceuthonectes* and *Ligulocamptus* Guo, 1998 (as suggested in Dussart and Defaye 1995, 2001) could be considered affiliated members is questionable. *Ceuthonectes* and *Stygepactophanes* do not display any of the diagnostic features shared by *Epactophanes* and *Epactophanoides* (i.e., the sole male P3 endopod and the female genital complex) and should reasonably be placed outside the epactophanid lineage. *Ligulocamptus* is apparently close to *Mesochra* Boeck, 1864 as proposed by Guo (1998) although this statement requires confirmation in order to establish their real affinities.

The alternative in which *Stygepactophanes* may enter the diagnosis of the Morariinae can only be partially supported. Borutzky (1952) originally included the genera *Moraria* T. and A. Scott, 1893, *Morariopsis* Borutzky, 1931 and *Ceuthonectes* in this subfamily. The group has gradually been expanded with the addition of *Pseudomoraria* Brancelj, 1994, *Paramorariopsis*, *Gulcamptus* Miura, 1969 and *Itunella* Brady, 1896 (see Dussart and Defaye 2001). Neither *Ceuthonectes* nor any of the recently added taxa are directly related to *Moraria* and *Morariopsis* as they are representatives of different lineages in the Canthocamptidae (Fiers and Jocque 2013).

However, *Stygepactophanes* displays some remarkable similarities with *Ceuthonectes*. In the latter, the sexually dimorphic P3 endopod is the main morphological trait shared with *Stygepactophanes.* Dimorphic traits of the male P2 and P4 endopods are limited (length/width of the segment, fusion of segments) and both legs have the same number of armature elements in males and females. In contrast, the male P3 endopod, 2-segmented in both sexes, is distinctly modified. The proximal segment is enlarged with one or more reinforcements of the proximal margin, the distal segment is quite short, has a globular aspect, and bears two narrow and spiked lanceolate armature elements. Moreover, the terminal segment of the male P3 possesses a long hyaline tubular expansion of the frontally located pore (originally interpreted as a spinule by Chappuis (1924)), a structure absent in the female. The dimorphic aspects of the endopods in *S. jurassicus* (the only species of the genus for which the male is known) are very similar to those of *Ceuthonectes*, but the tubular structure on the frontal surface of the terminal exopodal segment is absent. However, in *S. jurassicus* one of the spinules along the distal margin is conspicuously longer than in the female (see Fig. 7C, E). Although these terminal structures are not identical, their topology on (in *Ceuthonectes*) and near (in *Stygepactophanes*) the frontal pore supports the hypothesis that they may be homologous and likely have a similar function.

Conclusions

Stygepactophanes occitanus sp. n. is assigned to the genus *Stygepactophanes*. The new species shows several morphological characters in a primitive state, if compared to the type species of the genus *S. jurassicus*, weakening the attribution of the new species to a new genus, albeit closely related to *Stygepactophanes*. The morphological affinities of this genus to the other genera of the family Canthocamptidae have generated doubts since its original description. We have postulated that the genera *Ceuthonectes* and *Stygepactophanes* may represent a divergent lineage within the Canthocamptidae. Unfortunately, because of the complex systematics of the family still being in a state of flux, the relationships of this lineage to other members of the family remain unresolved. Presumably, *Stygepactophanes* entered the groundwater a very long time ago in the evolutionary history of the family Canthocamptidae, and has no representatives in surface waters (phylogenetic and distributional relict), as in the case of other harpacticoid genera, as well as the entire copepod order Gelyelloida.

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The Carabidae (Coleoptera) of Shada Al-A'Ala Nature Reserve, Southwestern Saudi Arabia, with description of a new species of Paussinae

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Abstract

We report the Carabidae collected at the Shada Al-A'Ala Nature Reserve (SANR) in Baha Province in southwestern Saudi Arabia during 2013–2015. In total, 62 carabid species and subspecies representing 39 genera, 17 tribes, and 10 subfamilies were identified, including one new species, *Paussus minutulus* Nagel & Rasool, **sp. n**, four new country records, and 24 species that are new provincial records for Baha. The carabid fauna was dominated by the Lebiini with 19 species. A high number of species were rarely collected (34 species) in comparison to the more abundant and common species (9 species). The highest number of species (52 species) was collected during autumn. The carabids of SANR are represented by a large component of Afrotropical faunal elements (28.1%) and smaller numbers of Oriental species (3.5%) and endemic taxa (5.3%). In comparison to Garf Raydah Nature Reserve in Asir Province, also in southwestern Saudi Arabia, SANR had an equal number of carabids sharing 64.5% of the species but with lower number of endemic elements. Our study can serve as a component for implementing a conservation plan for SANR using carabid beetles as sentinel taxa. These research results may support future ecological studies on SNAR carabids.

Keywords

Baha, ground beetles, Shada, endemics, faunistic inventory, new records, new species, nature reserve, Saudi Arabia, zoogeography

Introduction

Over the past three decades, numerous new wildlife protected areas have been established in Saudi Arabia (SA). Not only the number of national parks has increased but also newly established nature reserves, wildlife sanctuaries, and other protected landscapes and biosphere reserves (Abuzinada 2003). It is noted that SA has currently 16 protected areas and 12 national parks (Abuzinada 2003; SWA 2018). For this network of protected areas, biodiversity monitoring is fundamental for effective management. The invertebrate fauna of these protected areas has attracted relatively little attention as compared to those of vertebrates (Abuzinada et al. 2005), although international conventions signed by SA as a member of the Convention on Biological Diversity since 2001 (CBD 2011). Recent insect biodiversity survey and monitoring research projects in several protected areas in SA have been conducted by King Saud University Museum of Arthropods, Riyadh; resulting in several faunistic and ecological works being published (Aldhafer et al. 2012, 2016; Sharaf et al. 2013; Abdel-Dayem et al. 2016, 2017, 2018; El-Hawagry et al. 2016, 2017, 2018). One of these recent projects was focused on the insect biodiversity of Shada Al-A'Ala Nature Reserve (SANR) in Baha Province in southwestern SA. The location and elevation range (470–2,222 m) of the SANR provides relative high rainfall, diverse microclimates, and a distinct biodiversity (SWA 2018). The SANR is undoubtedly one of the most interesting protected areas in SA because of the existence of unique treasure trove of biological diversity. About 22% (495 plant species) of the total SA flora has been reported from the Shada Mountains including 19 endemic plant species and 43% endangered species (Thomas et al. 2017). The SANR harbours important faunas, including griffon vultures and other endemic birds of the southwestern mountains and carnivores (e.g. the rock fox, caracal, striped hyena, wolf, genet, and the Arabian leopard) (SWA 2018). Regarding insects, 119 species of flies (Diptera) have been reported from the SANR (El-Hawagry et al. 2016).

The Carabidae have a cosmopolitan distribution and form one of the most diverse and abundant families of insects constituting a considerable component of the soil fauna (Duchesne et al. 1999). These beetles play an important role in ecosystems as polyphagous predators, whereas others are phytophagous (Thiele 1977). Additionally, carabid beetles play significant role as bioindicators in habitat management, landscape ecology, conservation, pollution, climatic changes and soil characteristics (Rainio and Niemelä 2003; Avgın and Luff 2010; Koivula 2011; Kotze et al. 2011). There are currently 33,920 valid species (Lorenz 2005), 183 of which have been reported from SA (Abdel-Dayem et al. 2018). Unexpectedly, the number of previously published carabid species records from Baha Province are low (30 species) (Mateu 1986; Balkenohl 1994; El-Hawagry et al. 2013; Moore and Robertson 2014; Häckel and Azadbakhsh 2016; Rasool et al. 2017, 2018a, b). In the most recent study of the Baha insect fauna (El-

Hawagry et al. 2013), 17 carabid species were reported, with only ant nest beetle, *Paussus cephalotes* Raffray, 1886 known from SNAR. Rasool et al. (2017, 2018a, b) have reviewed the subtribes Cymindidina, Dromiusina and Lebiina of tribe Lebiini from southwestern SA and described *Lebia raeesae* Rasool, Abdel-Dayem & Felix, 2018 and reported *Calodromius mayeti* (Bedel, 1907), *Dromius buettikeri* Mateu, 1990, *Lebia auberti* Fairmaire, 1892, *L. nilotica* Chaudoir, 1871, *Matabele arabica* Mateu, 1986, *Mesolestes quadriguttatus* Mateu, 1979, *Metadromius arabicus* Mateu, 1979, *Met. brittoni* (Basilewsky, 1948), *Microlestes discoidalis* (Fairmaire, 1892), *Mic. infuscatus fragilis* Mateu, 1956 and *Zolotarevskyella rhytidera* (Chaudoir, 1876) from SANR.

However, despite the urgent conservation concerns associated with the SANR reserve, there have been no studies focused on beetles including the Carabidae. Thus, the objectives of this study are to provide a thorough baseline inventory of the carabid fauna of the SANR and to analyze its zoogeographical affinities. This information will assist in providing an essential cognitive basis for future management of this reserve. Additionally, our results will allow for future ecological studies of carabids of SNAR and will contribute to the overall knowledge of Carabidae of SA, the largest country of the Arabian Peninsula.

Methods

Study area

In Shada Al-A'Ala (Upper Shada) Mountain, an outlier of the Sarawat Mountains to the west, the SANR was established in 2002. The reserve is located (latitudes 19°48.894'–19°52.578'N and longitudes 41°17.130'–41°21.000'E) in Al-Mekhwa District (Baha Province); about 20 km southwest of Al-Mekhwa City, the capital of the district (Fig. 1). The SANR occupies an area of 67 km^2 and rises about 2,222 m. There is a perennial small freshwater stream in Wadi Neera at the west and southwest part of the reserve. Geologically the area belongs to the greater Afro-Arabian shield, which is a part of the Precambrian crust plate and is generally exposed and locally covered by tertiary volcanic rocks (Schmidt et al. 1972). There are terraced fields used by small local communities; these fields are very small scale and are used to grow distinctive varieties of coffee, banana, lemon, and natural figs (SWA 2018). The climate is similar to the uplands of southwestern SA. It is highly variable and characterized by cool winters, warmer partly cloudy summer, and high rainfall. The average annual temperature of 26.2 °C, and average annual rainfall of about 200 mm, and with wettest period concentrated between March and May (43% annual precipitation) (El-Hawagry et al. 2016).

The vegetation is rich, with the Leguminosae (Fabaceae) and composites (Asteraceae) having the highest contribution, followed by graminoides (Poaceae) (Al Zubaide et al. 2017, Thomas et al. 2017). The vegetation comprises 72.4% perennials and 27.6% annuals; represented by 17.2% trees, 51.8% shrubs, and 31.1% weeds (Al Zubaide et al. 2017). The vegetation at the foothills of Shada Al-A'Ala Mountain consists predominantly of subtropical *Acacia* thorn woodlands extending from the base up to

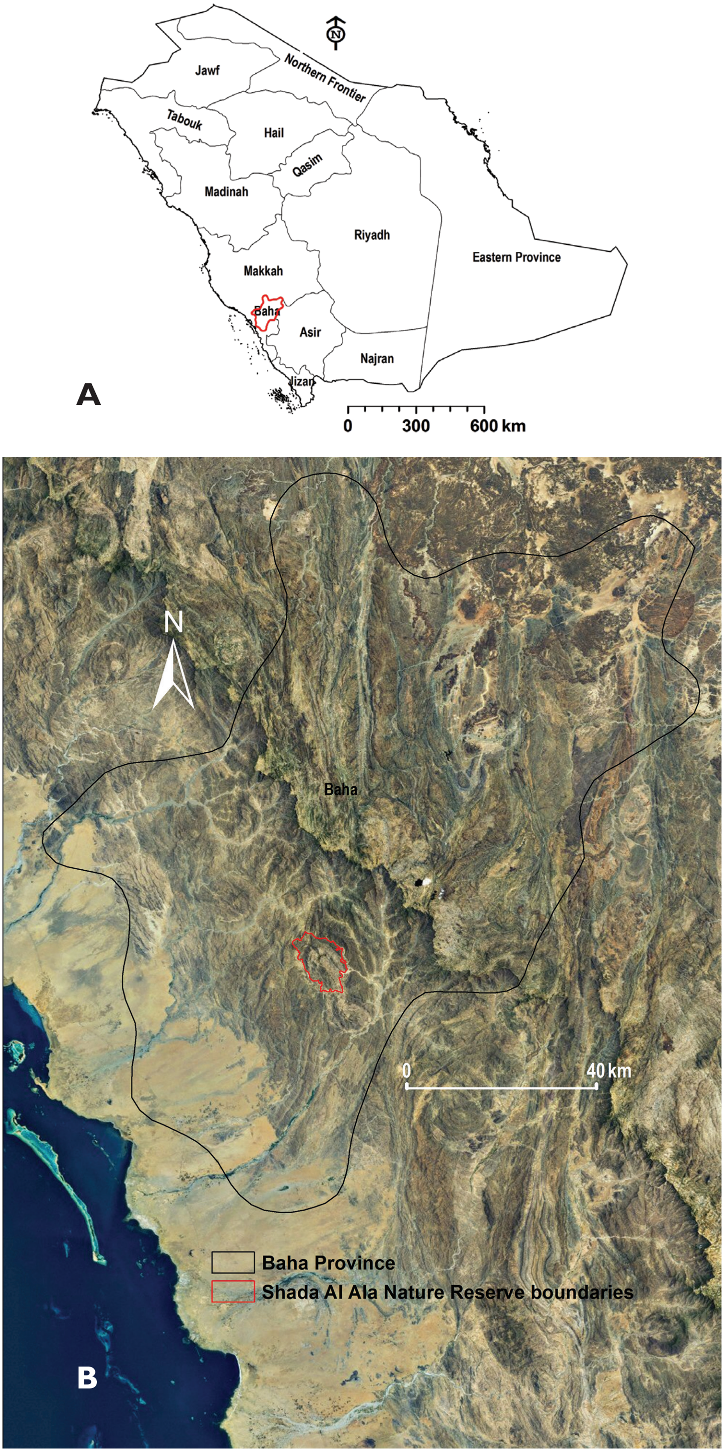

Figure 1. A Map of Saudi Arabia showing location of Baha Province **B** Location of Shada Al-A'Ala Nature Reserve within Baha Province.

1500 m a.s.l. The vegetation above 1000 m elevation is dominated by *Acacia asak* (Thomas et al. 2017). Higher up, above the *Acacia* zone, there are shrubs of Barbary fig or cactus pear, *Opuntia ficus-indica* (L.) Mill. (Cactaceae). For more details on the vegetation in SANR (see El-Hawagry et al. 2016).

Beetle collection

As part of a research project for studying the insect biodiversity in the SANR the adult ground beetles were sampled from 2013–2015. The sampling was conducted at various sites in varied habitats at 13 different elevation levels (Table 1) within the SANR. The geographical coordinate data of each collecting location were recorded using GPS Garmin, Montana 650 unit (Garmin Instruments Inc., Olathe, Kansas, USA).

Collected beetles were initially sorted to morphospecies level, mounted and then identified to species levels. Some species were sent to experts for identification or confirmation, as indicated in the remarks. The specimens are deposited in the collection of King Saud University Museum of Arthropods (KSMA), King Saud University, Riyadh, SA.

The description of the new species of *Paussus* was assisted using a Leica M205C dissecting microscope with 10× eyepieces and Planapo 1.0× and 1.6× front lenses, allowing magnification up to 240×. An eyepiece micrometer was used for measurements.

Classification and nomenclature

The subfamily and tribal classification of the family and nomenclature of the species in this study follows the Catalogue of Palaearctic Coleoptera (Löbl and Löbl 2017). However, the taxonomic order of species in the genus *Sphaerotachys* J. Miller, 1926 (Trechinae, Bembidiini) follows Sciaky and Vigna Taglianti (2003). The subfamilies, tribes, genera and species are listed alphabetically.

Table 1. List of collecting elevation levels and geographical coordinates, in Shada Al-A'Ala Nature Reserve, southwestern SA.

No.	Elevation (m)	Latitude (N)	Longitude (E)
1	471	19°44.870'	41°20.008'
2	825	19°52.717'	41°18.712'
3	851	19°52.685'	41°18.663'
4	892	19°52.598'	41°18.672'
5	1.008	19°52.023'	41°18.157'
6	1.225	19°51.762'	41°18.089'
7	1.325	19°51.066'	42°18.037'
8	1.388	19°51.387'	41°18.187'
9	1.448	19°47.511'	41°18.258'
10	1.474	19°50.710'	41°18.267'
11	1.563	19°50.329'	41°18.604'
12	1.611	19°50.411'	41°18.686'
13	1.666	19°50.575'	41°18.691'

Faunal list

For each species, the following information is provided: current nomenclatural combinations, material examined, zoogeography, distribution, published records in SA, and remarks. The label data for examined specimens are listed as follows: elevation level within the SANR, followed by the date of collection (months as Roman numerals), the collecting method (handpicking (HP), light traps (LT), malaise traps (MT), pitfall traps (PT), sweeping net (SW) and vacuuming (VC)) and the number of examined specimens followed by sex (♂ for male, ♀ for female, ex(s) for example with unidentified sex). The material examined is arranged in ascending order with respect to the elevation, then chronologically with respect to the month of collection. A semicolon separates different records; if these are from the same elevation, the elevation is listed only at the beginning with the older record.

General distribution and zoogeography

The zoogeography, which were used in the analysis of carabid faunal affinity, were assigned for each species using the zoogeographic realms of the world suggested by Holt et al. (2013). The zoogeography is based on their modern general geographical distributions (each country is represented by two capital letters according to ISO 3166 "ISO Alpha VC–2 Country code": The Nations Online Project: https://www.nationsonline.org/oneworld/country_code_list.htm :) provided by Löbl and Löbl (2017) and Lorenz (2017) unless otherwise stated.

Results

During this study, 3,287 adult carabid beetles were collected from SANR, comprising 62 species from 39 genera within 17 tribes and 10 subfamilies. These species include the description of a new species (*Paussus minutulus* sp. n.), three SA endemic species and six confined to Arabian Peninsula. Four species have not been previously recorded from SA, and 24 species recorded for the first time from Baha Province. The details of these species are provided in the faunal list below.

Of the carabids collected from SNAR, the most diverse tribe was the Lebiini, represented by 19 species (30.6% of the total species) in 13 genera (33.3% of the total genera) (Fig. 2). About 50% of the tribes are represented by one or two species. Nine species (14.5%) are classified as abundant and common species; *Lebia nilotica*, *Metadromius arabicus*, *Sphaerotachys conspicuus* (Schaum, 1863) were the most abundant species, comprising 50.4% of the total catch. Twenty species (32.3%) are considered rare, represented by four or fewer individuals collected over the two years. The maximum number of species were collected during autumn (52 species). The genus *Anthracus* Motschulsky, 1850 was recorded for the first time for SA. Three species have been identified to the genus level, belonging to *Amblystomus*, *Metadromius* and *Singilis*.

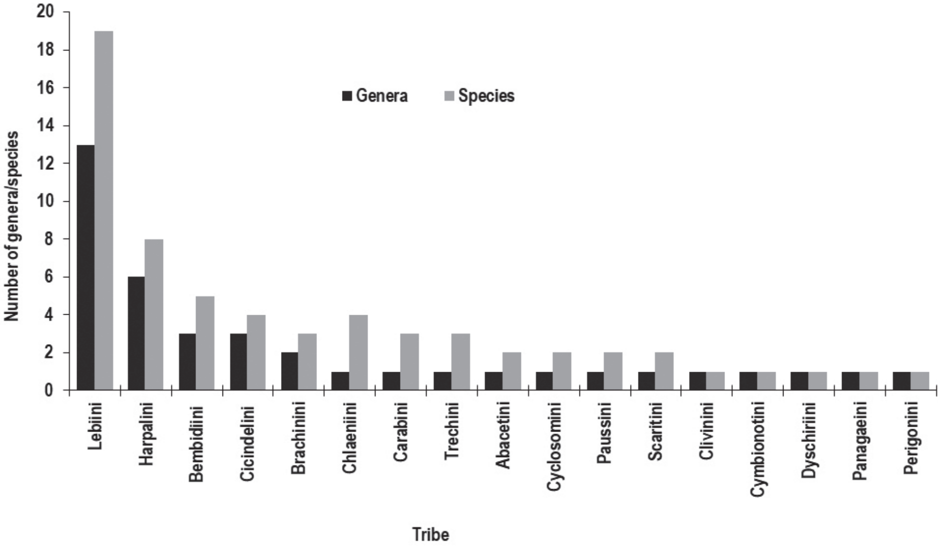

Figure 2. The number of genera and species for each tribe of ground beetles recorded between 2013–2015, from Shada Al-A'Ala Nature Reserve, southwestern Saudi Arabia.

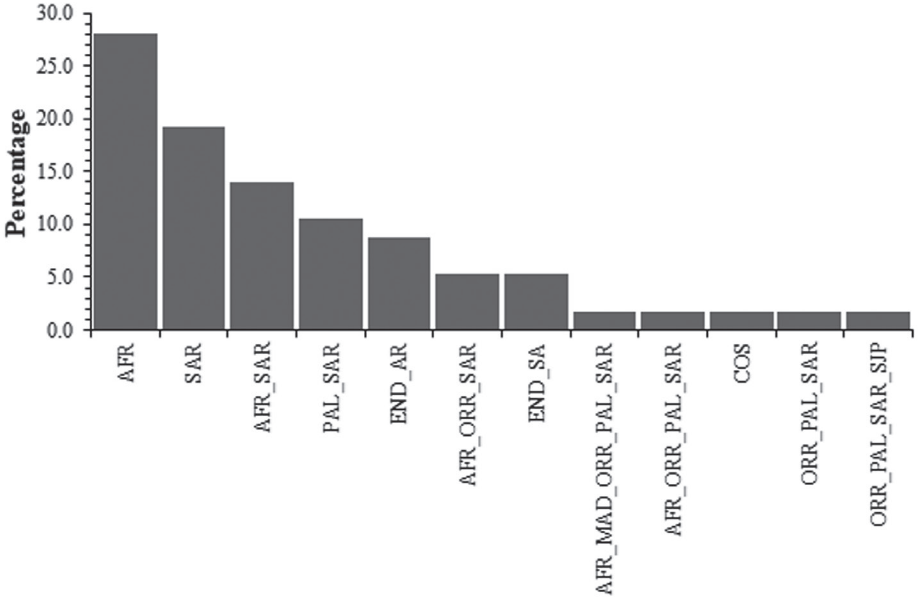

Figure 3. Zoogeographical affinities of the carabid fauna of Shada Al-A'Ala Nature Reserve, southwestern Saudi Arabia. AFR, Afrotropical; COS, Cosmopolitan; END_AR, endemic to the Arabian Peninsula; END_SA, endemic to Saudi Arabia; MAD, Madagascan; ORR, Oriental; PAL, Palaearctic; SAR, Saharo_Arabian; SJP, Sino_Japanese.

Faunal List

Brachininae
Brachinini

Brachinus crepitans (Linnaeus, 1758)

Material examined. 471 m: 10.XII.2014, LT, 1♂, 1♀.

General distribution and zoogeography. AL, DZ, AM, AT, AZ, BY, BE, BA, BG, HR, CY, CZ, DK, EE, FI, FR, GE, DE, GB, GR, HU, IR, IQ, IE, IT, JO (Nasir and Katbeh-Bader 2017), KZ, KG, LV, LB, LT, LU, MK, MD, ME, NL, NO, PL, PT, RO, RU, SA, SK, SI, ES, SE, CH, SY, TJ, TN, TR, TM, UA, UZ. This range exemplifies PAL_SAR.

Published records. Asir (Abdel-Dayem et al., 2018), Riyadh (Abdel-Dayem et al. 2017). New provincial records for Baha.

Remarks. A rare species. The adult beetles were collected during autumn by hand picking under stones along the edge of a freshwater stream flowing through *Acacia* thorn woodlands. Mahmoud Abdel-Dayem identified this species.

Brachinus dorsalis Dejean, 1831

Material examined. 892 m: 14.II.2014, LT, 1♂; 02. III.2014, LT, 1♂; 18.X.2014, LT, 1♀; 14.XI.2015, LT, 1♂; 08.XII.2014, LT. 1♂; 09.XII.2014, LT, 1♂.

General distribution and zoogeography. SA, YE. END_AR species.

Published records. Asir (Abdel-Dayem et al. 2018), Jizan (Mateu 1990). New provincial records for Baha.

Remarks. A rare species that was collected during autumn and winter by light trapping close to a freshwater stream flowing through *Acacia* thorn woodlands. Mahmoud Abdel-Dayem identified this species.

Pheropsophus africanus (Dejean, 1825)

Material examined. 471 m: 03.III.2015, LT, 1♂; 15.XI.2015, LT, 1♂, 3♀; 10.XII.2014, HP, 9♂, 2♀.

General distribution and zoogeography. AE, DZ, EG (including Sinai), ER, ET, IL, IQ, IR, JO (Nasir and Katbeh-Bader 2017), LY, MA, NE, SA, SD, TD, TN, YE. AFR_SAR species.

Published records. Asir (Basilewsky 1979), Baha (El-Hawagry et al. 2013), Makkah (Britton 1948; Basilewsky 1979).

Remarks. A frequent species, which was found only at low elevation (471 m) under stones and debris along the side of freshwater stream flowing through *Acacia* thorn woodlands. These adults were collected during autumn and winter by hand picking and by using a light trap. Mahmoud Abdel-Dayem identified this species.

Carabinae
Carabini

Calosoma imbricatum Klug, 1832

Material examined. 892 m: 26.I.2015, LT, 1♀; 14.II.2014, LT, 2♂, 1♀; 15.II.2014, LT, 1♀; 12.XI.2015, LT, 1♂; 14.XI.2015, LT, 1♂. 1,225 m: 12.XI.2015, LT, 1♀. 1,325 m: 15.XI.2015, 1♀. 1,448 m: 03.XI.2013, LT, 1♀; 03.XI.2013, HP, 1♂. 1,474 m: 02.III.2015, LT, 1♀. 1,611 m: 15.II.2014, LT, 1♀; 21.IV.2014, LT, 1♀.

General distribution and zoogeography. AE, BF, CV, DJ, DZ, EG, ER, ET, IQ, IR, KE, KW, LB, LY, ML, NE, OM, PK, QA, SA, SD, SN, SO, TD, YE. AFR_SAR species.

Published records. Asir (Abdel-Dayem et al. 2018), Baha (El-Hawagry et al. 2013), Eastern Province (Heinertz 1979), Jizan and Makkah (Britton 1948), Riyadh (Heinertz 1979; Abdel-Dayem et al. 2017).

Remarks. A rare species, which was collected during autumn, winter, and spring. The adults were collected by light trapping and hand picking in *Acacia* thorn woodlands and Barbary fig shrub community. Ali Elgharbawy identified this species.

Calosoma olivieri Dejean, 1831

Material examined. 892 m: 15.II.2014, LT, ♀; 23.IV.2014, LT, 1♂; 13.XI.2015, LT, 1♂; 14.XI.2015, LT, 1♂, 1♀. 1,225 m: 15.II.2014, PT, 1♀. 1,325 m: 15.II.2014, LT, 1♂, 3♀; 15.II.2014, PT, 1♀; 15.XI.2015, LT, 2♀. 1,474 m: 15.II.2014, LT, 1♂. 1,611 m: 15.II.2014, LT, 3♂, 6♀.

General distribution and zoogeography. AF, DZ, EG, IQ, IR, IT, JO, LY, MA, PK, SA, SY, TD, TM, TN, UZ, YE. PAL_SAR species.

Published records. Makkah (Britton 1948, Mateu 1990); Riyadh (Heinertz 1979; Abdel–Dayem et al. 2017). New provincial records for Baha.

Remarks. A frequent species that was collected during autumn, winter, and spring. The species was collected by light trapping and hand picking in *Acacia* thorn woodlands and Barbary fig shrub community. Ali Elgharbawy identified this species.

Calosoma senegalense Dejean, 1831

Material examined. 892 m: 15.XI.2015, LT, 1♀. 1,225 m: 14.XI.2015, LT, 1♂.

General distribution and zoogeography. AO, BF, BI, BJ, BW, CD, CF, CG, CI, CM, CV, ER, ET, GA, GH, GM, GN, GQ, GW, KE, LR, LS, MG, ML, MR, MW, MZ, NA, NE, NG, RE, RW, SL, SN, SO, SZ, TD, TG, TZ, UG, ZA, ZM, ZW. New to Arabian Peninsula. This range exemplifies the AFR realm.

Remarks. A rare species. The adults were collected only during autumn (November) by light trapping in an area dominated by *Acacia* trees. Mahmoud Abdel-Dayem identified this species.

Cicindelinae
Cicindelini

Calomera alboguttata (Klug, 1832)

Material examined. 471 m: 03.III.2015, LT, 24 exs; 15.XI.2015, LT, 2 exs; 10.XII.2014, LT, 7 exs. 1,611 m: 02.III.2015, LT, 1 ex.

General distribution and zoogeography. EG, ER, ET, KE, SA, SD, SO, YE. This range exemplifies the AFR realm.

Published records. Makkah (Britton 1948). New record for Baha Province.

Remarks. A frequent species collected during autumn and winter, with more individuals during late winter. These adult beetles were collected by light trapping in *Acacia* thorn woodlands and Barbary fig shrub community. Mahmoud Abdel-Dayem identified this species.

Cylindera rectangularis (Klug, 1832)

Material examined. 1,474 m: 14.XI.2015, LT, 1 ex. 1,611 m: 23.VIII.2014, LT, 2 exs.

General distribution and zoogeography. CD, ER, ET, KE, MW, MZ, SA, SD, SO, TZ, UG, YE. This range exemplifies the AFR realm.

Published records. Asir (Abdel-Dayem et al. 2018), Makkah (Abdel-Dayem and Kippenhan 2013). New record for Baha Province.

Remarks. A rare species. The adults were collected by light trapping during summer and autumn in *Acacia* thorn woodland community. Jürgen Wiesner identified this species.

Myriochila melancholica (Fabricius 1798)

Material examined. 1,325 m: 21.IV.2014, LT, 1♂. 1,474 m: 23.VIII.2014, LT, 1♀.

General distribution and zoogeography. AE, AF, AL, AM, AO, AZ, BF, BH, BJ, BW, CD, CF, CG, CI, CM, CN, CV, CY, CZ, DZ, EG, ER, ES, ET, FR, GA, GE, GH, GM, GN, GQ, GR, GW, IL, IN, IQ, IR, IT, JO, KE, KG, KZ, LB, LY, MA, MG, MW, MZ, NA, NG, NP, OM, PK, PT, QA, SA, SC, SD, SL, SN, SO, ST, SY, TD, TG, TJ, TM, TN, TR, TZ, UZ, YE, ZA, ZM, ZW. This range exemplifies AFR_MAD_ORR_PAL_SAR.

Published records. Asir (Abdel-Dayem et al. 2018), Jizan and Najran (Britton 1948), Riyadh (Abdel-Dayem et al. 2017). New record for Baha Province.

Remarks. A rare species. The two specimens were collected during summer and spring by light trapping in *Acacia* thorn woodlands. Mahmoud Abdel-Dayem identified this species.

Myriochila nudopectoralis (W. Horn, 1903)

Material examined. 1,325 m: 02.IX.2015, LT, 1♂, 1♀.

General distribution and zoogeography. ET, OM, YE. New country record. This range exemplifies the AFR realm.

Remarks. A rare species that was collected during summer by light trapping from *Acacia* thorn woodlands. Jürgen Wiesner identified this species.

Harpalinae

Chlaeniini

Chlaenius canariensis seminitidus Chaudoir, 1856

Material examined. 892 m: 15.II.2014, LT, 2♂; 21.IV.2014, LT, 1♂, 1♀; 14.XI.2015, LT, 1♂. 1,225 m: 15.II.2014, LT, 1♀; 14.XI.2015, LT, 1♂. 1,325 m: 15.II.2015, LT, 2♂; 3.VI.2014, LT, 1♂. 1,611 m: 15.II.2014, LT, 2♀; 21.IV.2014, LT, 3♂, 1♀. 1,666 m: 27.I.2014, LT, 1♂.

General distribution and zoogeography. AE, DZ, EG, IL, IQ, IR, JO, LB, LY, MA, SA, SY, TN, TR. This range exemplifies the SAR realm.

Published records. Asir (Abdel-Dayem et al. 2018). New record for Baha Province.

Remarks. A frequent species that was found at all elevation levels in the SANR in *Acacia* thorn woodlands and Barbary fig communities. The adults were collected by light trapping during autumn, winter, and spring. Erich Kirschenhofer identified this subspecies.

Chlaenius flavipes Ménétriés, 1832

Material examined. 1,008 m: 08.XII.2014, HP, 1♂.

General distribution and zoogeography. AF, AL, AM, AZ, BA, BG, GE, GR, HR, HU, IN, IQ, IR, KG, KZ, MD, MK, NP, PK, RO, RU, SA (Abdel-Dayem et al. 2017), TJ, TM, TR, UA, UZ. ORR_PAL_SAR species.

Published records. Asir and Riyadh (Abdel-Dayem et al. 2017, 2018). New record for Baha Province.

Remarks. A rare species that was collected during autumn. The only adult specimen was collected by hand under a stone at the edges of freshwater pools in *Acacia* thorn woodlands. Erich Kirschenhofer identified this species.

Chlaenius laeviplaga saudiarabica Mandl, 1980

Material examined. 471 m: 10.XII.2014, HP, 9♂, 5♀. 892 m: 10.XII.2014, LT, 1♂, 1♀.

General distribution and zoogeography. AE, SA. END_AR species.

Published records. Asir (Mandl 1980; Abdel-Dayem et al. 2018). New record for Baha Province.

Remarks. A frequent species that was collected during autumn. The adults were found under stones along the margins of a freshwater stream and collected by hand. Additional adults collected at night by using lights. Erich Kirschenhofer identified this subspecies.

Chlaenius pachys Chaudoir, 1876

Material examined. 471 m: 10.XII. 2014, HP, 2♀.

General distribution and zoogeography. ER, ET, JO (Nasir and Katbeh-Bader 2017), SD, SO, SA, YE. AFR species.

Published records. Jizan and Makkah (Britton 1948). New provincial records for Baha.

Remarks. A rare species that was collected during late autumn. The two adults were found under stones along the edge of freshwater stream collected by hand picking. Erich Kirschenhofer identified this species.

Cyclosomini

Tetragonoderus arcuatus Dejean, 1829

Material examined. 1611 m, 21.IV.2014, LT, 1♂; 03.VI.2014, LT, 1♀.

General distribution and zoogeography. BD, CN, EG, ET, IL (Assmann et al. 2015), IN, IQ, LA, MM, NE, NP, OM, PK, SD, TD, YE. AFR_ORR_SAR species.

Published records. known only from Baha Province (El-Hawagry et al. 2013).

Remarks. A rare species that was collected during spring by light trapping in a Barbary fig shrub community. Mahmoud Abdel-Dayem identified this species.

Tetragonoderus quadrum (Fabricius, 1792)

Material examined. 892 m: 14.II.2014, HP, 1♂; 15.II.2014, LT, 1♀. 1,325 m: 02.III.2015, LT, 1♀; 03.VI.2014, LT, 1♂; 05.VI.2014, PT, 1♀. 1563 m, 17.X.2014, LT,1♀.

General distribution and zoogeography. ER, ET, GM, MR, SA, SN, SO, TD. AFR species.

Published records. Jizan (Mateu 1990). New record for Baha Province.

Remarks. A rare species which was collected during autumn, winter, and spring from *Acacia* thorn woodlands and Barbary fig shrub communities. Mahmoud Abdel-Dayem identified this species.

Harpalini

Amblystomus orpheus (LaFerté-Sénectěre, 1853)

Material examined. 825 m: 14.XI.2015, LT, 2 exs; 15.XI.2015, LT, 1 ex. 892 m: 17.X.2014, LT, 1 ex; 14.XI.2015, LT, 1ex; 1,225 m: 2.IX.2015, LT, 2 exs. 1,325 m: 2.IX.2015, LT, 4 exs; 17.X.2014, LT, 1ex; 15.XI.2015, LT, 3 exs. 1,474 m: 14.XI.2015, LT, 1ex. 1,563 m: 20.IV.2014, LT, 2 exs; 14.XI.2015, LT, 4 exs. 1,611 m: 2.IX.2015, LT, 1 ex.

General distribution and zoogeography. AE, AO, BF, BI, CD, CF, CM, CV, ER, ET, KE, ML, MR, MW, MZ, NA, NE, SA (Abdel-Dayem et al. 2018), SN, SO, TD, TZ, UG, YE, ZA, ZW. AFR species.

Published records. Asir (Abdel-Dayem et al. 2018). New provincial records for Baha.

Remarks. A frequent species that was collected during late summer (September) to autumn, and spring. The adult specimens were collected only using light traps in *Acacia* thorn woodlands and cactus communities. David Wrase identified this species.

Amblystomus sp.

Material examined. 892 m: 16.X.214, LT, 1♂, 1♀; 15.XI.2015, LT, 1♂, 1♀; 08.XII.2014, LT, 1♀.1,611 m: 21.IX.2015, LT, 1♀.

Remarks. A rare species that was collected during late summer and autumn. The above specimens were collected by light traps set at lower altitude in *Acacia* thorn woodlands. David Wrase and Boris Kataev identified this taxon.

Anthracus angusticollis (Péringuey, 1908)

Material examined. 1,474 m: 17.X.2014, LT, 1♂.

General distribution and zoogeography. AE, CD, ET, GM, MG, MR, NA, SN, SO, TD, TZ, ZA. New country record. AFR species.

Remarks. A rare species. The single above male was collected during autumn at light in *Acacia* thorn woodlands. Bernd Jaeger identified this species.

Crasodactylus punctatus Guerin-Meneville, 1847

Material examined. 851 m: 15.II.2014, LT, 2 exs. 892 m: 26.I.2015, LT, 1 ex; 15.II.2014, LT, 2 exs; 20.IV.2014, LT, 1 ex; 15.X.2014, LT, 4 exs; 17.X.2014, LT, 3 exs; 18.X.2014, LT, 2 exs; 14.XI.2015, LT, 3 exs; 15.XI.2015, LT, 1 ex; 07.XII.2014, LT, 1 ex. 1,225 m: 02.IX.2015, LT, 1 ex; 17.X.2014, LT, 3 exs; 14.XI.2015, LT, 1 ex. 1,325 m: 17.X.2014, LT, 14 exs; 18.X.2014, PT, 1 ex; 15.XI.2015, LT, 9 exs. 1,474 m:

17.X.2014, LT, 1 ex; 18.X.2014, PT, 3 exs. 1,448 m: 03.XI.2013, LT, 31 exs. 1,563 m: 14.XI.2015, LT, 1 ex. 1,611 m: 02.IX.2015, LT, 1 ex. 1,666 m: 17.X.2014, LT, 10 exs. **General distribution and zoogeography.** AF, CD, DJ, ER, ET, KE, IQ, IR, NE, OM, PK, SA (Abdel-Dayem et al. 2018), SO, TD, TN, YE (including Socotra). AFR_SAR species.

Published records. Asir (Abdel-Dayem et al. 2018). New provincial records for Baha.

Remarks. A common species that was collected between an altitude of 851–1666 m in *Acacia* thorn woodlands and a Barbary fig shrub community using both light traps and pitfall traps. The highest number of individuals were collected during autumn. David Wrase identified this species.

Harpalus impressus Roth, 1851

Material examined. 892 m: 23.IV.2014, LT, 1 ex. 1,474 m: 2.XI.2013, HP, 3 exs.

General distribution and zoogeography. ER, ET, SD, SA, YE, UG. AFR species.

Published records. Asir (Basilewsky 1979; Abdel-Dayem et al. 2018).

Remarks. A rare species that was collected during summer and autumn from *Acacia* thorn woodlands. David Wrase identified this species.

Harpalus tenebrosus tenebrosus Dejean, 1829

Material examined. 1,225 m: 02.IX.2015, LT, 1♂, 2♀. 1,666 m: 02.IX.2015, LT, 1♀.

General distribution and zoogeography. AF, AL, AM, AT, AZ, BA, BE, BG, CH, CY, CZ, DE, DZ, EG, ES, FR, GB, GE, GR, HR, HU, IL, IQ, IR, IT, JO, MA, MD, ME, MK, MR, OM, PK, PL, PT, RO, RS, RU, SA (Abdel-Dayem et al. 2018), SI, SK, SY, TJ, TM, TN, TR, UA, UZ. PAL_SAR subspecies.

Published records. Asia (Abdel-Dayem et al. 2018). New provincial records for Baha.

Remarks. A rare species collected during late summer by light trapping in *Acacia* thorn woodlands and Barbary fig communities. David Wrase identified this species.

Progonochaetus planicollis (Putzeys, 1880)

Material examined. 471 m: 10.XII. 2014, HP, 1♀. 892 m: 26.I.2015, LT, 1♂, 1♀; 14.II.2014, LT, 3♂, 7♀; 15.II.2014, LT, 4♂, 5♀; 16.II.2014, LT, 1♂; 20.IV.2014, LT, 1♂; 21.IV.2014, LT, 1♀; 23.IV.2014, LT, 1♀; 15.XI.2015, LT, 1♀; 07.XII.2014, LT, 1♀; 08.XII.2014, LT, 1♂, 3♀; 10.XII.2014, LT, 1♂. 1,225 m: 27.I.2015, LT, 1♀. 1,325 m: 15.II.2014, LT, 4♂; 02.III.2015, LT, 1♀. 1,474 m: 15.II.2014, LT, 1♂. 1,448 m: 03.XI.2013, LT, 1♂. 1,563 m: 15.II.2014, LT, 1♀. 1,611 m: 15.II.2014, LT, 1♀; 21.IV.2014, LT, 1ex. 1,666 m: 21.IV.2014, LT, 1ex.

General distribution and zoogeography. AO, BF, CM, CD, CF, CG, CI, DJ, ER, ET, GA, GH, GN, GQ, GW, KE, ML, MZ, NA, NG, RW, SA, SN, SO, TD, TZ, YE, ZM, ZW. AFR species.

Published records. Asir (Basilewsky 1979; Abdel-Dayem et al. 2018). New record for Baha Province.

Remarks. A frequent species that was collected during all seasons with more individuals collected during winter. The adults were collected by light trapping and hand picking in *Acacia* thorn woodlands and Barbary fig communities. David Wrase identified this species.

Siopelus quadraticollis (Putzeys in Chaudoir, 1878)

Material examined. 892 m: 26.I.2015, LT, 1ex; 15.II.2014, LT, 1♂, 2♀; 18.X.2014, LT, 1♂, 2♀; 14.XI.2015, LT, 2♂, 5♀; 07.XII.2014, LT, 1♂, 1♀. 1,225 m: 15. II.2014, HP, 1♂; 17.X.2014, LT, 6♂, 4♀; 12.XI.2015, LT, 1♂. 1,325 m: 17.X.2014, LT, 14♀; 18.X.204, PT, 1♀; 15.XI.2015, LT, 2♀; 08.XII.2014, HP, 2♀. 1388 m: 08.XII.2014, HP, 1♀. 1,448 m: 03.XI.2013, HP, 3♀. 1,474 m: 17.X.2014, LT, 1♂; 18.X.2014, PT, 3♀; 02.IX.2015, LT, 2♀. 1,563 m: 02.IX.2015, LT, 3♀; 17.X.2014, LT, 1♂, 2♀. 1,611 m: 02.IX.2015, LT, 1♀.

General distribution and zoogeography. ET, SA (Abdel-Dayem et al. 2018), TZ. AFR species.

Published records. Asir (Abdel-Dayem et al. 2018). New record for Baha Province.

Remarks. A common species that was collected during late summer, autumn, and winter. The adults were collected by light and pitfall trapping in *Acacia* thorn woodlands and Barbary fig communities. David Wrase identified this species.

Lebiini

Apristus arabicus Mateu, 1986

Material examined. 1,325 m: 17.X.2014, LT, 1 ex. 1,474 m: 03.XI.2013, HP, 1 ex.

General distribution and zoogeography. AE, IQ, SA. SAR species.

Published records. Asir (Abdel-Dayem et al. 2018), Makkah (Mateu 1986). New record for Baha Province.

Remarks. A rare species collected during autumn from *Acacia* thorn woodlands by light trapping and hand picking. Ron Felix identified this species.

Calodromius mayeti (Bedel, 1907)

Material examined. 825 m: 13.XI.2015, LT, 1♂, 1♀; 15.XI.2015, LT, 1♂, 2♀. 851 m: 15.XI.2015, LT, 1♀. 892 m: 26.I.2015, LT, 1♀; 16.II.2014, LT, 2♂; 13.XI.2015, LT, 1♀. 1,225 m: 20.IV.2014, LT, 2♀; 12.XI.2015, LT, 1♂, 1♀. 1,325 m: 02.III.2015, LT, 1♂, 2♀; 14.XI.2015, LT, 1♂; 17.X.2014, LT, 1♀. 1,474 m: 17.X.2014, LT, 1♀.

General distribution and zoogeography. AE, DZ, IR, JO (Nasir and Katbeh-Bader 2017), LY, MA, SA, TN. SAR species.

Published records. Asir and Riyadh (Abdel-Dayem et al. 2017, 2018), Baha (Rasool et al. 2018b), Madinah and Makkah (Mateu 1986).

Remarks. A frequent species that was found in *Acacia* thorn woodlands during the four seasons of year, but peak populations occurred during autumn. The adults were collected by light trapping and hand picking. Ron Felix identified this species.

Dromius buettikeri Mateu, 1990

Material examined. 825 m: 13.XI.2015, LT, 1♀; 15.XI.2015, LT, 1♂. 851 m: 15.XI.2015, LT, 1♀. 892 m: 12.XI.2015, LT, 1♂, 1♀. 1,325 m: 27.I.2015, LT, 1♀; 14.XI.2015, LT, 1♂, 2♀; 15.XI.2015, LT, 1♀. 1,474 m: 27.I.2014, LT, 1♀; 15.II.2014, LT, 1♂, 1♀; 14.XI.2015, LT, 1♂; 08.XII.2014, LT, 1♂. 1,563 m: 27.I.2015, LT, 1♂. 1,611 m: 27.I.2015, LT, 1♀. 1,666 m: 27.I.2015, LT, 1♀.

General distribution and zoogeography. SA. END_SA species.

Published records. Asir and Baha (Rasool et al. 2018b), Riyadh (Mateu 1990).

Remarks. A frequent species. The adults were collected during autumn and winter from *Acacia* thorn woodlands and Barbary fig communities. The specimens were attracted only to light trap. Ron Felix identified this species.

Eremolestes sulcatus (Chaudoir, 1876)

Material examined. 1,474 m: 23.IV.2014, PT, 1♂. 1562 m: 03.XI.2013, HP, 1♂, 1♀.

General distribution and zoogeography. DJ, DZ, ER, ET, SA, SD, TD. This range exemplifies AFR realm.

Published records. Najran and Riyadh (Mateu 1979, 1986). New record for Baha Province.

Remarks. A rare species that was found during autumn and spring collected by pitfall trapping and hand picking in *Acacia* thorn woodlands and Barbary fig shrub communities. Ron Felix identified this species.

Lebia auberti Fairmaire, 1892

Material examined. 1,225 m: 08.XII.20114, LT, 1♀. 1,325 m: 02.IX.2015, LT, 1♂.

General distribution and zoogeography. DJ, SA (Rasool et al. 2018a). This range exemplifies AFR realm.

Published records. Asir (Abdel-Dayem et al. 2018, Rasool et al. 2018), Baha (Rasool et al. 2018a), Riyadh (Mateu 1986).

Remarks. A rare species with each sex represented by a single specimen collected from *Acacia* thorn woodlands during late summer and early autumn. Alexander Anichtchenko and Ron Felix identified this species.

Lebia nilotica Chaudoir, 1871

Material examined. 825 m: 14.XI.2015, LT, 7♂, 10♀; 15.XI.2015, LT, 4♂, 7♀. 892 m: 26.I.2015, LT, 2♂, 3♀; 14.II.2014, LT, 2♂, 3♀; 15.II.2014, LT, 2♂, 2♀; 20.IV.2014, LT, 4♂, 3♀; 21.IV.2014, LT, 1♂, 1♀; 16.X.2014, LT, 1♂; 18.X.2014, LT, 2♂, 4♀; 14.XI.2015, HP, 20♂, 26♀; 14.XI.2015, LT, 1♀; 15.XI.2015, LT, 2♂, 4♀; 09.XII.2014, LT, 1♀; 10.XII.2014, LT, 1♀; 11.XII.2014, LT, 1♀. 1,225 m: 27.I.2015, LT, 1♀; 15.II.2014, HP, 3♀; 15.II.2014, LT, 3♀; 21.IV.2014, LT, 3♂, 6♀; 02.IX.2015, LT, 19♂, 31♀; 14.XI.2015, LT, 3♀; 15.XI.2015, LT, 1♀. 1,325 m: 27.I.2015, LT, 1♀; 15.II.2014, LT, 1♂, 5♀; 15.II.2014, MT, 1♀; 21.IV.2014, LT, 4♂, 9♀; 03.VI.2014, LT, 1♂, 1♀; 23.VIII.2014, LT, 2♀; 02.IX.2015, LT, 14♂, 25♀; 15.XI.2015, LT, 4♂, 6♀. 1,474 m: 15.II.2014, LT, 1♂, 3♀; 21.IV.2014, LT, 2♂, 3♀; 05.V.2015, LT, 1♀; 23.VIII.2014, LT, 1♀; 02.IX.2015, LT, 41exs; 14.XI.2015, LT, 1♀. 1,563 m: 05.V.2015, MT, 2exs; 02.IX.2015, LT, 96exs; 17.X.2014, LT, 1♀. 1,611 m: 21.IV.2014, LT, 4♂, 4♀; 03.VI.2014, LT, 1♂; 03.VI.2014, SW, 1♂; 02.IX.2015, LT, 62exs; 17.X.2014, LT, 1♀; 15.XI.2015, LT, 1♀. 1,666 m: 21.IV.2014, LT, 2♂; 03.VI.2014, LT, 1♂, 2♀; 02.IX.2015, LT, 217exs.

General distribution and zoogeography. EG, IQ, SA. This range exemplifies SAR realm.

Published records. Asir (Mateu 1979, Abdel-Dayem et al. 2018, Rasool et al. 2018), Baha and Jizan (Rasool et al. 2018a).

Remarks. An abundant species that was recorded during all seasons of the year from a wide altitudinal range (471–1666 m). Most individuals were collected during late summer (September). Mahmoud Abdel-Dayem and Iftekhar Rasool identified this species.

Lebia raeesae Rasool, Abdel-Dayem & Felix, 2018

Material examined. 1,611 m: 21.IV.2014, LT, 1♀. 1,666 m: 27.I.2015, LT, 1♀; 02.III.2015, LT, 1♀; 21.IV.2014, LT, 1♀; 23.VIII.2014, LT, 1♂; 02.IX.2015, LT, 3♂, 4♀.

General distribution and zoogeography. SA, YE (Rasool et al. 2018a). This range exemplifies SAR realm.

Published records. Asir and Baha (Rasool et al. 2018a).

Remarks. A rare species that was collected during spring, summer, and winter by light trapping in Barbary fig shrubs communities. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Matabele arabica Mateu, 1986

Material examined. 1,225 m: 14.XI.2015, LT, 1♀. 1,563 m: 23.VII.2015, LT, 1♀.

General distribution and zoogeography. OM, SA. This range exemplifies SAR realm.

Published records. Asir (Mateu 1986), Baha (Rasool et al. 2018a).

Remarks. A rare species that was collected during autumn and summer, represented by a single specimen during each season. Ron Felix identified this species.

Merizomena buettikeri (Mateu, 1986)

Material examined. 825 m: 13.XI.2015, LT, 2♀; 15.XI.2015, LT, 20 exs. 851 m: 14.XI.2015, LT, 23 exs; 15.XI.2015, LT, 1♂, 2♀. 892 m: 13.XI.2015, LT, 1♀; 15.XI.2015, LT, 24 exs. 1,225 m: 17.X.2015, LT, 1♀; 12.XI.2015, LT, 1♂, 1♀; 14.XI.2015, LT, 1♂, 2♀;. 1611m: 23.VIII.2014, LT, 1♀.

General distribution and zoogeography. SA. END_SA species.

Published records. Asir and Riyadh (Abdel-Dayem et al. 2017, 2018), Madinah and Najran (Mateu 1986). New record for Baha Province.

Remarks. A common species with more individuals collected during autumn from *Acacia* thorn woodlands and relatively few individuals during late summer from a Barbary fig shrub community. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Metadromius arabicus Mateu, 1979

Material examined. 825 m: 13.XI.2015, LT, 2♂, 4♀; 15.XI.2015, LT, 5♂, 3♀. 851 m: 14.XI.2015, LT, 1♂; 15.XI.2015, LT, 2♂, 2♀. 892 m: 16.X.2015, LT, 1♂; 17.X.2014, LT, 6♀; 15.XI.2015, LT, 1♂, 1♀. 1,225 m: 02.III.2015, LT, 1♂, 1♀; 02.IX.2015, LT, 2♀; 17.X.2014, LT, 1♂, 2♀; 15.XI.2015, LT, 1♀. 1,325 m: 27.I.2015, LT, 1♀; 02.III.2015, LT, 1♂; 02.IX.2015, LT, 2♂, 1♀; 17.X.2014, LT, 1♂, 1♀; 14.XI.2015, LT, 3♂, 5♀. 1,474 m: 15.II.2014, LT, 1♂, 2♀; 02.IX.2015, LT, 38♂, 52♀; 17.X.2014, LT, 3♂, 2♀. 1562 m: 03.XI.2013, HP, 1♀. 1,563 m: 21.IV.2014, LT, 2♂, 4♀; 02.IX.2015, LT., 51♂, 71♀; 17.X.2014, LT, 3♂, 6♀; 14.XI.2015, LT, 2♂; 18.XI.2015, LT, 2♀. 1,611 m: 27.I.2015, LT, 1♂; 02.IX.2015, LT, 122♂, 146♀; 17.X.2014, LT, 4♂, 2♀. 1,666 m: 02.III.2015, LT, 3♂, 5♀; 05.V.2015, LT, 2♀; 02.IX.2015, LT, 21♂, 29♀.

General distribution and zoogeography. AE, IR, SA. This range exemplifies SAR realm.

Published records. Asir and Baha (Abdel-Dayem et al. 2018; Rasool et al. 2018b), Riyadh (Mateu 1979).

Remarks. An abundant species that was collected during all four seasons with most specimens caught during late summer (September). The adults of this species were only collected using light traps. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Metadromius brittoni (Basilewsky, 1948)

Material examined. 471 m: 3.III.2015, LT, 1♀; 10.XII.2014, LT, 1♀. 825 m: 13.XI.2015, LT, 5 exs; 15.XI.2015, LT, 7 exs. 851 m: 13.XI.2015, LT, 1♂; 15.XI.2015, LT, 17 exs. 892 m: 16.II.2014, LT, 1♀; 03.III.2015, LT, 2♀; 23.IV.2014, LT, 12♂, 8♀; 23.VIII.2014, LT, 1♂; 17.X.2014, LT, 24♂, 15♀. 16.X.2014, LT, 2♂, 4♀. 1,225 m: 27.I.2015, LT, 1♂; 02.III.2015, LT, 5♂, 3♀; 21.IV.2014, LT, 17♂, 10♀; 05.V.2015, LT, 9 exs; 03.VI.2014, LT, 21♂, 17♀; 23.VIII.2014, LT, 8♂, 5♀; 24.VIII.2014, LT, 1♂; 02.IX.2015, PT, 1 ex; 17.X.2014, LT, 2♂, 3♀; 18.X.2014, PT, 1♀. 1,325 m: 02.III.2015, LT, 6 exs; 21.IV.2014, LT, 2♂, 3♀; 05.V.2015, LT, 1 ex; 23.VIII.2014, LT, 13♂, 10♀; 02.IX.2015, LT, 3 exs; 17.X.2014, LT, 2♂, 5♀. 1,474 m: 20.IV.2014, LT, 1 ex; 23.VIII.2014, LT, 1♂; 02.IX.2015, LT, 1♂; 17.X.2014, LT, 1♂. 1562 m, 03.XI.2013, HP, 1♂. 1563 m, 21.IV.2014, LT, 1♂, 1♀; 03.VI.2014, LT, 3 exs. 1611 m 23.VIII.2014, VC, 1♂; 17.X.2014, LT, 1♂; 02.IX.2015, LT, 2 exs; 18.X.2014, PT, 1♀.

General distribution and zoogeography. JO, SA, YE. This range exemplifies SAR realm.

Published records. Asir, Baha and Jizan (Abdel-Dayem et al. 2018; Rasool et al. 2018b), Riyadh (Mateu 1979).

Remarks. A common species that was collected during all four seasons from different altitudinal ranges (471–1611 m) of the SANR. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Metadromius sp.

Material examined. 471 m: 03.III.2015, LT, 1♂. 1,325 m: 02.III.2015, LT, 1♀.

Remarks. A rare species that was collected during late winter. It is similar to *M. ephippiatus* (Fairmaire, 1884), which is known from North Africa (DZ, MA, TN) (Löbl and Löbl 2017). However, these specimens along with SA specimens identified by Mateu (1986) are rather different from *M. ephippiatus*. The specimens from SA probably represent a new species. These specimens have been included in a current taxonomic revision of the Middle East *Metadromius* (R. Felix, personal communication). Ron Felix identified this species.

Microlestes discoidalis (Fairmaire, 1892)

Material examined. 825 m: 13.XI.2015, LT, 1♂; 15.XI.2015, LT, 1♂. 851 m: 15.XI.2015, LT, 2♂, 1♀; 892 m: 26.I.2015, LT, 2♂, 1♀; 15.II.2014, LT, 3♂, 8♀; 23.IV.2014, LT, 1♂; 1,563 m: 21.IV.2014, LT, 2♂. 1,611 m: 02.IX.2015, LT, 1♂, 2♀.

General distribution and zoogeography. AE, AF, BD, CD, CV, DJ, ER, ET, IL, IN, IR, JO (Nasir and Katbeh-Bader 2017), KE, MR, NE, OM, SA, SD, SO, TD, TR, TZ, YE. AFR_ORR_SAR species.

Published records. Asir, Baha, Jizan and Riyadh (Abdel-Dayem et al. 2017; 2018; Rasool et al. 2018b), Makkah (Britton 1948; Mateu 1986).

Remarks. A frequent species that was collected during all seasons of the year with more individuals during the winter collecting dates. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Microlestes infuscatus fragilis Mateu, 1956

Material examined. 892 m, 23.IV.2014, LT, 7♂, 9♀. 1,325 m: 23.VIII.2014, LT, 1♀. 1,474 m: 02.IX.2015, LT, 1♀. 1,666 m: 02.II.2015, PT, 1♀; 23.VIII.2014, LT, 1♀; 02.IX.2015, LT, 1♂; 15.XI.2015, PT, 1♀.

General distribution and zoogeography. AF, SA, YE. This range exemplifies SAR realm.

Published records. Asir, Baha and Jizan (Mateu 1979; Abdel-Dayem et al. 2018; Rasool et al. 2018b).

Remarks. A frequent species, which recorded during all seasons with highest numbers collected during spring (April). Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Pseudomesolestes quadriguttatus Mateu, 1979

Material examined. 851 m: 15.XI.2015, LT, 1♀. 892 m: 26.I.2015, LT, 1♀; 17.X.2014, LT, 1♂; 18.X.2014, LT, 1♂, 2♀; 15.XI.2015, LT, 1♀. 1,325 m: 02.III.2015, LT, 1♂. 1,563 m: 17.X.2014, LT, 1♀.

General distribution and zoogeography. SA. END_SA species.

Published records. Asir and Baha (Rasool et al. 2018b), Riyadh (Mateu 1979).

Remarks. A rare species that was collected by light trapping during late summer (September), autumn, and winter. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Singilis discoidalis (Mateu, 1986)

Material examined. 851 m: 15.XI.2015, LT, 1♂. 1,225 m: 21.IV.2014, LT, 1♂; 03.VI.2014,1♀; 02.III.2015, 1♀. 1,325 m: 03.VI.2014, LT, 1♂, 2♀; 27.VII.2015, LT, 1♀.

General distribution and zoogeography. EG, IL, SA, YE. This range exemplifies SAR realm.

Published records. Asir (Abdel-Dayem et al. 2018), Madinah, Makkah and Najran (Mateu 1986). New record for Baha Province.

Remarks. A rare species that was caught by light trapping during the different seasons from *Acacia* thorn woodlands. Alexander Anichtchenko identified this species.

Singilis sp.

Material examined. 851 m: 15.XI.2015, LT, 1♀.

Remarks. A rare species that was collected by light trapping in *Acacia* thorn woodlands during autumn. This unidentified species is closely related to *S. cordiger* (Peringuey, 1896), which is known from NA, ZA and ZW. Unfortunately, only a single female was collected, and males are needed for identification. Alexander Anichtchenko identified this species.

Syntomus submaculatus (Wollaston, 1861)

Material examined. 892 m: 21.IV.2014, LT, 1♀. 1,225 m: 28.VIII.2014, LT, 1♀; 08.XII.2014, VC, 2♀. 1,325 m: 15.XI.2015, PT, 1♀. 1388 m: 08.XII.2014, HP, 5♂ 6♀. 1,474 m: 08.XII.2014, VC, 1♀. 1562 m: 03.XI.2013, 2♂ 4♀. 1,563 m: 05.VI.2014, PT, 1♀.

General distribution and zoogeography. CV, MR, SA TD, YE. This range exemplifies SAR realm.

Published records. Jizan (Mateu 1986). New record for Baha Province.

Remarks. A frequent species that was collected during spring, summer, and autumn. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Zolotarevskyella rhytidera (Chaudoir, 1876)

Material examined. 1,474 m: 18.X.2014, PT, 1♀. 1562 m: 03.XI.2013, HP, 1♀.

General distribution and zoogeography. CD, CV, ER, ET, GM, ML, NE, SA, SD, SN, TD, YE. This range exemplifies AFR realm.

Published records. Asir, Baha and Jizan (Rasool et al. 2018b), Najran (Mateu 1986).

Remarks. A rare species collected during the autumn from *Acacia* thorn woodlands and a Barbary fig shrub community. Iftekhar Rasool, Mahmoud Abdel-Dayem and Ron Felix identified this species.

Perigonini

Perigona nigriceps (Dejean, 1831)

Material examined. 1,225 m: 02.III.2015, LT, 2 exs; 24.VIII.2014, LT, 1 ex; 02.IX.2015, LT, 1 ex; 17.X.2014, LT, 1 ex. 1,325 m: 02.III.2015, LT, 5 exs; 23.VIII.2014, LT, 1 ex; 14.XI.2015, LT, 1 ex; 15.XI.2015, LT, 2 exs. 1,563 m: 05.V.2015, LT, 1 ex. 1,611 m: 02.III.2015, LT, 1 ex; 05.V.2015, LT, 2 exs; 02.IX.2015, LT, 130 exs. 1,666 m: 02.IX.2015, LT, 99 exs; 02.IX.2015, PT, 1 ex.

General distribution and zoogeography. AE, AO, AT, AU, AZ, BA, BB, BE, BG, BI, CA, CD, CG, CH, CI, CM, CN, CR, CU, CZ, DE, DK, ES, ET, FI, FR, GB, GH, GN, GP, GR, HR, HU, ID, IN, IQ, IR, IT, JP, KE, KH, KM, KP, KR, LK, LR, LU, LV, MD, MG, MM, MQ, MU, NC, NG, NL, NO, NZ, PG, PH, PL, PR, PT, RE, RU, RW, SA (Abdel-Dayem et al. 2018), SB, SC, SE, SI, SK, SL, SN, ST, TH, TW, TZ, UG, US, VN, YE, ZA, ZM, ZW. COS species.

Published records. Only reported from Asir (Abdel-Dayem et al. 2018). New record for Baha Province.

Remarks. A common species that was collected during all seasons with the highest number of individuals caught during late summer (September). The adults were collected mainly by light trapping in *Acacia* thorn woodlands and Barbary fig shrub communities. Mahmoud Abdel-Dayem identified this species.

Melaeninae
Cymbionotini

Cymbionotum microphthalmum Chaudoir, 1876

Material examined. 892 m: 23.IV.2014, LT, 1 ex.

General distribution and zoogeography. ET, NE, SA, SN, TR, YE. AFR_SAR species.

Published records. Known only from Eastern Province (Basilewsky 1979). New provincial records for Baha.

Remarks. A rare species that was collected during spring from *Acacia* thorn woodlands community. Mahmoud Abdel-Dayem identified this species.

Panagaeinae
Panagaeini

Microcosmodes arabicus Häckel & Azadbakhsh, 2016

Material examined. 825 m: 13.XI.2015, LT, 4♂, 8♀. 851 m: 14.XI.2015, LT, 2♂, 5♀. 892 m: 16.X.2014, LT, 1♂, 1♀; 17.X.2014, LT, 1♀; 14.XI.2015, LT, 6♂, 13♀; 15.XI.2015, LT, 1♂, 3♀; 10.XII.2014, LT, 1♀. 1,325 m: 15.XI.2015, PT, 1♂.

General distribution and zoogeography. OM, SA, YE. END_AR species.

Published records. Paratypes known from the SANR, Baha (Häckel and Azadbakhsh 2016).

Remarks. A frequent species that was collected during autumn from *Acacia* thorn woodlands with highest number of individuals collected by light traps at lower altitudes. Martin Häckel identified this species.

Paussinae
Paussini

Paussus cephalotes Raffray, 1886

Material examined. 1562 m, 03.XI.2013, HP, 1♀.

General distribution and zoogeography. SA, YE. END_AR species.

Published records. Asir (Abdel-Dayem et al. 2018), Baha (El-Hawagry et al. 2013; Moore and Robertson 2014), Hejaz Mountains (Nagel 1982).

Remarks. A rare species that was collected during autumn. The female of this species was found under a stone in a Barbary fig shrub community. Iftekhar Rasool identified this species and confirmed by Peter Nagel.

Paussus minutulus Nagel & Rasool, sp. n.

http://zoobank.org/EE392B1D-8B68-4CA1-A49C-61E4529093FD
Figs 4–6

Holotype (hereby designated). Female; dry-mounted, glued on pinned pointed card; head with antennal clubs, left middle tibia with tarsus, left hind leg detached, and glued on to same card.

Original labels. 1. White, rectangular, black printed text and thin frame (*verbatim, slash = line break*): "**KSA.** Baha; / Shada Al-A' Ala Nature Reserve; / 19°50.411'N, 41°18.686'E; / 1611 m; 2.IX.2015; PT.2; / Aldhafer, H., Fadl, H., Abdel-Dayem, M., / Elgharbawy, A., El Torkey, A., Soliman, A.". 2. White, rectangular, black handwriting: "Paussus sp. 3".

Added labels. 1. Red, rectangular, printed in black: "HOLOTYPUS/ *Paussus minutulus* / Nagel & Rasool, 2018". 2. White label, printed: "♀".

Holotype repository. King Saud University Museum of Arthropods (KSMA), Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.

Type locality. Arabian Peninsula, western Saudi Arabia, Baha Province, Upper Shada Mountain, Shada Al-A' Ala Nature Reserve 19°50.411'N, 41°18.686'E), 1611 m, pitfall trap no. 2, emptied on 2 September 2015 after 48 hours of exposure. The place of exposure of pitfall trap no. 2 is dominated by the cactus pear *Opuntia ficus-indica* (L.) Mill. (Cactaceae). This "cactus zone" covers the area of the nature reserve above approximately 1500 m. It is an extensively cultivated area, also characterized by small-scale terraced fields (Fig. 4). More details are given in "Study area" above.

Etymology. The specific epithet is the Latin adjective for very small, because it is the smallest known Arabian *Paussus*.

Diagnosis. *Paussus minutulus* sp.n. is a small *Paussus* of the *P. cucullatus* group *sensu lato* and is most similar to *P. abditus* Nagel, 2018 (SA) and *P. rougemontianus* Lorenz, 1998 (Yemen). It is distinguished from both by the tumid antennal club with its posterior basal angle large, thick, and apically truncate. Further specific characters are as

Figure 4. Photo of the cactus pear, *Opuntia ficus-indica* (L.) Mill. (Cactaceae) in the foreground and background, and the shrubs *Capparis cartilaginea* Decne. (Capparaceae) in the middle ground; the type locality for *Paussus minutulus* Nagel & Rasool, sp. n. at Shada Al-A' Ala Nature Reserve, Baha Province, southwestern Saudi Arabia at an elevation of 1611 m.

follows: antennal club with excavation ending far in front of apex; head with vertex produced, with two distinct pores at the top; collar of anterior pronotum low, with transverse edge rounded and with lateral projections absent; pronotal trichome well developed at both ends of transverse furrow; pygidium with lower (posterior) margin with dense fringe of hair; fore and middle legs robust, not compressed, hind femur dilated and flattened, hind tibiae little wider than thick; small, apically fringed setae present at several body parts, most obvious at antennomere 1 and all legs.

Description of female holotype specimen. (Fig. 5) Standardized body length from tip of head to tip of elytra 3.4 mm (3.5 mm total body length from tip of head to tip of pygidium), width across mid-elytra 1.5 mm. Body castaneous, appendices little darker, ventral abdomen and anterior part of pronotum little lighter; compacted or reinforced marginal areas of head, antennal clubs and pronotum narrowly blackish. Surface smooth and shining, except forehead matt and antennomere 1 (scape) with dense and coarse, yet shallow punctuation. Pubescence of elytra inconspicuous, restricted mostly to apical and lateral parts (abraded in the middle?), of short and upright as well as recumbent, narrowly lanceolate setae; elytra additionally with very few, long, thin, upright setae on lateral part of disc and as *series umbilicata*; head, pronotum, pygidium and appendices with scattered, short, mostly appressed setae; these short setae have a multiple split (fringed) apex and are most obvious at antennomeres 1, femora and tibiae, yet absent at

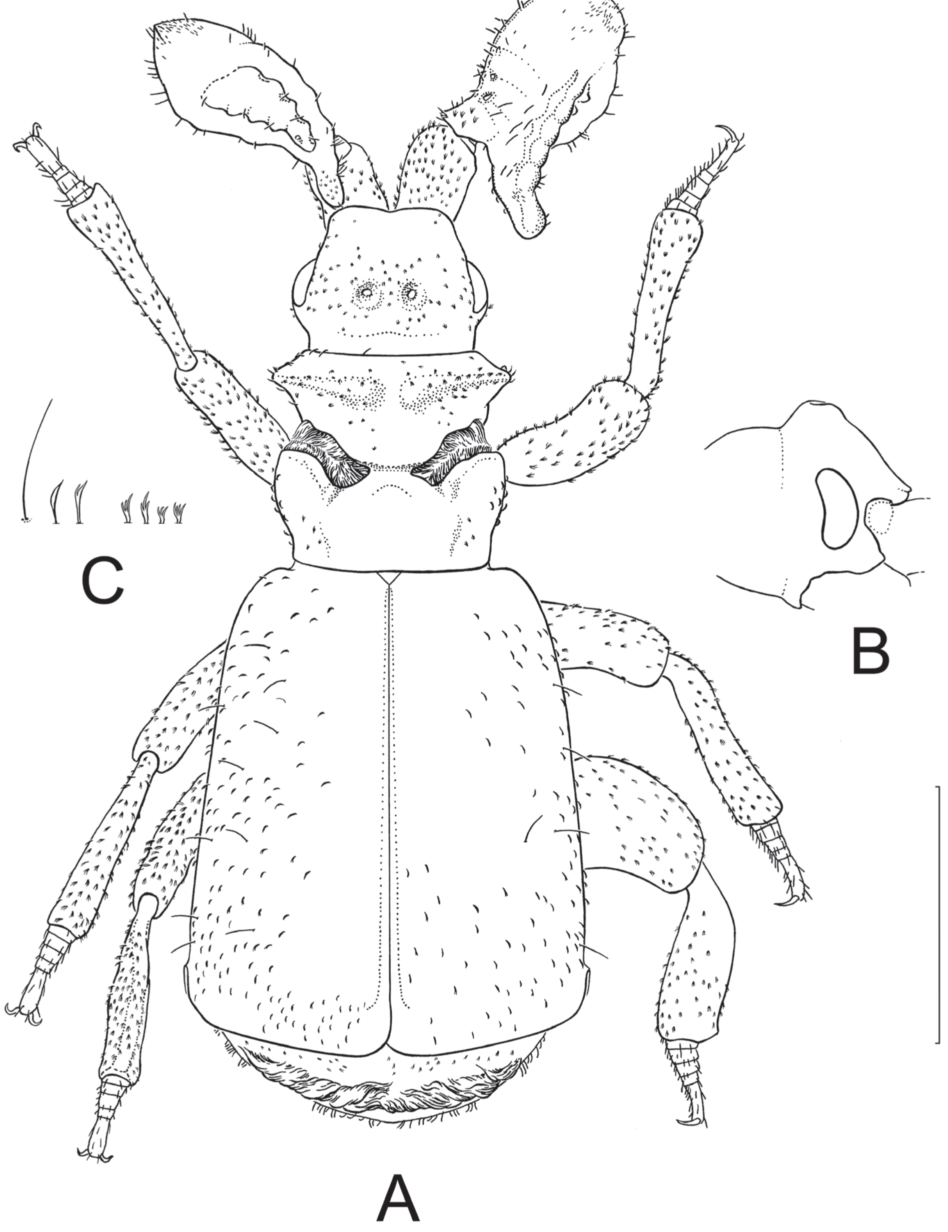

Figure 5. *Paussus* (*Hylotorus*) *minutulus* Nagel & Rasool, sp. n. **A** Habitus, dorsal view, appendices of right and left side at broadest and narrowest view respectively **B** Head, lateral view **C** Types of setae of dorsal pubescence, from left to right: one long, thin, hair-like seta; two shorter, lanceolate bristles, the right one with split apex; four short scaliform setae with multiply split, fringed apex; the setae are shown at enlarged view and at sizes relative to each other, yet without scale. Scale bar: 1 mm. Illustration: Adrian Gertsch. Copyright: Peter Nagel.

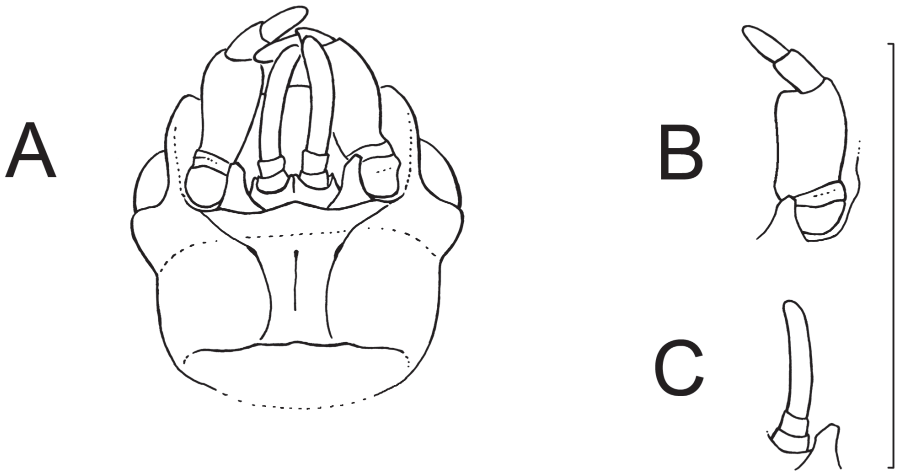

Figure 6. *Paussus* (*Hylotorus*) *minutulus* Nagel & Rasool, sp. n. **A** Head, ventral view **B** Left maxillary palpus, broadest view **C** Left labial palpus, broadest view. Scale bar: 1 mm. Illustration: Adrian Gertsch. Copyright: Peter Nagel.

elytra; antennal clubs with the normal apical sensory field and with scattered upright or slightly curved medium setae. Head 1.3 times wider than long, frontal margin broadly truncated, slightly biconvex; in dorsal view head in front of eyes little narrowed apically, gena and eye of equal length; temples not projecting; head with vertex produced, with two distinct pores at the top; pores broadly marginate with orifice slightly sunk. Antennomere 1 devoid of distinctly marked longitudinal edges; antennal club excavate on its exterior (posterior) side, 1.5 times longer than wide (basal tooth disregarded), tumid; frontal margin distinctly emarginate near base, with one small and one tiny fenestriform pit; anterior basal angle of club acute, marked; posterior basal projection large, thick, apically truncate; hind (exterior) side of the club with excavation limited by broad dorsal and ventral borders, and ending far before apex; at posterior view dorsal and ventral margins of excavation swollen, undulate with 3 or 4 low tubercles, each with one to three subapical setae; club without distinct trichome near ventral base, just an indistinct assemblage of three slightly thicker setae. Mouthparts as shown in Figure 6, not dissected; ligula at ventral view with longitudinal carinula in the middle of the disc (not shown in Fig. 6); (antepenultimate) maxillary palpomere II at broadest view 1.5 times as long as wide with mesal margin almost straight; (terminal) labial palpomere III long, narrow, five times as long as wide, apically rounded; gula with width/length ratio at narrowest point 0.9 (for measurement see Robertson and Moore (2016). Pronotum approximately as long as wide (1.1 times wider), transversely bipartite, with large trichomes at both ends of furrow; anterior part little wider than head (1.2 times), low, with transverse dorsal edge of collar broadly rounded, slightly indented in the middle, not angulate, lateral angles obscure; posterior part narrowed towards base. Elytral pubescence of two types: a few, very scattered, thin, long, erect setae on lateral parts of the disc (in addition to the similarly looking hairs of the *series umbilicornis*), plus scattered, recumbent, narrowly lanceolate setae on lateral and apical parts of disc; lateral subapical folds ("flange of Coanda") normal, without peculiarities. Hind wings present. Pygidium with central disc almost even, with indistinct microsculpture, weakly shining; lower (anatomically posterior) margin explanate; pygidial trichome of dense fringe of long hair set semicircular along lower margin; ventral part of explanate margin set with one row of short, appressed setae. Legs robust, femora and tibiae of fore and middle legs not compressed, hind femur and tibia slightly compressed and dilated, the whole inner side of hind tibia with longitudinal shallow groove; pubescence of femora and tibiae of scattered, small, apically fringed setae; all tibiae without terminal spurs; terminal tarsomere of posterior tarsus as long as three preceding ones together; tarsomeres ventrally with few lateral setae, and glabrous in the middle. Inner side of hind femur subbasally with file of stridulatory organ; the file consists of multiple parallel fine grooves and is located at both the anterior and posterior parts of a longitudinal, short carinula.

Male. Unknown.

Distribution. The new species is only known from the holotype female specimen from the type locality at Shada Al-A' Ala Nature Reserve.

Ecology. This single specimen of *Paussus* was found in an area with low impact small-scale agriculture. The vegetation is characterized by dominant cactus pear. The altitude is ca 1610 m, the winters are cool and most of the 200 mm annual rainfall is concentrated between March and May (see details in chapter "Study area" above). The specimen of *P. minutulus* sp.n. was taken by a pitfall trap which also caught ants of the following taxa: *Camponotus aegyptiacus* Emery, 1915; *Messor ebeninus* Santschi, 1927; *Monomorium jizane* Collingwood & Agosti, 1996; *Pheidole* Westwood, 1839, sp.; *Tetramorium simillimum* Smith, 1851) and *Tetramorium sericeiventre* Emery, 1877. Members of all these genera are known as host of one or more species of Paussini (Geiselhardt et al. 2007). *Paussus minutulus* sp.n. forms part of the Afrotropical *P. cucullatus* species group, of which several members are known to be associated with *Pheidole* sp., including the Arabian *P. rougemontianus* (see, for example, Luna de Carvalho 1989).

Remarks. The new species is assigned to *Paussus* subgenus *Hylotorus* Dalman, 1823, according to the phylogenetically based classification of Robertson and Moore (2016) (see Nagel et al. 2017a). The description of *P. minutulus* sp.n. given above agrees generally well the diagnosis and characters used in the key provided by Robertson and Moore (2016) and their subgenus description. The labial palpomere III is longer and narrower than described as usual for the Subgenus *Hylotorus*, yet still within the range of variation of this character, and similar to *P. abditus* Nagel, 2018 in Abdel-Dayem et al. (2018). The lacinia was not dissected. The fringed setae are conspicuous, despite their tiny size. Sometimes, individuals of a few *Paussus* species were found to show the same type of setae with both split and unsplit apices. In these cases, the splitting is most probably an artifact caused by a treatment during or after collecting (use of certain chemicals in the killing bottle or relaxing chamber, for example). In *P. minutulus* sp.n. this particular shape is obviously an intrinsic character, because they are alike at all body parts where they occur.

Paussus minutulus sp.n. forms part of the *P. cucullatus* group *sensu lato* and is most similar to and possibly part of the same clade as *P. abditus* Nagel, 2018 in Abdel-Dayem et al. (2018) (SA) and *P. rougemontianus* Lorenz, 1998 (Yemen) (replacement name for *Cochliopaussus rougemonti* Luna de Carvalho, 1989). It differs from *P. abditus* by the slightly shorter pronotum, the less compressed and dilated hind leg, the absence of a distinct subbasal antennal trichome, the smaller extension of the excavation at the posterior antennal club, the large, thick, apically truncated posterior basal projection of the antennal club, the presence of fringed setae, and the clearly marked, distinct cephalic orifices.

It differs from *P. rougemontianus* by the slightly shorter pronotum, the less compressed and dilated hind leg, the absence of a distinct subbasal antennal trichome, the dorsal hind margin of the antennal club retracted, the presence of fringed setae, and, above all, the large, thick, apically truncated posterior basal projection of the antennal club in the new species. The new species differs from the little-known Ethiopian *P. cyathiger* Raffray, 1886, among others, by the long, thin peg-like posterior basal angle of the antennal club, and the longitudinal crescent margin at the external part of the cephalic pores of the latter (see Abdel-Dayem et al. 2018).

It is the smallest known Arabian *Paussus* and at the same time it is among the smallest *Paussus* at global scale (smallest measurements 3.3–3.5 mm total body length): *Paussus* (Subg. *incertae sedis*) *exiguus* Reichensperger, 1929, Sudan; *P. (Anapaussus) asperulus* Fairmaire, 1898, Madagascar; *P. (Anapaussus) pipitzi* Dohrn, 1884 [ssp. *pictor* Reichensperger, 1922, and possibly further subspecies], Madagascar and *P. (Edaphopaussus) favieri* Fairmaire, 1851, southwestern Europe and northwestern North Africa).

The Arabian Peninsula and adjacent regions harbour ten (with *P. minutulus* sp.n. included) species of Paussinae, all members of the genus *Paussus* Linnaeus, 1775 (see Nagel et al. 2017b; Abdel-Dayem et al. 2018). The following eight species have been recorded from the montane ecoregion of southwestern SA and Yemen (linked to the Eastern Afromontane Highlands Hotspot, see Mittermeier et al. 2004). The presence of all of them is in line with the zoogeographical affiliation of southwestern Arabian Peninsula to the Afrotropical Region: *Paussus abditus* Nagel, 2018 (END_SA); *P. arabicus* Raffray, 1886 (AFR_SAR); *P. brittoni* Reichensperger, 1957 (END_YE); *P. cephalotes* Raffray, 1886 (END_AR); *P. cirenaicus* Fiori, 1914 (PAL_SAR); *P. minutulus* Nagel & Rasool, sp.n. (END_SA); *P. rougemontianus* Lorenz, 1998 (END_YE) and *P. thomsonii* Reiche, 1860 (PAL_AFR_SAR).

Pterostichinae
Abacetini

Abacetus crenulatus Dejean, 1831

Material examined. 892 m: 23.IV.2014, LT, 2 exs; 1,225 m: 05.V.2015, LT, 1 ex. 1,325 m: 03.VI.2015, LT, 1 ex.

General distribution and zoogeography. BF, BJ, CI, ML, MR, SA (Abdel-Dayem et al. 2018), SN, TD. AFR species.

Published records. Asir (Abdel-Dayem et al. 2018). New provincial records for Baha.

Remarks. A rare species collected during spring by light trapping in *Acacia* thorn woodlands. Mahmoud Abdel-Dayem identified this species.

Abacetus quadrisignatus Chaudoir, 1876

Material examined. 471 m, 10.XII.2014, HP, 2 exs. 892 m, 15.II.214, LT, 2 exs.

General distribution and zoogeography. ET, ER and YE (Socotra Island) (Felix et al. 2012). New country record. This range exemplifies AFR realm.

Remarks. A rare species that was collected during autumn and winter from lower elevations in area of *Acacia* thorn woodlands. Mahmoud Abdel-Dayem identified this species.

Scaritinae

Clivinini

Coryza beccarii Putzeys, 1873

Material examined. 471 m, 15.XI.2015, LT, 1 ex; 10.XII.2014, LT, 2 exs; 10.XII.2014, HP, 15 exs.

General distribution and zoogeography. EG (Sinai), ER, GN, IL, OM, SA, YE. AFR_SAR species.

Published records. Asir (Basilewsky 1979), Jizan and Makkah (Britton 1948, Balkenohl 1994). New provincial records for Baha.

Remarks. A frequent species that was collected during autumn at lower altitudes. The adults were caught by hand picking under stones and by light trapping along the edge of a freshwater stream. Michael Balkenohl and Ali Elgharbawy identified this species.

Dyschiriini

Dyschirius chalybeus gibbifrons Apfelbeck, 1899

Material examined. 825 m: 15.XI.2015, LT., 1 ex. 851 m: 15.XI.2015, LT., 1 ex. 892 m: 16.II.2014, LT, 1 ex; 23.IV.2014, LT, 1 ex; 15.XI.2015, LT, 2 exs. 1225 m, 17.X.2014, LT, 4 exs. 1,325 m: 02.IX.2015, LT, 2 exs; 17.X.2014, LT, 11 exs; 14.XI.2015, LT. 12 exs. 1,474 m: 15.II.2014, LT, 1 ex; 14.XI.2015, LT., 1 ex. PAL_SAR species.

General distribution and zoogeography. AL, AT, AZ, BG, CZ, GE, GR, IL, IR, IT, MD, RO, RS, RU (South European Territory), SA (Balkenohl 1994), SK, TM, TR, UA. PAL_SAR species.

Published records. Asir (Abdel-Dayem et al. 2018), Baha (Balkenohl 1994).

Remarks. A frequent species that was recorded during all seasons with more individuals were collected during autumn. The adults were caught using light traps set in *Acacia* thorn woodlands. Michael Balkenohl, Ali Elgharbawy and Mahmoud Abdel-Dayem identified this species.

Scaritini

Scarites striatus Dejean, 1825

Material examined. 1,225 m: 23.VIII.2014, PT, 3 exs. 1,325 m: 08.XII.2014, HP, 2 exs. 1,666 m: 02.IX.2015, PT, 1 ex; 15.XI.2015, HP, 5 exs.

General distribution and zoogeography. DZ, EG (including Sinai), LY, SA, TN, YE. SAR species.

Published records. Asir, Makkah (Balkenhol 1994; Abdel-Dayem et al. 2018). New provincial records for Baha.

Remarks. A rare species that was collected during late summer and autumn from *Acacia* thorn woodlands and Barbary fig shrub communities. Michael Balkenohl, Ali Elgharbawy and Mahmoud Abdel-Dayem identified this species.

Scarites terricola aethiopicus Bänninger, 1933

Material examined. 471 m: 15.XI.2015, LT, 1 ex; 10.XII.2014, HP, 1 ex.

General distribution and zoogeography. DZ, EG (Sinai), ER, ET, IL, OM, SA, YE. AFR_SAR species.

Published records. Baha, Eastern Province, Jizan, Madinah, Makkah, Riyadh (Britton 1948; Balkenohl 1994).

Remarks. A rare species collected during autumn at lower altitudes in *Acacia* thorn woodlands. The adults were caught along the edge of a freshwater stream by hand picking under stones and by using light traps. Michael Balkenohl, Ali Elgharbawy and Mahmoud Abdel-Dayem identified this species.

Trechinae

Bembidiini

Bembidion atlanticum atlanticum Wollaston, 1854

Material examined. 892 m, 16.II.2014, LT, 1♂; 17.X.2014, LT, 1♀. 1,225 m: 02.IX.2015, LT, 1♀. 1,325 m: 15.XI.2015, LT, 1♀. 1,611 m: 27.I.2015, LT, 3♂, 1♀.

General distribution and zoogeography. AE, AF, AM, AZ, BG, CV, CY, DZ, EG (including Sinai), FR, GE, GR, IL, IN, IQ, IR, KG, KZ, MA, MD, MR, MT, NE, PT, RU (South European Territory), SA, SY, TD, TJ, TM, TN, TR, UA, UZ, YE. AFR_ORR_PAL_SAR species.

Published records. Asir, Baha and Riyadh (Basilewsky 1979; El-Hawagry et al. 2013; Abdel-Dayem et al. 2018).

Remarks. A rare species that was collected during late summer, autumn, and winter by light trapping in *Acacia* thorn woodlands and Barbary fig shrub communities. Paolo Neri and Mahmoud Abdel-Dayem identified this subspecies.

Bembidion niloticum niloticum Dejean, 1831

Material examined. 825 m, 15.XI.2015, LT, 2♂, 3♀. 851 m: 15.XI.2015, LT, 2♂, 2♀. 892 m: 16.II.2014, LT, 2♂; 15.XI.2015, LT, 1♀. 1,225 m: 03.VI.2014, LT, 1♀; 14.XI.2015, LT, 1♂, 2♀.

General distribution and zoogeography. AE, AF, AM, AZ, BG, CN, CY, EG, GE, GR, IL, IN, IQ, IR, JO, JP, KG, KH, KP, KR, KZ, MM, NP, OM, PH, PK, RU (South European Territory), SA, SY, TM, TR, TW, UZ, VN. ORR_PAL_SAR_SJP species.

Published records. Asir (Abdel-Dayem et al. 2018), Makkah (Britton). New record for Baha Province.

Remarks. A frequent species that was sporadically collected during autumn, winter, and spring in *Acacia* thorn woodlands. Paolo Neri and Mahmoud Abdel-Dayem identified this subspecies.

Sphaerotachys conspicuus (Schaum, 1863)

Material examined. 471 m: 2.III.2015, LT, 4 exs. 825 m, 15.XI.2015, LT, 15 exs. 851 m, 14.XI.2015, LT, 3 exs; 15.XI.2015, LT, 17 exs. 892 m: 23.IV.2014, LT, 1 ex. 1,225 m: 2.III.2015, LT, 41 exs; 05.V.2015, LT, 1 ex; 03.VI.2014, LT, 1 ex; 2.IX.2015, LT, 2 exs; 14.XI.2015, LT, 4 exs. 1,325 m: 27.I.2015, LT, 27 exs; 15.II.2014, LT, 1 ex; 2.III.2015, LT, 108 exs; 21.IV.2014, LT, 1 ex; 05.V.2015, LT, 1 ex; 03.VI.2014, LT, 6 exs; 17.X.2014, LT, 2 exs; 15.XI.2015, LT, 5 exs. 1,474 m: 15.II.2014, LT, 1 ex; 2.III.2015, LT, 7 exs; 05.V.2015, LT, 1 ex. 1,563 m: 27.I.2015, LT, 1 ex; 2.III.2015, LT, 4 exs; 05.V.2015, LT, 2 exs. 1,611 m: 27.I.2015, LT, 2 exs; 2.III.2015, LT, 1 ex; 05.V.2015, LT, 1 ex. 1614 m, 20.X.2014, LT, 3 exs. 1,666 m: 27.I.2015, LT, 1 ex; 05.V.2015, LT, 1 ex.

General distribution and zoogeography. AE, AO, DZ, EG, ER, ET, IL, KE, LY, MR, NE, SA, SD, SO, TD, YE. AFR_SAR species.

Published records. Asir (Basilewsky 1979; Abdel-Dayem et al. 2018), Baha (El-Hawagry et al. 2013).

Remarks. An abundant species, which was collected during all four seasons, with highest number of individuals collected during winter (March) and lowest numbers during the late summer (September). The adults were caught by light traps set at various altitudinal zones (471–1666 m) in *Acacia* thorn woodlands and Barbary fig shrub communities. Mahmoud Abdel-Dayem identified this species.

Sphaerotachys tetraspilus variabilis (Chaudoir, 1876)

Material examined. 471 m: 03.III.2015, LT, 11 exs. 851 m, 15.XI.2015, LT, 1 ex. 892 m: 23.IV.2014, LT, 2 exs. 1,325 m: 27.I.2015, LT, 53 exs. 15.II.2015, LT, 1 ex. 17.X.2014, LT, 1 ex. 1,474 m: 26.I.2015, PT, 5 exs. 27.I.2015, LT, 57 exs; 15.II.2014, LT, 2 exs; 02.III.2015, PT, 1 ex; 05.V.2015, LT, 5 exs; 18.X.2014, PT, 1 ex. 1,563 m: 26.I.2015, PT, 2 exs; 27.I.2015, LT, 7 exs; 02.III.2015, LT, 8 exs; 05.V.2015, LT, 3 exs. 1,611 m: 27.I.2015, LT, 25 exs; 02.III.2015, LT, 5 exs; 05.V.2015, LT, 25 exs; 03.VI.2014, LT, 1 ex; 17.X.2014, LT, 1 ex. 1,666 m: 27.I.2015, LT, 1 ex; 02.III.2015, LT, 1 ex; 05.V.2015, LT, 5 exs.

General distribution and zoogeography. AE, AO, BF, CD, CI, CV, DZ, ER, ET, GM, IN, KE, ML, MR, NE, PK, SA, SD, SN, SO, TD, UG. AFR_ORR_SAR species.

Published records. Asir (Basilewsky 1979; Abdel-Dayem et al. 2018), Baha (El-Hawagry et al. 2013), Makkah (Britton 1948).

Remarks. A common species that was collected during autumn, winter, and spring, with a peak during winter. The adults were collected at various altitudinal zones in *Acacia* thorn woodlands and Barbary fig communities. Mahmoud Abdel-Dayem identified this subspecies.

Tachyura biblis (Britton, 1948)

Material examined. 471 m: 03.III.2015, LT, 3 exs. 963 m: 03.XI.2013, HP, 1 ex. 1225 m, 02.III.2015, LT, 6 exs; 05.V.2015, LT, 2 exs. 1,325 m: 27.I.2015, LT, 2 exs; 02.III.2015, LT, 24 exs; 21.IV.2014, LT, 2 exs; 05.V.2015, LT, 19 exs; 23.VIII.2014, LT, 1 ex. 1,474 m: 27.I.2015, LT, 1 ex; 02.III.2015, LT, 14 exs; 05.V.2015, LT, 8 exs. 1,563 m: 02.III.2015, LT, 3 exs; 05.V.2015, LT, 6 exs. 1,611 m: 02.III.2015, LT, 2 exs; 21.IV.2014, LT, 1 ex; 05.V.2015, LT, 3 exs; 03.VI.2014, SW, 1 ex; 27.VII.2015, LT, 1 ex. 1,666 m: 05.V.2015, LT, 1 ex; 27.VII.2015, LT, 1 ex; 17.X.2014, LT, 1 ex.

General distribution and zoogeography. AE, DJ, DZ, IR, MR, NE, SA, TD, YE. AFR_SAR species.

Published records. Asir (Abdel-Dayem et al. 2018), Riyadh (Basilewsky 1979).

Remarks. A common species, which was collected during all seasons of the year with the peak reached during winter (March). The adults were collected from both major plant communities in the SANR and from a wide altitudinal range (471–1666 m). Mahmoud Abdel-Dayem identified this species.

Trechini

Perileptus areolatus Creutzer, 1799

Material examined. 1,225 m: 27.I.2015, LT, 2 exs; 02.III.2015, LT, 11 exs; 21.IV.2014, LT, 1 ex. 1,325 m: 27.I.2015, LT, 1 ex; 02.III.2015, LT, 2 exs; 05.V.2015, 2 exs. 1,474 m: 27.I.2015, LT, 2 exs; 02.III.2015, LT, 2 exs. 1,563 m: 21.IV.2014, LT, 1 ex; 05.V.2015, LT, 1 ex. 1,611 m: 27.I.2015, LT, 1 ex. 1,666 m: 03.V.2015, PT, 1 ex.

General distribution and zoogeography. AL, AM, AT, AZ, BA, BE, BG, CH, CZ, DE, DZ, ES, FR, GB, GE, GR, HR, HU, IE, IL, IR, IT, LT, LV, MA, MD, MK, NO, PL, PT, RO, RU, SA, SE, SI, SK, SY, TN, TR, UA. PAL_SAR species.

Published records. Makkah (Britton 1948). New record for Baha Province.

Remarks. A frequent species that was collected during winter and spring from *Acacia* thorn woodlands and Barbary fig communities. Mahmoud Abdel-Dayem identified this species.

Perileptus rutilus Schaum, 1860

Material examined. 471 m: 03.III.2015, LT, 2 exs; 10.XII.2014, HP, 1 ex; 10.XII.2014, LT, 1 ex. 1,325 m: 02.III.2015, LT, 2 exs; 05.V.2015, LT, 2 exs. 1,474 m: 02.III.2015, PT, 1 ex; 02.IX.2015, LT, 1 ex.

General distribution and zoogeography. EG, SA (Abdel-Dayem et al. 2018), SD, TD, YE. SAR species.

Published records. Asir (Abdel-Dayem et al. 2018). New provincial record for Baha.

Remarks. A rare species that was collected during all seasons from *Acacia* thorn woodlands. Mahmoud Abdel-Dayem identified this species.

Perileptus testaceus Putzeys, 1870

Material examined. 825 m: 15.XI.2015, LT, 3 exs. 851 m: 15.XI.2015, LT, 7 exs. 1,225 m: 14.XI.2015, LT, 1 ex. 1,325 m: 14.XI.2015, LT, 1 ex; 15.XI.2015, LT, 1 ex. 1,474 m: 05.V.2015, LT, 1 ex. 1,563 m: 21.IV.2014, LT, 1 ex.

General distribution and zoogeography. AE, DJ, ET, OM, SA (Abdel-Dayem et al. 2018), SO, YE. AFR species.

Published records. Asir (Abdel-Dayem et al. 2018). New provincial record for Baha.

Remarks. A frequent species that was collected during spring and autumn by light trapping in *Acacia* thorn woodlands and Barbary fig communities. Mahmoud Abdel-Dayem identified this species.

Discussion

The Carabidae of SA, comprising the ground and tiger beetles, has been reviewed with currently approximately 183 recognized species (Abdel-Dayem et al. 2018). However, not every SA province has been equally surveyed and studied. The highlands of the southwestern SA are a major hotspot of biodiversity (Hegazy et al. 1998; Heneidy and Bidak 2001), yet, the knowledge of the carabid diversity in this region is incompletely understood. This includes Baha Province, which includes SANR. Thirty species have been documented from Baha Province (Mateu 1986; Balkenohl 1994; El-Hawagry et al. 2013; Moore and Robertson; 2014; Häckel and Azadbakhsh 2016; Rasool et al. 2017, 2018a, b). The study of El-Hawagry et al. (2013) included the first recorded carabid species in the SANR, reporting only *Paussus cephalotes*. Häckel and Azadbakhsh (2016) and Rasool et al. (2018) documented 13 species in which *Lebia raeesae* and *Microcosmodes arabicus* were newly described. It should be noted that none of these three studies specifically targeted this family for the SANR.

This study represents the first baseline inventory of the carabid beetles in SNAR, within the mountains in the southwestern Saudi Arabia. The study revealed 62 species belonging to 39 genera, 17 tribes and 10 subfamilies. This number of species represent about 33% of the total known carabid fauna of SA. Also, our study includes a new species, three species endemic to SA, six confined to Arabian Peninsula, four new country records, and 24 species recorded for the first time from Baha Province. The result expands the number of carabid species recorded from Baha to 67. The number of species in this current list is similar to that of Garf Raydah Nature Reserve (GRNR) in Asir Province (61 species), a much smaller area as compared to SANR (Abdel-Dayem et al. 2018). This may be due to the wide altitudinal range (1,150–2,820 m), high annual rainfall range (600–800 mm/annum), cool temperatures, relatively high humidity, and the presence of the last remnants of dense African pencil cedar forest, *Juniperus procera* Hochst. ex Endl. (Cupressaceae) in GRNR (El-Juhany 2015, SWA 2018). Both nature reserves sharing about 64.5% (40 species) of the recorded carabid species. The Lebiini species are prevailed the carabid fauna of SNAR (30.6% of the total species), a similar finding was recently being reported from GRNR (Abdel-Dayem et al. 2018).

Biogeographically, SA is heterogeneous region that hosts an interesting mixture of biodiversity from Afrotropical, Palaearctic, and traces of Oriental realms due to its position between Africa and Eurasia (Büttiker 1979; Larsen 1984; Hölzel 1998). This mixture of taxa is also apparent in Baha Province, including SANR (El-Hawagry et al. 2016, 2017). The carabid fauna of the SANR is characterized by the prevalence of Afrotropical (28.1%) and Saharo-Arabian (19.3%) elements. The influence of the Palaearctic species is moderate (10.5%) and Oriental species is noticeably smaller (3.5%) (Fig. 3). Based on the zoogeographical analysis of the insect fauna, El Hawagry et al. (2013) suggested that the fauna of Baha Province is biologically related to the Afrotropical region rather than to the Palaearctic or Eremic zone and has little Oriental affinity. The specificity of the SANR carabid fauna is enhanced by a small fraction of endemics (5.3%). This percentage of endemic species is low compared to the percentage endemic species of the carabid fauna in Garf Raydah Nature Reserve (Abdel-Dayem et al. 2018).

In conclusion, our study provides a first account of the carabid beetle fauna of the SANR, Baha Province, in the southwestern SA. The SNAR has a relatively diverse carabid fauna (62 species), reflecting its rich flora. In its composition, the carabid fauna of SANR has almost the same number of species as GRNR, in Asir Province (Abdel-Dayem et al. 2018), and shares with the GRNR 64.5% of its species. The SNAR carabid fauna is mostly of Afrotropical origin with high influence of Saharo–Arabian and relatively little influence of the Oriental elements. The carabid fauna of the SNAR has a low level of endemism and high number of Lebiini species. Extensive surveying of the highlands in southwestern SA, may reveal further species. Beyond enhancing our knowledge of the SA carabid fauna, these results will provide useful information for guiding the conservation activities (Koivula 2011; Kotze et al. 2011) in the SANR and starting point to the future more detailed investigation on the fauna in the southwestern SA.

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RESEARCH ARTICLE

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Description of two cryptic species of the *Amolops ricketti* group (Anura, Ranidae) from southeastern China

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Abstract

Two cryptic species, which were previously reported as *Amolops ricketti*, are revealed on the basis of significant morphological and genetic divergences. *Amolops sinensis* **sp. n.** from central Guangdong, northeastern Guangxi and southwestern Hunan can be distinguished by the longitudinal glandular folds on the skin of the shoulders and other character combinations. *Amolops yatseni* **sp. n.** from the coastal hills of west Guangdong can be distinguished by the dense tiny round translucent, or white, spines on the dorsal skin of the body, dorsal and dorsolateral skin of the limbs, and other character combinations. The phylogenetic interrelationships of the *A. ricketti* group have been inferred as (*A. wuyiensis* + *A. ricketti*) + (*A. yunkaiensis* + (*A. albispinus* + (*A. sinensis* **sp. n.** + *A. yatseni* **sp. n.**))). This work indicates that the current records of *A. ricketti* might be a species complex composed of multiple species, and further work is needed to figure out this puzzle.

Keywords

Amolops sinensis sp. n., *Amolops yatseni* sp. n., mitochondrial DNA, morphology, phylogeny, species complex, torrent frog

Introduction

The torrent frog genus *Amolops* Cope, 1865, which is comprised of 55 species, is widespread from the southern and eastern Himalayas, eastward to southeastern mainland China, and southward to Malay Peninsula (Frost 2018). Of these, 28 species from China were assigned into six species groups (Fei et al. 2009, 2017; Sun et al. 2013; Lu et al. 2014; Jiang et al. 2016; Sung et al. 2016; Yuan et al. 2018; Lyu et al. 2018). Among them, the *A. ricketti* group is a monophyletic species group containing four recognized species from southeastern China (Fei et al. 2012; Sung et al. 2016; Lyu et al. 2018): *A. yunkaiensis* Lyu, Wang, Liu, Zeng & Wang, 2018 from southwestern Guangdong, *A. albispinus* Sung, Wang & Wang, 2016 from Shenzhen City, Guangdong, *A. wuyiensis* (Liu & Hu, 1975) distributed in Fujian, Zhejiang, Anhui and Jiangxi in eastern China, and *A. ricketti* (Boulenger, 1899) reported to be widely distributed in Sichuan, Chongqing, Yunnan, Guizhou, Hubei, Hunan, Jiangxi, Fujian, Guangdong and Guangxi in southern China and even to northern and central Indochina. It is worth noting that the species *A. albispinus* and *A. yunkaiensis* were recognized as other known species for a long time and recognition as new species represented the beginning of uncovering the cryptic diversity within the *A. ricketti* group (Sung et al. 2016; Lyu et al. 2018).

During our herpetological surveys in Guangdong, Guangxi and Hunan provinces in southeastern China, we have collected a series of *Amolops* specimens which were recorded as *A. ricketti* (Fei et al. 2009, 2012; Li et al. 2011). However, morphological examinations indicated that these specimens belong to two different undescribed species that can be markedly and reliably distinguished from all congeners, especially from specimens of *A. ricketti* collected from the type locality, Mt. Wuyi, Fujian. Molecular analyses further well supported the morphological result, showing that these specimens formed two lineages within the *A. ricketti* group; in this study we describe them as two new species of genus *Amolops*.

Material and methods

Sampling

A total of 28 muscle samples of the two new species were collected for molecular analyses, encompassing six from Zhongshan City, Guangdong, two from Shangchuan Island, Guangdong, two from Mt. Gudou, Guangdong, three from Ehuangzhang Nature Reserve, Guangdong, two from Yunkaishan Nature Reserve, Guangdong, seven from Shimentai Nature Reserve, Guangdong, two from Mt. Nankun, Guangdong, two from Mt. Dupangling, Guangxi, one from Mt. Yangming, Hunan, and one from Mt. Hengshan, Hunan. In addition, 36 samples from nine known species of the genus *Amolops*, namely *A. albispinus*, *A. ricketti*, *A. wuyiensis*, *A. yunkaiensis*, *A. daiyunensis* (Liu & Hu, 1975), *A. hongkongensis* (Pope & Romer, 1951), *A. hainanensis*

(Boulenger, 1900), *A. torrentis* (Smith, 1923) and *A. chunganensis* (Pope, 1929), were also collected and incorporated into our dataset. All muscle samples were attained from euthanasia specimens and then preserved in 95% ethanol and stored at -40 °C. Detail information for these materials is shown in Table 1 and Figure 1.

DNA Extraction, PCR and sequencing

Genomic DNA was extracted from muscular tissue by using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Partial 16S ribosomal RNA gene (16S) and partial cytochrome C oxidase 1 gene (CO1) were amplified. Primers used for 16S were L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3') following Simon et al. (1994), and L2A (5'-CCAAACGAGCCTAGTGATAGCTGGTT-3') and H10 (5'-TGATTACGCTACCTTTGCACGGT-3') following Chen et al. (2013), and for CO1 were Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3') following Che et al. (2012), and dgLCO (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO (5'-AAACTTCAGGGTGACCAAARAAYCA-3') following Meyer et al. (2005). PCR amplifications were processed in a 20 reaction volume with the cycling conditions as follows: an initial denaturing step at 95 °C for 4 min, 35 cycles of denaturing at 94 °C for 40 s, annealing at 53 °C (for 16S) / 48 °C (for CO1) for 40 s and extending at 72 °C for 1 min, and final extending step of 72 °C for 10 min. PCR products were purified with spin columns. The purified products were sequenced with the same primers using a BigDye Terminator Cycle Sequencing Kit as per the guidelines, on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd and Beijing Genomics Institute. All sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

DNA sequences were aligned by the Clustal W algorithm with default parameters (Thompson et al. 1997) and trimmed with the gaps partially deleted in MEGA 6 (Tamura et al. 2013), while within highly variable regions, all gaps were removed. Two gene segments, 637 base pairs (bp) of CO1 and 1032 bp of 16S, were concatenated seriatim into a 1669-bp sequence, and further divided into two partitions based upon each gene. Two partitions were tested respectively in jmodeltest v2.1.2 (Darriba et al. 2012) with Akaike and Bayesian information criteria, all resulting in the best-fitting nucleotide substitution models of GTR + I + G. Sequenced data were analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012) and maximum likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in a BI analysis, each of which was performed for 2,000,000 generations and sampled every 1000 generations with the first 25% samples discarded as

Table 1. Localities, voucher number and GenBank numbers for all samples used in this study.

ID	Species	Localities (* type localities)	Voucher	16S	CO1
1	*Amolops sinensis* sp. n.	* China: Shimentai Nature Reserve, Guangdong	SYS a004165	MK263262	MK263314
2		* China: Shimentai Nature Reserve, Guangdong	SYS a004722	MK263278	MK263318
3		* China: Shimentai Nature Reserve, Guangdong	SYS a007105	MK263297	MK263329
4		* China: Shimentai Nature Reserve, Guangdong	SYS a007106	MK263298	MK263330
5		* China: Shimentai Nature Reserve, Guangdong	SYS a007107	MK263299	MK263331
6		* China: Shimentai Nature Reserve, Guangdong	SYS a007108	MK263300	MK263332
7		* China: Shimentai Nature Reserve, Guangdong	SYS a007109	MK263301	MK263333
8		China: Mt. Nankun, Guangdong	SYS a005710	MK263287	MK263321
9		China: Mt. Nankun, Guangdong	SYS a005712	MK263288	MK263322
10		China: Mt. Dupangling, Guangxi	SYS a005089	MK263279	MK263319
11		China: Mt. Dupangling, Guangxi	SYS a005111	MK263280	MK263320
12		China: Mt. Yangming, Hunan	SYS a007268	MK263302	MK263334
13		China: Mt. Hengshan, Hunan	SYS a004257	MK263265	MK263315
14	*Amolops yatseni* sp. n.	* China: Zhongshan City, Guangdong	SYS a006806	MK263289	MK263323
15		* China: Zhongshan City, Guangdong	SYS a006807	MK263290	MK263324
16		* China: Zhongshan City, Guangdong	SYS a006808	MK263291	MK263325
17		* China: Zhongshan City, Guangdong	SYS a006810	MK263292	MK263326
18		* China: Zhongshan City, Guangdong	SYS a006811	MK263293	MK263327
19		* China: Zhongshan City, Guangdong	SYS a006857	MK263296	MK263328
20		China: Shangchuan Island, Guangdong	SYS a003633	MK263250	MK263304
21		China: Shangchuan Island, Guangdong	SYS a003634	MK263251	MK263305
22		China: Mt. Gudou, Guangdong	SYS a006818	MK263294	MK263306
23		China: Mt. Gudou, Guangdong	SYS a006819	MK263295	MK263307
24		China: Ehuangzhang Nature Reserve, Guangdong	SYS a003978	MK263252	MK263308
25		China: Ehuangzhang Nature Reserve, Guangdong	SYS a003980	MK263254	MK263309
26		China: Ehuangzhang Nature Reserve, Guangdong	SYS a003981	MK263255	MK263310
27		China: Yunkaishan Nature Reserve, Guangdong	SYS a004642	MK263269	MK263316
28		China: Yunkaishan Nature Reserve, Guangdong	SYS a004643	MK263270	MK263317
29	*Amolops albispinus*	* China: Mt. Wutong, Guangdong	SYS a003452	MK263247	KX507332
30		* China: Mt. Wutong, Guangdong	SYS a003453	MK263248	KX507333
31		* China: Mt. Wutong, Guangdong	SYS a003454	MK263249	KX507334
32	*Amolops ricketti*	* China: Mt. Wuyi, Fujian	SYS a004141	MK263259	MG991927
33		* China: Mt. Wuyi, Fujian	SYS a004142	MK263260	MG991928
34		* China: Mt. Wuyi, Fujian	SYS a004143	MK263261	MG991929
35		China: Mt. Emeifeng, Fujian	SYS a002492	MK263244	KX507329
36		China: Shanghang County, Fujian	SYS a003342	MK263246	KX507331
37		China: Shanghang County, Fujian	SYS a004106	MK263256	MK263311
38	*Amolops wuyiensis*	* China: Mt. Wuyi, Fujian	SYS a001716	MK263239	KX507324
39		* China: Mt. Wuyi, Fujian	SYS a001717	MK263240	KX507325
40		* China: Mt. Wuyi, Fujian	SYS a004139	MK263257	MK263312
41		* China: Mt. Wuyi, Fujian	SYS a004140	MK263258	MK263313
42		China: Jingning County, Zhejiang	SYS a002723	MK263245	MK263303
43	*Amolops yunkaiensis*	* China: Ehuangzhang Nature Reserve, Guangdong	SYS a003979	MK263253	MG991907
44		* China: Ehuangzhang Nature Reserve, Guangdong	SYS a004705	MK263275	MG991906
45		* China: Ehuangzhang Nature Reserve, Guangdong	SYS a004706	MK263276	MG991908
46		* China: Ehuangzhang Nature Reserve, Guangdong	SYS a004707	MK263277	MG991909
47		China: Yunkaishan Nature Reserve, Guangdong	SYS a004681	MK263271	MG991910

48	*Amolops yunkaiensis*	China: Yunkaishan Nature Reserve, Guangdong	SYS a004682	MK263272	MG991911
49		China: Yunkaishan Nature Reserve, Guangdong	SYS a004683	MK263273	MG991912
50		China: Yunkaishan Nature Reserve, Guangdong	SYS a004684	MK263274	MG991913
51	*Amolops daiyunensis*	* China: Mt. Daiyun, Fujian	SYS a001737	MK263241	KX507326
52		* China: Mt. Daiyun, Fujian	SYS a001738	MK263242	KX507327
53		* China: Mt. Daiyun, Fujian	SYS a001739	MK263243	KX507328
54	*Amolops hongkongensis*	* China: Hong Kong	SYS a004577	MK263266	MG991919
55		* China: Hong Kong	SYS a004578	MK263267	MG991920
56		* China: Hong Kong	SYS a004579	MK263268	MG991921
57	*Amolops hainanensis*	* China: Mt. Wuzhi, Hainan	SYS a005281	MK263281	MG991916
58		* China: Mt. Wuzhi, Hainan	SYS a005282	MK263282	MG991917
59		* China: Mt. Wuzhi, Hainan	SYS a005283	MK263283	MG991918
60	*Amolops torrentis*	* China: Mt. Wuzhi, Hainan	SYS a005289	MK263284	MG991930
61		* China: Mt. Wuzhi, Hainan	SYS a005290	MK263285	MG991931
62		* China: Mt. Wuzhi, Hainan	SYS a005291	MK263286	MG991932
63	*Amolops chunganensis*	China: Mt. Jinggang, Jiangxi	SYS a004212	MK263263	MG991914
64		China: Mt. Jinggang, Jiangxi	SYS a004213	MK263264	MG991915

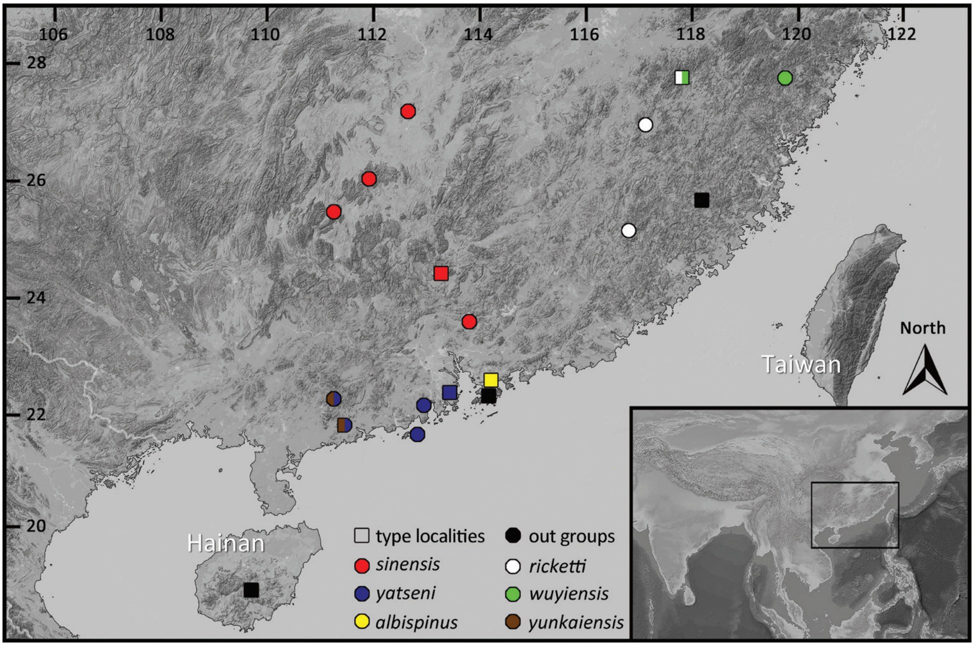

Figure 1. Collecting localities of samples used in this study.

burn-in, resulting a potential scale reduction factor (PSRF) of < 0.01. In ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Pairwise distances (*p*-distance) were calculated in MEGA 6 using the uncorrected p-distance model.

Morphology

We obtained diagnostic characters of known species of the genus *Amolops* from the literature for comparison. In addition, a total of 67 museum specimens of *A. ricketti* group were examined for comparison, which are listed in Appendix 1.

Measurements follow Fei et al. (2009) and Lyu et al. (2018), and were taken with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. These measurements are as follows:

SVL	snout-vent length (from tip of snout to posterior margin of vent);
HDL	head length (from tip of snout to the articulation of the jaw);
HDW	head width (head width at the commissure of the jaws);
SNT	snout length (from tip of snout to the anterior corner of the eye);
IND	internasal distance (distance between nares);
IOD	interorbital distance (minimum distance between upper eyelids);
ED	eye diameter (from the anterior corner of the eye to posterior corner of the eye);
TD	tympanum diameter (horizontal diameter of tympanum);
TED	tympanum-eye distance (from anterior edge of tympanum to posterior corner of the eye);
HND	hand length (from distal end of radioulna to tip of distal phalanx III);
RAD	radioulna length (from the flexed elbow to the base of the outer palmar tubercle);
FTL	foot length (from distal end of tibia to tip of distal phalanx IV);
TIB	tibial length (from the outer surface of the flexed knee to the heel);
F3W	width of digital disc on finger III;
T4W	width of digital disc on toe IV.

Sex was determined by observation of secondary sexual characters, i.e. the presence of nuptial spines in males, following Fei et al. (2009) and Lyu et al. (2018).

All specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol, and deposited in The Museum of Biology, Sun Yat-sen University (SYS) and Chengdu Institute of Biology, the Chinese Academy of Sciences (CIB), P.R. China.

Results

The phylogenetic trees strongly supported that the *Amolops ricketti* group was a monophyletic species group, which can be further divided into six well-supported clades with marked divergences (Fig. 2; Table 2). The four known species of this group, *A. wuyiensis*, *A. ricketti*, *A. yunkaiensis* and *A. albispinus*, formed the four basal clades respectively. The unnamed specimens from Shimentai Nature Reserve and Mt. Nankun in Guangdong, Mt. Dupangling in Guangxi, and Mt. Yangming and Mt. Hengshan in Hunan clustered into a clade (clade V) with highly supported node values (BS = 100, BPP = 1.00) and small divergences (*p*-distance 0.0–0.6); the speci-

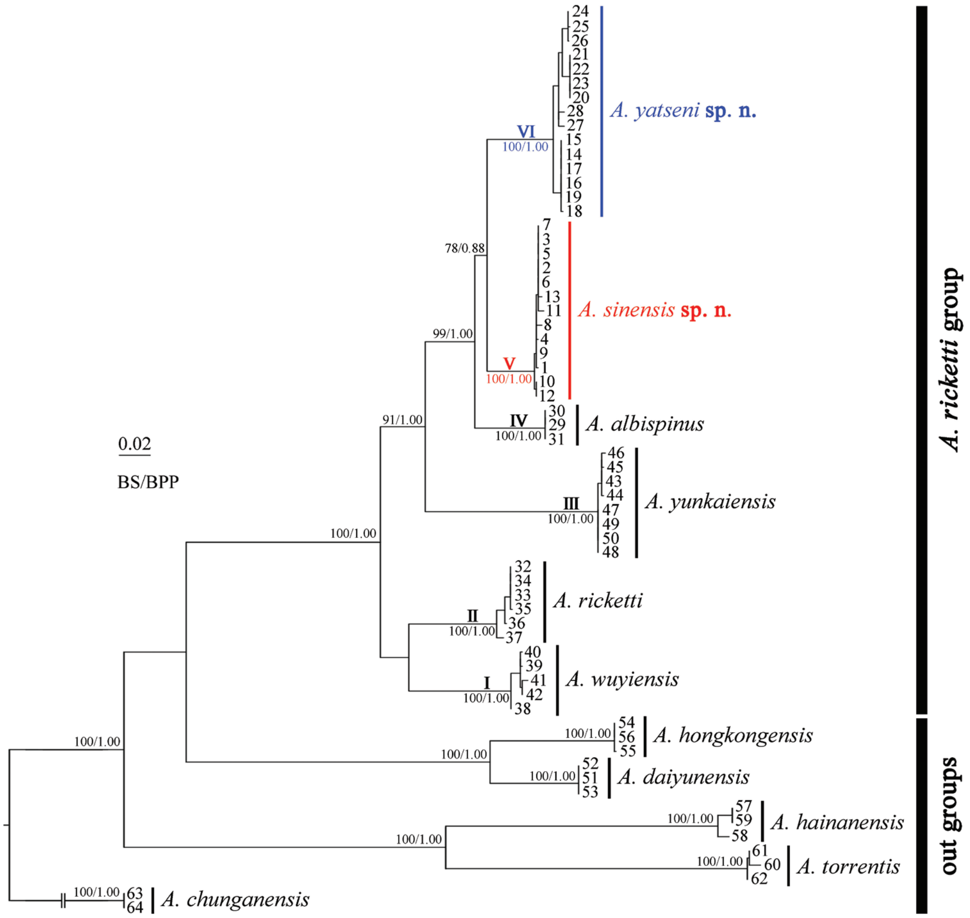

Figure 2. Bayesian inference and maximum-likelihood phylogenies. The bootstrap supports for maximum likelihood analysis (BS) > 75 and the Bayesian posterior probabilities (BPP) > 0.85 were retained.

mens from Zhongshan City, Mt. Gudou, Shangchuan Island, Ehuangzhang Nature Reserve and Yunkaishan Nature Reserve clustered into a clade (clade VI) with highly supported node values (BS = 100, BPP = 1.00) and small divergences (*p*-distance 0.0–0.8). These two clades are sister taxa to each other with significant divergences (*p*-distance 3.5–4.2).

Morphologically, the specimens clustered into clade V from central Guangdong, northeastern Guangxi and southwestern Hunan can be distinguished from all known *Amolops* species by having unique longitudinal glandular folds on the skin of the shoulders and other characters (see diagnosis below). Therefore this clade represents a distinct evolutionary lineage, and is described below as a new species, *Amolops sinensis* sp. n. The specimens grouped in clade VI from the coastal hills of west Guangdong differ from all known *Amolops* species by having unique dense

Table 2. Uncorrected *p*-distances (in %) among the *Amolops* species in this study.

ID	Species	I	II	III	IV	V	VI	VII	IIX	IX	X	XI
I	*Amolops sinensis* sp. n.	0.0–0.6	–	–	–	–	–	–	–	–	–	–
II	*Amolops yatseni* sp. n.	3.5–4.2	0.0–0.8	–	–	–	–	–	–	–	–	–
III	*Amolops albispinus*	3.8–4.1	4.1–4.4	0.0–0.0	–	–	–	–	–	–	–	–
IV	*Amolops ricketti*	6.7–7.2	6.4–7.0	6.4–6.7	0.0–0.7	–	–	–	–	–	–	–
V	*Amolops wuyiensis*	6.2–6.8	6.2–6.8	6.8–7.0	5.1–5.5	0.1–0.5	–	–	–	–	–	–
VI	*Amolops yunkaiensis*	6.4–6.9	6.9–7.7	6.7–7.0	7.9–8.2	8.0–8.3	0.0–0.2	–	–	–	–	–
VII	*Amolops daiyunensis*	12.5–12.9	12.6–12.7	12.4–12.4	12.3–12.8	12.6–12.8	12.4–12.6	0.0–0.0	–	–	–	–
IIX	*Amolops hongkongensis*	12.5–12.9	12.2–12.4	12.2–12.3	11.8–12.3	12.1–12.4	12.4–12.7	5.5–5.5	0.0–0.1	–	–	–
IX	*Amolops hainanensis*	15.1–15.5	15.4–15.8	14.6–14.7	14.9–15.2	14.5–14.8	15.6–16.0	14.8–14.9	15.7–15.8	0.0–0.8	–	–
X	*Amolops torrentis*	15.3–15.7	15.5–15.8	15.5–15.5	15.1–15.5	15.3–15.5	16.3–16.7	14.8–15.0	14.6–14.7	10.9–11.0	0.1–0.4	–
XI	*Amolops chunganensis*	15.8–16.2	15.6–16.0	15.8–15.8	14.8–15.2	14.8–15.0	15.3–15.5	15.3–15.3	15.8–15.9	17.7–17.9	17.3–17.5	0.0

tiny round translucent, or white, spines on the dorsal skin of the body, dorsal and dorsolateral skin of the limbs and other characters (see diagnosis below). Therefore, this clade represents a distinct evolutionary lineage, and is described below as a new species *Amolops yatseni* sp. n.

Taxonomy accounts

Amolops sinensis Lyu, Wang & Wang, sp. n.

http://zoobank.org/DF35246E-39C3-46E7-8C16-F2ACD366C01C

Chresonymy. *Amolops ricketti* (Boulenger, 1899): Fei et al. 2009 (Hengshan, Hunan); Li et al. 2011 (Guangdong); Fei et al. 2012 (Guangxi; Hengshan, Hunan).

Holotype. SYS a007107 (Fig. 3), adult male, collected by Hong-Hui Chen (HHC) and Yuan-Qiu Li (YQL) on 22 June 2018 from Qianjin (24.49N, 113.11E; ca 510 m a.s.l.), Shimentai Nature Reserve, Guangdong.

Paratypes. Ten adult specimens. Male SYS a007105, 7106 and 7108, and female SYS a007109, collected at the same time from the same stream as holotype. Female SYS a004165/CIB 110004, collected by Zhi-Tong Lyu (ZTL) and YQL on 26 July 2015 from Hengshitang, Shimentai Nature Reserve, Guangdong. Male SYS a005710 and female SYS a005712, collected by ZTL and Jian Wang (JW) on 8 April 2017 from Mt. Nankun, Guangdong. Male SYS a005089, collected by JW on 18 July 2016 from Mt. Dupangling, Guangxi. Female SYS a007268, collected by ZTL and Yu-Long Li on 21 June 2018 from Mt. Yangming, Hunan. Female SYS a004257, collected by ZTL and JW on 19 August 2015 from Mt. Hengshan, Hunan.

Other examined material. Juvenile SYS a004722 (Fig. 4A), collected by ZTL, JW and YQL on 1 May 2016 from the same stream as holotype.

Diagnosis. The new species was assigned to genus *Amolops* and further to the *A. ricketti* group morphologically based on the absence of dorsolateral folds, the presence of a circummarginal groove on the disk of the first finger, the absence of tarsal glands, and the presence of nuptial pads with conical nuptial spines on the first finger in males.

Amolops sinensis sp. n. is distinguished from its congeners by a combination of the following morphological characteristics: (1) body stout and robust, SVL 40.2–46.5 (43.1±2.2, n=6) mm in adult males, 47.7–52.7 (50.5±2.0, n=5) mm in adult females; (2) dorsal body olive-brown to dark brown, with irregular light strip-shaped patches or not; (3) ventral surface creamy white or beige, with dark gray patches; (4) dorsal skin of body very rough, granular and scattered with conical tubercles and raised large warts in males; (5) vomerine teeth strong, tongue cordiform, deeply notched posteriorly; (6) absence of dorsolateral folds; (7) a longitudinal glandular fold on skin of shoulder on each side; (8) supernumerary tubercles below base of fingers III and IV indistinct; (9) heels overlapping; (10) absence of outer metatarsal tubercles and tarsal glands; (11) absence of vocal sacs; (12) nuptial pad on first finger prominent with beige spines in

Figure 3. Morphological features of the adult male holotype SYS a007107 of *Amolops sinensis* sp. n. in life. **A** Dorsolateral view **B** Ventral view **C** A longitudinal glandular folds on skin of shoulder **D** Beige nuptial pad and nuptial spines **E** Left hand **F** Left foot.

breeding males; and (13) white conical spines present on skin of temporal region (including tympanum in several individuals) and loreal region in breeding males.

Description of holotype. Body stout, SVL 43.3 mm. Head width slightly smaller than head length (HDW/HDL = 1.04); snout short (SNT/HDL = 0.45) and rounded in profile, projecting beyond lower jaw; nostril closer to tip of snout than eye; loreal region concave; top of head flat; eye large (ED/HDL = 0.32) and convex; canthus rostralis distinct; pineal body distinct; tympanum small, edge distinct; tympanum-eye distance smaller than tympanum, TED/TD 0.90; supratympanic fold distinct, start from back of eye and extending to shoulder, a well-developed gland on end of supratympanic fold; choanae moderate; vomerine teeth present; tongue cordiform, deeply notched posteriorly.

Forelimbs moderately robust; hands moderately long (HND/SVL = 0.33); relative finger lengths I = II < IV < III; finger tips dilated to wide oval disks with circummarginal grooves, relative width of finger disks I < II < III = IV; subarticular tubercles prominent, rounded; supernumerary tubercles below base of fingers III and IV indistinct, below base of fingers I and II absent; inner metacarpal tubercle small, outer metacarpal tubercle prominent and slightly separated; absence of webbing and presence of weak lateral fringes on fingers.

Hindlimbs long and robust (TIB/SVL = 0.60); tibio-tarsal articulation reaching tip of snout when hindlimb stretched alongside of body; relative toe lengths I < II < III = V < IV; tips of all toes expanded to well-developed oval discs with circummarginal grooves; subarticular tubercles oval and distinct; inner metatarsal tubercle prominent, elongated; outer metatarsal tubercles absent; toes fully webbed; lateral fringes of toes I and V developed; tarsal glands absent; heels overlapping when hindlimbs flexed at right angles to axis of body.

Dorsal skin of body rough, granular and scattered with raised tubercles and warts; underdeveloped conical spines on skin of loreal region and temporal region except tympanum; flanks very rough and granular with raised warts; dorsal limbs rough with numerous tubercles; several longitudinal dermal ridges on dorsal surfaces of thigh, tibia and tarsus; dorsolateral fold absent; a longitudinal glandular fold on skin of shoulder; posterior part of upper lip swollen; rictal gland prominent and ellipsoidal, posterior to corner of mouth.

Ventral surface slightly wrinkled with granules; ventral surface of hand smooth, ventral surface of foot granular; large warts surrounding cloaca.

Measurements of holotype (in mm). SVL 43.3; HDL 15.4, HDW 16.0; SNT 6.9; IND 6.2; IOD 4.3; ED 4.9; TD 1.9; TED 1.7; HND 14.3; RAD 9.4; FTL 36.9; TIB 26.0; F3W 2.9; T4W 2.9.

Color of holotype in life. Dorsal body olive-brown with irregular light yellow patches; longitudinal glands on occipital region light yellow; warts on flanks dark or grayish white; irregular dark patches on dorsal surface of forearms, distinct dark transverse bars on dorsal surface of lower arms and hindlimbs; dorsal discs of digits brown or white; nuptial pads and nuptial spines beige; posterior edge of upper lip and rictal gland light maize-yellow; throat and chest creamy white; belly beige; several dark gray mottling on surface of throat, chest and anterior part of abdomen; ventral surfaces of limbs gray pink grounding; ventral hands and feet dark grey; warts around cloaca yellowish white tubercles and olive-brown.

Color of holotype in preservative. Dorsal surface dark brown with irregular gray patches, transverse bars on limbs more distinct; longitudinal glands on occipital region more distinct; webs on toes gray, mottling with olive-brown; ventral surface grayish white, mottling on surface of throat, chest and anterior part of abdomen become more distinct; ventral surface of limbs beige.

Figure 4. A Juvenile SYS a004722 of *Amolops sinensis* sp. n. in life **B** Female paratype SYS a007109 in life **C** Male paratype SYS a005710 in life, eating an earthworm.

Variations. Measurements of type series specimens are given in Table 3. All specimens are very similar in morphology except that: dorsal skin dark brown without any patterns in SYS a007109 (Fig. 4B); skin of tympanum with white conical spines in SYS a005710 (Fig. 4C); nuptial spines are conical in SYS a005710; tibia-tarsal articulation reaching forward to the loreal region in SYS a004257, 5712, 7109 and 7268.

Sexual dimorphism. *Amolops sinensis* sp. n. possesses distinct sexual dimorphism: (1) larger body size in females with SVL 47.7–52.7 mm (vs. SVL 40.2–46.5 mm in males); (2) beige nuptial spines on beige nuptial pads in breeding males; (3) dense white conical spines present on skin of temporal region and loreal region in males during breeding season (vs. absent in females); and (4) females bearing light yellow oocytes.

Comparisons. The character of longitudinal glandular folds on skin of shoulders makes *Amolops sinensis* sp. n. unique when compared with all known congeners within the genus. The new species is further compared with the four recognized species of the *A. ricketti* species group below (Fig. 5).

Amolops sinensis sp. n. was previously reported as *A. ricketti*, but significantly differs from the topotype of *A. ricketti* by the presence of longitudinal glandular folds on the skin of the shoulders (vs. absent), large raised warts on the dorsal surface of body (vs. relatively smooth), the presence of white conical spines on skin of temporal region and loreal region in breeding males (vs. absent), and nuptial pad and nuptial spines beige (vs. white).

Figure 5. Comparisons of morphological characteristics among species in the *Amolops ricketti* group. **A** *A. sinensis* sp. n. **B** *A. yatseni* sp. n. **C** *A. albispinus* **D** *A. ricketti* **E** *A. wuyiensis* **F** *A. yunkaiensis* **1** dorsolateral view **2** close-up of the head **3** ventral view of the hand **4** ventral view of the foot.

Amolops sinensis sp. n. is phylogenetically close to *A. albispinus*, but can be distinguished from the later by the presence of longitudinal glandular folds on the skin of the shoulders (vs. absent), the presence of supernumerary tubercles below the base of fingers III and IV (vs. absent), outer metacarpal tubercle slightly separated (vs. completely divided into two tubercles), pineal body distinct (vs. indistinct), ventral surface smooth (vs. presence of tiny, transparent and dispersive conical spines on surface of chest in males), and nuptial spines beige (vs. white).

Amolops sinensis sp. n. can be easily distinguished from *A. wuyiensis* by the presence of longitudinal glandular folds on skin of shoulders (vs. absent), vomerine teeth present (vs. absent), lacking vocal sacs (vs. present), and nuptial spines beige (vs. black).

Amolops sinensis sp. n. further differs from *A. yunkaiensis* by the presence of longitudinal glandular folds on the skin of the shoulders (vs. absent), larger body size, SVL 40.2–46.5 mm in adult males and 47.7–52.7 mm in adult females (vs. SVL 31.8–34.1 mm in males and 35.2–39.0 mm in females), vomerine teeth present (vs. absent), lacking vocal sacs (vs. present), and ventral surface smooth (vs. presence of tiny transparent spines on surface of chest).

Etymology. The specific name "*sinensis*" refers to "Chinese", for this new species takes a wide distribution in southern China. We suggest its English common name "Chinese Torrent Frog" and Chinese name "Zhong Hua Tuan Wa (中华湍蛙)".

Distribution and habits. Currently, the Chinese Torrent Frog is recognized from the Shimentai Nature Reserve and Mt. Nankun in Guangdong, Mt. Dupangling in Guangxi, and Mt. Yangming and Mt. Hengshan in Hunan, which indicates the potential distribution area of *Amolops sinensis* sp. n. is from central Guangdong, to northeastern Guangxi and southwestern Hunan.

Amolops sinensis sp. n. inhabits rocky, fast-flowing streams (ca 500–1300 m a.s.l.) surrounded by moist subtropical secondary evergreen broadleaved forests. All individuals were observed from April to August. Males bear nuptial spines from April to July; females bear mature light yellow oocytes from April to August. Nevertheless, much of the ecology and behavior of this species remains unknown.

Amolops yatseni Lyu, Wang & Wang, sp. n.

http://zoobank.org/44B205CF-7C89-40BC-9E20-E9FEED5937C8

Chresonymy. *Amolops ricketti* (Boulenger, 1899): Fei et al. 2009 (Xinyi, Guangdong); Li et al. 2011 (Guangdong); Fei et al. 2012 (Xinyi, Guangdong).

Holotype. SYS a006807 (Fig. 6), adult male, collected by JW and HHC on 27 March 2018 from Mt. Wugui (22.45N, 113.49E; ca 260 m a.s.l.), Zhongshan City, Guangdong.

Paratypes. Sixteen adult specimens. Male SYS a006806, and female SYS a006811/CIB 110005 and SYS a006808–6810, collected at the same time from the same stream as holotype. Male SYS a003634 and 3638, and female SYS a003633, collected by ZTL and JW on 22 April 2015 from Shangchuan Island, Guangdong. Female SYS a006819, collected by JW and HHC on 28 March 2018 from Mt. Gudou, Guangdong. Female SYS a003978 and 3981, collected by ZTL and Chao Huang on 14 May 2015 from Ehuangzhang Nature Reserve, Guangdong. Male SYS a004643 and 4676, and female SYS a004640, 4642 and 4994, collected by ZTL and JW on 14–15 April 2016 from Yunkaishan Nature Reserve, Guangdong.

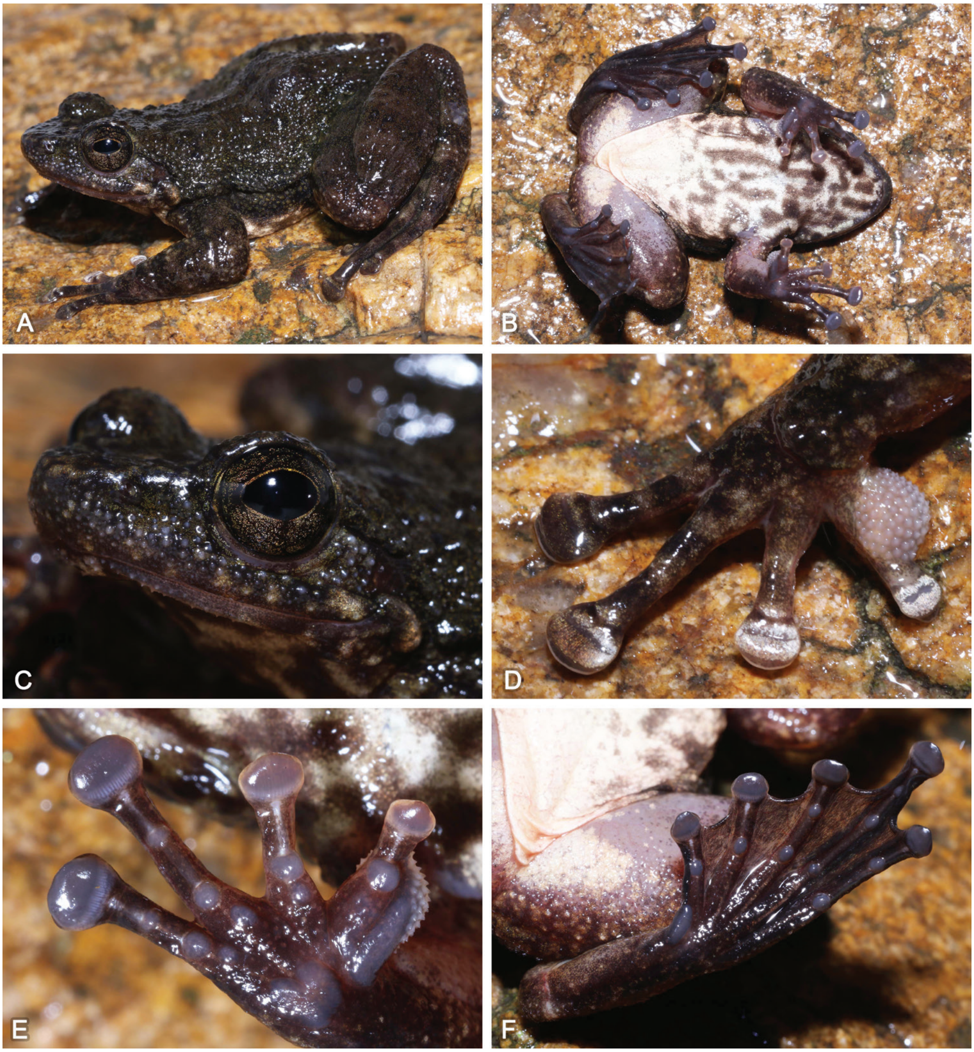

Figure 6. Morphological features of the adult male holotype SYS a006807 of *Amolops yatseni* sp. n. in life. **A** Dorsolateral view **B** Ventral view **C** Dense white conical spines on skin of temporal region, loreal region, snout, lips and chin **D** Nuptial pad and nuptial spines **E** Right hand **F** Left foot.

Other examined material. Juvenile SYS a006857, collected by Yuan-Peng Cen on 26 March 2018 from Daliao, Zhongshan City, Guangdong.

Diagnosis. The new species was assigned to genus *Amolops* and further to the *A. ricketti* group morphologically based on the absence of dorsolateral folds, the presence of a circummarginal groove on the disk of the first finger, the absence of tarsal glands, and the presence of nuptial pads with conical nuptial spines on the first finger in males.

Amolops yatseni sp. n. is distinguished from its congeners by a combination of the following morphological characteristics: (1) body stout and robust, SVL 39.3–44.7

(42.5±2.1, n=6) mm in adult males, 42.1–48.9 (46.4±2.0, n=11) mm in adult females; (2) dorsal body olive-brown or light brown, with irregular light strip-shaped patches or not; (3) ventral surface creamy white, with nebulous dark gray patches or not; (4) dorsal skin of body very rough, granular and scattered with tubercles and raised large warts, lacking warts on central back of trunk in females; (5) dense tiny round translucent, or white, spines present on dorsal skin of body, dorsal and dorsolateral skin of limbs in males, denser in females; (6) vomerine teeth strong, tongue cordiform, deeply notched posteriorly; (7) absence of the dorsolateral folds; (8) supernumerary tubercles below the base of fingers II, III and IV distinct and prominent; (9) heels just meeting; (10) absence of outer metatarsal tubercles and tarsal glands; (11) absence of vocal sacs; (12) nuptial pad on the first finger prominent with developed white conical spines in breeding males, tip of nuptial spines brown; and (13) dense white conical spines present on the skin of the temporal region (including the tympanum in several individuals), loreal region, snout, lips and chin in males during breeding season, and such spines less developed and rounded only on skin of temporal region except the tympanum and lower lips in females.

Description of holotype. Body stout, SVL 41.0 mm. Head width slightly smaller than head length (HDW/HDL = 1.05); snout short (SNT/HDL = 0.38) and rounded in profile, projecting beyond lower jaw; nostril closer to tip of snout than eye; loreal region concave; top of head flat; eye large (ED/HDL = 0.31) and convex; canthus rostralis distinct; pineal body distinct; tympanum small, edge faintly distinct, upper margin of tympanum in contact with supratympanic fold; tympanum-eye distance larger than tympanum, TED/TD 1.08; supratympanic fold distinct, start from back of eye and extending to shoulder; choanae moderate; vomerine teeth present; tongue cordiform, deeply notched posteriorly.

Forelimbs moderately robust; hands moderately long (HND/SVL = 0.31); relative finger lengths I < II < IV < III; finger tips dilated to wide oval disks with circummarginal grooves, relative width of finger disks I < II < III = IV; subarticular tubercles prominent, rounded; supernumerary tubercles below the base of fingers II, III and IV distinct and prominent, below base of fingers I absent; inner metacarpal tubercle elongated and prominent, outer metacarpal tubercle prominent and slightly separated; absence of webbing and presence of weak lateral fringes on fingers.

Hindlimbs long and robust (TIB/SVL = 0.55) ; tibio-tarsal articulation reaching tip of snout when hindlimb stretched alongside of body; relative toe lengths I < II < III = V < IV; tips of all toes expanded to well-developed oval discs with circummarginal grooves; subarticular tubercles oval and distinct; inner metatarsal tubercle prominent, elongated; outer metatarsal tubercles absent; toes fully webbed; lateral fringes of toes I and V developed; tarsal glands absent; heels just meeting when hindlimbs flexed at right angles to axis of body.

Dorsal skin of body very rough, granular and scattered with raised large warts; dense rounded spines present on dorsal body, dorsal limbs, and many well developed and denser ones on sacral region; dense conical spines present on skin of temporal region except tympanum, loreal region, snout, lips and chin, conical spines on skin of lower lips much smaller; flanks very rough and granular with raised warts; dorsal limbs rough with numerous tubercles; several longitudinal dermal ridges on dorsal surfaces of thigh, tibia and tarsus; dorsolateral fold absent; posterior part of upper lip swollen; rictal gland prominent and ellipsoidal, posterior to corner of mouth.

Ventral surface slightly wrinkled with round spines on chest; ventral surface of hand and foot granular; large warts surrounding the vent.

Measurements of holotype (in mm). SVL 41.0; HDL 15.3, HDW 16.1; SNT 5.8; IND 5.7; IOD 4.0; ED 4.8; TD 1.7; TED 1.8; HND 12.5; RAD 9.0; FTL 31.2; TIB 22.5; F3W 2.7; T4W 2.1.

Color of holotype in life. Dorsal body dark green; faint dark transverse bars on dorsal surface of limbs; dorsal discs of digits yellowish brown; posterior edge of upper lip and rictal gland light maize-yellow; all round spines and conical spines on skin grayish white; throat, chest, and belly creamy white; several dark gray nebulous mottling on surface of throat, chest and anterior part of abdomen; ventral surfaces of limbs gray pink grounding; creamy white blotches on ventral thighs; rear of thighs mottled with dark brown; ventral hands and feet dark grey; yellowish white tubercles and olive-brown warts around cloaca.

Color of holotype in preservative. Dorsal surface dark brown, irregular light strip-shaped patches present, transverse bars indistinct; ventral surface grayish white, mottling on surface of throat, chest and anterior part of abdomen become more distinct; ventral surface of limbs light brown.

Variations. Measurements of type series specimens are given in Table 3. All specimens are very similar in morphology except that: dorsal skin without any patterns in the specimens from Zhongshan City (vs. dorsal skin with irregular light strip-shaped patches in the remaining specimens); skin of tympanum with white conical spines in SYS a004643 (Fig. 7A) and 4676; tibia-tarsal articulation reaching anterior corner of eye in SYS a003633, 3634, 3678, 3680, 4994, 6806 and 6809).

Sexual dimorphism. *Amolops yatseni* sp. n. possesses significantly-distinct sexual dimorphism: (1) larger body size in females with SVL 42.1–48.9 mm (vs. SVL 39.3–44.7 in males); (2) white nuptial spines with brown tips on white nuptial pads in breeding males; (3) rounded spines on dorsal skin denser and more distinct in females (Fig. 7B); (4) skin of central back bearing raised large warts in males (vs. such warts absent in females); (5) dense white conical spines on skin of temporal region (including the tympanum in several individuals), loreal region, snout, lips and chin in males during breeding season (vs. spines underdeveloped and rounded only on skin of temporal region and lower lips in females); and (6) females bearing light yellow oocytes.

Comparisons. The dense tiny round translucent, or white, spines on dorsal skin of body, dorsal and dorsolateral skin of the limbs makes *Amolops yatseni* sp. n. unique when compared with all known congeners within the genus. *Amolops yatseni* sp. n. is further compared with *Amolops sinensis* sp. n. and other four recognized species within the *A. ricketti* species group below (Fig. 5).

Amolops yatseni sp. n. is a sister taxon to *A. sinensis* sp. n. in our phylogenetic trees (Fig. 2), and differs from the later by a significant genetic divergence of 3.5–4.2%. Mor-

Table 3. Measurements (in mm; minimum-maximum, mean±1SD) of the type series of *Amolops sinensis* sp. n. and *A. yatseni* sp. n.

	***A. sinensis* sp. n.**		***A. yatseni* sp. n.**	
	Males (n=6)	**Females (n=5)**	**Males (n=6)**	**Females (n=11)**
SVL	40.2–46.5 (43.1±2.2)	47.7–52.7 (50.5±2.0)	39.3–44.7 (42.5±2.1)	42.1–48.9 (46.4±2.0)
HDL	14.6–16.7 (15.5±0.7)	16.1–18.6 (17.1±1.2)	14.2–16.8 (16.0±1.0)	15.4–18.1 (16.7±0.9)
HDW	15.2–18.4 (16.1±1.2)	16.2–19.5 (17.7±1.6)	16.1–17.8 (16.9±0.7)	16.1–19.0 (17.5±0.9)
SNT	6.0–7.5 (6.6±0.6)	6.6–7.9 (7.1±0.5)	5.6–7.4 (6.4±0.6)	6.2–7.2 (6.7±0.4)
IND	5.3–6.9 (5.9±0.5)	5.6–7.4 (6.6±0.8)	5.7–6.4 (6.1±0.3)	5.7–6.6 (6.3±0.3)
IOD	4.2–4.7 (4.4±0.2)	4.5–5.4 (4.8±0.4)	3.9–4.8 (4.3±0.3)	4.1–4.5 (4.3±0.1)
ED	4.8–5.6 (5.1±0.4)	5.4–6.4 (6.0±0.4)	4.7–5.3 (5.1±0.3)	4.9–6.2 (5.5±0.4)
TD	1.8–2.2 (2.1±0.2)	1.8–2.2 (2.1±0.1)	1.5–1.9 (1.7±0.1)	1.8–2.3 (2.0±0.2)
TED	1.4–1.8 (1.7±0.1)	1.5–2.0 (1.7±0.2)	1.4–2.0 (1.8±0.2)	1.8–2.9 (2.1±0.3)
HND	12.0–14.3 (13.0±0.8)	12.5–14.7 (13.7±0.9)	11.6–13.8 (12.7±0.9)	11.8–14.0 (12.9±0.6)
RAD	8.4–10.6 (9.3±0.7)	9.3–10.9 (10.0±0.7)	7.8–10.9 (8.9±1.1)	8.2–9.9 (8.8±0.5)
FTL	30.7–37.1 (34.3±2.4)	34.8–39.5 (36.9±1.9)	27.1–37.0 (32.4±3.3)	29.1–35.6 (33.2±2.4)
TIB	22.4–26.9 (24.7±1.7)	24.6–29.2 (26.9±1.8)	20.6–27.0 (24.5±2.5)	21.5–26.3 (24.6±1.5)
F3W	2.4–2.9 (2.7±0.2)	2.5–3.2 (2.9±0.3)	2.6–3.4 (2.8±0.3)	2.2–3.1 (2.8±0.3)
T4W	2.3–2.9 (2.5±0.2)	2.5–3.1 (2.8±0.3)	2.1–3.2 (2.4±0.4)	2.0–2.8 (2.4±0.3)
HDL/SVL	0.35–0.37 (0.36±0.01)	0.33–0.36 (0.34±0.01)	0.36–0.38 (0.38±0.01)	0.35–0.37 (0.36±0.01)
HDW/SVL	0.35–0.40 (0.37±0.02)	0.33–0.38 (0.35±0.02)	0.39–0.42 (0.40±0.01)	0.36–0.39 (0.38±0.01)
HDW/HDL	1.00–1.11 (1.04±0.04)	1.01–1.05 (1.03±0.02)	1.02–1.13 (1.06±0.04)	1.02–1.09 (1.05±0.02)
SNT/HDL	0.39–0.45 (0.43±0.02)	0.40–0.43 (0.42±0.01)	0.38–0.44 (0.40±0.02)	0.36–0.44 (0.40±0.02)
SNT/SVL	0.14–0.16 (0.15±0.01)	0.13–0.15 (0.14±0.01)	0.14–0.17 (0.15±0.01)	0.13–0.15 (0.15±0.01)
IND/HDW	0.34–0.39 (0.37±0.02)	0.35–0.39 (0.38±0.02)	0.33–0.38 (0.36±0.02)	0.34–0.39 (0.36±0.01)
IOD/HDW	0.26–0.30 (0.28±0.02)	0.26–0.28 (0.27±0.01)	0.23–0.28 (0.25±0.02)	0.24–0.26 (0.25±0.01)
ED/HDL	0.31–0.37 (0.33±0.02)	0.33–0.38 (0.35±0.02)	0.31–0.33 (0.32±0.01)	0.30–0.36 (0.33±0.02)
ED/SVL	0.11–0.13 (0.12±0.01)	0.11–0.12 (0.12±0.00)	0.12–0.12 (0.12±0.00)	0.11–0.13 (0.12±0.01)
TD/ED	0.36–0.46 (0.40±0.04)	0.29–0.39 (0.35±0.04)	0.28–0.37 (0.34±0.03)	0.32–0.40 (0.36±0.03)
TED/TD	0.78–0.90 (0.82±0.04)	0.74–0.97 (0.83±0.08)	0.81–1.18 (1.05±0.13)	0.86–1.61 (1.08±0.21)
HND/SVL	0.28–0.33 (0.30±0.02)	0.24–0.28 (0.27±0.02)	0.28–0.32 (0.30±0.01)	0.26–0.29 (0.28±0.01)
RAD/SVL	0.21–0.23 (0.22±0.01)	0.19–0.21 (0.20±0.01)	0.18–0.24 (0.21±0.02)	0.18–0.21 (0.19±0.01)
FTL/SVL	0.76–0.85 (0.80±0.03)	0.71–0.75 (0.73±0.02)	0.69–0.83 (0.76±0.04)	0.66–0.77 (0.72±0.03)
TIB/SVL	0.56–0.60 (0.57±0.01)	0.50–0.55 (0.53±0.02)	0.52–0.61 (0.57±0.03)	0.48–0.56 (0.53±0.02)

phologically, *A. yatseni* sp. n. differs from *A. sinensis* sp. n. by the presence of dense tiny round translucent, or white, spines on the dorsal skin of the body, dorsal and dorsolateral skin of limbs (vs. absent), the presence of rounded spines on the skin of the temporal region and lower-lips in females (vs. absent), the absence of longitudinal glandular folds on the skin of the shoulders (vs. present), supernumerary tubercles below the base of fingers II, III and IV distinct and prominent (vs. indistinct below the base of fingers III and IV, absent below the base of finger II), and heels just meeting (vs. overlapping).

Amolops yatseni sp. n. was previously reported as *A. ricketti*, but significantly differs from the topotype *A. ricketti* by the presence of dense tiny round translucent or

Figure 7.A Male paratype SYS a004643 of *Amolops yatseni* sp. n. in life **B** Female paratype SYS a003981 in life, showing denser and more distinct rounded spines on dorsal skin **C** Habits on Shangchuan Island.

white spines on the dorsal skin of the body, dorsal and dorsolateral skin of the limbs (vs. absent), large raised warts on dorsal surface of body (vs. relatively smooth), supernumerary tubercles below the base of fingers II, III and IV distinct and prominent (vs. indistinct below the base of fingers III and IV, absent below the base of finger II), and the presence of white conical spines on skin of temporal region and loreal region in breeding males (vs. absent).

Amolops yatseni sp. n. differs from *A. albispinus* by the presence of dense tiny round translucent, or white, spines on the dorsal skin of the body, dorsal and dorsolateral skin of the limbs (vs. absent), pineal body distinct (vs. indistinct), the presence of conical spines on skin of the tympanum (vs. absent), the presence of rounded spines on skin of temporal region and lower-lips in females (vs. absent), the presence of supernumerary tubercles below the base of fingers II, III and IV (vs. absent), and ventral surface smooth (vs. presence of tiny, transparent and dispersive conical spines on surface of chest in males).

Amolops yatseni sp. n. can be easily distinguished from *A. wuyiensis* by the presence of dense tiny round translucent or white spines on dorsal skin of body, dorsal and dorsolateral skin of limbs (vs. absent), vomerine teeth present (vs. absent), lacking vocal sacs (vs. present), and conical nuptial spines white (vs. black).

Amolops yatseni sp. n. further differs from *A. yunkaiensis* by the presence of dense tiny round translucent or white spines on dorsal skin of body, dorsal and dorsolateral skin of limbs (vs. absent), a significantly larger body size, SVL 39.3–44.7 mm in adult males and 42.1–48.9 mm in adult females (vs. SVL 31.8–34.1 mm in males and 35.2–39.0 mm in females), vomerine teeth present (vs. absent), lacking vocal sacs (vs. present), and ventral surface smooth (vs. presence of tiny transparent spines on surface of chest).

Etymology. The specific name "*yatseni*" refers to the founder of Sun Yat-sen University, Dr. Sun Yat-sen, who was born in Cuiheng Village, Zhongshan City, about five kilometers from the type locality, Mt. Wugui. We suggest its English common name "Yat-sen's Torrent Frog" and Chinese name "Yi Xian Tuan Wa (逸仙湍蛙)".

Distribution and habits. Currently, the Yat-sen's Torrent Frog is known from the Zhongshan City, as well as from Mt. Gudou, Shangchuan Island, Ehuangzhang Nature Reserve, and Yunkaishan Nature Reserve. All these localities are situated in the coastal hills of west Guangdong, indicating the potential distribution area of *Amolops yatseni* sp. n. is from the west border of Pearl River Delta to the Yunkai Mountains. However, the five known localities of the new species are being threatened by hydropower station construction and tourism development respectively, and surveys are needed in western Guangdong to investigate the accurate population status and the distribution of this species.

Amolops yatseni sp. n. inhabits rocky, fast-flowing streams (ca 250–1000 m a.s.l.) surrounded by moist subtropical secondary evergreen broadleaved forests (Fig. 7C). All individuals were observed from March to August when males bear nuptial spines and females bear mature oocytes. Nevertheless, much of the ecology and behavior of this species remains unknown.

Discussion

The species *Amolops ricketti* was originally described based on two specimens from Mt. Wuyi, Fujian (Boulenger 1899), and was recorded subsequently over wide area from southern China to northern and central Indochina (Bourret 1942; Liu 1950; Fei et al. 2012; Frost 2018). In this work, we have found that the recorded population of *A. ricketti* from central Guangdong, northeastern Guangxi and southwestern Hunan (now recognized as *A. sinensis* sp. n.) and from coastal hills of west Guangdong (now recognized as *A. yatseni* sp. n.), are markedly different from the topotype of *A. ricketti* from Fujian, both morphologically and genetically. This indicates that the current records of *A. ricketti* might be a species complex (designated here as *A. ricketti* sensu lato) composed of multiple species. Further surveys and studies are required to clarify the concept of *A. ricketti*, especially for the reported populations from southwestern China and Indochina and to determine the accurate distribution of *A. sinensis* sp. n. and *A. yatseni* sp. n.

Southwestern China has been considered as hotspot area with highest species diversity over time, while southeastern China, which suffers from more human activities, is considered as much less diverse, which may reflect the lack of biodiversity surveys over time. Recently, a number of new amphibian species were described from southeastern China (Lyu et al. 2017; Wang et al. 2017; Yuan et al. 2017; Zeng et al. 2017; Lyu et al. 2018; Wang et al. 2018a; Wang et al. 2018b; this study), to be the greatest number of new amphibian species in China in recent times. These discoveries indicate that the species diversity in southeastern China is highly underestimated. Comprehensive and careful surveys are urgently demanded to investigate the biodiversity status in this area, especially for herpetological species which are sensitive to rapid environmental changes.

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Appendix 1

Specimens examined

Amolops albispinus (n=10): China: Guangdong Province: Shenzhen City: Mt. Wutong (type locality): SYS a003270–3271, 3452–3454, 4511, 5643; Mt. Paiya: SYS a002436, 6898–6899.

Amolops ricketti (n=16): China: Fujian Province: Wuyishan City: Mt. Wuyi (type locality): SYS a004141–4143, 5922–5923; Taining County: Mt. Emeifeng: SYS a002492; Shanghang County: Gutian Town: SYS a003342, 4106; Jiangxi Province: Yanshan County: Wuyishan Nature Reserve: SYS a001605, 1342–1343; Guixi City: Yangjifeng Nature Reserve: SYS a000214, 0240, 0314, 0354–0355.

Amolops wuyiensis (n=20): China: Fujian Province: Wuyishan City: Mt. Wuyi (type locality): SYS a001716–1717, 4139–4140, 5936–5938; Shaowu City: Longhu Forest Station: SYS a004129, 4131; Ninghua County: Mt. Yashu: 5897–5900; Jiangxi Province: Yanshan County: Wuyishan Nature Reserve: SYS a001606; Guixi City: Yangjifeng Nature Reserve: SYS a000324, 0358–0360; Guangfeng County: Tongboshan Nature Reserve: SYS a001668; Zhejiang Province: Jingning County: Makeng Village: SYS a002723.

Amolops yunkaiensis (n=21): China: Guangdong Province: Yangchun City: Ehuangzhang Nature Reserve (type locality): SYS a000773–0774, 3979, 3982, 4696–4701, 4703–4707; Xinyi City: Yunkaishan Nature Reserve: SYS a004674, 4681–4684, 4992.