

Redescription of *Potamonautes sidneyi* (Rathbun, 1904) (Decapoda, Potamonautidae) and description of a new congeneric species from KwaZulu-Natal, South Africa

Nasreen Peer¹, Gavin Gouws², Eric Lazo-Wasem³,
Renzo Perissinotto¹, Nelson A.F. Miranda¹

1 DST/NRF Research Chair in Shallow Water Ecosystems, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa **2** South African Institute for Aquatic Biodiversity (SAIAB), Private Bag 1015, Grahamstown, 6140, South Africa **3** Division of Invertebrate Zoology, Peabody Museum of Natural History, Yale University, PO Box 208118, New Haven, CT 06520-8118, USA

Corresponding author: Nasreen Peer (peer.nasreen@gmail.com)

Academic editor: S. De Grave | Received 23 December 2016 | Accepted 31 January 2017 | Published 16 February 2017

<http://zoobank.org/570CDA53-5D30-4B63-83CA-52E5FEFB9A08>

Citation: Peer N, Gouws G, Lazo-Wasem E, Perissinotto R, Miranda NAF (2017) Redescription of *Potamonautes sidneyi* (Rathbun, 1904) (Decapoda, Potamonautidae) and description of a new congeneric species from KwaZulu-Natal, South Africa. ZooKeys 657: 1–28. <https://doi.org/10.3897/zookeys.657.11623>

Abstract

A new species of freshwater crab, *Potamonautes danielsi* sp. n., is described from the southern region of the KwaZulu-Natal Province, South Africa. *Potamonautes danielsi* most closely resembles *P. sidneyi* which is re-described here, but can be distinguished by a suite of key morphological characters including carapace shape and width, slim pereopods, inflated propodi of the chelipeds, and the shape and terminal segment length:subterminal segment length ratio of the 1st gonopod. In a previous study (Gouws et al. 2015), a 9.2–11.8 % divergence was found in the mitochondrial COI and 16S genes of the *P. sidneyi* clade, allowing for the delineation of a new species. Despite the clear molecular distinction between the two species, it is difficult to separate them based on individual morphological characters, as there is a great deal of overlap even among key features. The new species is found in slow-moving mountain streams and pools at high altitudes between Umhlanga and Mtamvuna, in KwaZulu-Natal.

Keywords

Taxonomy, Brachyura, freshwater, morphometrics, KwaZulu-Natal

Introduction

Potamonautes sidneyi (Rathbun, 1904) was first described from “Natal, southern Africa” (presently KwaZulu-Natal Province, South Africa). The original description was sourced from the Muséum National d’Histoire Naturelle, Paris, allowing us to locate the syntypes. Two type specimens are known and are lodged at the Peabody Museum, Yale University (original catalogue number 1191). These were collected by Sarah Abraham in 1871, but no accurate locality data were provided. Rathbun (1904) listed another individual, collected from Port Natal (presently Durban), as *P. sidneyi*, but pointed out certain differences from the types, notably the lack of a concave ridge behind the eyes. Although the description (Rathbun, 1904) highlighted the main difference between *P. sidneyi* and *P. perlatus* (H. Milne-Edwards, 1837), it was brief and relatively vague with few quantifiable or measurable distinctions between the two species.

Potamonautes sidneyi is regarded as one of the most widespread potamonautid species, occurring from the eastern parts of South Africa, northwards to Zimbabwe and Malawi (Gouws et al. 2002). Within the province of KwaZulu-Natal (KZN), *P. sidneyi* was thought to occur in the low-lying midlands regions from the Drakensberg to the coast and to inhabit the entire coastal zone from the Maputaland (northern KZN) to the northern border of Pondoland (northern Eastern Cape ending at the southern KZN border) (Gouws and Stewart 2001, Gouws et al. 2015). However, recent genetic analyses have shown that these KwaZulu-Natal populations include two distinct genetic lineages, i.e. a northern lineage and a southern lineage, with the divergences warranting recognition of these lineages as separate species (Gouws et al. 2015). The issue of which lineage corresponds to the described *P. sidneyi* was difficult to resolve due to various morphological similarities between the two lineages, the vagaries of the original species description and the lack of a precise locality for the type material (Rathbun 1904).

Examination of high resolution photographs of key diagnostic features, including the carapace, chelipeds and male gonopods of *P. sidneyi* type specimens alongside specimens collected from both lineages revealed that specimens from the northern Maputaland lineage likely represent *P. sidneyi* s. str. as they match the type specimens in terms of the following: the slim propodi of the chelipeds, the stout pereopods, the shape and terminal segment length:subterminal segment length ratio of the 1st gonopod; and its larger size. The southern lineage, thus, represents a new species and is described in this paper by NP and GG while RP and NAFM contributed to the information on its ecology and natural history, and EL-W contributed to the redescription of *P. sidneyi*. The delineation of a new species at the northern border of the Pondoland region is significant, based on the distribution of the lineages revealed by Gouws et al. (2015), as it interrupts the unclear and often confusing transition between *P. sidneyi* and *P. perlatus* at locations where the two species overlap and are often morphologically indistinct (Barnard 1950, Gouws and Stewart 2001).

Materials and methods

Collection of crabs

Detailed photos of the original syntype and additional specimens of *P. sidneyi* were obtained from the Invertebrate Zoology Division at the Yale Peabody Museum (CT, USA) and the Muséum National d'Histoire Naturelle (Paris, France) respectively.

Crab specimens were collected from various localities around KwaZulu-Natal (Fig. 1). For the taxonomic description and morphometric analyses, crabs from localities 1 (Lake Sibaya), 2 (Mpophomeni Stream), 3 (Hluhluwe), 5 (Siyayi), 6 (Mhlanga), 7 (Oribi Gorge), and 8 (Mtamvuna) were used. The crabs from Mtamvuna, Mhlanga and Oribi Gorge were used to describe the new species, while the morphology and morphometric analyses of *Potamonautes sidneyi* were conducted using the syntype specimens, as well as crabs from Lake Sibaya, Mpophomeni Stream, Hluhluwe and Entumeni. Crabs were collected by hand or by net and preserved in 70% ethanol. Photographs were taken using a Canon Powershot G12 digital camera.

Morphological and morphometric analyses

For examination of *P. danielsi* type specimens, a pair of Vernier callipers was used to measure morphological variables. A Nikon SMZ25 microscope fitted with a Nikon Digital Sight DS-Fi2 camera was used for macro-examination and to take photos of gonopods and mouthparts. A Canon Powershot G12 was used to photograph the carapace and appendages.

For the redescription of *P. sidneyi* s. str., a lectotype was designated from the syntypes housed at the Peabody Museum.

Abbreviations for repositories and provinces:

| | |
|-------------|---|
| YPM | Yale Peabody Museum, New Haven, Connecticut, United States of America |
| SAM | Iziko South African Museum, Cape Town, South Africa |
| AM | Albany Museum, Grahamstown, South Africa |
| DNM | Ditsong National Museum of Natural History, Pretoria, South Africa |
| NMMU | Nelson Mandela Metropolitan University, Port Elizabeth, South Africa |
| MNHN | Muséum National d'Histoire Naturelle |
| EC | Eastern Cape Province, South Africa |
| WC | Western Cape Province, South Africa |
| KZN | KwaZulu-Natal Province, South Africa |

Abbreviations for all morphological and morphometric characters (following Gouws et al. 2001):

| | |
|------------|------------------------|
| CL | Carapace length; |
| CWW | Carapace widest width; |

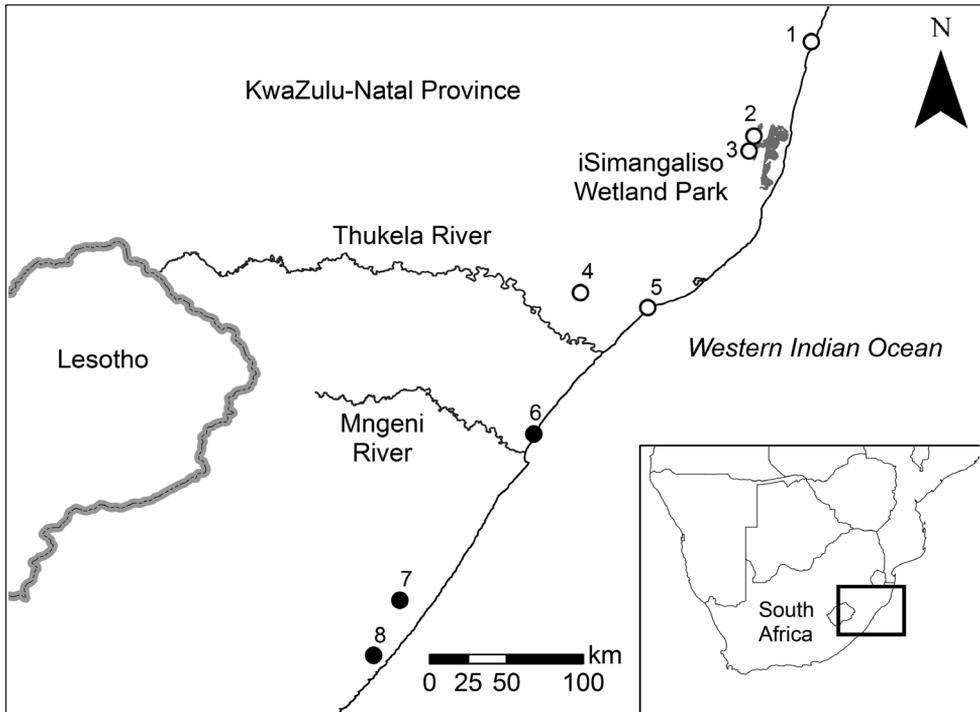


Figure 1. Sampling localities for the present study of *Potamonautes* in KwaZulu-Natal Province on the east of South Africa. The collection localities of *P. danielsi* sp. n. are indicated by black dots, while those of *P. sidneyi* are indicated by white open dots. Sites are indicated by numbers which correspond to localities as follows: (Site 1) Lake Sibaya, (Site 2) Mpophomeni Stream, (Site 3) Hluhluwe, (Site 4) Entumeni, (Site 5) Siyayi, (Site 6) Mhlanga, (Site 7) Oribi Gorge and (Site 8) Mtamvuna. Map modified from Gouws et al. (2015; copyright Gouws et al. 2015; licensee: AOSIS Publishing).

| | |
|--------------|--|
| CWP | Carapace posterior width; |
| PFC | Distance between postfrontal crest and anterior margin; |
| ED | Distance between orbits; |
| CWA | Distance between exorbital teeth; |
| CH | Carapace height; |
| AW6 | Width of sixth abdominal segment; |
| MCPL | Major cheliped propodus length; |
| MCPH | Major cheliped propodus height; |
| P2ML | Pereopod 2, merus length; |
| P2MW | Pereopod 2, merus width; |
| s2/s3 | First sternal groove (suture between the second and third sulci); |
| s3/s4 | Second sternal groove (suture between the third and fourth sulci); |
| CRDL | Right cheliped, dactyl length; |
| CLDL | Left cheliped, dactyl length; |
| CRPL | Right cheliped, propodus length; |
| CRPW | Right cheliped, propodus width. |

Eight variables, including six carapace variables and dimensions of the propodus of the right cheliped (CL, CWW, CH, PFCD, ED, CWA, CRPW and CRPL), were log transformed and used to statistically analyse morphometric differences between the two species, by means of a discriminant functions analysis in STATISTICA v12.5 (Statsoft Inc., Tulsa, OK, USA; www.statsoft.com). Classification functions were calculated and individuals were then reassigned to groups based on *a-priori* probabilities. Canonical scores were plotted for both species as a frequency histogram to examine distinctions between the two forms. Lastly, linear regression analyses were used to examine variation among the two species for combinations of specific variables.

Taxonomic description

Suborder Brachyura Linnaeus, 1758

Superfamily Potamoidea Ortmann, 1896

Family Potamonautidae Bott, 1970

Subfamily Potamonautinae Bott, 1970

Genus *Potamonautes* Macleay, 1838

***Potamonautes sidneyi* (Rathbun, 1904)**

Table 1; Figs 4, 5, 6A

Potamon (*Potamonautes*) *perlata* var. *a* Krauss 1843: 37

Potamon (*Potamonautes*) *sidneyi* Rathbun, 1904: plate 14, fig. 3.-Rathbun, 1905: 163-166.-Stebbing 1910: 295.-Lenz 1912: 7.-Barnard 1935: 438, fig 1c.-Chace 1942: 222. Barnard 1950: 184, 187, fig 34b.-Chace 1953: 440.-Cumberlidge 1998: 204.

Potamonautes (*Orthopotamonautes*) *sidneyi* Bott 1955: 278-279, 235, fig 46, pl XX, figs 1a-d.

Thelphusa perlata Milne Edwards 1837: 13.-Kingsley 1880: 36

Thelphusa corrugata Heller 1865: 32, pl IV, fig 1.-Milne-Edwards 1869: 181.

Type series. Lectotype: male, CL = 35.6 mm, CWW = 52.4 mm (Table 1), Port Natal, 1871, S. Abraham legit (YPM IZ 001191).

Paralectotype: CL = 27 mm, CWW = 36 mm, Port Natal, 1871, S. Abraham legit (YPM IZ 078196)

Additional material examined. Locality unknown, MNHN B3841 (MNHN-IU-2000-3841) (1♀), CWW = 50 mm; Ikhamanzi River, SAM A43967 (2♂, 2♀), CWW = 45 mm (♂), 61 mm (♀), 29°16'13"S, 30°38'30"E; Mseleni River Bridge near Lake Sibaya, SAM A41112 (2♂, 2♀), CWW = 50 mm (♂), 50 mm (♀), 27°21'50"S, 32°31'35"E, coll. M. Coke on 19 Aug 1997; Linwood (Kwa Gqishi Stream near Midmar Dam), SAM A43960 (1♀), CWW = 36 mm, 29°33'50"S, 30°05'40"E, coll. O. Bourquin on 4 May 1998; Pinetown (Durban), SAM A41139 (2♂, 2♀), CWW =

Table 1. Ranges of measurements (mm) for 12 morphometric variables of the *P. danielsi* sp. n. holotype and paratypes collected from Mtamvuna, Oribi Gorge and Mhlanga, as well as *P. sidneyi* (Rathbun, 1904) specimens collected from Lake Sibaya, Hluhluwe, Mpophomeni Stream and Entumeni.

| Variable | <i>Potamonautes danielsi</i> sp. n. | | | <i>Potamonautes sidneyi</i> | |
|----------|-------------------------------------|---------------|-----------------|-----------------------------|-----------------|
| | Holotype | Males (n= 14) | Females (n= 22) | Males (n=12) | Females (n= 10) |
| CL | 18.5 | 12.4–23.6 | 10.4–34.3 | 13.64–34.2 | 13.3–41.6 |
| CWW | 25.8 | 16.1–34.0 | 13.2–45.5 | 19.11–43.5 | 19.1–54.8 |
| CWP | 12.8 | 9.2–15.2 | 7.4–23.1 | 9.97–21.3 | 10.4 - 29 |
| PFC D | 2.6 | 1.4–3.0 | 1.3–4.7 | 2.3–4.2 | 1.9–5.5 |
| ED | 8.9 | 6.0–10.5 | 5.7–15.8 | 7.8–16.4 | 7.4–19.52 |
| CWA | 18.8 | 13.2–24.4 | 11.5–26.5 | 16.5–33.1 | 15.9–39.6 |
| CH | 9.3 | 5.7–12.5 | 5.3–13.4 | 7.0–16.0 | 6.9–20.3 |
| AW6 | 5.4 | 1.1–6.9 | 4.6–24.0 | 4.1–12.6 | 5.1–32.2 |
| MCPL | 16.6 | 7.6–33.1 | 7.8–22.3 | 11.3–29.6 | 11.3–34.0 |
| MCPH | 9.41 | 4.81–19.5 | 2.9–9.7 | 4.3–12.8 | 4.4–16.0 |
| P2ML | 10.29 | 6.1–15.7 | 5.6–14.5 | 8.0–16.7 | 7.9–20.5 |
| P2MW | 4.25 | 2.1–6.5 | 2.3–6.2 | 3.4–7.2 | 3.3–8.7 |

45 mm (♂), 70 mm (♀); Mtunzini (Otungulu Pan, Umlalazi Nature Reserve), coll. B. Stewart and P. Cook on 10 Apr 1992; SAM A41171 (1 ♀), CWW = 35 mm, 29°03'17"S, 31°39'52"E, coll. M. Coke and A. Wood on 08 Mar 1994; Mdumbeni River, SAM A41979 (1 ♂), CWW = 42 mm, 28°58'07"S, 30°22'20"E, coll. M. Coke on 10 Jan 1995; Mvudi River (University of Venda), SAM A41993 (1 ♂), CWW = 37 mm, 22°55'S 30°30'E, coll. B.C.W. van de Waal on 06 Mar 1994; Phongolo River (Rivierplaats, Luneburg), SAM A41966 (1 ♂, 1 ♀), CWW = 39 mm (♂), 39 mm (♀), 27°21'20"S, 30°27'17"E, coll. M. Coke and S. de Jager on 19 Jan 1995; Mariti River (The Gums), SAM A41131 (1 ♀), CWW = 37 mm, 24°56'18"S, 31°04'43"E, coll. D. Weeks and V. Makunyane on 17 Aug 1992; Hlimbitwa River (Klipnek near Hermannsburg), SAM A43965 (1 ♂), CWW = 41 mm, 28°57'56"S, 30°45'20"E, coll. M. Coke on 20 Dec 1996; Syzygium swamp forest (University of Zululand), SAM A43937 (1 ♂, 1 ♀), CWW = 33 mm (♂), 40 mm (♀), 28°51'25"S, 31°51'02"E, coll. B.A Stewart, P.A Cook, P.E. Reavell, L. Hoenson and G. Gouws on 24 Jan 1997; Bilanyoni River (Koppie Alleen near Luneburg), SAM A41959 (1 ♂, 1 ♀), CWW = 43 mm (♂), 42 mm (♀), 27°17'25"S, 30°34'55"E, coll. M. Coke on 19 Jan 1995; Manzibomvu River (Upper reaches, Hluhluwe Game Reserve), SAM A43935 (1 ♂, 1 ♀), CWW = 53 mm (♂), 50 mm (♀), 28°02'20"S, 32°05'10"E, coll. B.A Stewart, L. Hoenson and G. Gouws on 28 Jan 1997; Shinane River (Tributary of Mutshindini, Venda), SAM A41994 (1 ♂, 1 ♀), CWW = 27 mm (♂), 40 mm (♀), 22°53'S 30°31'E, coll. B.C.W. van de Waal on 25 Oct 1995; Stream behind dunes at Amatikulu Hatchery, SAM A41104 (1 ♂, 1 ♀), CWW = 36 mm (♂), 36 mm (♀), 29°04'30"S, 31°38'35"E, coll. M. Coke on 10 Mar 1994; Palmiet River (Westville, Durban), SAM A41114 (1 ♀), CWW = 37 mm, 29°49'53"S, 30°54'37"E, coll. M. Coke and D. Coutts on 10 Feb 1994; Wekeweke River (plunge pool, Nshongweni),

SAM A43948 (1 ♀), CWW = 35 mm, 29°50'07"S, 30°43'20"E, coll. M. Coke and J. Craigie on 22 May 1997; Nsuze River (R614 Bridge, Fawnleas, Tongaat Road), SAM A43969 (1 ♂), CWW = 33 mm, 29°22'28"S, 30°56'25"E, coll. M. Coke and M. Peters on 06 Feb 1996; Mhlathuze River (D223 Bridge near Babanango), SAM A43976 (1 ♀), CWW = 60 mm, 28°28'46"S, 31°04'09"E, coll. M. Coke and M. Peters on 14 June 1996; Blood River (at laager behind monument), SAM A43941 (1 ♀), CWW = 71 mm, 28°06'15"S, 30°32'40"E, coll. T. Ridgway and G. Gouws on 16 May 1997; Amagoda River (outside Vryheid), SAM A43939 (1 ♂, 1 ♀), CWW = 52 mm (♂), 56 mm (♀), 27°46'45"S, 30°46'07"E, coll. T. Ridgway and G. Gouws on 15/16 Oct 1997; Mhulumbela River (Onverwacht picnic site, Itala Game Reserve), SAM A43949 (1 ♂, 1 ♀), CWW = 40 mm (♂), 44 mm (♀), 27°32'00"S, 31°19'02"E, coll. M. Coke on 06 Nov 1997; Ifaye River (Mount Elias, Fawnleas), SAM A43968 (1 ♀), CWW = 40 mm, 29°19'12"S, 30°46'53"E, coll. M. Coke and M. Protheroe on 09 Oct 1995; Macabuzela Stream (near Dakaneni, Hluhluwe Game Reserve), SAM A43942 (1 ♂, 1 ♀), CWW = 56 mm (♂), 40 mm (♀), 28°02'32"S, 32°09'51"E, coll. T. Ridgway and G. Gouws on 19 May 1997; Mvuzane River (D50 Bridge), SAM A43977 (1 ♂, 1 ♀), CWW = 45 mm (♂), 45 mm (♀), 28°49'41"S, 31°11'23"E, coll. Coke, Eckard and Louw on 20 Aug 1997; Mahai Stream (Royal Natal National Park), SAM A41311 (1 ♀), CWW = 37 mm, 28°41'15"S, 28°56'30"E, coll. E. Dickson on 04 Dec 1994; Mgeni River (Albert Falls Dam), SAM A43963 (1 ♂), CWW = 36 mm, 29°26'47"S, 30°20'50"E, coll. M. Coke and M. Peters on 15 May 1996; Bilanyoni River (D27 Bridge, Luneburg), SAM A41965 (1 ♂, 1 ♀), CWW = 47 mm (♂), 45 mm (♀), 27°19'20"S, 30°38'00"E, coll. M. Coke on 19 Jan 1995; Crocodile Rover (Rietvlei Farm, AM GEN256J) (2 ♂), CWW = 28 mm, 25°22'49"S, 30°33'03"E, coll. unknown on 21 Nov 1959; Inyamvubu River (5km above Craigieburn), AM GEN843 (1 ♀), CWW = 64 mm (♀), 29°11'24"S, 30°16'12"E, coll. M. Coke and P. Couldon on 12 Apr 1989; Mkomas River (Nhlavini Stream, Coothill Farm), AM GEN847 (1 ♂, 1 ♀), CWW = 40 mm (♂), 35 mm (♀), 30°11'24"S, 30°09'00"E, coll. C. Arter and M. Coke on 03 Oct 1988; Nontshibongo River (below Gala Forest), AM GEN848 (1 ♂, 1 ♀), CWW = 37 mm (♀), 29°59'24"S, 29°48'36"E, coll. C. Arter and M. coke on 05 Oct 1988; KwaCota River (The Springs Farm), AM GEN849 (1 ♀), CWW = 31 mm (♀), 30°04'48"S, 29°52'12"E, coll. C. Arter and M. Coke on 07 Oct 1988; Manzana River (Tributary at Rondspring Farm), AM GEN953 (1 ♂, 1 ♀), CWW = 73 mm (♂), 27°33'00"S, 31°00'36"E, coll. M. Coke on 23 Apr 1991; Nosonto River Headwaters (near Vryheid), AM GEN954 (1 ♂, 1 ♀), CWW = 57 mm (♂), 35 mm (♀), 27°44'24"S, 30°37'48"E, coll. M. Coke on 21 Mar 1991; Grantleighspruit (near Mooi River), AM GEN955 (1 ♂, 1 ♀), CWW = 57 mm (♀), 29°10'48"S, 29°58'48"E, coll. M. Coke on 25 Apr 1991; Lynspruit (Waterhoek Farm, Vryheid), AM GEN956 (1 ♂, 1 ♀), CWW = 36 mm (♂), 27°44'24"S, 30°36'36"E, coll. M. Coke on 21 Mar 1991; Bells Spruit (Ladysmith), AM GEN957 (1 ♂, 1 ♀), CWW = 37 mm (♂), 32 mm (♀), 28°32'24"S, 29°48'00"E, coll. M. Coke on 20 Mar 1991; Hlambizandla Stream (Gluckstad), AM GEN959 (2 ♂, 1 ♀), CWW = 57 mm (♂), 70 mm (♀), 27°58'48"S, 31°03'36"E, coll. M. Coke on 22 Mar 1991; KwaMbizankulu River (KwaMbizankulu

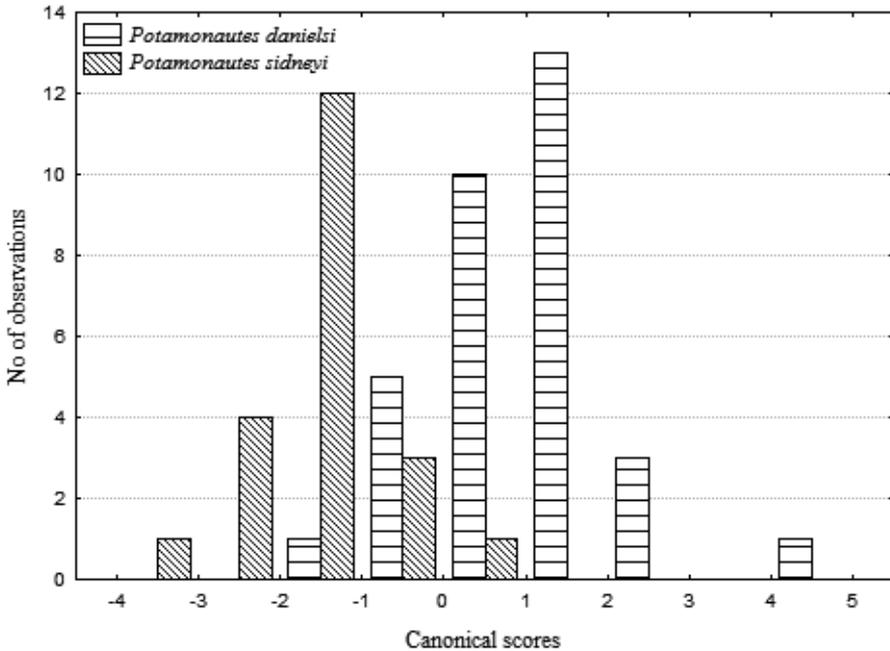


Figure 2. Histogram of canonical scores for *P. danielsi* sp. n. and *P. sidneyi* calculated from a discriminant function analysis, based on six carapace and two right cheliped variables.

Stream, Bevenson Farm), AM GEN960 (1 ♂, 1 ♀), CWW = 37 mm (♂), 36 mm (♀), 27°59'24"S, 31°07'12"E, coll. M. Coke on 22 Mar 1991; Bivane River (Frischgewaagd Farm), AM GEN962 (1 ♂, 1 ♀), CWW = 27 mm (♀), 27°32'24"S, 30°47'24"E, coll. M. Coke and J. van Niekerk on 24 Mar 1991; Bivane River (Kruger Bridge), AM GEN963 (1 ♂, 1 ♀), CWW = 28 mm (♂), 46 mm (♀), 27°31'12"S, 30°49'12"E, coll. M. Coke and J. van Niekerk on 24 Mar 1991; Bridal Veil Falls (near Sabie), AM GEN141A (1 ♂, 1 ♀), CWW = 48 mm (♂), 25°04'48"S, 30°44'24"E, coll. unknown on 08 Jul 1959; Lake Sibaya (North bank, South basin, East shore), AM SIB46H (2 ♂, 1 ♀), CWW = 36 mm (♂), 29 mm (♀), 27°23'24"S, 32°42'36"E, coll. B. Allanson on 18 Jan 1967; Waterpoort (Salt pan), DNM TM5061 (1 ♀), CWW = 36 mm, 22°53'50"S, 29°37'44"E; Lake Fundusi, DNM TM5063 (1 ♂), CWW = 33 mm (♂), 22°51'04"S, 30°18'35"E. Wakkerstroom, DNM TM5015 (1 ♀), CWW = 41 mm, 27°21'23"S, 30°07'47"E, collected by P. Simons and G. van Dam; Wakkerstroom, DNM TM5041 (1 ♀), CWW = 45 mm, 27°21'23"S, 30°07'47"E, Ratomba, DNM TM5183 (1 ♂), CWW = 48 mm, 23°04'00"S, 30°09'59"E.

Diagnosis. Carapace flat and scabrous. Anterolateral margin heavily granulated. Postfrontal crest complete bearing concavity behind orbital regions. Propodi of chelipeds straight and slim. Pereopods 2–5 stout. Gonopod 1 displaying high terminal segment length:subterminal segment length ratio of 0.31.

Description of lectotype. *Carapace* (Figs 4A, C, 9A). Cephalothorax flat (CH/CL = 0.54), wide (CWW/CL = 1.33), ovoid in frontal aspect. Branchial region flat forming angle with anterolateral margin. Anterior margin straight bearing concavity behind orbital regions, heavily granulated. Epigastric lobes poorly defined above postfrontal crest; two slight indentations present, forked from midpoint of postfrontal crest. Postfrontal crest heavily granulated, straight and distinct from epibranchial region to midpoint, curving downward at epibranchial region. Exorbital teeth present; no epibranchial teeth present, anterolateral margin serrated. Flank of carapace scabrous, well-defined epimeral sutures dividing pterygostomial region from subhepatic and suborbital regions, well-defined pleural groove dividing subhepatic region from suborbital region.

Sternites (Fig. 4B). Sternites 1 and 2 fused, first sulcus (s1/s2) absent. Second sulcus (s2/s3) prominent, running completely across sternum, third sulcus (s3/s4) projecting downwards medially to abdominopelvic region. Sulci and episternal sulci thereafter well-defined but shallow.

Third maxillipeds (Figs 4C, 5E). Filling entire buccal frame except for oval respiratory openings medially above maxilliped. Ischium scabrous, with wide groove running vertically. Flagellum on exopod very long, straight.

Mandibular palp (Fig. 5C, D). Consisting of two segments; terminal segment smooth and undivided, with hirsute margins; dense tuft of long setae arising from base. Subterminal segment bulbous in appearance.

Pereopods (Figs 4A, B, 5A, B). No substantial heterochely (CRDL/CLDL > 0.91. CRDL broken at the tip. Refer to fig. 4B). Dactyl of major cheliped slightly arched. Small slim interspace formed when closed in minor chelipeds; not possible to establish if same applies to major cheliped due to broken tip. Propodus slim (CRPW/CRPL = 0.40), exhibiting ~21 cutting teeth. Carpus on either side containing one prominent tooth followed by one small tooth. Meri granulated with strong medial tubercle on inner lateral face. Pereopods 2 (ML/MW = 2.29) and 5 (ML/MW = 2.39) moderately stout; pereopod 3 longest among pereopods; pereopod 5 shortest. Ventral margins of meri smooth; ventral margins of propodi serrated; dactyli serrated, ending in sharp points.

Pleon (Figs 4B, 6A). Somites 1-6 four-sided with distally-rounded triangular terminal somite (telson). First 5 somites broad and short; somite 6 longer, about 1.7 times as wide as long, distal margins concave, lateral margins slanted towards medial line, swelling slightly at articulation with somites 5 and 7; telson terminally rounded, lateral margins concave, swell at articulation with somite 6; hirsute lateral margins.

Pleopods (Fig. 5F, G, H, I). Gonopod 1 widest at base; both subterminal and terminal segments tapering, ending with sharp point. Medial margin fairly straight displaying extrusion near base; lateral margin concave relative to midline; both margins hirsute. Groove extending almost entire length of gonopod, visible on dorsal surface, lined with setae. Gonopod 1 terminal segment long (0.31 times length of subterminal segment), curving outwards (i.e. away from midline) when viewed dorsally. Gonopod 2 consisting of two segments. Distal segment very long (0.57 times length of basal seg-

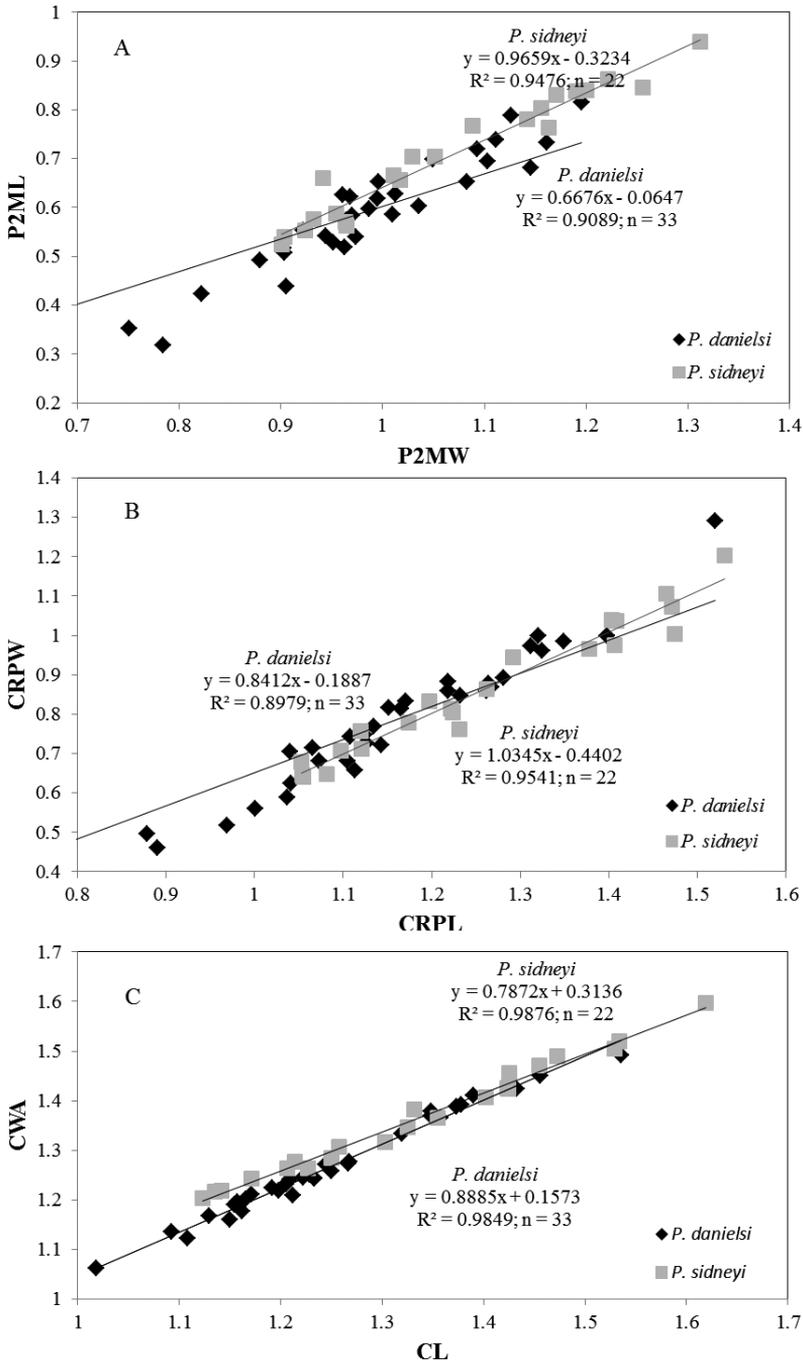


Figure 3. Regression analyses of morphometric measurements of *P. danielsi* sp. n. and *P. sidneyi*. Regression analyses of: **A** LogP2ML over LogP2MW **B** LogCRPW over LogCRPL; and **C** LogCWA over LogCL between the two species, *P. danielsi* sp. n. and *P. sidneyi*. All differences between regressions were statistically significant ($p < 0.001$).

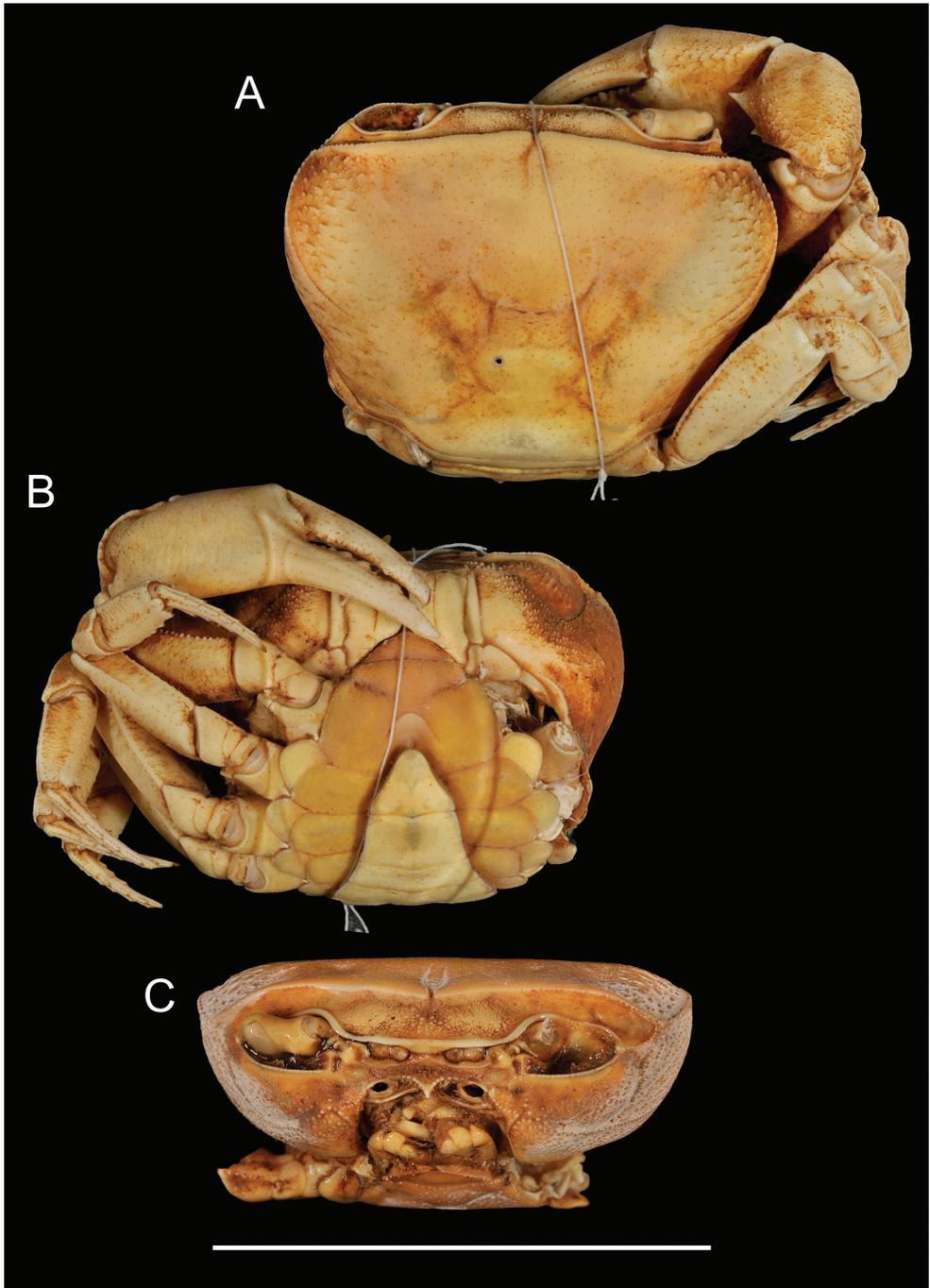


Figure 4. *Potamonautes sidneyi* (Rathbun, 1904). Male holotype CWW 47 mm (Yale Peabody Museum catalogue number 1191) **A** dorsal view **B** ventral view, and **C** cephalothorax, frontal aspect. Scale bar: 50 mm. Photos: Eric Lazo-Wasem.

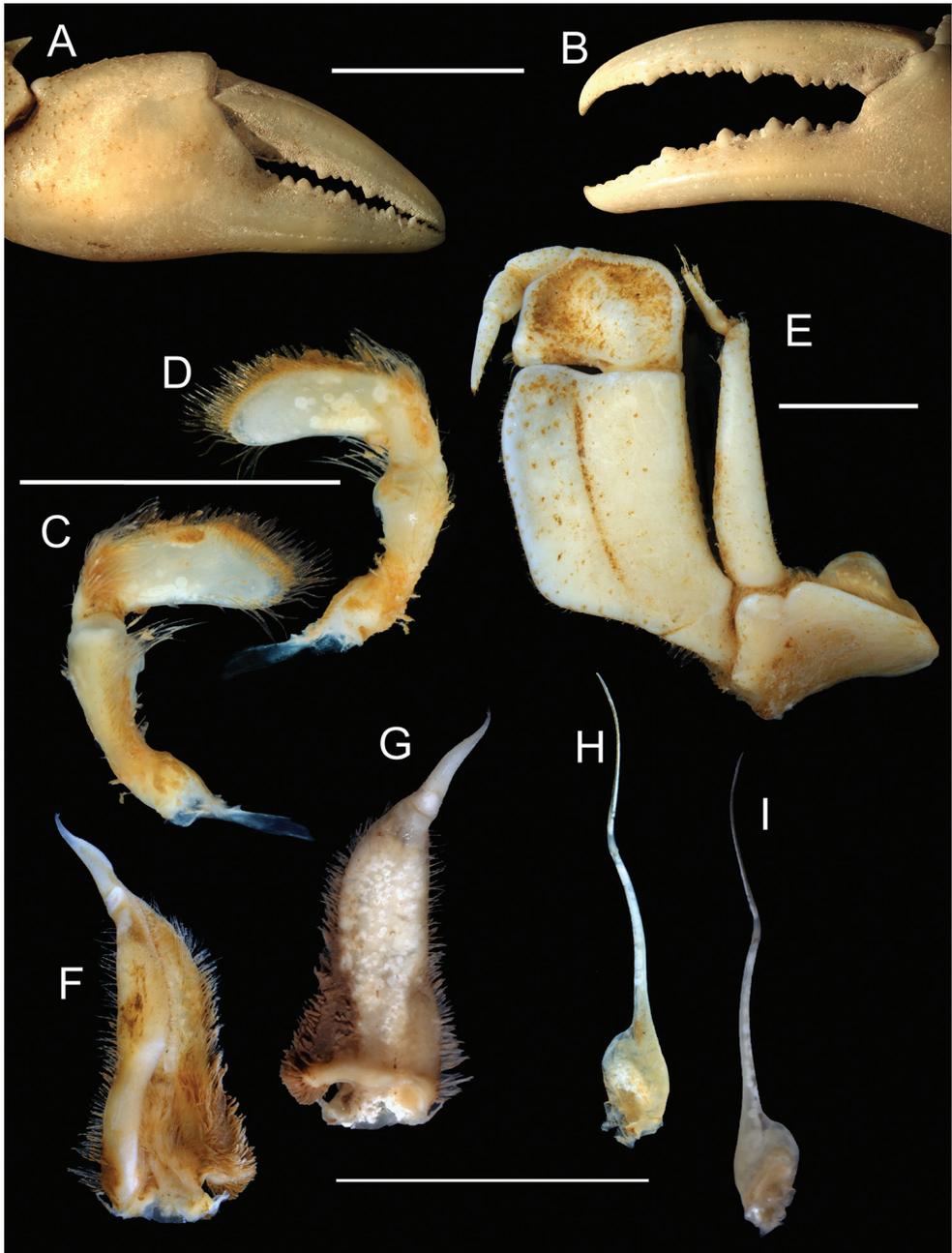


Figure 5. *Potamonautes sidneyi* (Rathbun, 1904). male holotype CWW 47 mm (Yale Peabody Museum catalogue number 1191) **A** major cheliped **B** minor cheliped **C** right mandibular palp posterior view **D** right mandibular palp anterior view **E** 3rd maxilliped **F** left gonopod 1 anterior view **G** left gonopod 1 posterior view **H** left gonopod 2 anterior view, and **I** left gonopod 2 posterior view. Scale bars: 5 mm (**A**, **B**), 5 mm (**C**, **D**), 5 mm (**E**), 10 mm (**F**, **G**, **H**, **I**). Photos: Eric Lazo-Wasem.

ment), slim; basal segment with wide elongated base sharply becoming narrow around 0.4 of length; narrow process forming at this point leading to distal segment.

Variation. The species appears to be extremely variable, with the northern populations displaying a more pronounced scabrosity and granulation on the chelipeds and carapace with fine hairs in some cases. Pereopods range from slender to stout. The inflation of the propodi on the chelipeds varies too, with some specimens bearing slim chelipeds while others possess more inflated propodi similar to that of *P. danielsi*.

Live colouration. Usually a variety of brown, ranging from chocolate brown to light brown or beige. In some cases pereopods are lighter than the carapace itself, a feature seen more in northern populations.

Distribution. Known to extend from Lake Sibaya in the north to Durban along the coast (Fig. 1), based on recent sampling and Gouws et al. (2015). This species has also been recorded in the Mpumalanga Province.

Type locality. South Africa, Port Natal. Collected by Mme Sarah Abraham. The specimens were received by the Peabody Museum (Yale) in 1871, along with other material. There is a possibility that the crab was among material accumulated for years, before it was sent to Yale and therefore it is uncertain as to whether the specimens were actually collected in 1871. While Mme Abraham was known to reside in Maphumulo (approximately 55 km north of Port Natal), it is unknown where exactly the specimens were from.

Etymology. The species was named by Miss M.J. Rathbun in honour of Professor Sidney I. Smith from Yale University.

Remarks. Only two type specimens were indicated in the original description, i.e. one male and one female from Port Natal both repositated at the Yale Peabody Museum. The lectotype was chosen on the basis of its designation as the only male type specimen in the original description. Compared to the lectotype, the paralectotype appears to be the same on the basis of the carapace scabrosity and granulation, the stout pereopods and the slim propodus of the cheliped. Although two additional specimens were mentioned, they were not designated as types. The first, a single female belonging to the Muséum National d'Histoire Naturelle, was uncertainly classified as *Thelphusa corrugata* Heller (Milne-Edwards, 1869). Following examination, it resembles *P. sidneyi* s. str. based on the stout limbs and slender propodi of the chelipeds. Despite the wide distribution and lack of specific locality all three specimens represent the same species i.e. *P. sidneyi* s. str.

***Potamonautes danielsi* Peer & Gouws, sp. n.**

<http://zoobank.org/D1C2B8F4-6903-4BC6-8798-36BF711B6033>

Type series. Holotype: male, CL = 18.5 mm (Table 1), mountain stream running into the Mtamvuna River, Mtamvuna Nature Reserve (31°03'31.60"S, 30°10'26.11"E; elevation 140 m), 18 November 2015, N. Miranda and N. Peer legit (SAMC A83487).

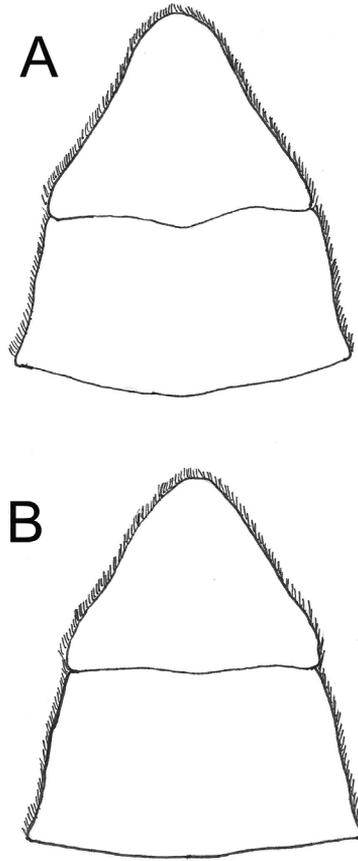


Figure 6. Morphological comparison of somites 5 and 6 between **A** *P. sidneyi* (Rathbun, 1904) and **B** *P. danielsi* sp. n.

Allotype: female, CL = 22.8 mm (Table 1), collection details as per holotype (SAMC A83488).

Paratypes: (Table 1) collection data same as above, SAMC A83489 (1 ♂, 1 ♀); collection data same as above, NMMU (2 ♀); Umhlanga Nature Reserve, SAMC A83490 (1 ♂, 1 ♀), 29°42'13"S, 31°05'27"E, 28 January 2013, N. Miranda and N. Peer legit; Oribi Gorge Nature Reserve, SAMC A83491 (2 ♂), 30°40'55"S, 30°18'26"E, 30 January 2013, N. Peer and J. Raw legit. Mvoti River (Makabeleni), SAM A43970 (1 ♂), CWW = 53 mm, 29°14'45"S, 30°56'15"E, coll. M. Coke on 09 Nov 1995; Pongola Bush Nature Reserve, SAM A41984 (1 ♂, 1 ♀), CWW = 46 mm (♂), 47 mm (♀), 27°19'40"S, 30°29'15"E, coll. M. Coke and M. Protheroe on 05 Apr 1995; Mvoti River ('Canema' below falls), SAM A43966 (1 ♂, 1 ♀), CWW = 36 mm (♂), 51 mm (♀), 29°10'00"S, 30°41'15"E, coll. M. Coke and M. Peters on 02 May 1996; Mtamvuna Nature Reserve, SAM A44982 (1 ♂), CWW = 40 mm, 31°02.704'S 30°10.080'E, coll. S. van Noort on 10 Nov 2000; Nwaku River (D356 Causeway near Nwaku Store), SAM A43974 (2 ♂, 1 ♀), CWW = 69 mm (♂), 55

mm (♀), 28°56'35"S, 31°23'50"E, coll. M. Coke on 19 Aug 1997; Umhlanga Nature Reserve, SAM A41179 (1 ♀), CWW = 41 mm, 29°42'40"S, 31°05'35"E, coll. M. Coke on 04 Aug 1994; Pandana River (Schuilhoek, near Luneburg), SAM A41976 (1 ♂), CWW = 30 mm, 27°23'22"S, 30°30'55"E, coll. M. Coke on 20 Jan 1995; Giant's Castle (Path to Bannerman's Hut), SAM A44178 (1 ♀), coll. M. Hamer on 27 Mar 1995; CWW = 35 mm, Spartelspruit (above D251 Bridge), SAM A43952 (1 ♂), CWW = 62 mm, 27°50'02"S, 30°32'45"E, coll. M. Coke on 07 Sep 1997; Pandana River (Welbedacht, near Luneburg), SAM A41970 (1 ♂, 1 ♀), CWW = 52 mm (♂), 42 mm (♀), 27°22'40"S, 30°34'30"E, coll. M. Coke on 20 Jan 1995; Nuwejaarspruit tributary (Oliviershoek Pass summit, near waterfall, Drifters Inn), SAM A43944 (1 ♂, 1 ♀), CWW = 39 mm (♂), 40 mm (♀), 28°33'25"S, 29°03'21"E, coll. B.A Stewart, P.A Cook, I. Hoenson and G. Gouws on 21 Jan 1997; Mfongosi (Zululand), SAM A41100 (1 ♂, 1 ♀), CWW = 37 mm (♂), 45 mm (♀), coll. W.C. Jones on May 1918; Mzinto River (Esperanza Bridge, near Umzinto), SAM A43946 (1 ♀), CWW = 40 mm, 30°20'28"S, 30°58'50"E, coll. Coke and Murray on 14 Jan 1998; Mgeni River (causeway near Nagle Dam), SAM A41968 (2 ♂), CWW = 65, 51 mm, 29°39'10"S, 30°41'10"E, coll. M. Coke and J. Craigie on 11 May 1995; Phongolo River (Rivierplaats, Luneburg), SAM A41969 (1 ♂, 1 ♀), CWW = 49 mm (♂), 55 mm (♀), 27°21'10"S, 30°27'13"E, coll. M. Coke on 19 Jan 1995; Greytown pond, SAM A41972 (1 ♂), CWW = 53 mm, 29°04'03"S, 30°35'25"E, coll. M. Coke on 10 Jan 1995; Mlaas River (Maybole, near Baynesfield), SAM A41971 (1 ♂, 1 ♀), CWW = 40 mm (♂), 41 mm (♀), 29°44'20"S, 30°15'22"E, coll. M. Coke on 07 Mar 1995; Tsakwe River (Protest, near Kempslust), SAM A41962 (1 ♂), CWW = 38 mm, 27°27'00"S, 30°31'15"E, coll. M. Coke on 20 Jan 1995; Mbango River (Port Shepstone), SAM A43945 (1 ♀), CWW = 44 mm, 30°45'05"S, 30°26'40"E, coll. M. Coke on 14 Mar 1996; Umgababa River (below dam wall), SAM A43947 (1 ♀), CWW = 59 mm, 30°08'52"S, 30°48'52"E, coll. J. Craigie on 02 Oct 1997; Inzimuke River (Utrecht), SAM A43953 (1 ♂), CWW = 33 mm, 27°37'47"S, 30°21'35"E, coll. Coke and Murray on 26 Jan 1998; Upper Bouthosloop (near Mount Carmel), AM GEN246A (1 ♂, 1 ♀), CWW = 35 mm (♂), 25°24'00"S, 30°43'48"E, coll. unknown on 21 Nov 1959; Nkonzo and Mzimkulu Rivers (Underbush Farm, near Creighton), AM GEN837 (2 ♂), CWW = 39, 50 mm, 29°58'12"S, 29°48'36"E, coll. C. Arter on 04 Oct 1988; Nkonzo River (at Nxumeni River confluence), AM GEN842 (1 ♂, 1 ♀), CWW = 46 mm (♂), 29°58'48"S, 29°51'00"E, coll. C. Arter on 04 Oct 1988; Mgeweni River (at Mgeni River confluence), AM GEN908 (1 ♂), CWW = 47 mm, 29°39'36"S, 30°40'48"E, coll. C. Dickens and M. Coke on 04 Jan 1991.

Diagnosis. *Potamonautes danielsi* exhibits smooth to lightly granulated carapace flanks and epibranchial regions compared to those of *P. sidneyi* s. str. which often display a pronounced scabrosity and heavy granulation. The anterolateral margin is straight and complete. *Potamonautes danielsi* has long slender pereopods and the propodi of the chelipeds are inflated in contrast to the stout pereopods and slim propodi of *P. sidneyi* s. str. *Potamonautes danielsi* has a low terminal segment length:subterminal segment length ratio of gonopod 1 compared to that of *P. sidneyi* s. str.

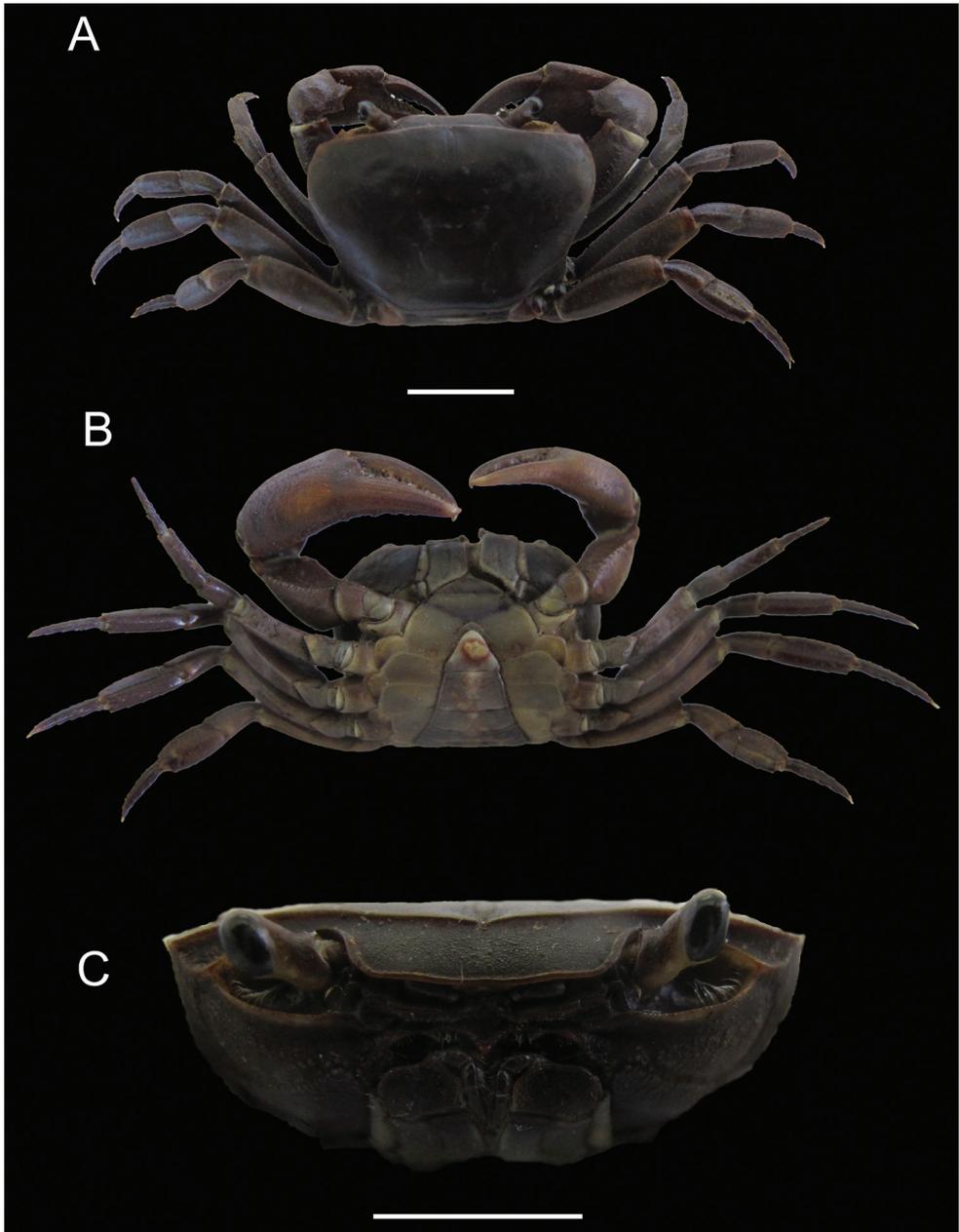


Figure 7. *Potamonautes danielsi* sp. n. male holotype CWW 25.8 mm (SAMC A83487) **A** dorsal view, **B** ventral view, and **C** cephalothorax, frontal aspect. Scale bar: 10 mm. Photos: Nasreen Peer.

Description of holotype. *Carapace* (Figs 7A, C, 9B). Cephalothorax flat (CH/CL = 0.49), wide (CWW/CL = 1.34), almost rectangular in frontal aspect. Branchial region flat forming angle with anterolateral margin. Anterior margin straight, smooth

with occasional faint granulation. Urogastric, cervical and intestinal grooves well-defined; cardiac and branchial grooves well-defined where attached to urogastric and cervical grooves, becoming poorly defined and faint towards edge of carapace. Epigastric lobes poorly defined above postfrontal crest; two slight indentations present, forked from midpoint of postfrontal crest. Postfrontal crest slightly granulated at branchial region, straight and distinct from epibranchial region to midpoint, curving downward at epibranchial region. Exorbital teeth present; no epibranchial teeth present. Flank of carapace scabrous, well-defined epimeral sutures dividing pterygostomial region from subhepatic and suborbital regions, well-defined pleural groove dividing subhepatic region from suborbital region.

Sternites (Fig. 7B). Sternites 1 and 2 fused, first sulcus (s1/s2) absent. Second sulcus (s2/s3) prominent, running completely across sternum, third sulcus (s3/s4) projecting downwards medially to abdominopelvic region. Sulci and episternal sulci thereafter well-defined but shallow.

Third maxillipeds (Fig. 7C, 8E). Filling entire buccal frame except for oval respiratory openings medially above maxilliped. Ischium scabrous, with wide groove running vertically. Flagellum on exopod very long, curved to form a loop.

Mandibular palp (Fig. 8C, D). Consisting of two segments; terminal segment smooth and undivided, with hirsute margins; dense tuft of setae emerging from base. Subterminal segment enlarged distally then compressed at joint with terminal segment.

Pereopods (Figs 7A, B, 8A, B). No substantial heterochely (CRDL/CLDL = 1.04). Dactyl of major cheliped slightly arched; small slim interspace formed when closed in major and minor chelipeds. Twenty-five cutting teeth present on dactyl of major cheliped; 4 larger and more prominent than the rest. Propodus inflated (CRPW/CRPL = 0.46), exhibiting 20 cutting teeth. Carpus on either side containing one prominent tooth followed by one small tooth. Meri strongly granulated at margins. Pereopods 2 (ML/MW = 2.59) and 5 (ML/MW = 2.66) moderately slender; pereopods 3 and 4 equal in length and longest among pereopods; pereopod 5 shortest. Ventral margins of meri smooth; ventral margins of propodi serrated; dorsal margins of meri and propodi bearing fine bristles; dactyli serrated, ending in sharp points.

Pleon (Figs 6B, 7B). Somites 1-6 four-sided with distally-rounded triangular terminal somite (telson). First 5 somites broad and short; somite 6 longer, about 1.6 times as wide as long, distal margins straight or slightly concave, lateral margins slanted towards medial line, lateral margins swell slightly at articulation with somite 5; telson terminally rounded, lateral margins concave, swell at articulation with somite 6; hirsute lateral margins

Pleopods (Fig. 8F, G, H, I). Gonopod 1 widest at base; both subterminal and terminal segments tapering, ending with sharp point. Medial margin fairly straight; lateral margin concave relative to midline; both margins hirsute. Groove extending to almost entire length of gonopod, visible on dorsal surface, lined with setae. Gonopod 1 terminal segment short (0.21 times length of subterminal segment), curving away from midline when viewed dorsally. Gonopod 2 consisting of two segments. Distal segment very long (0.67 times length of basal segment), slim; basal segment with wide

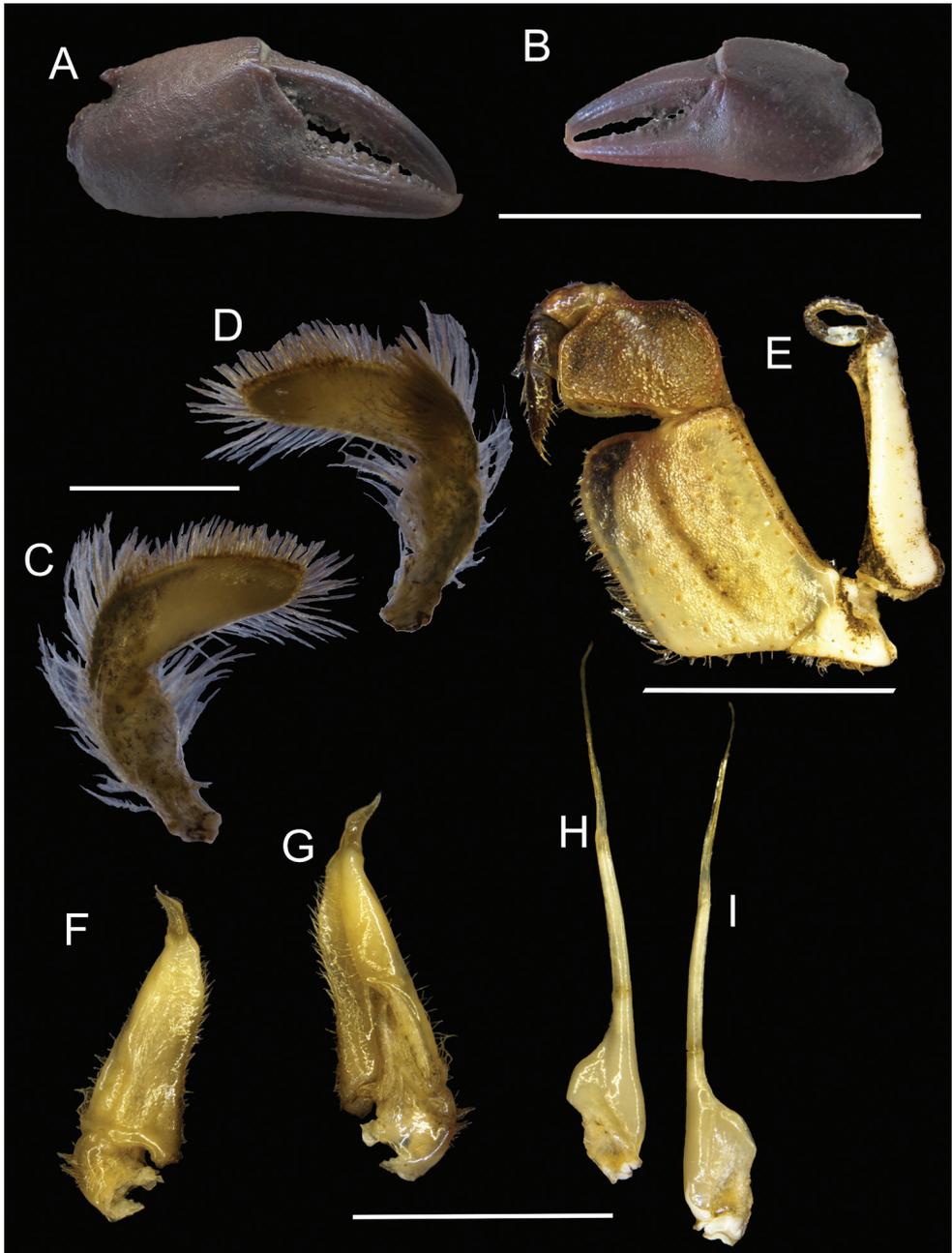


Figure 8. *Potamonautes danielsi* sp. n. male holotype CWW 25.8 mm (SAMC A83487). **A** major cheliped **B** minor cheliped **C** right mandibular palp posterior view **D** right mandibular palp anterior view **E** 3rd maxilliped **F** left gonopod 1 anterior view **G** left gonopod 1 posterior view **H** left gonopod 2 anterior view, and **I** left gonopod 2 posterior view. Scale bars: 10 mm (**A**, **B**), 2 mm (**C**, **D**), 5 mm (**E**), 5 mm (**F**, **G**, **H**, **I**). Photos: Nasreen Peer.

elongated base sharply becoming narrow around 0.4 of length; narrow process forming at this point leading to distal segment. Gonopod 2 fairly straight, barely curving outward when viewed ventrally; curving slightly inward towards medial line at tips of distal segment.

Variation. The species appears to be extremely variable, with the northernmost Mhlanga population more closely resembling *P. sidneyi* s. str. rather than the *P. danielsi* sp. n. holotype. The epibranchial corners of the Mhlanga type are scabrous and granulated. Granulation, however, is not as pronounced as in *P. sidneyi* s. str. and no fine hairs are observed on the carapace. The terminal segments of both gonopods in the Mhlanga type are also more curved (typical of *P. sidneyi* s. str.), as opposed to straight (typical of *P. danielsi*). The flagellum on the exopod of the third maxilliped is highly variable. In the Mtamvuna population, the flagellum is long and curves backward to form a loop in some specimens. In both the Oribi and Mhlanga populations this is not seen. Instead, the flagellum curves upwards, similar to the pattern observed in most other potamonautid species.

Live colouration. Variable. Carapace colour ranges from purple to reddish-brown to greenish-brown. Carapace and pereopods are fairly uniform in colour with tips of dactyli and chelipeds usually displaying a lighter orange colour.

Distribution. Currently known to extend from Mhlanga (Durban North) to the Mtamvuna River on the northern border of Pondoland (southern KZN), based on recent sampling and the results published in Gouws et al. (2015). Morphological examination of museum specimens shows that this species is also present in the Mpumalanga Province.

Holotype locality. South Africa, KwaZulu-Natal: Mtamvuna Nature Reserve (31°00'23"S, 30°09'12"E).

Etymology. The species is named after Professor Savel Daniels in recognition of his valuable contribution to knowledge of freshwater crabs in southern Africa.

Remarks. *Potamonautes danielsi* sp. n. is easily distinguished from most other South African *Potamonautes* species. *Potamonautes dentatus* Stewart, Coke & Cook, 1995, *P. parvispina* Stewart, 1997, *P. unispinus* Stewart & Cook, 1998, *P. warreni* Calman, 1918 and *P. calcaratus* (Gordon, 1929) all bear dentate anterolateral margins or epibranchial corners (cf. Stewart et al. 1995: figs 1, 2J; cf. Stewart 1997: figs 2A, 8, 9A; cf. Stewart and Cook 1998: figs 2A, 2D, 6, 7A; cf. Gordon 1929: fig. 1), while *P. danielsi* has an angular epibranchial corner and slightly scabrous anterolateral margin (Figs 7, 9B).

Potamonautes clarus Gouws, Stewart & Coke, 2000, *P. depressus* (Krauss, 1843), *P. tuerkayi* Wood & Daniels, 2016, *P. brincki* (Bott, 1960), *P. flavusjo* Daniels, Phiri & Bayliss, 2014, *P. isimangaliso* Peer & Gouws, 2015 and *P. lividus* Gouws, Stewart & Reavell, 2001 all have smooth anterolateral margins and smooth, rounded epibranchial corners (cf. Gouws et al. 2000: figs 5G, 6, 7A; cf. Krauss 1843: table II fig. 4; cf. Bott 1960: figs 1–4; cf. Daniels et al. 2014: figs 6, 7; cf. Peer et al. 2015: fig. 4; cf. Gouws et al. 2001: figs 5A, 6, 7A), while *P. danielsi* sp. n. has an angular epibranchial corner and a slightly scabrous anterolateral margin (Figs 7, 9B). Additionally, *P. danielsi*



Figure 9. Morphological comparison of carapace features between **A** *P. perlatus* and **B** *P. danielsi* sp. n.

has a more vaulted carapace (CL/CH = 1.9-2.3) compared to *P. depressus* (CL/CH = 1.6-1.8), but a flatter carapace compared to *P. isimangaliso* (CL/CH = 2.3-2.6). *Potamonautes brincki* is confined to the Western Cape. *Potamonautes clarus* and *P. tuerkayi* are typically bright orange with highly arched dactyls creating a large interspace when chelipeds are closed (cf. Gouws et al. 2000: figs 6A, B, 7C). Conversely, *P. danielsi* varies in colour from brown to purple but no orange specimens have been collected, while dactyls are moderately arched forming a slim interspace when closed (Figs 7A, 8A, B). *Potamonautes flavusjo* and *P. lividus* are distinctly coloured (yellow pereopods, and orange pereopods with a blue carapace respectively), with *P. flavusjo* occurring in the Mpumalanga Highveld and *P. lividus* mainly in northern KwaZulu-Natal and the Eastern Cape (Gouws et al. 2001, 2015, Daniels et al. 2014), but also in Swaziland (Daniels and Bayliss 2012). *Potamonautes parvicorpus* Daniels, Stewart & Burmeister, 2001 also has rounded epibranchial corners, although it bears a slightly granulated anterolateral margin (cf. Daniels et al. 2001: figs 10, 11A). It also differs in location occurring only in the Western Cape.

Potamonautes danielsi shares outward similarities with *P. perlatus* (H. Milne Edwards, 1837), *P. granularis* Daniels, Stewart & Gibbons, 1998, *P. sidneyi* Rathbun, 1904, *P. barbarai* Phiri & Daniels, 2014, and *P. barnardi* Phiri & Daniels, 2014. All the above-mentioned species display an angular epibranchial corner with granulation or scabrosity and prominent postfrontal crests (fig. 4A; cf. Daniels et al. 1998: figs 2A, 10, 11A; cf. Rathbun 1904: plate XIV fig. 5; cf. Daniels et al. 1998: fig. 2F). Additionally, these five species are typically widespread, large, robust species occurring from the middle to lower reaches of rivers. *Potamonautes granularis* differs from *P. danielsi* in that it consistently exhibits orange-tipped chelipeds, the branchial region is highly convex and the anterior margin curves heavily inwards at the midpoint (cf. Daniels et

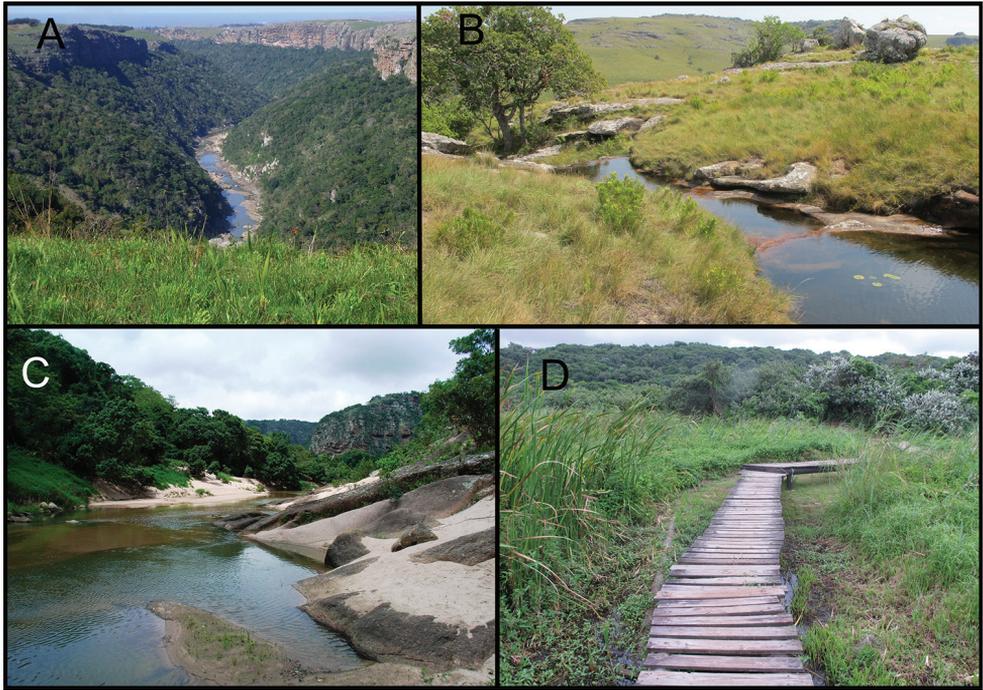


Figure 10. Habitat types of *P. danielsi* sp. n. Various habitat types of *P. danielsi* sp. n. **A** and **B** Mtamvuna Nature Reserve showing the dense canopy cover under which specimens were collected as well as open streams and pools **C** Oribi Gorge Nature Reserve, and **D** Umhlanga Nature Reserve. Photos: A, B, C-Lynette Clennell; D-Nasreen Peer.

al. 1998: figs 2A, 10, 11A), while *P. danielsi* does not always have orange-tipped chelipeds, has a flatter branchial region and a fairly straight anterior margin (Fig. 7A). *Potamonautes perlatus*, *P. barbarai*, and *P. barnardi* are morphologically indistinct (Phiri and Daniels 2014) and all differ from *P. danielsi* slightly (Fig. 9). The main difference lies in the anterior margin, which is similar in *P. sidneyi* s. str., *P. perlatus*, *P. barbarai* and *P. barnardi*. When viewed dorsally, the anterior margin of *P. danielsi* sp. n. lies relatively straight with a slight forward projection medially (Fig. 9B). The anterior margins of *P. perlatus*, *P. barbarai* and *P. barnardi* all contain a concavity in the crest behind each orbit so that a wide “W” is formed (Fig. 9A). The orbits of *P. danielsi* are deeper set than the orbits of *P. perlatus*, *P. barbarai* and *P. barnardi*. Additionally, the epibranchial corner of the former is more angular while those of the latter species group are slightly more rounded. However, even here variation across populations makes it difficult to differentiate between these species based solely on morphology.

Potamonautes danielsi and *P. sidneyi* s. str. are difficult to distinguish based on morphology alone, as key characters often overlap. The type specimens of the two species exhibit marked differences i.e.: 1) *P. danielsi* sp. n. has a smoother or slightly granulated anterolateral margin with a smoother or scabrous epibranchial region (fig. 7A), while *P. sidneyi* s. str. typically has a heavily granulated anterolateral margin with a

highly scabrous branchial region (plate XIV fig 5-Rathbun, 1904; fig. 4A) and even bears fine hairs on the carapace in some populations; 2) the propodi of *P. danielsi* sp. n. are inflated (Fig. 8A, B) while those of *P. sidneyi* s. str. are slender (Fig. 5A, B); 3) *P. danielsi* sp. n. has a high terminal segment length: subterminal segment length of gonopod 2 (Fig. 8H, I) and a low terminal segment length: subterminal segment length of gonopod 1 (Fig. 8F, G), while *P. sidneyi* s. str. has a lower terminal segment length: subterminal segment length of gonopod 2 (Fig. 5H, I) and a higher terminal segment length: subterminal segment length of gonopod 1 (Fig. 5F, G); 4) *P. danielsi* sp. n. bears slim pereopods (Figs 7A, B) as opposed to the stout limbs of *P. sidneyi* s. str. (Figs 4A, B). However, across the known range of distribution these individual characters vary significantly, with one species resembling the other on occasions. Their separation, thus, requires the inclusion of the whole suite of key characters including the shape and width of the carapace, the inflation of the propodi, the slenderness of the pereopods, the terminal segment length:subterminal segment length ratio of gonopod 1, and the shape of gonopod 1. The granulation of the carapace alone is not a reliable distinguishing character to tell these two species apart. Following morphometric analyses, the new species was distinguished from *P. sidneyi* s. str. mainly by the carapace variables CWA, CL and CH, which contributed the most to distinguishing between the two forms in the discriminant analysis (Fig. 2). The classification functions for both species were as follows:

$$Y(P. danielsi \text{ sp. n.}) = 620.17(\text{LogCWA}) - 1349.21(\text{LogCL}) - 362.50(\text{LogCH}) - 382.53$$
$$Y(P. sidneyi \text{ s. str.}) = 765.23(\text{LogCWA}) - 1491.17(\text{LogCL}) - 305.94(\text{LogCH}) - 420.01$$

Individuals were then reassigned to groups based on a priori probabilities, using these classification functions. Ninety-one percent (91%) of the *P. danielsi* sp. n. individuals and 95% of the *P. sidneyi* s. str. individuals were correctly classified, with only three and one individuals being reassigned to the other species, respectively. The following three regressions were used to support the distinction between the two species: A. P2ML/P2MW, B. CRPW/CRPL, and C. CWA/CL (Fig. 3a, b, c). Regression analyses showed that the two species are significantly distinct using these morphological regressions (P2ML/P2MW-SS = 0.62, df = 1, F = 581, p < 0.001; CRPW/CRPL-SS = 1.11, df = 1, F = 939, p < 0.001; CWA/CL-SS = 0.59, df = 1, F = 2923, p < 0.001).

Discussion

In a previous study (Gouws et al. 2015), a 9.2–11.8 % divergence was found in the mitochondrial COI and 16S genes of the *P. sidneyi* clade. Based on the genetic delineation of the two lineages, now *P. danielsi* sp. n. and *P. sidneyi* s. str., in Gouws et al. (2015), the distribution of *P. danielsi* sp. n. is currently known to encompass the coastal zone of southern KwaZulu-Natal, i.e., the northern Pondoland region. However, genetic analyses of recently collected specimens from a wider range of localities (Gouws, unpublished data) suggest a larger distribution of the species. It is likely that it also occurs

in Swaziland, but this is yet to be confirmed. With more extensive inland sampling it is possible that the discrete distributions of these species suggested in the earlier study (Gouws et al. 2015) may not be consistent. Furthermore, the huge morphological variation and overlap in key characters obscure purely visual differentiation between these two species in the field. A whole suite of key characters, and possibly even molecular analyses, might be necessary to tell them apart with reasonable confidence.

Phylogenetically, within the southern African potamonautid fauna, *P. sidneyi* s. str. belongs to the clade of large-bodied, robust freshwater crabs, including *P. perlatus*, *P. granularis*, *P. barbarai* and *P. barnardi* (Daniels et al. 2002, 2015), that are mostly confined to the middle to lower reaches of rivers (Daniels et al. 2002). The derivation of these species from a common ancestor explains their overall morphological similarities. *Potamonautes danielsi*, however, appears to belong to a clade that includes burrowing species such as *P. isimangaliso*, *P. lividus* and *P. flavusjo* (Gouws et al. 2015), all three of which are easily distinguishable from *P. danielsi*. While the phylogenetic placement of these taxa requires more rigorous testing, it would appear that the morphological similarity between *P. danielsi* sp. n. and *P. sidneyi* s. str. is not phylogenetically determined and may well reflect habitat similarities and environmental drivers, given their current distributions. This may support the lack of distinctive species-specific characters that appears to be a widespread trend for African potamonautids (Daniels et al. 2003, Jesse et al. 2010, Cumberlidge and Daniels 2014, Phiri and Daniels 2014, Phiri and Daniels 2016).

South Africa is fairly well-studied regarding the taxonomy of freshwater crab fauna (Daniels et al. 2014, Phiri and Daniels 2016) and yet ongoing molecular work still yields novel undescribed species of *Potamonautes*, many of which are cryptic and thus easily mistaken for previously recorded species in the past (Phiri and Daniels 2014). The large number of freshwater habitat types present through South Africa, ranging from the headwaters of the Drakensberg region to coastal freshwater seepage points, is associated with the high diversity and endemism of freshwater brachyurans found in the country (Darwall et al. 2009). Some species appear to be quite adaptable, inhabiting a variety of habitat types, such as *P. perlatus* which extends from the inland region of the Northern Cape Province to freshwater seepage barrage pools along the Eastern Cape coast (Perissinotto et al. 2014), withstanding a wide range of altitude, salinity and temperature throughout its distribution. Conversely, certain species occupy very specific niches, such as *P. isimangaliso* which is currently only known from ephemeral pans along the western shore of False Bay, iSimangaliso Wetland Park (Peer et al. 2015a).

Potamonautes danielsi sp. n. does not appear to be habitat-specific but seems to prefer purely freshwater habitats established in areas with summer rainfall (Fig. 10). In Mtamvuna and Oribi Gorge, specimens were found under boulders and logs in mountain streams (altitude = 140-150 m) flowing into or connected to the main rivers. The Umhlanga Reserve consists mostly of KwaZulu-Natal coastal belt (CB3 vegetation unit-Mucina and Rutherford 2006) with a small portion of Northern Coastal forest (FOz7 vegetation unit). While the latter is classified as least threatened, its location next to an endangered CB3 habitat type within a growing urban area means that the surrounding habitat is already heavily transformed. The crab populations in this reserve were found close to sea level in the coastal belt habitat, burrowing under

dominant grasses and shrubs near a freshwater seepage area which is connected to the Mhlanga River Mouth. Both the Mtamvuna and Oribi Gorge reserves consist of Scarp Forest (FOz 5), which is well-known for housing many endemic tree species and forms a core component of the Pondoland Centre of Endemism (van Wyk and Smith 2001). These forests exhibit tall and well-developed canopy and understorey tree layers that provide moist damp areas during the rainfall season (spring-summer), with adequate shade and shelter for the crab populations dwelling in the streams.

The species is sympatric with *Chiromantes eulimene* (de Man in Weber, 1898) in all three habitats, although the latter prefers the reed-like habitat adjacent to the main river, while *P. danielsi* appears to prefer the slower-flowing streams running into the main river body.

A greater number of *P. danielsi* adults were found under boulders and detritus in the water, as opposed to in burrows, although no feeding behaviour was observed at the time of collection. Generally, the feeding ecology of all *Potamonautes* spp. is supposedly opportunistic and thought to shift with age. Gut content analyses and stable isotope analyses have been conducted on *P. perlatus* (see Hill and O’Keeffe 1992) and *P. sidneyi* (see Peer et al. 2015b), respectively. While adults of these two species are mainly herbivorous and detritivorous, juveniles appear to favour a carnivorous diet. This could possibly relate to the ontogenetic shift in habitats, where juveniles occupy the water body, while adults reside in burrows on the banks of streams or rivers. Juveniles encounter more potential prey items on the benthos than adults do near their burrows. Thus, the presence of *P. danielsi* adults in the water body means that a wider range of prey is available to them for consumption. However, the overall ecology of the southern African potamonautids remains highly understudied. As new species are described, interspecific ecological differences are becoming apparent highlighting the need for more ecological research in this field.

Currently, 20 species of *Potamonautes* have been described in South Africa with six additional new but undescribed species (Phiri and Daniels 2016). Daniels et al. (2014) highlighted the relatively poor exploration of high altitude mountainous freshwater habitats and predicted that future collections from these understudied areas will yield new undescribed species. This sheds new light on inland freshwater habitats, in terms of conservation, as highly endemic or specialist species are often a priority in earmarking areas for protection. As recent national biodiversity assessments regarding South Africa’s inland water systems have highlighted the threat faced by these largely unprotected systems (Nel et al. 2011), exploring and documenting their rich biodiversity and specialised ecology should be prioritised.

Acknowledgements

We are grateful to Professor D Guinot, Dr P Martin-Lefevre and Ms N Mollaret of the Muséum National d’Histoire Naturelle (Paris, France) who were all instrumental in helping us to locate the original descriptions of Rathbun as well as providing photographs

and measurement of specimen MNHN B3841. We would like to thank Mr A Bosman and Dr W Florence of the Iziko South African Museum (Cape Town, South Africa); Ms A Ndaba of the Ditsong National Museum of Natural History (Pretoria, South Africa); and Dr H James and Mr M Mlambo of the Albany Museum (Grahamstown, South Africa) who were all exceptional in providing smooth access to museum specimens. We also extend our gratitude to Mr W Coetzer from the South African Institute of Aquatic Biodiversity (Grahamstown, South Africa) for the editing of the map. This work is based on the research supported by the South African Research Chairs Initiative of the Department of Science and Technology (DST) and National Research Foundation (NRF) of South Africa. Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard.

References

- Barnard KH (1935) Scientific results of the Vernay-Lang Kalahari expedition, March to September, 1930: Crustacea. *Annals of the Transvaal Museum* 16: 481–492.
- Barnard KH (1950) Descriptive catalogue of South African decapod Crustacea (crabs and shrimps). *Annals of the South African Museum* 38: 1–837.
- Bott R (1955) Die Süßwasserkrabben von Afrika (Crust., Decap.) und ihre Stammesgeschichte. *Annalen van het Museum van Belgisch Congo, Dierkunde* 1: 209–352.
- Bott R (1960) Crustacea (Decapoda): Potamonidae. In Hanström B, Brinck P, Rudebeck G (Eds) *South African animal life: Results of the Lund University expedition in 1950–1951*. Almqvist & Wiksells, Uppsala, 13–18.
- Bott R (1970) Betrachtungen über die Entwicklungsgeschichte der Süßwasserkrabben nach der Sammlung des Naturhistorischen Museums in Genf/Schweiz. *Revue Suisse Zoologie* 77: 327–344. [Pl. 1, 2]
- Calman WT (1918) A new river crab from the Transvaal. *Annals and Magazine of Natural History* 9: 234–236. <https://doi.org/10.1080/00222931808562306>
- Chace FA (1942) Scientific results of a fourth expedition to forested areas in eastern Africa III: Decapod Crustacea. *Bulletin of the Museum of Comparative Zoology, Harvard College* 91: 185–233.
- Chace FA (1953) Zoological results of a fifth expedition to East Africa VI. Decapod Crustacea. *Bulletin of the Museum of Comparative Zoology, Harvard College, Harvard* 110: 427–443.
- Cumberlidge N (1998) The African and Madagascan freshwater crabs in the Zoologische Staatssammlung, Munich (Crustacea, Decapoda, Brachyura, Potamoidea). *Spixiana* 21: 193–214.
- Cumberlidge N, Daniels SR (2014) Recognition of two new species of freshwater crabs from the Seychelles based on molecular evidence (Potamoidea: Potamonautidae). *Invertebrate Systematics* 28: 17–31. <https://doi.org/10.1071/IS13017>
- Daniels SR, Bayliss J (2012) Neglected refugia of biodiversity: mountainous regions in Mozambique and Malawi yield two novel freshwater crab species (Potamonautidae: *Potamonautes*). *Zoological Journal of the Linnean Society* 164: 498–509. <https://doi.org/10.1111/j.1096-3642.2011.00773.x>

- Daniels SR, Phiri EE, Klaus S, Albrecht C, Cumberlidge N (2015) Multilocus phylogeny of the Afrotropical freshwater crab fauna reveals historical drainage connectivity and transoceanic dispersal since the Eocene. *Systematic Biology* 64: 549–567. <https://doi.org/10.1093/sysbio/syv011>
- Daniels SR, Phiri EE, Bayliss J (2014) Renewed sampling of inland aquatic habitats in southern Africa yields two novel freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*). *Zoological Journal of the Linnean Society* 171: 356–369. <https://doi.org/10.1111/zoj.12139>
- Daniels SR, Gouws G, Stewart BA, Coke M (2003) Molecular and morphometric data demonstrate the presence of cryptic lineages among freshwater crabs (Decapoda: Potamonautidae: *Potamonautes*) from the Drakensberg Mountains, South Africa. *Biological Journal of the Linnean Society* 78: 129–147. <https://doi.org/10.1046/j.1095-8312.2003.00143.x>
- Daniels SR, Stewart BA, Gouws G, Cunningham M, Matthee CA (2002) Phylogenetic relationships of the southern African freshwater crab fauna (Decapoda: Potamonautidae: *Potamonautes*) derived from multiple data sets reveal biogeographic patterning. *Molecular Phylogenetics and Evolution* 21: 511–523. [https://doi.org/10.1016/S1055-7903\(02\)00281-6](https://doi.org/10.1016/S1055-7903(02)00281-6)
- Daniels SR, Stewart BA, Burmeister L (2001) Geographic patterns of genetic and morphological divergence amongst populations of a river crab (Decapoda, Potamonautidae) with the description of a new species from mountain streams in the Western Cape, South Africa. *Zoologica Scripta* 30: 181–197. <https://doi.org/10.1046/j.1463-6409.2001.00061.x>
- Daniels SR, Stewart BA, Gibbons MJ (1998) *Potamonautes granularis* sp. n. (Brachyura, Potamonautidae), a new cryptic species of river crab from the Olifants River System, South Africa. *Crustaceana* 71: 885–903. <https://doi.org/10.1163/156854098X00905>
- Darwall WRT, Smith KG, Tweddle D, Skelton P (2009) The status and distribution of freshwater biodiversity in southern Africa. Gland, Switzerland: IUCN and Grahamstown, South Africa: South African Institute of Aquatic Biodiversity. <https://doi.org/10.1080/00222932908672988>
- Gordon I (1929) A new river-crab of the subgenus *Potamonautes* from Portuguese East Africa. *Annals and Magazine of Natural History* 3: 405–411.
- Gouws G, Stewart BA (2001) Potamonautid river crabs (Decapoda, Brachyura, Potamonautidae) of KwaZulu-Natal, South Africa. *Water SA* 27: 85–98.
- Gouws G, Peer N, Perissinotto R (2015) MtDNA lineage diversity of a potamonautid freshwater crab in KwaZulu-Natal, South Africa. *Koedoe* 57: 1–12. <https://doi.org/10.4102/koedoe.v57i1.1324>
- Gouws G, Daniels SR, Stewart BA (2002) Allozyme electrophoresis demonstrates the presence of a species boundary in freshwater crabs (Decapoda: Potamonautidae). *Journal of Natural History* 36: 1199–1222. <https://doi.org/10.1080/00222930110045425>
- Gouws G, Stewart BA, Reavell PE (2001) A new species of freshwater crab (Decapoda, Potamonautidae) from the swamp forests of KwaZulu-Natal, South Africa: biochemical and morphological evidence. *Crustaceana* 74: 137–160. <https://doi.org/10.1163/156854001750096256>
- Gouws G, Stewart BA, Coke M (2000) Evidence for a new species of river crab (Decapoda, Brachyura, Potamonautidae) from the Drakensberg, South Africa. *Journal of Crustacean Biology* 20: 743–758. <https://doi.org/10.1163/20021975-99990096>

- Heller C (1865) Crustaceen. In: Reise der österreichischen Fregatte Novara um die Erde in den Jahren 1857, 1858, 1859 unter den Befehlen des Commodore B. von Wüllerstorff-Urbair. Zoologischer Theil. Kaiserliche Akademie der Wissenschaften, Vienna, 280 pp.
- Hill MP, O’Keeffe JH (1992) Some aspects of the ecology of the freshwater crab (*Potamonautes perlatus* Milne Edwards) in the upper reaches of the Buffalo River, Eastern Cape Province, South Africa. South African Journal of Aquatic Science 18: 42–50. <https://doi.org/10.1080/10183469.1992.9631323>
- Jesse R, Schubart CD, Klaus S (2010) Identification of a cryptic lineage within *Potamon fluviatile* (Herbst) (Crustacea: Brachyura: Potamidae). Invertebrate Systematics 24: 348–356. <https://doi.org/10.1071/IS10014>
- Kingsley JS (1880) Carcinological Notes No. 1. In: Nolan EJ (Ed.) Proceedings of the Academy of Natural Sciences in Philadelphia. Academy of Natural Sciences, Philadelphia, 34–37.
- Krauss F (1843) Südafrikanischen Crustaceen. Eine Zusammenstellung aller bekannten Malacostraca, Bemerkungen über deren Lebensweise und geographische Verbreitung, nebst beschreibung und Abbildung mehrerer neuen arten. E. Schweizerbart’sche Verlagsbuchhandlung, Stuttgart, Germany.
- Lenz H (1912) Afrikanische Crustaceen aus schwedischen Sammlungen. Arkiv för Zoologi 7: 1–10.
- Linnaeus C (1758) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis locis (10th edn). Impensis Direct. Laurentii Salvii, Stockholm, 824 pp.
- MacLay WS (1838) Brachyurous Decapod Crustacea, Illustrations of the zoology of South Africa 5; being a portion of the objects of natural history chiefly collected during an expedition into the interior of South Africa, under the direction of Dr. Andrew Smith, in the years 1834, 1835, and 1836; fitted out by “The Cape of Good Hope Association for Exploring Central Africa.” In: Smith A (Ed.) Illustrations of the Zoology of South Africa; Consisting Chiefly of Figures and Descriptions of the Objects of Natural History Collected During an Expedition into the Interior of South Africa, in the Years 1834, 1835, and 1836; Fitted Out by “The Cape of Good Hope Association for Exploring Central Africa” 5. Invertebrata 3: 53–71.
- Milne-Edwards H (1837) Histoire naturelle des Crustacés, comprenant l’anatomie, la physiologie et la classification des ces animaux (2nd edn). Librairie Encyclopédique de Roret, Paris.
- Milne-Edwards A (1869) Revision du genre *Thelphuse* et description de quelques espèces nouvelles faisant partie de la collection du Muséum. Nouvelles Archives du Muséum d’Histoire naturelle, Paris 5, 161–191. <https://doi.org/10.5962/bhl.title.10050>
- Mucina L, Rutherford MC (2006) The Vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19. Pretoria, South Africa: South African National Biodiversity Institute: Pretoria, South Africa, 807 pp.
- Nel JL, Driver A, Swartz E (2011) National Biodiversity Assessment 2011: Freshwater component. CSIR Natural Resources and the Environment, Stellenbosch.
- Ortmann AE (1896) Das System der Decapoden-Krebse. Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie de Thiere 9: 409–453.

- Peer N, Perissinotto R, Gouws G, Miranda NAF (2015a) Description of a new species of *Potamonautes* Macleay, 1838, from the iSimangaliso Wetland Park, South Africa. *ZooKeys* 503: 23–43. <https://doi.org/10.3897/zookeys.503.9532>
- Peer N, Perissinotto R, Miranda NAF, Raw JL (2015b) A stable isotopic study of the diet of *Potamonautes sidneyi* (Brachyura: Potamonautidae) in two coastal lakes of the iSimangaliso Wetland Park, South Africa. *Water SA* 41: 549–558. <https://doi.org/10.4314/wsa.v41i4.15>
- Perissinotto R, Bornman T, Steyn P-P, Miranda NAF, Dorrington RA, Matcher GF, Strydom N, Peer N (2014) Tufa stromatolite ecosystems on the South African south coast. *South African Journal of Science* 110: 1–8. <https://doi.org/10.1590/sajs.2014/20140011>
- Phiri EE, Daniels SR (2014) Disentangling the divergence and cladogenesis in the freshwater crab species (Potamonautidae: *Potamonautes perlatus sensu lato*) in the Cape Fold Mountains, South Africa, with the description of two novel cryptic lineages. *Zoological Journal of the Linnean Society* 170: 310–332. <https://doi.org/10.1111/zoj.12103>
- Phiri EE, Daniels SR (2016) Multilocus coalescent species delimitation reveals widespread cryptic differentiation among Drakensberg mountain-living freshwater crabs (Decapoda: *Potamonautes*). *Invertebrate Systematics* 30: 60–74. <https://doi.org/10.1071/IS15035>
- Rathbun MJ (1904) Les crabes d'eau douce (Potamonidae). *Nouvelles Archives du Muséum d'Histoire Naturelle* 6: 163–166.
- Rathbun MJ (1905) Les crabes d'eau douce (Potamonidae). *Nouvelles Archives du Muséum d'Histoire Naturelle* 7: 159–322.
- Stebbing TRR (1910) General catalogue of South African Crustacea. *Annals of the South African Museum* 6: 281–593.
- Stewart BA (1997) Biochemical and morphological evidence for a new species of river crab *Potamonautes parvispina* sp. n. (Brachyura, Potamonautidae). *Crustaceana* 70: 737–753. <https://doi.org/10.1163/156854097X00168>
- Stewart BA, Cook PA (1998) Identification of a new species of river crab (Decapoda: Brachyura: Potamonautidae) from South Africa using morphological and genetic data. *Journal of Crustacean Biology* 18: 556–571. <https://doi.org/10.1163/193724098X00386>
- Stewart BA, Coke M, Cook PA (1995) *Potamonautes dentatus*, new species, a fresh-water crab (Brachyura: Potamoidea: Potamonautidae) from KwaZulu-Natal, South Africa. *Journal of Crustacean Biology* 15: 558–568. <https://doi.org/10.2307/1548776>
- van Wyk AE, Smith GF (2001) Regions of floristic endemisms in southern Africa. A review with emphasis on succulents. Umdaus Press, Pretoria.
- Weber M (1898) 3. Die decapoden Crustaceen des Süßwassers von Süd Afrika (Mit Beiträgen von Dr JCH de Meijere und Dr JG de Man). In Weber M (1898) *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere*. V.G Fischer, Jena, 156–180.
- Wood LE, Daniels SR (2016) Genetic and morphological evidence for a new mountain-living freshwater crab species (Decapoda : Potamonautidae : *Potamonautes*) from the Western Cape province of South Africa. *Invertebrate Systematics* 30: 219–230. <http://dx.doi.org/10.1071/IS15051>

Designation of a neotype and redescription of *Hesione reticulata* von Marenzeller, 1879 from Japan (Annelida, Hesionidae)

Naoto Jimi¹, Sergio I. Salazar-Vallejo², Hiroshi Kajihara¹

1 Department of Natural History Sciences, Graduate School of Science, Hokkaido University, N10 W8, Sapporo 0060-0810, Japan **2** El Colegio de la Frontera Sur, Depto. Sistemática y Ecología Acuática, Chetumal, México

Corresponding author: Sergio I. Salazar-Vallejo (savs551216@hotmail.com; ssalazar@ecosur.mx)

Academic editor: Chris Glasby | Received 4 November 2016 | Accepted 2 February 2017 | Published 17 February 2017

<http://zoobank.org/26EFA2AD-8E78-47BF-B9D5-7F8A79672ECF>

Citation: Jimi N, Salazar-Vallejo SI, Kajihara H (2017) Designation of a neotype and redescription of *Hesione reticulata* von Marenzeller, 1879 from Japan (Annelida, Hesionidae). ZooKeys 657: 29–41. <https://doi.org/10.3897/zookeys.657.11064>

Abstract

The hesionid polychaete *Hesione reticulata* von Marenzeller, 1879 was described from Enoshima Island, Japan and has been recorded also from the Red Sea. Depending on researchers, it has been regarded as either a distinct species or synonymous with older established ones. The type specimen has been lost. In order to clarify its taxonomic status, *H. reticulata* is herein redescribed, illustrated, and a neotype is proposed based on recent material collected near the type locality. The diagnostic features include the presence of several dorsal, discontinuous longitudinal bands, interrupted by pale segmental spots; prostomium with tiny antennae; a tuberculated dorsal integument; acicular lobes double; and neurochaetal blades with guards approaching the distal tooth. The dorsal color pattern in life enables a clear distinction from similar species such as *H. intertexta* Grube, 1878 amongst others. Mitochondrial COI barcoding sequences are deposited in the DNA Data Bank of Japan. A key to *Hesione* species from Japan is also included.

Keywords

Neurochaetal blades, parapodial features, pigmentation pattern, polychaetes, taxonomy

Introduction

The hesionid polychaete *Hesione reticulata* von Marenzeller, 1879 is in need of re-description, in particular incorporating observations of the living pigmentation. This is because delimitation of the species from similar forms, such as *H. splendida* Savigny in Lamarck, 1818, *H. pantherina* Risso, 1826, and *H. intertexta* Grube, 1878, involves the color pattern on the dorsal surface, which unfortunately disappears immediately after fixation. Neither the original description (von Marenzeller 1879) nor subsequent re-descriptions (Izuka 1912; Imajima and Hartman 1964; Imajima 1997) furnished any illustration that depicts the dorsal color pattern with sufficient quality. Text descriptions on the color pattern, such as “white transverse spots merging to reticulations” (Imajima and Hartman 1964), can refer to a variety of different states, and thus were insufficient to delineate the species and to separate similar forms. Although some color images have been published (Uchida 1992, 2000), their resolution is insufficient for reliable identification.

Further, the morphological features of the currently valid *Hesione* species (after Read and Bellan 2016) have not been standardized nor revised. Dorsal pigmentation can be roughly separated into three patterns: 1) bright grayish in *H. splendida* Savigny in Lamarck, 1818, 2) transverse bands as in *H. genetta* Grube, 1867 and *H. picta* Müller, 1858, but in the former there are also round spots along body, and 3) longitudinal lines such as in *H. intertexta* Grube, 1878, *H. pantherina* Risso, 1826, *H. reticulata* von Marenzeller, 1879, and *H. steenstrupi* de Quatrefages, 1866. For *H. eugeniae* Kinberg, 1866, no pigmentation was given in the description. On the other hand, there are three conditions for the size of neurochaetal guards in comparison with blade’s teeth (Chamberlin 1919:190, Monro 1926:312, 1931:10): approaching the subapical tooth (*H. pantherina*, *H. genetta*), approaching the apical tooth (*H. intertexta*, *H. reticulata*), and surpassing the apical tooth (*H. eugeniae*). More information is needed in order to prepare a key to all species, and this is an expected result of an ongoing revision by one of us (SISV).

The holotype specimen of *H. reticulata* was collected by the German naturalist Carl Koerbl on the east coast of Enoshima Island, Kanagawa, during his visit to Japan during 1875–1876. The type material was supposedly deposited in the Natural History Museum in Vienna by Richard von Drasche-Wartinberg (Sato and Sattmann 2009), but is not likely to be extant (Sattmann pers. comm. 2016 email to SISV).

Due to the uncertainties pertaining to some characters in *H. reticulata*, the taxonomic status of the species has been doubted by some researchers. Augener (1913) and Hessle (1925) regarded it as synonymous with *H. splendida* (type locality: Red Sea); Fauvel (1937: 59) synonymized it with *H. pantherina* (type locality: Mediterranean Sea); Wu et al. (1975: 75) viewed it as conspecific with *H. intertexta* (type locality: Philippines). Grube (1880: 227) and Hartman (1959:185) regarded *H. reticulata* as a distinct species, and von Marenzeller even identified his own species from the Red Sea (Stagl et al. 1996:34). There has been no clarification about the morphological

features of *H. reticulata* and, by extension, its taxonomic status, and so species delineations are ill-defined.

In this paper, *H. reticulata* is redescribed as a distinct species. We designate a neotype as there is no existing type material, and, moreover, there are apparently two different species occurring in Kanagawa Province, which closely resemble each other. Uchida (2009) reported "*Hesione* cf. *ehlersi*" and *H. reticulata* from Kanagawa, but von Marenzeller's (1879) original description applies equally well to both of these forms. Uchida (2009: 36) separated these two species in his keys because of differences in pigmentation and chaetotaxy. For *H. reticulata*, he indicated yellow dorsal cirrophores, no middorsal reddish brown line, and long blade neurochaetae in chaetigers 1–3, and for *H. cf. ehlersi* pale cirrophores, a middorsal reddish brown line present, and long blade neurochaetae present in chaetigers 1–7. These differences deserve further evaluation because we have noted that, for example, the pigmentation of dorsal cirrophores fades even after being anesthetized, and the presence of long bladed neurochaetae might be size-dependent.

Photographs of the dorsal color pattern in the living state are also provided, as well as of other morphological characters, and the COI barcoding sequence on the basis of freshly-collected material from a place near to the type locality. The key to species of *Hesione* from Japan by Uchida (2009) has been modified and is included below.

Material and methods

Four specimens were collected at a depth of 1 m by hand in Zaimokuza (35°18'02.9"N, 139°33'02.9"E), Kanagawa Prefecture, Japan. Two specimens were fixed in a 10% formalin sea water solution, later washed and preserved in 70% ethanol (NSMT Pol N-620, NSMT Pol 113205), the other two specimens were fixed and preserved in 70% ethanol (NSMT Pol 113206, NSMT Pol 113207). All specimens were anesthetized with menthol before fixation.

Live and preserved specimens were examined under stereoscopic microscopes (Leica MZ 16F and OLYMPUS BX51); photographs were taken with a digital camera (Nikon D5200). Morphology of chaetae and parapodial features were described from chaetigers 7–9. Neurochaetal blade length was measured from the level of the articulation membrane attachment to chaetal tip; the width was measured at the widest part and expressed as a length:width ratio or by indicating how many times the length corresponds to the width.

Tissue from the dorsal cirri was used for DNA extraction from the two specimens, NSMT Pol N-620 and NSMT Pol 113205. Methods for DNA extraction, PCR amplification, and sequencing followed those of Jimi et al. (2016). Newly obtained sequences were deposited in DNA Data Bank of Japan (DDBJ) (accession nos. LC169753, LC169754). The neotype and other specimens from the neotype locality, referred to here as paraneotypes (term not regulated by the ICZN 1999) were deposited in the National Museum of Nature and Science, Tsukuba (NSMT), Japan.

Systematics

Hesione reticulata von Marenzeller, 1879

Japanese name: otohime-gokai

Figs 1–3

Hesione reticulata von Marenzeller, 1879: 129–131, pl. 3, fig. 4; Izuka 1912: 192–194, pl. 2, fig. 7; Imajima and Hartman 1964: 80; Uchida 2009: 36–37, fig. 1.

Hesione splendida Hessle 1925: 13–15 (*non* Savigny in Lamarck, 1818; *partim*, smallest specimen with transverse white bands belongs elsewhere).

Hesione pantherina Fauvel 1937: 59–60 (*non* Risso, 1826).

Hesione intertexta Wu et al. 1975: 75, pl. 2, figs 7–8 (*non* Grube, 1878).

Type material. Northwestern Pacific, Japan. Neotype NSMT Pol N-620, and three paraneotypes NSMT Pol 113205, NSMT Pol 113206, NSMT Pol 113207, Zaimokuza (35°18'02.9"N, 139°33'02.9"E), rocky bottom, 1 m depth, 19 Mar. 2016, N. Jimi & H. Tanaka, coll. Paraneotypes (NSMT Pol 113205 – NSMT Pol 113207) 40–47 mm long, 4 mm wide).

Neotype locality. Zaimokuza (35°18'02.9"N, 139°33'02.9"E), rocky bottom, 1 m depth.

Description. Neotype (NSMT Pol N-620) complete. Body cylindrical, medially swollen (Fig. 1A), damaged, 43 mm long, 4 mm wide in chaetigers 8–9 (not including parapodia), 16 chaetigers (chaetae and parapodia of 2nd left, 8th right, and 9th right chaetigers removed for observation; dorsal cirri of 3rd and 7th chaetigers removed for DNA extraction).

Dorsal pigmentation pattern consisting of longitudinal, brownish, subcontinuous, irregular lines; no reddish brown longitudinal broken line on median line; single, irregularly-shaped spot (formed by absence of brown pigment, through which basement pale tan to wheat body color seen) on each chaetiger except 2nd, arranged mid-dorsally (larger anteriorly; reduced medially and posteriorly); and additional row of similar but smaller spots on lateral cushion on each side; silvery white spots absent. Cirrophores yellow; cirrostyles yellow to whitish; parapodial lobes whitish (Fig. 1B–E). After six months in ethanol, pigmentation limited to dorsal, pale brown, discontinuous longitudinal bands (Fig. 2A).

Integument smooth, annulated, giving impression of being tuberculated, especially along posterior region; longitudinal ridges absent in lateral cushions.

Prostomium heart-shaped, wider than long (Fig. 1B, E); anterior margin truncated; lateral margins rounded in anterior body, but expanded posteriorly; posterior margin cleft, as long as 1/6 prostomial length; longitudinal furrow shallow; dark transverse line present on prostomial anterior margin. Antennae digitate, twice longer than wide. Eyes blackish, on center of prostomium; anterior and posterior eyes in trapezoidal arrangement; anterior eyes slightly more separated than posterior eyes; anterior eyes ovoid (appearing longer than wide), posterior eyes rounded.

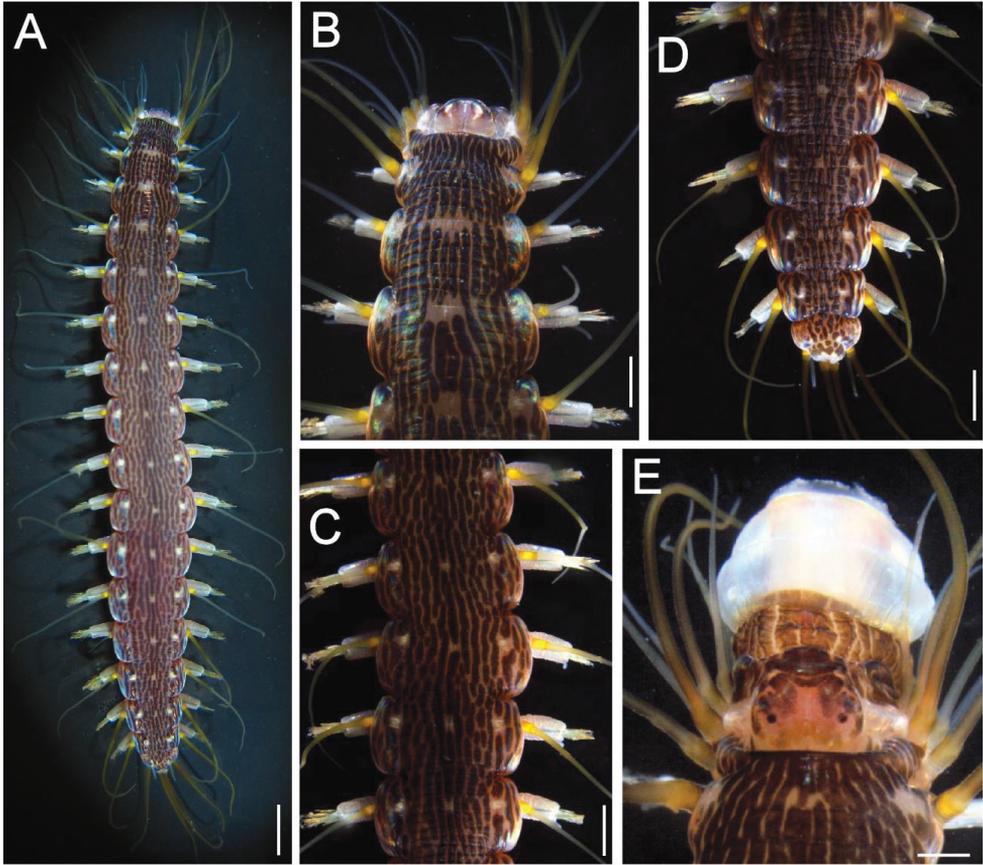


Figure 1. *Hesione reticulata* von Marenzeller, 1879, anesthetized living specimens. **A–D** (NMST Pol N-620) **E** (NSMT Pol-113206). **A** Dorsal view **B** Anterior end, dorsal view **C** Medial part **D** Posterior end, dorsal view **E** anterior end, dorsal view. Scale bars: 3.2 mm (**A**); 1.1 mm (**B–D**); 0.7 mm (**E**).

Tentacular cirri tapered, longest complete anterior cirri reaching chaetiger 5. Lateral cushions slightly projected, entire, with smooth surface.

Parapodia with dorsal cirrophore twice longer than wide, articulated (Fig. 3A, E). Cirrostyle basally cylindrical, medially and distally articulated, as long as body width, including parapodia (Fig. 3A, F). Neuropodia with parallel sides, cylindrical (Fig. 3A). Acicular lobe double; upper tine twice larger than lower one, digitate (Fig. 3B); lower tine of 8th chaetiger of NSMT Pol N-620 and 9th chaetiger of NSMT Pol 113205 adhered or fused to upper tine and difficult to observe (Fig. 3C), it can be clearly confirmed on other four parapodia examined. One acicula present, blackish. Neurochaetae 19–28 per bundle, blade size decreasing ventrally (Fig. 3C); neurochaetal blades bidentate, 3–4 times longer than wide, subdistal tooth shorter and wider than distal one; guard reaching apical tooth (Fig. 3D). Ventral cirrophore three times wider than long; cirrostyle articulated, surpassing chaetal lobe tip.

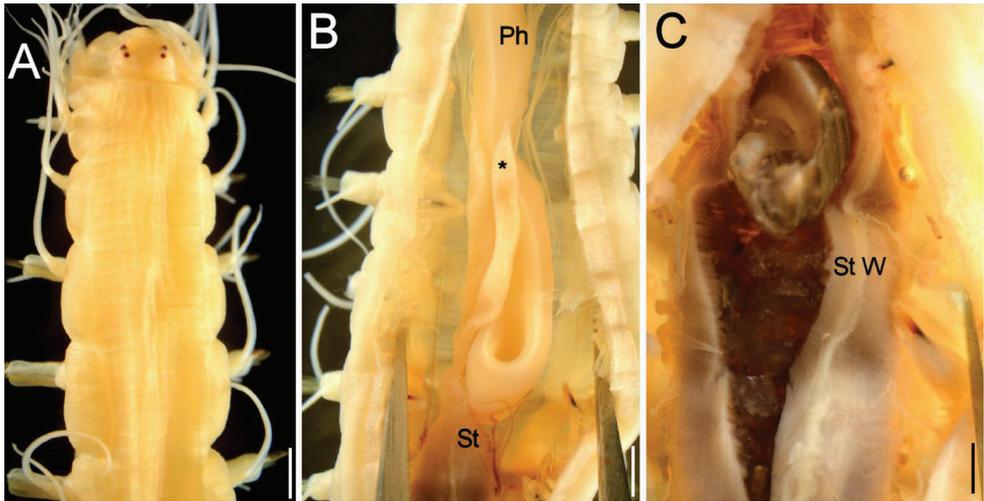


Figure 2. *Hesione reticulata* Marenzeller, 1879, **A** (NSMT Pol N-620) **B–C** (NSMT Pol-113205) **A** Anterior end, dorsal view, six months after fixation **B** Medial part, ventral view after longitudinal dissection (*: enteric caecum, Ph: pharynx, St: stomach) **C** Same, close up after dissecting stomach, with a gastropod prey (St W: stomach wall). Scale bars: 1.5 mm (**A**); 1.2 mm (**B**); 0.6 mm (**C**).

Prepygidial segment with two cirri, three times as long as body width of previous chaetiger (chaetiger 16). Pygidium smooth, trapezoidal, as long as wide, cylindrical (Fig. 1D); anus with two anal cirri; anal cirri tapered.

Venter without pigmentation, with longitudinal midventral depression.

Pharynx divided into three rings, with relative lengths 1.5:1.5:1; basal ring with similar pigmentation as anterior end (Fig. 1E); dorsal papilla pale, longer than wide (length: width 1.5:1).

Oocytes not visible.

Inner anatomy (observed in paraneotypes NSMT Pol 113205–113207). Pharynx and esophagus thick, muscular, yellowish, continuing into darker, shorter stomach; enteric caeca extending anteriorly along two or three chaetigers (Fig. 2B). Stomach contents included gastropod (Fig. 2C) and amphipod remains as prey items.

Remarks. The nomenclatural status of *Hesione reticulata* von Marenzeller, 1879 has been unclear due to several reasons: there is no type material, some diagnostic features were not clarified in the original description, the species has been recorded from the Red Sea, and some authors have regarded it as a junior synonym of other species within the genus. Consequently, in order to comply with the International Code of Zoological Nomenclature (ICZN 1999, Art. 75.3) we are herein proposing a neotype.

The above description and illustrations will clarify the taxonomic status of *H. reticulata* (Art.75.3.1), and its diagnostic and differential features have been included in the description and illustrations (Arts 75.3.2, 75.3.2), and will be contrasted below. Our enquiries on the existence of type material of *H. reticulata* to Dr. Helmut Sattmann, Curator of Marine Invertebrates, in the Naturhistorisches Museum, Vienna, where

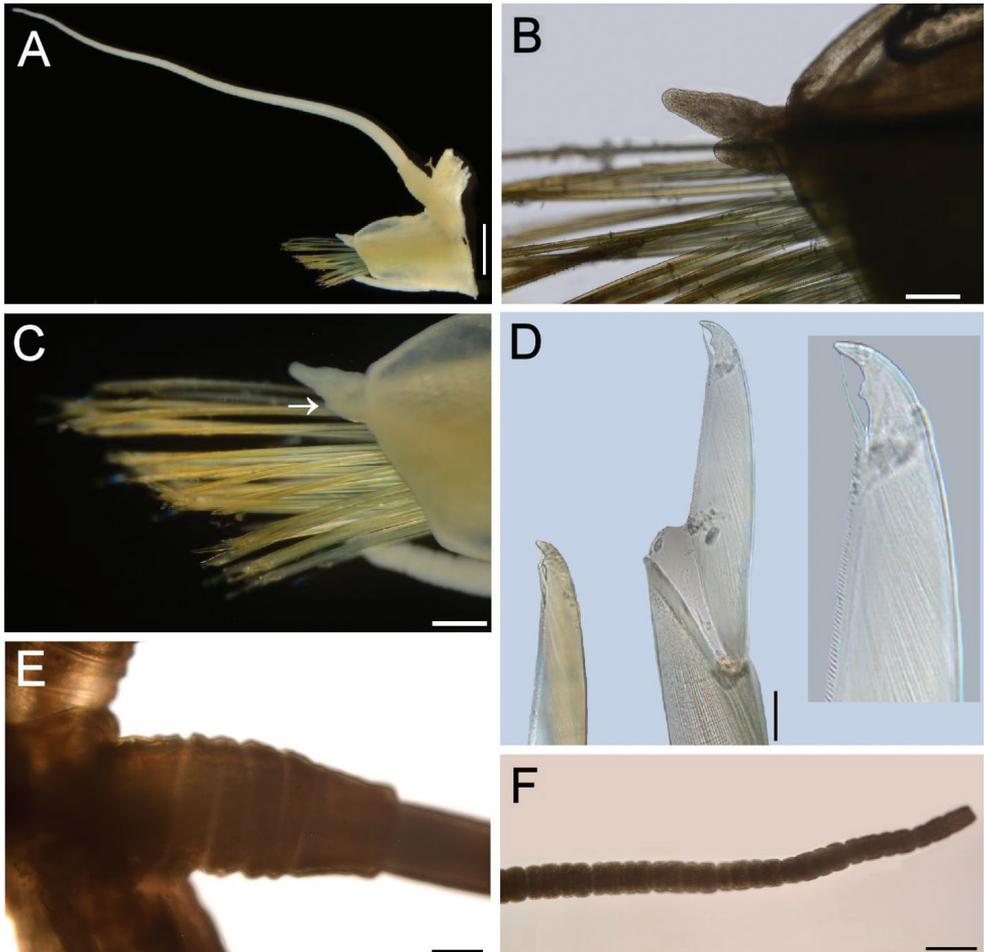


Figure 3. *Hesione reticulata* Marenzeller, 1879, **A–F** (NSMT Pol N-620). **A** Chaetiger 8, right parapodium, anterior view **B** Chaetiger 9, right parapodium, anterior view, close-up of acicular lobe **C** Chaetiger 8, right parapodium, anterior view, close-up of acicular lobe (arrow points to lower tine) and neurochaetal bundle **D** Same, tip of neurochaetae (inset: blade tip) **E** Same, close-up of dorsal cirrophore **F** Same, close-up of cirrostyle distal region. Scale bars: 0.78 mm (**A**); 0.15 mm (**B**); 0.17 mm (**C**), 25 μ m (**D**); 0.13 mm (**E**, **F**).

Emil von Marenzeller used to work and produced all of his publications, indicate that type material is absent (Art. 75.3.4), probably destroyed or never deposited. The original collector was Carl Koerbl (von Marenzeller 1879: 131) and some of his specimens were donated to the Vienna Museum by Richard von Drasche-Wartinberg (Sato and Sattmann 2009), but there is no type material available there.

The neotype fits the original description and because it was recently collected, it even matches the general pigmentation pattern which is not long-lasting in ethanol. Further, as happens in some other species of *Hesione*, they are simultaneous hermaphrodites

(Bergmann 1902, 1903), so that differences in pigmentation pattern among different specimens cannot be attributed to sex (Art. 75.3.5). Thus, the morphology of our material does not contradict von Marenzeller's (1879) original description of *H. reticulata*, nor the general features subsequently described by Izuka (1912: 192), Imajima and Hartman (1964: 80), and Uchida (2009: 36–37).

The original specimen was collected in the east coast of Enoshima (35°18'07"N, 139°29'00"E), and the neotype was found in Zaimokuza (35°18'02.9"N, 139°33'02.9"E), nearly four kilometers away, such that we are confident these two localities belong to the same ecological unit (Art. 75.3.6). The neotype of *H. reticulata* has been deposited in the National Science Museum, Tokyo, which holds the most important polychaete collection in Japan, and has a very important tradition in the scientific study of polychaetes from Japan and elsewhere (Art. 75.3.7).

Another taxonomic relevance of our study lies in the identification of the dorsal color pattern in the living state as a clear distinguishing feature between *H. reticulata*, *H. intertextata*, and *H. cf. ehlersi* sensu Uchida (2009). The color pattern agrees with von Marenzeller's (1879) description of the holotype which had, over a reddish-brown background, irregular spots fused into wide bands along some anterior segments continuing to the end of the body. von Marenzeller (1879) mostly relied on this complex reticulated pigmentation pattern for justifying the establishment of *H. reticulata*.

Ngamniyom et al. (2014) and Lee and Ong (2015) characterized the two western Pacific species, *H. cf. picta* and *H. intertextata*. The former has wide dorsal transverse bands, by which *H. cf. picta* can be separated from *H. intertextata* and *H. reticulata*, because the latter two have dorsal, longitudinal, discontinuous dark bands with paler spots mid-dorsally and along dorsal surface of lateral cushions. Furthermore, *H. intertextata* and *H. reticulata* also have tiny antennae and neurochaetal blades with guards approaching distal tooth. Based on these shared characteristics, Wu et al. (1975) viewed *H. reticulata* as a junior synonym of *H. intertextata*. Our observation, however, clearly shows that they differ in pigmentation pattern: in *H. reticulata* the paler spots are smaller, and the mid-dorsal ones tend to be round, whereas in *H. intertextata* they are longer than wide and markedly larger.

Uchida (2009) described *H. cf. ehlersi*, a species with similar morphological features to *H. reticulata*. Indeed, von Marenzeller's original description of *H. reticulata* could apply to both species. As Uchida (2009) stated, dorsal pigmentation in life is useful for discrimination of the two species; *H. cf. ehlersi* has a reddish brown longitudinal broken line on the median line, whereas *H. reticulata* lacks this line. Further study is needed to resolve the taxonomic position of *H. cf. ehlersi*.

The vivid images of the dorsal color pattern in *H. reticulata*, along with the COI barcoding sequence provided in this paper, will contribute to future taxonomic revision of the genus *Hesione*.

Hesione reticulata was regarded as a distinct species by Hartman (1959: 185) and it can be distinguished from its former synonyms *H. intertextata*, *H. splendida* as indicated by Augener (1913) and Hesse (1925), or from *H. pantherina* as suggested by Fauvel (1937) as follows: from *H. splendida*, *H. reticulata* can be separated by the dorsal pigmentation; it is brownish in *H. reticulata*, but pearly gray in *H. splendida* (Savigny,

1822), whereas from *H. pantherina*, *H. reticulata* can be distinguished because the guard tooth in *H. reticulata* reaches the apical tooth, whereas those in *H. pantherina* do not (Monro 1926).

One of the important discoveries in our observation of the specimens of *H. reticulata* is that the acicular lobe in this species is doubled, comprised of the upper and lower tines, a character state that separates *Hesione* species in two groups, each with approximately the same number of species (SISV pers. obs.). von Marenzeller (1879, fig. 4) illustrated a parapodium excised from the middle part of the body in the holotype specimen, indicating that there was a single, thick, finger-shaped acicular lobe, unlike the doubled lobe that we observed in this study. Izuka (1912) and Imajima and Hartman (1964) also described the acicular lobe as a single lobe. In two of the six parapodia examined (left one on the 2nd, right one on the 8th, and right one on the 9th chaetigers from NSMT Pol N-620; right one on the 9th chaetiger from NSMT Pol 113205; and left ones on the 3rd and 9th chaetigers from NSMT Pol 113207), the lower tine adhered to the upper tine. It appeared as if it were a single parapodial lobe, but a careful observation showed that it actually represents a doubled lobe. The reason the acicular lobe was described as ‘single’ in the previous studies may be that the lower tine in their material was deformed in preservation to lie below the upper tine, or to contact closely to the upper tine. The original illustration (von Marenzeller 1879, fig. 4) clearly indicates that the acicular lobe was placed under the chaetal bundles on the glass slide. This must have made the acicular lobe difficult to be observed, which would also explain why the adjacent upper and lower tines were hardly detected. This feature further adds to the distinction between *H. reticulata* and *H. intertexta*: the acicular lobe in *H. reticulata* is double whereas it is single in *H. intertexta*.

The record of *H. reticulata* by Imajima (1997: 171) might not belong to the same species because he indicated that the acicular lobe was single (“a superior conical papilla”), and because unlike our specimens, his material was collected from 230–250 m depth in Suruga Bay. Other specimens recorded as *H. reticulata* by Imajima (2003: 132–134), collected in shallow water, were characterized as having acicular lobe single (“a superior conical papilla”), and are regarded as belonging to another species.

Distribution. *Hesione reticulata* has so far been recorded only from Japan: Kanagawa (von Marenzeller 1879; Izuka 1912; this study), Shizuoka and Wakayama (Izuka 1912), and the middle of Honshu to Kyushu (Uchida 2009).

Key to species of *Hesione* from Japan

(modified from Uchida 2009)

- 1 Antennae present; eyes positioned centrally on prostomium 2
- Antennae absent; eyes displaced anteriorly *Hesione?* sp.
- 2 Neurochaetal blades with guard 3
- Neurochaetal blades without guard *H. splendida?* sensu Monro, 1931

- 3 Dorsum with transverse bands; neurochaetal guards approaching subdistal tooth..... **4**
- Dorsum with longitudinal bands; neurochaetal guards approaching distal tooth..... **5**
- 4 Chaetiger 2 pale; dorsal pigmentation without spots; second tentacular cirri markedly longer than fourth..... ***H. genetta* Grube, 1867**
- Chaetiger 2 with a black band; dorsal pigmentation includes spots; second tentacular cirri as long as fourth..... ***Hesione* sp.**
- 5 Longitudinal bands short; silvery white spot present mid-dorsally.....
..... ***H. intertexta* Grube, 1878**
- Longitudinal bands long; silvery white spot absent **6**
- 6 Dorsal cirrophores pale; neurochaetae with long blades in chaetigers 1–7; acicular lobe as long as chaetal lobe width; mid-dorsal reddish brown, sub-continuous line present..... ***H. cf. ehlersi***
- Dorsal cirrophores yellow; neurochaetae with long blades in chaetigers 1–3; acicular lobe shorter than chaetal lobe width; mid-dorsal reddish brown line absent ***H. reticulata* von Marenzeller, 1879**

Acknowledgments

Dr Helmut Sattmann at the Natural History Museum in Vienna provided information about the collections studied by Emil von Marenzeller, and indicated there are no type specimens of *H. reticulata*. Mr Hayate Tanaka helped sampling at Zaimokuza and Enoshima, Japan. Drs Alexandra Rizzo, Yen-ling Lee, and Chris Glasby carefully read a previous draft and made positive recommendations to improve this final form, which was carefully line-edited by Nathalie Yonow.

References

- Augener H (1913) Polychaeta I, Errantia. Die Fauna Südwest – Australiens 4(5): 63–304. [pls 2–3] <http://biodiversitylibrary.org/page/7160888>
- Bergmann W (1902) Untersuchungen über die Eibildung bei Anneliden und Cephalopoden. Zeitschrift für wissenschaftliche Zoologie 73: 278–301. [pls 17–19] <http://biodiversitylibrary.org/page/43239588>
- Bergmann W (1903) Über das spätere Schicksal der Zwitterdrüsen von *Hesione sicula*. Zoologischer Anzeiger 26: 415–417. <http://biodiversitylibrary.org/page/30125385>
- Chamberlin RV (1919) The Annelida Polychaeta of the Albatross Tropical Pacific Expedition, 1891–1905. Memoirs of the Museum of Comparative Zoology of Harvard College 31: 1–514. <http://dx.doi.org/10.5962/bhl.title.49195>
- de Quatrefages A (1866) Histoire Naturelle des Annélés marins et d’Eau Douce. Annélides et Gephyriens. Librairie Encyclopédique de Roret, Paris, volume 1, 588 pp.

- Fauvel P (1937) Annélides polychètes du Japon. Memoirs of the College of Science, Kyoto Imperial University, Series B 12[1936]: 41–92.
- Grube AE (1867) Neue Anneliden aus den Gattungen Eunice, Hesione, Lamprophaës und Traviaia. Jahres-Bericht der Schlesischen Gesellschaft für vaterländische Cultur 44[1866]: 64–66. <http://biodiversitylibrary.org/page/46548206>
- Grube AE (1878) Annulata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen. Memoires de l'Academie Imperiale des Sciences de St. Petersbourg, Septième Série 25(8): 1–300. <http://dx.doi.org/10.5962/bhl.title.85345>
- Grube AE (1880) Mittheilungen über die Familie der Phyllocoeen und Hesioneen. Jahresbericht der Schlesischen Gesellschaft für vaterländische Cultur 57: 204–228. <http://biodiversitylibrary.org/page/46547075>
- Hartman O (1959) Catalogue of the Polychaetous Annelids of the World. Allan Hancock Foundation Publications, Occasional Paper 23: 1–628. <http://digitallibrary.usc.edu/cdm/ref/collection/p15799coll82/id/19573>
- Hessle C (1925) Einiges über die Hesioniden und die Stellung der Gattung *Ancistrosyllis*. Arkiv för Zoologi 17: 1–36.
- Imajima M (1997) Polychaetous annelids of Suruga Bay, Central Japan. National Science Museum Monographs (Tokyo) 12: 149–228. http://ci.nii.ac.jp/els/110004312462.pdf?id=ART0006480764&type=pdf&lang=en&host=cinii&order_no=&ppv_type=0&lang_sw=&no=1476474983&cp=
- Imajima M (2003) Polychaetous annelids from Sagami Bay and Sagami Sea collected by the Emperor Showa of Japan and deposited at the Showa Memorial Institute, National Science Museum, Tokyo, 2. Orders included within the Phyllococida, Amphinomida, Spintherida and Eunicida. National Science Museum Monographs 23: 1–221. <http://ci.nii.ac.jp/naid/110004708004/en>
- Imajima M, Hartman O (1964) The polychaetous annelids of Japan, 1. Allan Hancock Foundation Publications, Occasional Paper 26: 1–166. <http://cdm15799.contentdm.oclc.org/cdm/ref/collection/p15799coll82/id/18946>
- ICZN (1999) International Code of Zoological Nomenclature (4th edn). International Trust for Zoological Nomenclature, London.
- Izuka A (1912) The errantiate Polychaeta of Japan. Journal of the College of Science (Tokyo) 30: 1–262. <http://hdl.handle.net/2261/32884>
- Jimi N, Tanaka M, Fujiwara Y (2016) *Diplocirrus nicolaji* (Annelida: Flabelligeridae) from Japan, detailed morphological observation and DNA barcoding. Marine Biodiversity Records 9(1): 1–8. <https://doi.org/10.1186/s41200-016-0024-7>
- Kinberg JGH (1866) Annulata nova (Nephythidea, Phyllocoea, Alciopaea, Hesionida, Gycerea, Goniadea, Syllidea, Ariciea, Spiodea, Aonidea, Cirratulida, Opheliacea). Öfversigt af Kongelige Vetenskaps-Aakademiens Förhandlingar 22[1865]: 239–258. <http://biodiversitylibrary.org/page/32339515>
- Lamarck JBPA de (1818) Histoire naturelle des animaux sans vertèbres, présentant les caractères généraux et particuliers de ces animaux, leur distribution, leurs classes, leurs familles, leurs genres, et la citation des principales espèces qui s'y rapportent; précédés d'une introduction offrant la détermination des caractères essentiels de l'animal, sa distinction du végétal et des

- autres corps naturels, en fin, l'exposition des principes fondamentaux de la zoologie, Vol. 5. Deterville & Verdiere, Paris, 612 pp. <http://dx.doi.org/10.5962/bhl.title.12712>
- Lee Y, Ong R (2015) New records of two hesionid polychaetes from the Singapore Strait. Singapore Biodiversity Records 2015: 201–204. <https://lkcnhm.nus.edu.sg/nus/images/pdfs/sbr/2015/sbr2015-201-204.pdf>
- Monro CCA (1926) Polychaeta of the 'Alert' Expedition. Families Hesionidae and Nereidae. Journal of the Linnean Society of London, Zoology 36(243): 311–323. <https://doi.org/10.1111/j.1096-3642.1926.tb02172.x>
- Monro CCA (1931) Polychaeta, Oligochaeta, Echiuroidea, and Sipunculoidea. Great Barrier Reef Expedition 1928–29, Scientific Reports 4: 1–37. <http://biodiversitylibrary.org/page/49516539>
- Müller F (1858) Einiges über die Annelidenfauna der Insel Santa Catharina an der brasilianischen Küste. Archiv für Naturgeschichte 24: 211–220. [pls 6–7] <http://biodiversitylibrary.org/page/7460059>
- Ngamniyom A, Silprasit K, Sriyapai T (2014) Morphological and molecular evidence for a new record of *Hesione* cf. *picta* (Polychaeta: Hesionidae) from the Western coast of the Gulf of Thailand. Kasetsart Journal, Natural Sciences 48: 719–728. <http://www.thaiscience.info/journals/Article/TKJN/10961404.pdf>
- Read G, Bellan G (2016) *Hesione* Savigny in Lamarck, 1818. In: Read G, Fauchald K (Eds) World Polychaeta Database. <http://www.marinespecies.org/aphia.php?p=taxdetails&id=129308> [Jan. 7, 2017]
- Risso A (1826) Histoire naturelle des principales productions de l'Europe méridionale et particulièrement de celles des environs de Nice et des Alpes maritimes, Vol. 4. F.G. Levrault, Strasbourg, 439 pp. <http://biodiversitylibrary.org/page/39428699>
- Sato M, Sattmann H (2009) Extirpation of *Hediste japonica* (Izuka, 1908) (Nereididae, Polychaeta) in Central Japan, evidenced by a museum historical collection. Zoological Science 26: 369–372. <https://doi.org/10.2108/zsj.26.369>
- Savigny JC (1822) Système des annélides, principalement de celles des côtes de l'Égypte et de la Syrie, offrant les caractères tant distinctifs que naturels des ordres, familles et genres, avec la description des espèces. Description de l'Égypte. Histoire naturelle, Paris 21: 1–128. <http://dx.doi.org/10.5962/bhl.title.66284>
- Stagl V, Sattmann H, Dworschak PC (1996) The material of the *Pola* Red Sea expeditions (1895–1898) in the collections of the Natural History Museum in Vienna. Biosystematics and Ecology Series 11: 29–41. http://www.nhm-wien.ac.at/jart/prj3/nhm/data/uploads/mitarbeiter_dokumente/dworschak/Stagl_et_al1996_Pola.pdf
- Uchida H (1992) [Annelida, Polychaeta. In: Nishimura S (Ed.) Guide to Seashore Animals of Japan with Color Pictures and Keys (Vol. 1). 310–373. [pls 63–72, In Japanese]
- Uchida H (2000) [Animals of Chiba Prefecture 2, Marine Animals of Chiba Prefecture: Annelida]. In: The Foundation of Chiba Prefecture for the Study of Historical Materials (Ed.) Natural History of Chiba Prefecture 7. The Foundation of Chiba Prefecture for the Study of Historical Materials, Chiba, 278–291. [In Japanese]
- Uchida H (2009) [Polychaetologica 55: Key to genus and description of species (40), Hesionidae 4]. Marine Pavilion, Kushimoto Marine Park 38: 36–37. [In Japanese]

Von Marenzeller E (1879) Sudjapanische Anneliden, 1. Amphinomea, Aphroditea, Lycoridea, Phyllodocea, Hesionea, Syllidea, Eunicea, Glycera, Sternaspidea, Chaetoptera, Cirratulea, Amphictenea. Denkschriften der Mathematisch-Naturwissenschaftlichen classe der Kaiserlichen Akademie der Wissenschaften 41: 109–154.

Wu B, Shen S, Chen M (1975) [Preliminary report of polychaetous annelids from Xisha Islands, Guangdong Province, China]. *Studia Marina Sinica* 10: 65–104.

On some new species of Ancorabolidae Sars, 1909 from the Gulf of California: the genera *Ceratonotus* Sars, 1909, and *Dendropsyllus* Conroy-Dalton, 2003 (Crustacea, Copepoda, Harpacticoida)

Samuel Gómez¹, Karen Díaz²

1 Instituto de Ciencias del Mar y Limnología, Unidad Académica Mazatlán, Universidad Nacional Autónoma de México; Joel Montes Camarena *s/n*, Fracc. Playa Sur, Mazatlán, 82040, Sinaloa, México **2** Posgrado en Ciencias del Mar y Limnología, Unidad Académica Mazatlán, Universidad Nacional Autónoma de México; Joel Montes Camarena *s/n*, Fracc. Playa Sur, Mazatlán, 82040, Sinaloa, México

Corresponding author: Samuel Gómez (samuelgomez@ola.icmyl.unam.mx)

Academic editor: D. Defaye | Received 5 October 2016 | Accepted 26 January 2017 | Published 17 February 2017

<http://zoobank.org/7C348352-0727-4F90-915A-5D5CBA9A9F83>

Citation: Gómez S, Díaz K (2017) On some new species of Ancorabolidae Sars, 1909 from the Gulf of California: the genera *Ceratonotus* Sars, 1909, and *Dendropsyllus* Conroy-Dalton, 2003 (Crustacea, Copepoda, Harpacticoida). ZooKeys 657: 43–65. <https://doi.org/10.3897/zookeys.657.10725>

Abstract

Two new species of two genera of the family Ancorabolidae, *Ceratonotus elongatus* **sp. n.** and *Dendropsyllus californiensis* **sp. n.**, found at 1642 m and 1759 m depth, respectively, in the Southern Trough of Guaymas Basin, are described. *Ceratonotus elongatus* **sp. n.** was attributed to that genus by a series of character states of which the lack of dendroid dorsal processes on the P6-bearing somite and the presence of such processes on the first abdominal somite were definitive. This species was observed to be very close to *C. thistlei* Conroy-Dalton, 2003 from the San Diego Trough, and can be separated by a number of traits of which the elongated sensilla-bearing dorsal tubercles on the second abdominal somite in the new species was definitive. *Dendropsyllus californiensis* **sp. n.** has been placed within that genus given the presence of four geniculate setae on P1EXP2 and one seta on P1ENP2, one inner seta on P3EXP3, and lack of inner armature on P4EXP3. *Dendropsyllus californiensis* **sp. n.** appears to be more closely related to *D. thomasi* Conroy-Dalton, 2003 and *D. magellanicus* (George & Schminke, 1998) on account of the spinulose nature of the basis of the maxilliped, the two-segmented P4ENP, and the fused condition of the P5 baseopod and exopod, and seems to be even more closely related to *D. thomasi* by the degree of development of the lateroventral processes of the cephalothorax. *Dendropsyllus californiensis* **sp. n.** can be separated from its congeners by the relative length of the first antennular segment, relative length of the caudal rami, and by the armature formula of P3ENP2.

Keywords

Deep sea, Gulf of California, Harpacticoida, taxonomy

Introduction

The macrofauna diversity of the Gulf of California is fairly well known. The Gulf is home to more than 4,916 named species of macroinvertebrates, comprising approximately 70% of the invertebrate fauna of the Gulf of California. In contrast, the open sea and the deep sea below the continental shelf are regions more poorly known (Brusca and Hendrickx 2012). For example, a quick search in The Gulf of California Invertebrate Database (Brusca and Hendrickx 2008) yielded only around 508 benthic macroinvertebrate species reported below 200 m depth. The biodiversity of meiofauna of the Gulf of California is even less known, and is based on few studies available, mostly from sublittoral situations (e.g. Álvarez-Castillo et al. 2015 for kinorhynchs, Holovachov et al. 2008, 2009, Mundo-Ocampo et al. 2007, Pereira et al. 2010 for nematodes, and see Gómez and Morales-Serna 2014 for harpacticoid copepods), while only a handful of described meiofaunal species are known from the deep Gulf of California below 200 m depth (e.g. Álvarez-Castillo et al. 2015 for kinorhynchs, and both Gómez and Conroy-Dalton 2002 and Gómez and Morales-Serna 2014 for harpacticoids). The low number of studies on the diversity of deep-sea meiofauna of the Gulf of California is due mainly to a difficult taxonomy, special sample processing techniques required for the different taxa, and above all to a limited expertise and lack of experts (Gómez and Morales-Serna 2014, Álvarez-Castillo et al. 2015, Pereira et al. 2010). A series of intensive oceanographic cruises, Talud IV–XVI, have been carried out in the deep-sea of the Gulf of California since the late 90's. These samplings include, among other components, the meiofaunal communities, of which harpacticoid copepods are the only meiofaunal group studied so far with one described species (*Ancorabolus hendrickxi* Gómez & Conroy-Dalton, 2002), from a depth of 1985 m off Sinaloa state (Gómez and Conroy-Dalton 2002). Described herein are two more species of two genera of the family Ancorabolidae, *Ceratonotus elongatus* sp. n. and *Dendropsyllus californiensis* sp. n. found at 1642 m and 1759 m depth in the Southern Trough of Guaymas Basin (see Fig. 1), with closely related species, *C. thistlei* Conroy-Dalton, 2003 and *D. thomasi* Conroy-Dalton, 2003, respectively, from the San Diego Trough.

Material and methods

Sediment samples for meiofaunal analyses were taken in February 2007 during the Talud X oceanographic cruise in the Southern Trough of Guaymas Basin, on board the research vessel “El Puma” of the Universidad Nacional Autónoma de México (UNAM). The sediment samples were collected using a box corer, and triplicate sub-samples were taken with 69 cm² cores of 20 cm in length. The upper 3 cm layer of sediment was

recovered and preserved in 70% alcohol, sieved through 500 and 38 μm sieves to separate macro- and meiofauna, and stained with Rose Bengal. Meiofauna was sorted and quantified at a magnification of 40 \times using an Olympus SZX12 stereomicroscope equipped with DF PLAPO 1 \times objective and WHS10X eyepieces. The specimens of the species presented herein were partly dissected as indicated in “Material examined” for each species. Illustrations and figures were made from whole individuals and its dissected parts using a Leica DMLB microscope equipped with L PLAN 10 \times eyepieces, N PLAN 100 \times oil immersion objective, and drawing tube. The dissected parts were mounted on separate slides using lactophenol as mounting medium. Terminology of Huys and Boxshall (1991), Conroy-Dalton (2003), and George (2006b) were adopted for descriptive morphology. Abbreviations used in the text:

| | |
|-----------------------|---|
| P1-P6 | first to sixth legs; |
| EXP | exopod; |
| ENP | endopod; |
| EXP(ENP)1(2,3) | first (second, third) exopodal (endopodal) segment; |
| ae | aesthetasc; |
| mya | million years ago. |

The type material was deposited in the Copepoda collection of the Instituto de Ciencias del Mar y Limnología, Unidad Académica Mazatlán (**ICML-EMUCOP**).

The map showing the sampling locations where the new species were found were prepared with GeoMapApp (<http://www.geomapapp.org/>) and the Global Multi-Resolution Topography (GMRT) default basemap (Ryan et al. 2009).

Results

Taxonomy

Family Ancorabolidae Sars, 1909

Subfamily Ancorabolinae Sars, 1909

Genus *Ceratonotus* Sars, 1909

Ceratonotus elongatus sp. n.

<http://zoobank.org/1A041782-E9FC-4455-9D57-82A8A2A07DE1>

Material examined. One female holotype as follows: body partially dissected (leaving cephalothorax with right first antennular segment and antenna, first thoracopod to fifth urosomite, and right P5 intact) and preserved in alcohol (ICML-EMUCOP-100207-01), left antennule and antenna, pair of mandibles, maxillules, maxillae and maxillipeds, P1-P4 and left P5, and anal somite with caudal rami dissected and mounted on four slides (ICML-EMUCOP-100207-04).

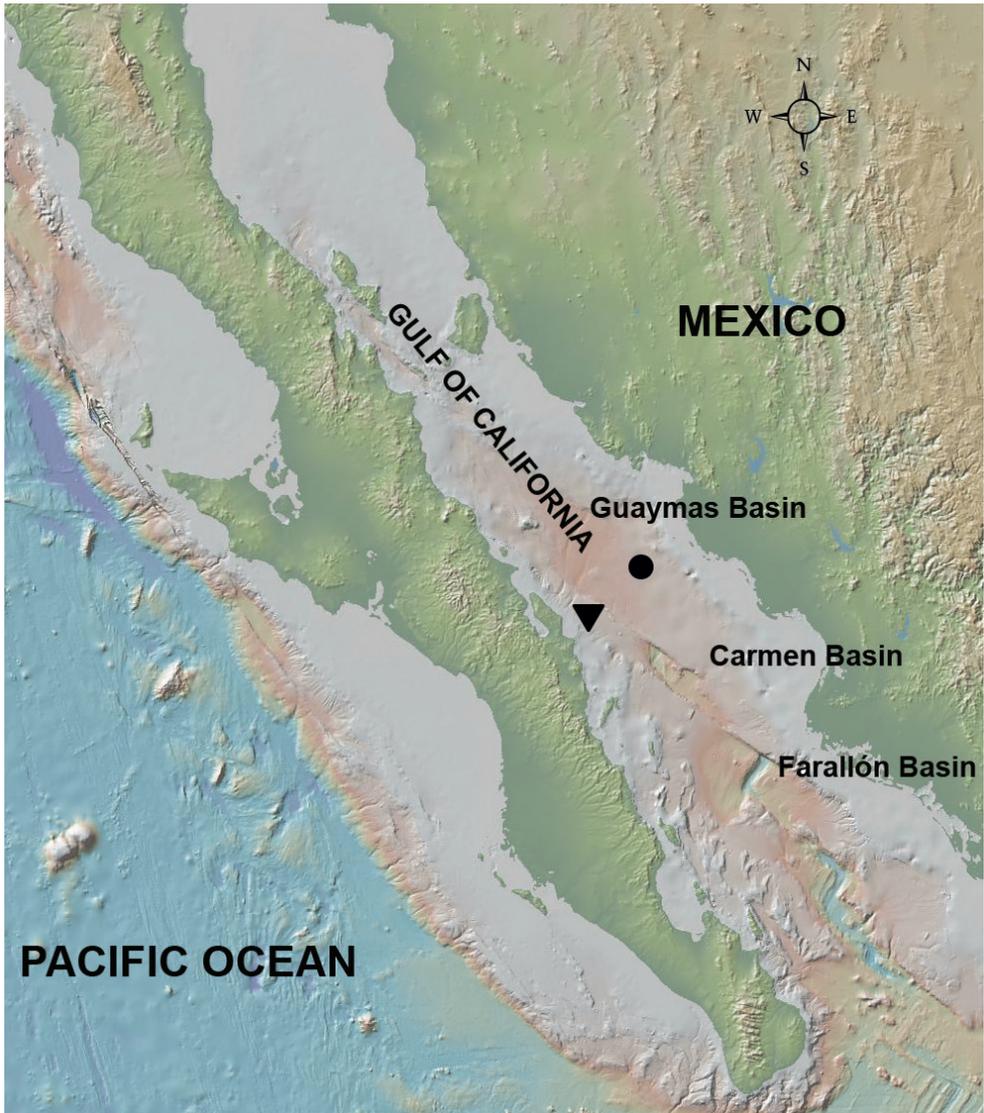


Figure 1. Sampling sites and type localities of *Ceratonotus elongatus* sp. n. (circle) and *Dendropsyllus californiensis* sp. n. (inverted triangle). Figure prepared with GeoMapApp (<http://www.geomapp.org/>) and the Global Multi-Resolution Topography (GMRT) default basemap (Ryan et al. 2009).

Type locality. Southern Trough of Guaymas Basin, Gulf of California, México ($27^{\circ}01'N$, $110^{\circ}53'04''W$), 1642 m depth (see Fig. 1); coll. S. Gómez.

Diagnosis (based on the female only). Ancorabolidae. Cephalothorax without anterior horn-like processes, with bilateral constriction in anterior half. First half of genital double-somite without, posterior half with well-developed dendroid processes. With dorsal elongate tubercles and paired tube-pores on fourth urosomite. Caudal rami divergent, approximately 11 times as long as wide, with seven setae of which

seta IV fused at base of seta V. Antennule three-segmented. Antenna with allobasis bearing one abexopodal seta; without exopod; endopod with nine setae/spines. Palp of mandible one-segmented, with five setae. Maxillule with two surface setae and seven spines on pracoxal arthrite; coxal endite with two elements; basis with six setae; exopod represented by two, endopod by three elements. Maxilla with two syncoxal endites, each with three setae; endopod represented by two setae. Maxilliped with one seta on syncoxa; endopodal claw with one accessory seta. Exopod of P1 two-segmented, of P3-P4 three-segmented. Endopod of P1-P4 two segmented; endopod of P1 as long as exopod, second endopodal segment approximately 1.7 times as long as first endopodal segment; first endopodal segment of P2 and P3 reduced, smaller than second endopodal segment; endopod of P4 much smaller than in P2 and P3, first endopodal segment twice as long as second. P5 with exopod and endopodal lobe distinct; endopodal lobe a tiny pedestal with one seta and one tube-pore; exopod elongate, slender, with three elements.

Description of female. Total body length, 920 μm measured from anterior outer corner of cephalothorax to posterior margin of caudal rami; length of caudal rami, 222.5 μm (ca. 24% total body length). Body (Fig. 2A) cylindrical, rather slender, tapering slightly posteriorly, without clear demarcation between prosome and urosome; integument moderately chitinised; general pattern of dendroid processes as for the genus; dendroid processes well developed. Cephalothorax without anterior horn-like processes, with bilateral constriction in anterior half; with large tube-pore medially along anterior margin; anterior corners of cephalothorax with two anterior sensilla associated with a tube-pore (Fig. 3A); dorsal dendroid processes well developed (Fig. 2A); lateroventral processes situated rather anteriorly. Rostrum fused to cephalothorax, absorbed into anteroventral surface of cephalothorax, with paired sensilla and well-developed midventral tube-pore (Figs 2A, 3A).

P2-P4-bearing somites with medial tube-pore and two posterior sensilla; dorsal dendroid processes well developed. P5-bearing somite with dorsal tube-pore, without posterior sensilla; with well-developed dorsal processes, nearly as long as in preceding somites and without backwardly directed excrescent.

Original segmentation of genital double-somite indicated by bilateral constriction; first half of genital double-somite with dorsal tube-pore, without dendroid processes, without spinular ornamentation ventrally, genital field as shown (Fig. 3C); posterior half without dorsal tube-pore, with well-developed dendroid process, though smaller than those of P5 bearing-somite (Fig. 2A), ventrally without spinular ornamentation but with paired tube-pores (Fig. 3C). Fourth urosomite (second abdominal somite) with elongate tubercles and paired tube-pores dorsally (Figs 2A, 3B), ventrally with medial short spinular row close to posterior margin and with paired tube-pores (Fig. 3C). Fifth urosomite (third abdominal somite) with paired tube-pores and with fine spinules along posterior margin dorsally (Fig. 2A), with four medial sets of spinules and paired tube-pores ventrally (Fig. 3C). Anal somite cleft medially (Fig. 2A, C); with tube-pore and small spinules ventrally as shown (Fig. 2C); rounded anal operculum smooth (Fig. 2A).

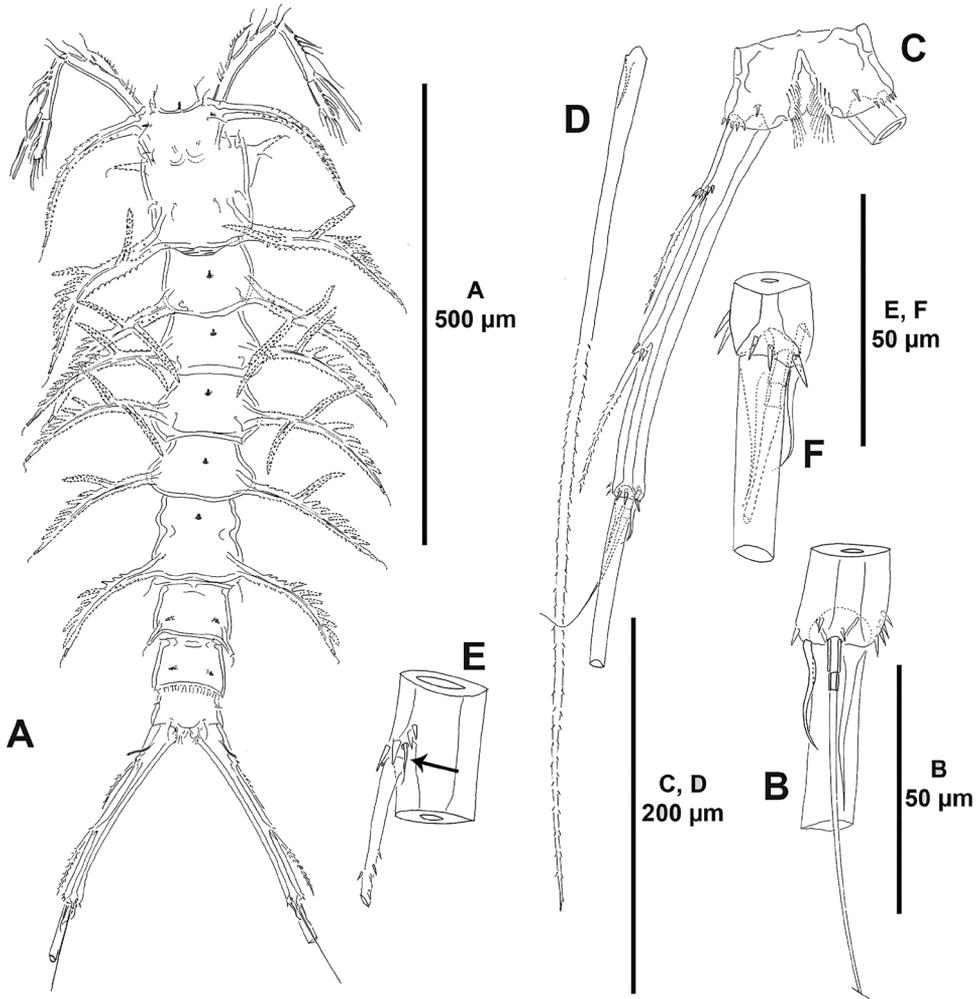


Figure 2. *Ceratontotus elongatus* sp. n., female holotype. **A** habitus, dorsal **B** distal part of caudal ramus, dorsal **C** anal somite and right caudal ramus, ventral **D** caudal setae IV and V **E** caudal setae I and II, indicating position of reduced seta I **F** distal part of caudal ramus, ventral.

Caudal rami elongate, divergent, cylindrical, approximately 11 times as long as wide (Fig. 2A, C); with some spinules at base of setae I, II and III, and close to posterior margin ventrally; with conspicuous tube-pore proximally (Fig. 2A); with seven setae; seta I minute, ventral to seta II (Fig. 2A, C, E), both situated on proximal fifth of ramus; seta II bipinnate; seta III inserted on proximal margin of distal third, as long as seta II, bipinnate (Fig. 2A, C); seta IV fused at base of seta V (Fig. 2B, D, F), the latter longest, ornamented as shown (Fig. 2D); seta VI shorter than seta IV, inserted on distal inner corner of ramus (Fig. 2B, C, F); dorsal seta VII triarticulate, arising from small pedestal close to posterior margin (Fig. 2B).

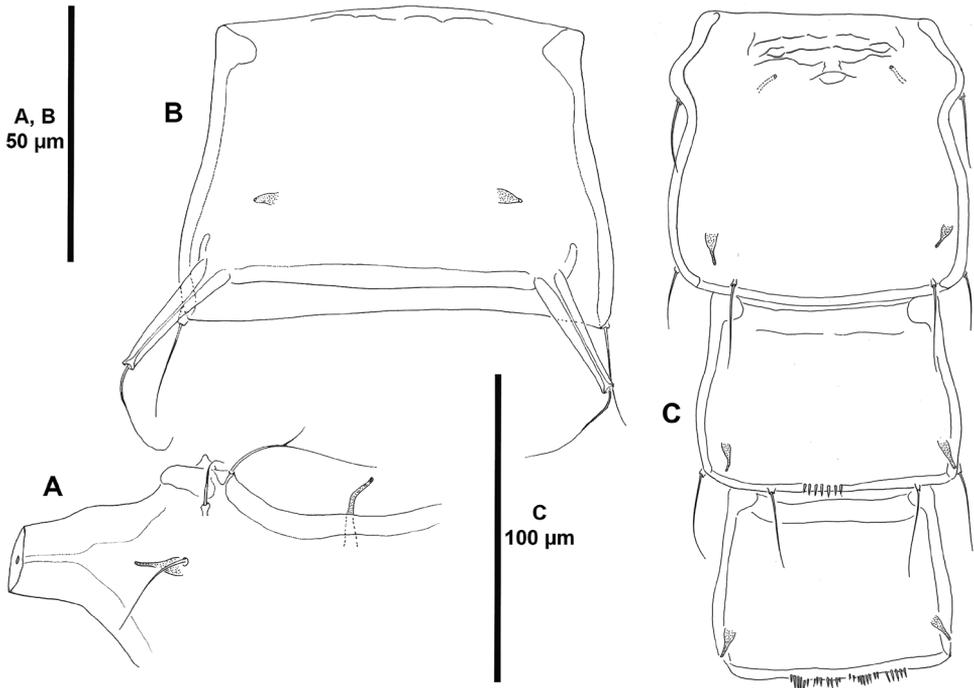


Figure 3. *Ceratonotus elongatus* sp. n., female holotype. **A** anterior corner of cephalothorax **B** fourth urosomite (second abdominal somite), dorsal **C** genital double-somite, and fourth and fifth urosomites (second and third abdominal somites), ventral.

Antennule (Fig. 4A) three-segmented, segments elongate and slender. Armature formula as follows: 1-[9], 2-[7+(1+ae)], 3-[9+acrothek].

Antenna (Fig. 4B). Coxa represented by sclerite; with allobasis and one-segmented endopod. Allobasis with membranous insert indicating original division between basis and first endopodal segment; with small spinules along inner margin of proximal half; with well-developed pinnate abexopodal seta in endopodal half. Exopod absent. Endopod with small spinules along inner margin of proximal half; with two inner lateral spines and one slender seta; with two outer subdistal frills; apically with two pinnate spines, two geniculate single setae, and one geniculate element fused to tiny seta basally; with additional distal tube-pore (arrowed in Fig. 4B).

Mandible (Fig. 5A) with robust coxa. Gnathobase with distal teeth as shown, with one lateral pinnate seta accompanied by spine-like element. Palp one-segmented, well developed, with two inner (basal) setae, and three apical (endopodal) setae.

Maxillule (Fig. 5B). Praecoxal arthrite with two surface setae and some posterior spinules (some of them very long), distally with seven spines (two of them spinulose) and two pinnate setae. Coxal endite with some spinules distally, with one strong and spinulose element and one slender seta ornamented with very few spinules. Proximal endite of basis with four, distal endite with two setae. Exopod and endopod incorpo-

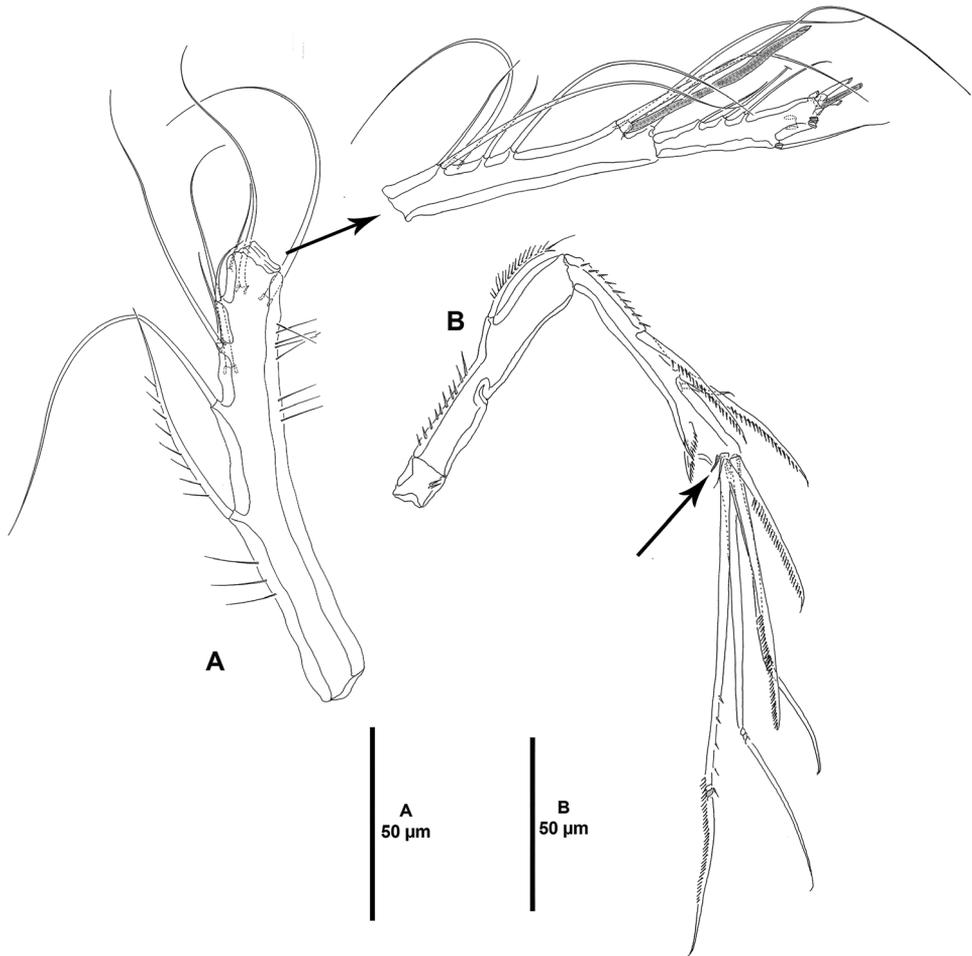


Figure 4. *Ceratonotus elongatus* sp. n., female holotype. **A** antennule **B** antenna.

rated into basis, the former represented by one small and one strong seta, the latter represented by three pinnate elements.

Maxilla (Fig. 5C). Syncoxa with spinulose patches as shown, with two endites; proximal endite with one strong spinulose element fused to endite, and two spinulose setae, distal endite with three spinulose elements. Allobasis drawn out into strong claw; accessory armature consisting of one spinulose strong spine, and one bare and one pinnate seta. Endopod represented by two setae.

Maxilliped (Fig. 5D) subchelate, slender. Syncoxa with one pinnate seta. Basis with spinules as figured. Endopod drawn into long, curved claw finely pinnate with one accessory small seta.

P1 (Fig. 6A). Coxa with one outer spinule. Basis transversely elongate, with anterior tube-pore, with some spinules at base of outer seta, the latter well-developed, inner seta lost during dissection. Exopod two-segmented; first segment with pinnate

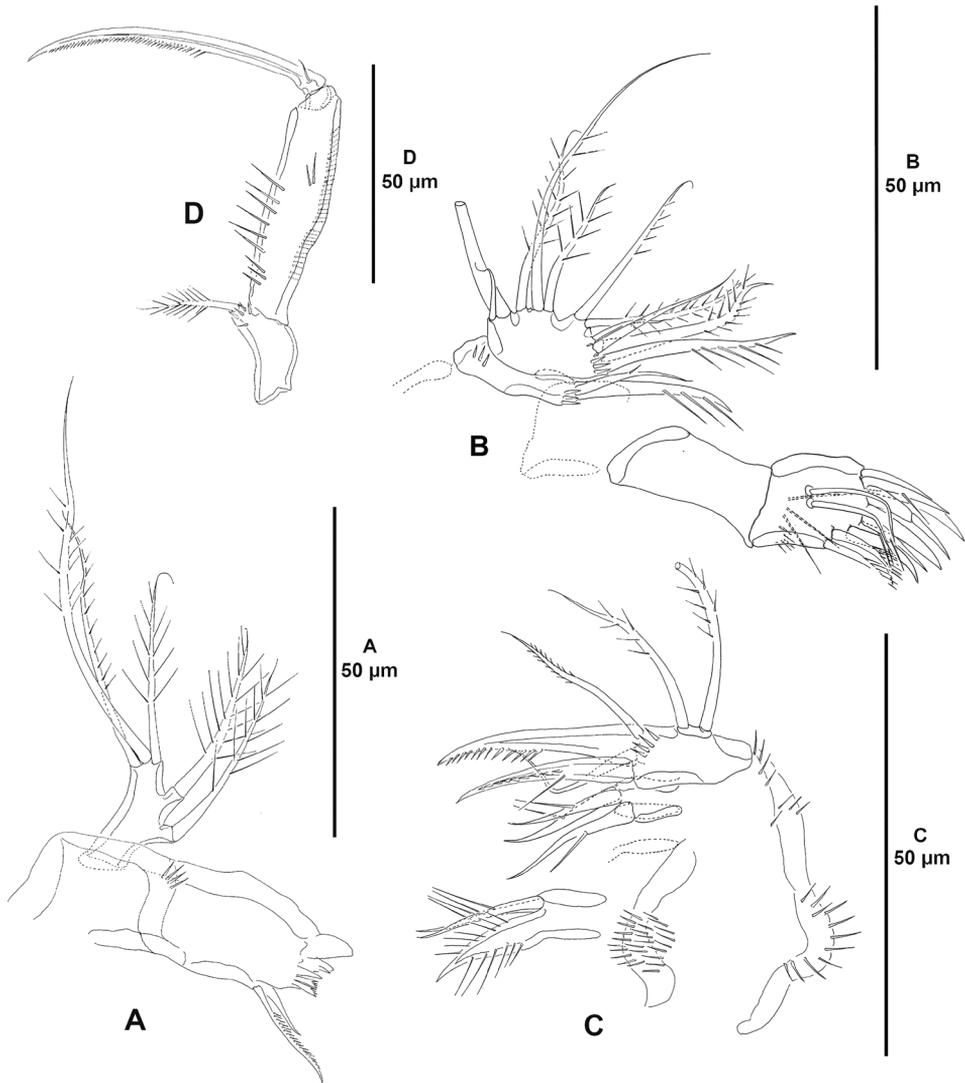


Figure 5. *Ceratonotus elongatus* sp. n., female holotype. **A** mandible **B** maxillule **C** maxilla, showing insertion of proximal endite **D** maxilliped.

spine longer than entire ramus; second segment with two pinnate outer spines and three geniculate apical setae. Endopod two-segmented, nearly as long as exopod; first segment unarmed; second segment 1.7 times as long as first one, with two apical setae.

P2-P4 (Fig. 6B–D) with trapezoid coxa ornamented with spinules on lobate outer process. Basis transversely elongate, with anterior tube-pore midway length of basis, with some spinules at base of outer seta. Exopod three-segmented; first segment with outer bipinnate elongate spine; second segment with outer bipinnate elongate spine and inner seta; third segment with two outer elongate spines, two apical elements and

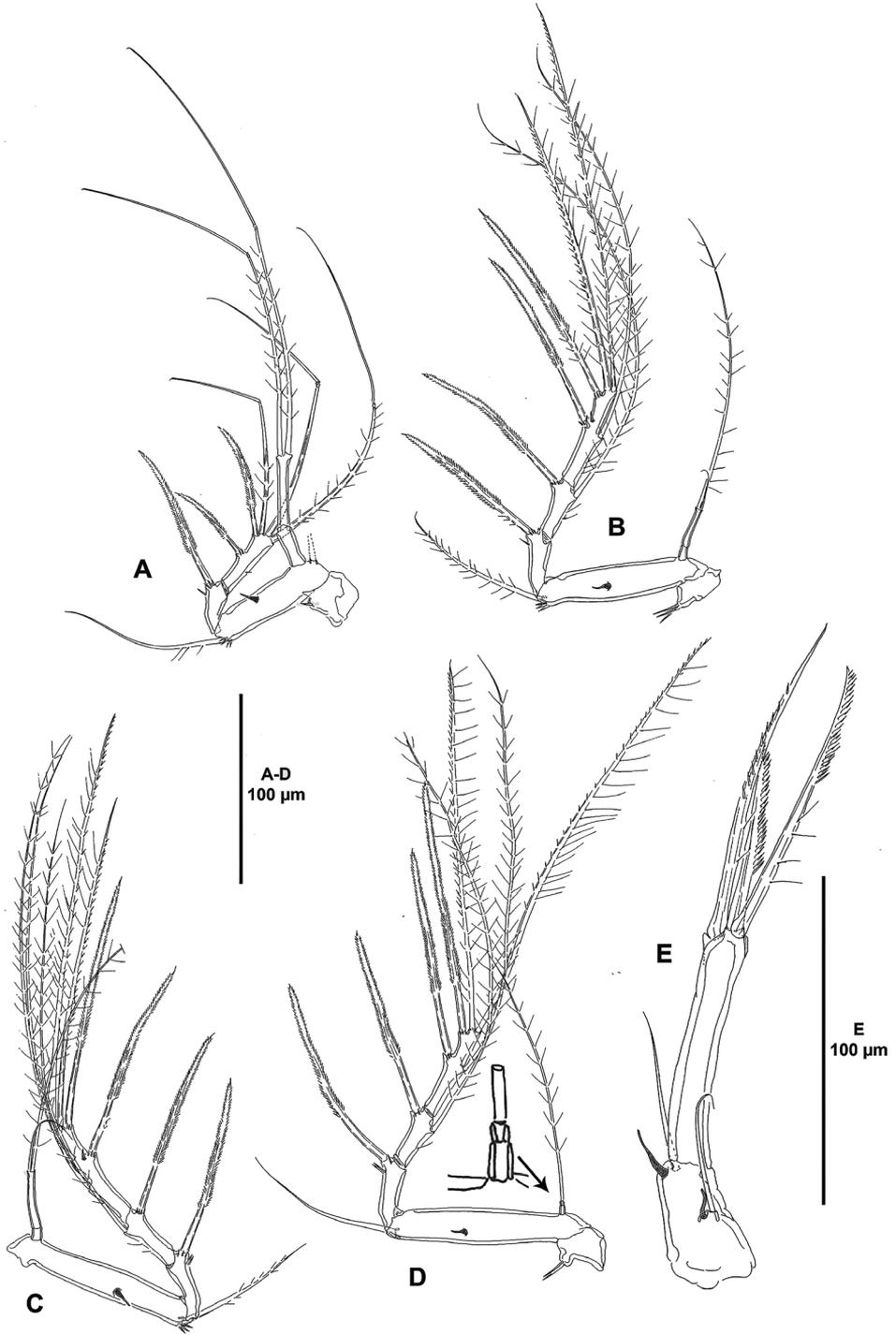


Figure 6. *Ceratonotus elongatus* sp. n., female holotype. **A** P1, anterior **B** P2, anterior **C** P3, anterior **D** P4, anterior, showing insert of endopod **E** P5, lateral.

one (P2 and P4) or two (P3) inner setae, with (P2 and P3) or without (P4) tube-pore. Endopod two-segmented; P2ENP1 and P3ENP1 reduced, smaller than ENP2, the latter with two setae, inner one smaller; P4ENP much smaller than in preceding legs, ENP1 twice as long as ENP2, the latter with one seta.

Armature formula as follows:

| | EXP | ENP |
|----|---------|-------|
| P1 | 0.023 | 0.020 |
| P2 | 0.1.122 | 0.020 |
| P3 | 0.1.222 | 0.020 |
| P4 | 0.1.122 | 0.010 |

P5 (Fig. 6E) without baseopodal setophore; outer basal seta bare, accompanied by tube-pore. Endopodal lobe represented by tiny pedestal armed with one seta and accompanied by tube-pore. Exopod distinct, long, slender, with three elements as figured.

Description of male. Unknown.

Etymology. The specific epithet, *elongatus*, makes reference to the elongate dorsal tubercles on the second abdominal somite.

Genus *Dendropsyllus* Conroy-Dalton, 2003

Dendropsyllus californiensis sp. n.

<http://zoobank.org/4730BB01-67A4-4AF9-9942-27E5FA1B3580>

Material examined. One female holotype as follows: body partially dissected (leaving cephalothorax, left antennule and antenna, left P1-P5, abdomen, anal somite and caudal rami intact) and preserved in alcohol (ICML-EMUCOP-100207-02), pair of mandibles, maxillules, maxillae and maxillipeds, and right P1-P5 dissected and mounted on four slides (ICML-EMUCOP-100207-03).

Type locality. Southern Trough of Guaymas Basin, Gulf of California, México (26°41'06"N, 111°12'W), 1759 m depth (see Fig. 1); coll. S. Gómez.

Diagnosis (based on the female only). Ancorabolidae. Cephalothorax with bilateral anterior constriction; with two sensilla and one tube-pore on distal corners; with paired dorsal processes anteriorly, lateroventrally, and posteriorly. Rostrum fused to cephalothorax. P2-P5-bearing somites with paired dorsal dendroid processes. Second and third urosomites fused ventrally, distinct dorsally, without dendroid processes. Caudal rami divergent, around 7.5 times as long as wide; with seven setae. Antennule three-segmented. Antenna with allobasis bearing a reduced abexopodal seta; without exopod; free endopodal segment with eight setae/spines. Mandible with one-segmented palp bearing five setae. Maxillule with two surface setae and five spines on praecoxal arthrite; coxal endite with two setae; basis with six setae; exopod represented by two, endopod by three elements. Maxilla with two syncoxal endites bearing three setae each; allobasis drawn out into strong claw, accompanied by five elements; endopod one-segmented, with two

setae. Maxilliped with one seta on syncoxa; endopodal claw with one accessory seta. Exopod of P1 two-segmented, of P2-P4 three-segmented. First endopodal segment of P1 small, second segment elongate, close to 4.3 times as long as first segment, and 7.6 times as long as wide. P2 without endopod. First endopodal segment of P3 and P4 very small, second segment around 8.6 and 4.4 times as long as first segment, and 8.6 and 4 times as long as wide, respectively. P5 with baseoendopod and exopod fused; endopodal lobe a small pedestal with one naked seta and one tube-pore; exopod slender, 7.7 times as long as wide, with long subdistal tube-pore and three elements.

Description of female. Total body length, 670 μm measured from anterior outer corner of cephalothorax to posterior margin of caudal rami; length of caudal rami, 145 μm (ca. 22% total body length). Body cylindrical, tapering posteriorly, without clear demarcation between prosome and urosome; integument moderately chitinised; well-developed dendroid processes as for the genus (Fig. 7A). Cephalothorax with bilateral anterior constriction (Fig. 7A); anterior corners with sensory triplet consisting of two sensilla and associated tube-pore (Fig. 8C); with paired sensillate processes as follows: paired dorsal dentate conical processes anteriorly, pair of dentate processes lateroventrally accompanied by anterior small sensillum-bearing processes, and paired dorsal dendroid processes posteriorly seemingly without tube-pore (Fig. 7A). Rostrum fused to cephalothorax, absorbed into anteroventral surface of cephalothorax, with paired sensilla-bearing tubercles, and with well-developed midventral tube-pore (Figs 7A, 8C). P2-P4-bearing somites with conspicuous dorsal tube-pore; with paired dorsal dendroid processes as shown, each with a sensillum halfway along length of process (Fig. 7A). P5-bearing somite seemingly without dorsal tube-pore; with paired dorsal dendroid processes less developed than in preceding somites.

Second and third urosomites fused ventrally forming genital double-somite, distinct dorsally, with dorsal sensilla-bearing tubercles as shown (Fig. 7A, C); proximal half (second urosomite) of genital double somite without sensory ornamentation, genital field as shown (Fig. 7C); distal half (third urosomite) of genital double-somite with paired tube-pores and posterior sensilla as shown. Fourth urosomite with dorsal and ventral sensilla as shown, with set of four strong ventral spinules medially (Fig. 7C). Fifth urosomite without sensilla, posterior margin with fine spinules (Fig. 7A, B), dorsally with paired pores as shown, ventrally with set of medial strong spinules close to posterior margin (Fig. 7C). Anal somite (Fig. 7A, B, C) partly cleft medially; dorsally with rounded and smooth anal operculum, and two sensilla; with two anterolateral, and two posteroventral tube-pores; with few small spinules on ventral hind margin.

Caudal rami (Fig. 7A, B) elongate, divergent, close to 7.5 times as long as wide; with lateral tube-pore on proximal third of ramus; ornamented with spinules as shown; with seven setae; seta I and II arising half way along lateral margin of ramus, the former minute and ventral to the latter; seta III somewhat longer than seta II, arising in distal seventh; setae IV and V broken off in Fig. 7B; seta VI small, arising on distal inner corner; dorsal seta VII triarticulate, situated close to distal margin of ramus.

Antennule (Fig. 8A) three-segmented, segments elongate and slender. Armature formula as follows: 1-[9], 2-[8+(1+ae)], 3-[8+acrothek].

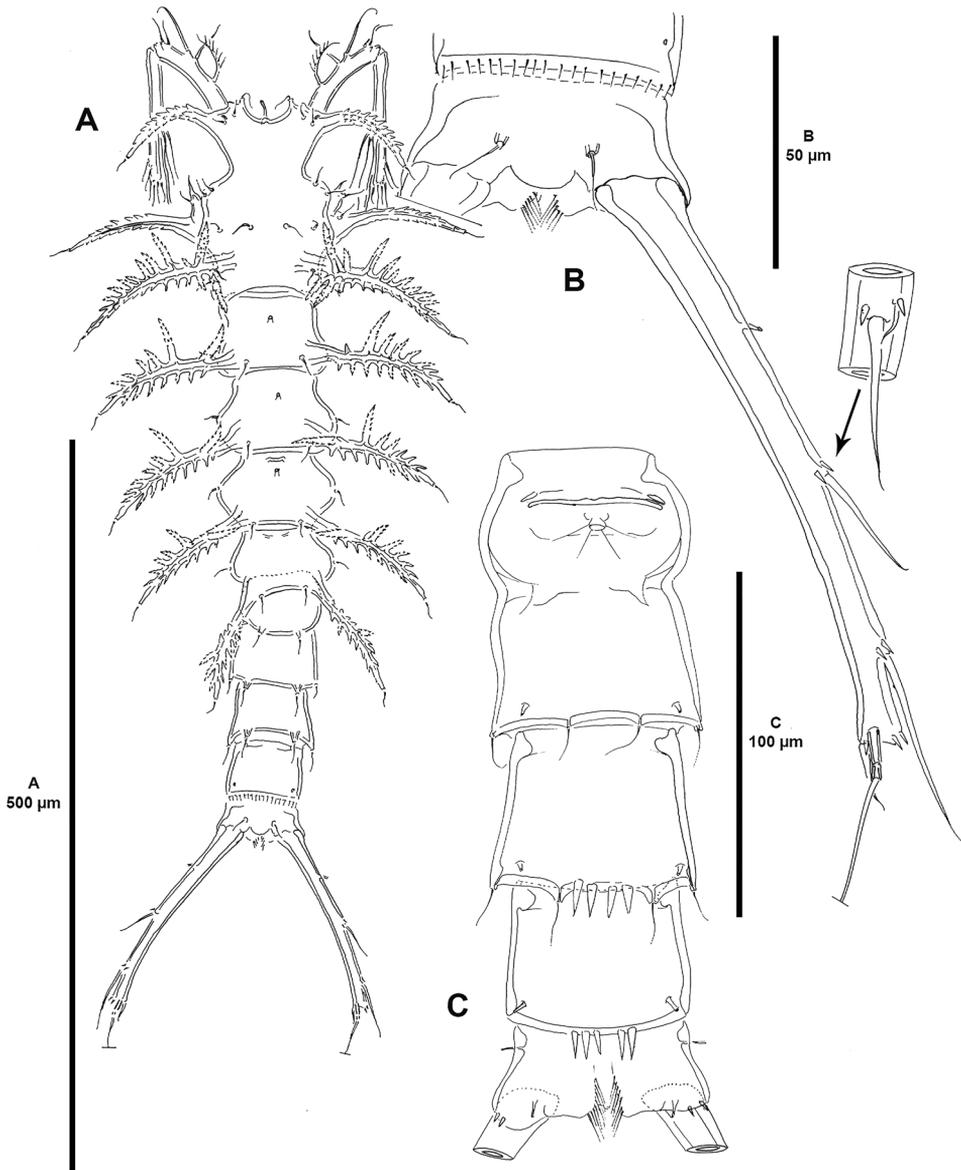


Figure 7. *Dendropsyllus californiensis* sp. n., female holotype. **A** habitus, dorsal **B** anal somite and right caudal ramus, dorsal, showing insert of lateral view of seta I and II **C** urosome, ventral, P5 bearing-somite and caudal rami omitted.

Antenna (Fig. 8B), with allobasis; original division of basis and first endopodal segment indicated by membranous insert; basal and endopodal halves with small inner spinules as shown; endopodal half with one reduced abexopodal seta. Exopod absent. Free endopodal segment with inner spinules and two pinnate spines; outer margin with two frills subdistally; apically with two pinnate spines, two pinnate geniculate

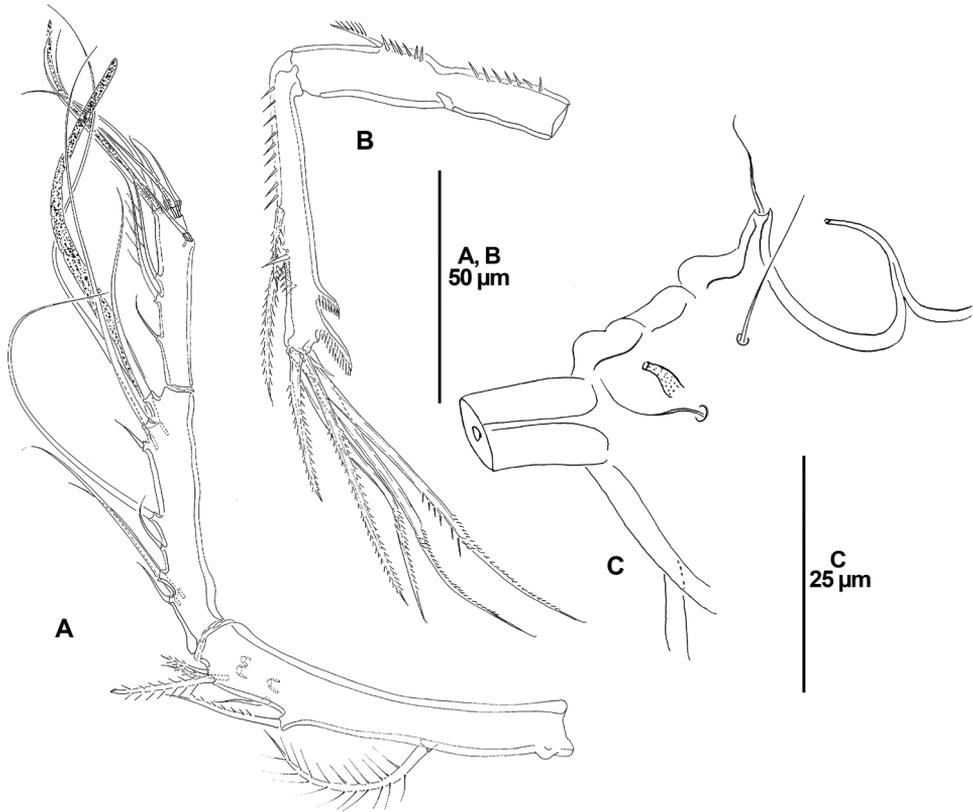


Figure 8. *Dendropsyllus californiensis* sp. n., female holotype. **A** antennule **B** antenna **C** distal outer corner of cephalothorax.

setae, and one pinnate geniculate seta with additional outer spinules halfway its length and fused basally to small seta.

Mandible (Fig. 9A) with robust coxa; gnathobase with teeth as figured, with two setae one of which bifid. Palp one-segmented, with spinules as shown, with two inner (basal), and three apical (endopodal) setae.

Maxillule (Fig. 9B) with quadrate praecoxal arthrite bearing two surface setae and five distal spines. Coxal endite with one spinulose and one bare seta, with some spinules distally. Proximal endite of basis with four, distal endite with two setae. Exopod represented by one long and one tiny seta. Endopod represented by three elements.

Maxilla (Fig. 9C). Syncoxa with spinulose patches as depicted; with two endites; proximal endite with three setae, one of which spinulose and basally fused to endite; distal endite with three spinulose elements. Allobasis drawn out into strong claw, the latter with subdistal spinules, accompanied by two outer elements, one strong spine, and two naked setae. Endopod very small, one-segmented, with two setae.

Maxilliped (Fig. 9D) subchelate. Syncoxa with some inner spinules apically and one spinulose seta on distal inner corner. Basis with spinules as depicted. Endopod drawn out into long spinulose spine with one accessory seta.

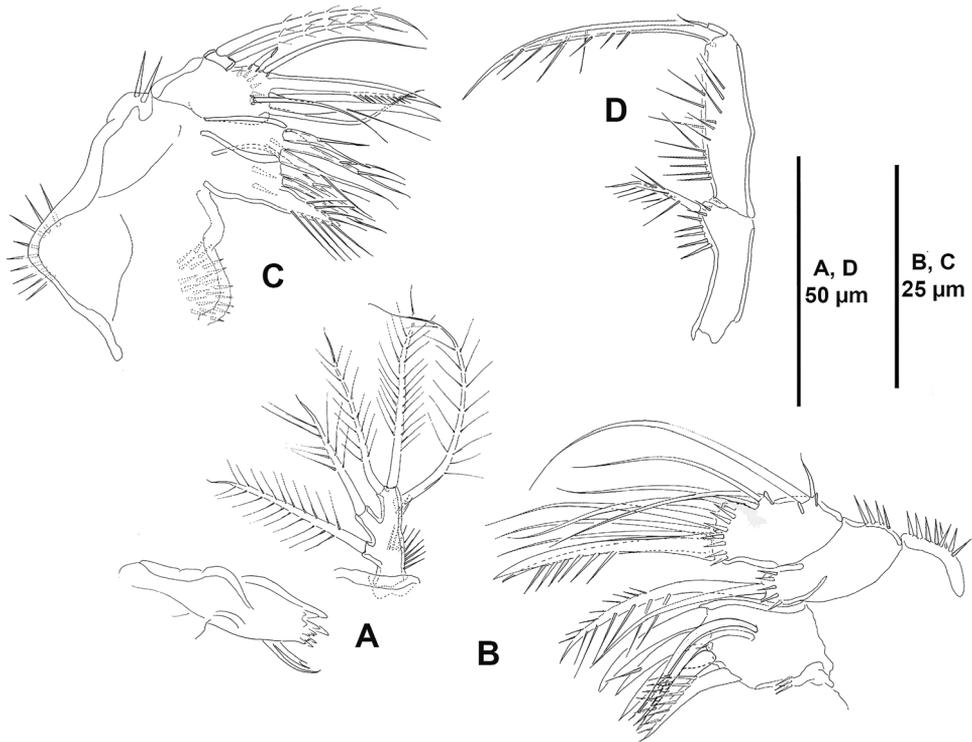


Figure 9. *Dendropsyllus californiensis* sp. n., female holotype. **A** mandible, showing detached palp **B** maxillule **C** maxilla **D** maxilliped.

P1 (Fig. 10A). Coxa trapezoid, with small lobate process bearing several spinules. Basis transversely elongate, with tube-pore midway along anterior margin, with one outer and one inner setae. Exopod two-segmented, ornamented with spinules and setules as depicted; first segment visibly shorter than second, with long outer pinnate spine; second segment elongate, without inner armature, with two apical geniculate setae, and with two outer geniculate elements and one bipinnate spine. Endopod two-segmented; first segment small, slightly longer than wide; second segment elongate, nearly 4.3 times as long as first segment, and almost 7.6 times as long as wide, with one apical seta.

P2-P4 (Fig. 10B–D). Coxa trapezoid, with outer lobate process ornamented with some spinules (as for P3, see Fig. 10C). Basis transversely elongate, with tube-pore close to outer seta, the latter bipinnate. Exopod three-segmented, exopodal segments with spinular ornamentation as shown; first segment without inner armature, with long bipinnate outer spine; second segment with inner seta and outer bipinnate spine; third segment of P2 and P3 with, of P4 without inner seta, with two apical setae and two outer bipinnate spines. Endopod of P2 absent; endopod of P3 and P4 two segmented, first segment very small, nearly as long as wide, second segment elongate, the

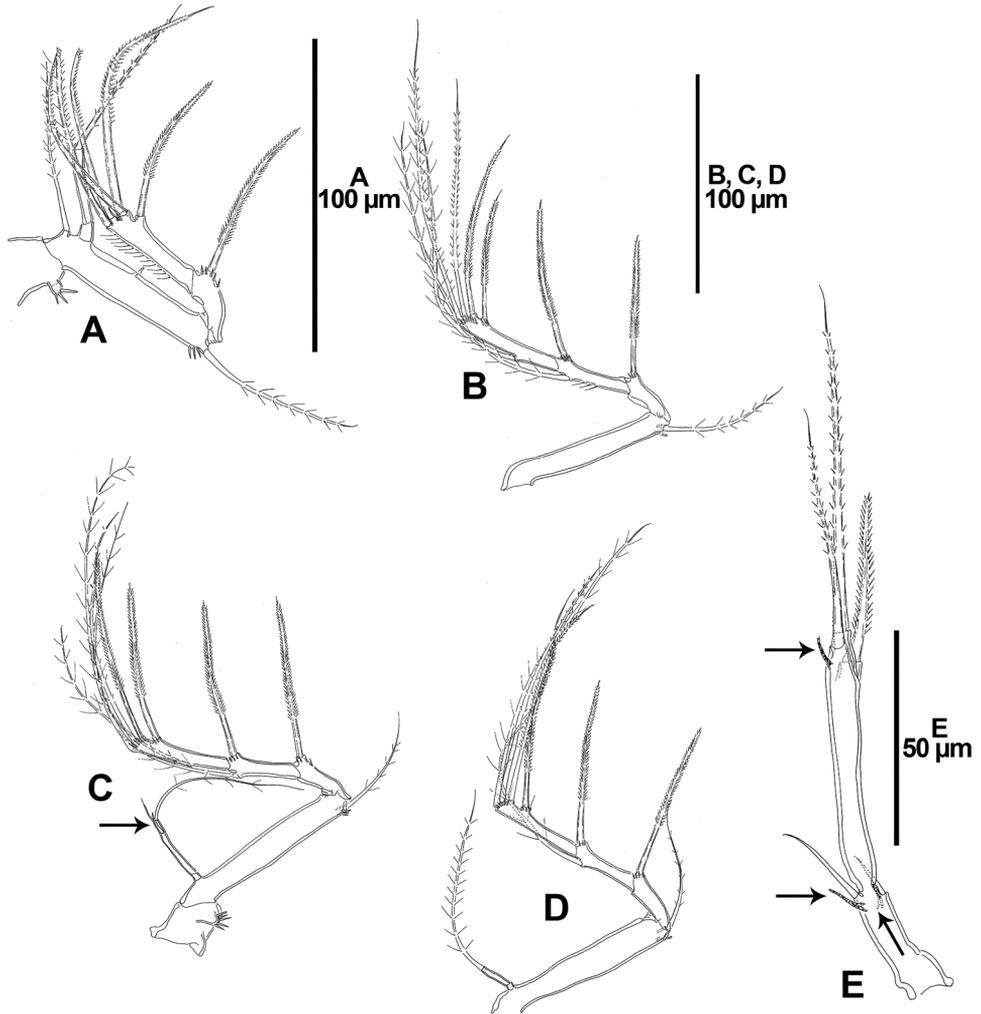


Figure 10. *Dendropsyllus californiensis* sp. n., female holotype. **A** P1, anterior **B** P2, anterior **C** P3, anterior, outer spine-like element of second endopodal segment indicated with an arrow **D** P4, anterior **E** P5, lateral, arrows showing tube pores.

latter 8.6 and 4.4 times as long as first segment and 8.6 and 4 times as long as wide in P3 and P4, respectively.

Armature formula as follows:

| | EXP | ENP |
|----|---------|--------|
| P1 | 0.023 | 0.010 |
| P2 | 0.1.122 | absent |
| P3 | 0.1.122 | 0.021 |
| P4 | 0.1.022 | 0.010 |

P5 (Fig. 10E) with fused baseopod and exopod; outer basal seta naked, with accompanying tube-pore. Endopodal lobe represented by small pedestal with one naked seta accompanied by tube-pore. Exopod slender, elongate, 7.7 times as long as wide, with long subdistal tube-pore, with one outer, one distal (longest) and one inner (shortest) element.

Description of male. Unknown.

Etymology. The specific epithet, *californiensis*, makes reference to the Gulf of California, where the species was found.

Discussion

Conroy-Dalton and Huys (2000) defined the *Ancorabolus*-group (*Ancorabolus*, *Arthropysyllus*, *Breviconia*, *Juxtaramia* and *Uptionyx*) and one year later, Conroy-Dalton (2001) defined the *Ceratonotus*-group composed by nine species and one subspecies within the genera *Ceratonotus*, *Dorsiceratus* and *Polyascophorus*, sharing a suite of seven synapomorphies (Conroy-Dalton 2001: 182). In that same paper, Conroy-Dalton (2001) provided enough evidence to support the monophyly of the genus *Ceratonotus* for which she identified nine apomorphies (Conroy-Dalton 2001, Fig. 8, Table 1) and divided the genus into two geographically separated clades, the sub-Antarctic *antarcticus-magellanicus* pair composed of *C. antarcticus* George & Schminke, 1998 and *C. magellanicus* George & Schminke, 1998 described from the Straits of Magellan (Chile) and an European group composed of *C. coineau* Soyer, 1965, *C. pectinatus pectinatus* Sars, 1909, and *C. pectinatus elaphus* Por, 1965 (Conroy-Dalton 2001: 187, Fig. 8). The *antarcticus-magellanicus* pair was defined by the relative size and general shape of the processes on the cephalic shield, the armature formula of the P1ENP (with one seta only), the loss of the P2ENP, and the lack of the inner seta on the P4EXP3 (and probably the P3EXP3 also) (Conroy-Dalton 2001). On the other hand, the European group was defined by the presence of dorsal dendroid processes on the posterior half of the female genital double-somite (Conroy-Dalton 2001).

Later, Conroy-Dalton (2003) created the genus *Dendropsyllus* based on the combination of seven apomorphies (Conroy-Dalton 2003: 92) to accommodate *C. magellanicus* (= *D. magellanicus*) and *C. antarcticus* (= *D. antarcticus*) from the Straits of Magellan, and a new species, *D. thomasi*, found near the base of the Coronado Escarpment, San Diego Trough in the north Pacific Ocean (Conroy-Dalton 2003). Also, Conroy-Dalton (2003) presented the diagnosis for the genus *Ceratonotus* in which she included the Norwegian type species, *C. pectinatus*, *C. coineau* from Banyuls-sur-mer (French Mediterranean coast), and two new species, *C. concavus* Conroy-Dalton, 2003 from the East Mediterranean coast of Hadera (Israel), and *C. thistlei* from the Coronado Escarpment (San Diego Trough, north Pacific Ocean). Still later, George (2006a, b, c) published a series of articles on the taxonomy and systematics of the Ancorabolinae. In George (2006b), three new species of *Ceratonotus* are described, *C. tauroides* George,

2006 from the Arctic Laptev Sea, and *C. steiningeri* George, 2006 and *C. vareschii* George, 2006 from the Angola deep-sea basin in the south Atlantic off Namibia, as well as the male of *D. magellanicus* found in the Chilean Pacific continental slope off Chiloé Island. As a result, George (2006b: 118) made some amendments to Conroy-Dalton's (2003) generic diagnosis of *Ceratonotus* (at present composed of seven species, *C. coineaui*, *C. concavus*, *C. steiningeri*, *C. tauroides*, *C. thistlei*, *C. vareschii*, and its type species, *C. pectinatus*), and reduced the number of apomorphies for the genus to only one, namely, the presence of dendroid dorsal processes on the male first abdominal somite (abdominal half of genital double-somite in the female). George (2006b) concluded also that the seven apomorphies identified for *Dendropsyllus* by Conroy-Dalton (2003) should be reduced to four, namely, the presence of four geniculate setae on P1EXP2, one seta only on P1ENP2, one inner seta only on P3EXP3, and lack of inner armature on P4EXP3. The other three apomorphies for *Dendropsyllus* identified by Conroy-Dalton (2003) turned out to be synapomorphies for *Ceratonotus* and *Dendropsyllus* (George 2006b: 120). Moreover, given the high number of synapomorphies for *Ceratonotus* and *Dendropsyllus*, and the low number of autapomorphies for each genus, George (2006b) questioned the preservation of the latter genus.

The first species proposed herein, *C. elongatus* sp. n., can be undoubtedly attributed to *Ceratonotus* given a) the presence of dendroid processes on the posterior margin of the cephalothorax and on pedigerous somites 2-5, b) the presence of a simple conical lateroventral processes on each side of the cephalothorax, c) the presence of three geniculate setae on P1EXP2, d) the two-segmented condition of P2ENP, e) the presence of two apical setae on P2ENP2, f) the presence of two inner elements on P3EXP3, g) the discrete condition of the P5EXP, h) the insertion site of the caudal setae I and II (inserted in proximal third of caudal rami), and i) the presence of dendroid processes on the first abdominal somite (second half of the double genital-somite).

Ceratonotus elongatus sp. n. from the Guaymas Basin and *C. thistlei* from the San Diego Trough are similar in several respects, and a close relationship between these two species is hypothesised. The description of *C. elongatus* sp. n. is based on one female only, making the assessment of intraspecific variability impossible. Based on the present description, *C. elongatus* sp. n. and *C. thistlei* can be separated by the relative length of the two segments of the P2ENP (ENP2 1.6 times as long as ENP1 in *C. thistlei*, but ENP2 nearly 2.7 times as long as ENP1 in the new species) and P4ENP (subequal in *C. thistlei*, but ENP1 twice as long as ENP2 in *C. elongatus* sp. n.), by the armature formula of the second antennary segment ($7+(1+ae)$ in the new species, but $6+(1+ae)$ in *C. thistlei*), by the relative length of the caudal rami (11 times as long as wide in *C. elongatus* sp. n., but nine times as long as wide in *C. thistlei*), by the total body length (920 μm in *C. elongatus* sp. n., but 677 μm in *C. thistlei*), and above all, by the elongated sensilla-bearing dorsal tubercles on the second abdominal somite. Similar but somewhat smaller dorsal tubercles are known also for *C. pectinatus*, and comparatively longer tubercles have been observed for *C. concavus*, *C. tauroides*, *C. vareschii*, and *C. steiningeri*, being extremely elongated in the latter. The presence of these tubercles is not exclusive for *Ceratonotus*, and similar but smaller tubercles have

been observed also for *D. thomasi* and *D. magellanicus*. Similar tubercles have not been observed for *C. coineaui*, *C. thistlei*, and *D. antarcticus*. The dorsal dendroid processes of *C. elongatus* sp. n. seems to be longer than in *C. thistlei*. However, the comparatively shorter processes of *C. thistlei* may be an artefact of the position of the body when observed in dorsal view, making the processes look shorter than they really are.

Conroy-Dalton (2003) did not observe any intraspecific variability in the four *Ceratonotus* females (one female holotype, and three female paratypes) upon which she based her description of *C. thistlei*. Unfortunately, the description of *C. elongatus* sp. n. is based on a single female and nothing can be said about the intraspecific variability of the species. Besides the differences noted above between *C. thistlei* and *C. elongatus* sp. n., the proposal of a new species of *Ceratonotus* from the Southern Trough of the Guaymas Basin is founded also on its relatively isolated situation, assuming that the Baja California Peninsula acts as an effective geographical barrier for the dispersal of these two species, thus preventing any gene flow.

Ceratonotus elongatus sp. n. and *C. thistlei* can be separated from their congeners by a combination of several characters, i.e. the presence/absence of anterior horn-like processes of the cephalothorax (absent in *C. elongatus* sp. n., *C. thistlei*, *C. coineaui* and *C. pectinatus*, but present in *C. tauroides*, *C. steiningeri*, *C. vareschii* and *C. concavus*), the general shape and degree of development of the dorsal dendroid processes (visibly more developed in *C. elongatus*, *C. thistlei*, and *C. concavus*, than in the other species), the armature formula of the second and third antennular segments of the female (the females of *C. concavus* and *C. vareschii* remain unknown; the armature formula of second segment, 7+(1+ae) in *C. elongatus* and *C. tauroides*, but 6+(1+ae) in *C. pectinatus*, *C. thistlei*, and *C. steiningeri*; the armature formula of third segment, 9+acrothek in *C. elongatus*, *C. pectinatus*, and *C. thistlei*, but 8+acrothek in *C. tauroides* and *C. steiningeri*), by the spinulose nature of the outer margin of the endopodal segment of the antenna (without spinules in *C. elongatus*, *C. thistlei*, *C. coineaui*, and *C. pectinatus*, but with dense patch of fine spinules in *C. tauroides*, *C. steiningeri* and *C. vareschii*), the number of elements on the first endite of the maxillary syncoxa (with two elements in *C. vareschii*, but with three elements in the other species), by the nature of the spinular ornamentation of the basis of the maxilliped (densely covered with fine spinules in *C. vareschii* and *C. steiningeri*, but with comparatively fewer spinules in the other species), by the general shape of their antennules and antennary segments (comparatively more elongate and slenderer in *C. elongatus* sp. n. and *C. thistlei* than in the other species), the relative length of the outer basal element of P1 (visibly longer than basis in *C. elongatus* sp. n., *C. thistlei*, *C. tauroides*, *C. vareschii*, and probably *C. steiningeri*, but relatively shorter in the other species), by the presence/absence of P2ENP (present in *C. elongatus* sp. n., *C. thistlei*, *C. coineaui*, *C. pectinatus*, *C. concavus*, *C. tauroides*, and *C. vareschii*, but absent in *C. steiningeri*), by the one- or two-segmented condition of P4ENP (one-segmented in *C. coineaui* and *C. pectinatus*, but two-segmented in *C. elongatus* sp. n., *C. thistlei*, *C. tauroides*, *C. steiningeri*, *C. vareschii* and *C. concavus*), by the armature formula of P4ENP (with one seta on P4ENP2 in *C. elongatus* sp. n., *C. thistlei*, *C. tauroides*, and *C. steiningeri*, with two setae on P4ENP2 in *C. concavus* and

C. vareschii, and with one seta on the only segment of P4ENP in *C. coineau* and *C. pectinatus*), and by the relative length of the caudal rami (11 times as long as wide in *C. elongatus* sp. n. and *C. steiningeri*, but nine and eight times as long as wide *C. vareschii* and *C. concavus*, respectively, 7.6 times as long as wide in *C. thistlei*, and 6.8, 6.6 and 6.5 times as long as wide in *C. coineau*, *C. pectinatus* and *C. tauroides*, respectively).

The second species proposed herein, *D. californiensis* sp. n., has been unequivocally placed within the genus *Dendropsyllus* given a suite of characters defined by George (2006b: 120) as the only four apomorphies for the genus, namely, the presence of four geniculate setae on P1EXP2 and only one seta on P1ENP2, one inner seta only on P3EXP3, and lack of inner armature on P4EXP3. As noted above, the genus *Dendropsyllus* is composed of three species only, *D. antarcticus* from the Straits of Magellan (George and Schminke 1998), *D. magellanicus* known from the Straits of Magellan (George and Schminke 1998) and from the Chilean Pacific continental slope off Chiloé Island (George 2006b), and *D. thomasi* known only from the base of the Coronado Escarpment in the San Diego Trough (Conroy-Dalton 2003). Conroy-Dalton (2003) suggested a close relationship between *D. thomasi* and *D. magellanicus* based on the nature of the anterolateral and lateroventral cephalic processes, the spinulose nature of the maxilliped, the two-segmented P4ENP, and general shape of the female P5 (fused condition of the baseoendopod and exopod). Nevertheless, Conroy-Dalton (2003) was able to separate *D. thomasi* from the other two congeners by the elongate dendroid body processes (comparatively longer in *D. thomasi*, than in the Chilean species), by the slender and elongate first antennular segment (7.8, 4.9, and 7.1 times as long as wide in *D. thomasi*, *D. magellanicus*, and *D. antarcticus*, respectively), and by the extreme elongation of the caudal rami (12.8, 8, and 7 times as long as wide in *D. thomasi*, *D. magellanicus*, and *D. antarcticus*, respectively). *Dendropsyllus californiensis* sp. n. seems to be more closely related to *D. thomasi* and *D. magellanicus* than to *D. antarcticus* on account of the spinulose nature of the basis of the maxilliped, the two-segmented P4ENP, and the fused condition of the P5 baseoendopod and exopod. On the other hand, *D. californiensis* sp. n. seems to be more related to *D. thomasi* by the degree of development of the lateroventral processes of the cephalothorax which seem to be longer than the posterodorsal processes in these two species than in the two Chilean representatives. The new species proposed herein, *D. californiensis* sp. n., can be separated from its congeners by the relative length of the first segment of the antennule (6.5 times as long as wide) and by the relative length of the caudal rami (7.5 times as long as wide), and above all, by the presence of a small outer spine-like element on the second endopodal segment of P3. Note that in Fig. 10C, this element appears to be located just in front of the segment. This could be an artefact of the mounting process since this spine-like element is clearly situated along the outer margin of the segment as observed on the not dissected P3.

The genus *Dendropsyllus*, when found, occurs at very low densities (for example, one single specimen of *D. californiensis* sp. n. was found in the present study), and most species of the genus are known from one sex only (Conroy-Dalton 2003), which prevents any phylogenetic analysis (George 2006b). So far, *Dendropsyllus magellanicus* is the only species for which both sexes are known. The species was originally described based on one female only (George and Schminke 1998), and some years later George

(2006b) described the male. With this record, George (2006b) was able to observe the expression of sexual dimorphism in the antennule (six-segmented, subchirocer), P3ENP (three-segmented, with long inner apophysis on ENP2), P4ENP (two-segmented, both segments small and subequal), and P5 (baseoendopod and exopod separated). The outer spine-like element observed on the P3ENP2 of *D. californiensis* sp. n., is considered here as a novel, autapomorphic element for the species and not homologous to the inner apophysis observed for the male of *D. magellanicus*.

The formation of the Gulf of California is a very recent and complicated process that began between 130 and 90 mya during the Cretaceous when the Farallon Plate started to subduct eastward from the East Pacific Rise under the North American Plate, while the latter was moving slowly westward (Ledesma-Vázquez and Carreño 2012). Following Ledesma-Vázquez and Carreño's (2012) scheme, it seems reasonable to hypothesise that during the Cretaceous, the location where Conroy-Dalton (2003) found *C. thistlei* and *D. thomasi* was situated 330–400 km south of its current position. This suggests that these two species may be present off the entire Baja California Peninsula. The original populations of these species may have invaded the northern Gulf of California through an early proto-Gulf of California marine incursion during the early late Miocene from 14 to 12 mya (Helenes and Carreño 1999, Ledesma-Vázquez 2002) and during the first great incursion of the Pacific Ocean in the late Miocene-earliest Pliocene 8.2-7.5 mya (Ledesma-Vázquez and Carreño 2012). Following Nagy and Stock (2000), spreading of the Gulf of California began in the early Pliocene around the mouth of the Gulf, and the Guaymas Basin opened 2.1 mya. If this scenario is assumed to be correct, the speciation of the genera *Ceratonotus* and *Dendropsyllus* may have been potentiated on one hand, by the formation of the Gulf of California and subsequent geographic isolation by the consolidation of the Baja California Peninsula, and on the other hand, by the subsequent movement of the Pacific Plate north-westwards.

Acknowledgements

This study was financed by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) of the Dirección General de Asuntos del Personal Académico of the Universidad Nacional Autónoma de México (UNAM-DGAPA-PAPIIT), project IN202116 Distribución y riqueza de comunidades de microinvertebrados poco conocidos del Golfo de California, and IN217306-3 Biocenosis de invertebrados bentónicos y pelágicos en aguas profundas del Pacífico Mexicano en relación con las condiciones ambientales. Ship time was provided by the Coordinación de la Investigación Científica, UNAM. The authors thank all scientists, students and crew members for their help and support during the Talud X cruise. The authors are grateful to Dr. Ray Gerber (Saint Joseph's College of Maine, Department of Biology) for his style and grammar review to the first draft of this manuscript, and to Dr. Kai Horst George and one anonymous referee for their constructive criticism and review of the first version of the manuscript.

References

- Álvarez-Castillo L, Hermoso-Salazar M, Estradas-Romero A, Prol-Ledesma RM, Pardos F (2015) First records of Kinorhyncha from the Gulf of California: Horizontal and vertical distribution of four genera in shallow basins with CO₂ venting activity. *Cahiers de Biologie Marine* 56: 271–281.
- Brusca RC, Hendrickx ME (2008) The Gulf of California Invertebrate Database: The invertebrate portion of the Macrofauna Golfo Database. <http://www.desertmuseum.org/center/seaofcortez/database.php> [September 30, 2016]
- Brusca RC, Hendrickx ME (2012) Invertebrate biodiversity and conservation in the Gulf of California. In: Brusca RC (Ed.) *The Gulf of California, biodiversity and conservation*. The University of Arizona Press and The Arizona-Sonora Desert Museum, Tucson, 72–95.
- Conroy-Dalton S (2001) Systematics and phylogeny of the Ancorabolidae (Copepoda: Harpacticoida). II. Polyphyly of *Polyascophorus* and description of *Arthuricornua*, new genus. *Journal of Crustacean Biology* 21: 170–191. <https://doi.org/10.1163/20021975-99990115>
- Conroy-Dalton S (2003) Systematics and phylogeny of the Ancorabolidae (Copepoda: Harpacticoida). III. Description of two new species of *Ceratonotus* Sars and *Dendropsyllus*, new genus. *Journal of Crustacean Biology* 23: 69–93. <https://doi.org/10.1163/20021975-99990319>
- Conroy-Dalton S, Huys R (2000) Systematics and Phylogeny of the Ancorabolidae (Copepoda: Harpacticoida). I. The *Ancorabolus*-lineage, with the description of three new genera. *Cahiers de Biologie Marine* 41: 343–397.
- George KH (2006a) Ancorabolinae Sars (Copepoda: Harpacticoida: Ancorabolidae) of the deep Atlantic Ocean. *Ancorabolina chimaera* gen. et sp. nov. including remarks to ancorabolid phylogeny and to the evolution of the first natatorial leg in comparison with Laophon-toidea T. Scott. *Meiofauna Marina* 15: 157–176.
- George KH (2006b) New Ancorabolinae Sars, 1909 (Copepoda: Harpacticoida: Ancorabolidae) of the Atlantic and the Pacific Ocean. The taxa *Ceratonotus* Sars, and *Dendropsyllus* Conroy-Dalton*. *Meiofauna Marina* 15: 87–122.
- George KH (2006c) New Ancorabolinae Sars, 1909 (Copepoda: Harpacticoida: Ancorabolidae) of the Atlantic Ocean. Description of *Pseudechinopsyllus sindemarkae* gen. et sp. nov. and *Dorsiceratus ursulae* sp. nov. from the Great Meteor Seamount, and redescription of *D. octocorni*. *Meiofauna Marina* 15: 123–156.
- George KH, Schminke HK (1998) First records of the genus *Ceratonotus* G.O. Sars, 1909 (Copepoda, Ancorabolidae) from the Southern Hemisphere, with the description of two new species. *Crustaceana*. 71: 801–817. <https://doi.org/10.1163/156854098X00059>
- Gómez S, Conroy-Dalton S (2002) Description of *Ancorabolus hendrickxi* sp. nov. (Copepoda: Harpacticoida: Ancorabolidae) from the neotropics and notes on caudal ramus development within oligoarthran harpacticoids. *Cahiers de Biologie Marine* 43: 111–129.
- Gómez S, Morales-Serna FN (2014) Updated checklist of published and unpublished records of harpacticoid copepods (Crustacea: Copepoda: Harpacticoida) from Mexico. *Proceedings of the Biological Society of Washington* 127: 99–121. <https://doi.org/10.2988/0006-324X-127.1.99>

- Helenes J, Carreño AL (1999) Neogene sedimentary evolution of Baja California in relation to regional tectonics. *Journal of South American Earth Sciences* 12: 589–605. [https://doi.org/10.1016/S0895-9811\(99\)00042-5](https://doi.org/10.1016/S0895-9811(99)00042-5)
- Holovachov O, De Ley IT, Mundo-Ocampo M, Gingold R, De Ley P (2009) Nematodes from the Gulf of California. Part 3. Three new species of the genus *Diplopeltoides* Gerlach, 1962 (Nematoda: Diplopeltoididae) with overviews of the genera *Diplopeltis* Gerlach, 1962 and *Diplopeltula* Gerlach, 1950. *Russian Journal of Nematology* 17: 43–57.
- Holovachov O, Tandingan De Ley I, Mundo-Ocampo M, Baldwin JG, Rocha-Olivares A, De Ley P (2008) Nematodes from the Gulf of California. Part 1. The genera *Ceramonema* Cobb, 1920, *Pselionema* Cobb in Cobb, 1933 and *Pterygonema* Gerlach, 1954 (Nematoda: Ceramonematidae). *Nematology* 10: 347–373. <https://doi.org/10.1163/156854108783900311>
- Huys R, Boxshall GA (1991) Copepod evolution. The Ray Society, London, 468 pp.
- Ledesma-Vázquez J (2002) A gap in the Pliocene invasion of seawater, Gulf of California. *Revista Mexicana de Ciencias Geológicas* 19: 145–151.
- Ledesma-Vázquez J, Carreño AL (2012) Origin, age, and geological evolution of the Gulf of California. In: Brusca RC (Ed.) *The Gulf of California, biodiversity and conservation*. The University of Arizona Press and The Arizona-Sonora Desert Museum, Tucson, 7–23.
- Mundo-Ocampo M, Lamshead PJD, Debenham N, King IW, De Ley P, Baldwin JG, De Ley IT, Rocha-Olivares A, Waumann D, Thomas WK, Packer M, Boucher G (2007) Biodiversity of littoral nematodes from two sites in the Gulf of California. *Hydrobiologia* 586: 179–189. <https://doi.org/10.1007/s10750-006-0624-z>
- Nagy A, Stock M (2000) Structural controls on the continent-ocean transition in the northern Gulf of California. *Journal of Geophysical Research* 105: 16251–16269. <https://doi.org/10.1029/1999JB900402>
- Pereira TJ, Fonseca G, Mundo-Ocampo M, Guilherme BC, Rocha-Olivares A (2010) Diversity of free-living marine nematodes (Enoplida) from Baja California assessed by integrative taxonomy. *Marine Biology* 157: 1665–1678. <https://doi.org/10.1007/s00227-010-1439-z>
- Por FD (1965) Harpacticoida (crustacea, Copepoda) from muddy bottoms near Bergen. *Sarsia* 21: 1–16. <https://doi.org/10.1080/00364827.1965.10409556>
- Ryan WBF, Carbotte SM, Coplan JO, O'Hara S, Melkonian A, Arko R, Weissel RA, Ferrini V, Goodwillie A, Nitsche F, Bonczkowski J, Zensky R (2009) Global multi-resolution topography synthesis. *Geochemistry, Geophysics, Geosystems* 10: Q03014. <https://doi.org/10.1029/2008GC002332>
- Sars, GO (1909) Copepoda Harpacticoida Parts XXVII & XXVIII. Cletodidae (concluded), Anchorabolidae, Cyliandrocyllidae, Tachidiidae (part). *An account of the Crustacea of Norway with short descriptions and figures of all the species* 5: 305–336.
- Soyer J (1965) Copépodes harpacticoides de l'étage bathyal de la région de Banyuls-sur-mer. IV. La sous-famille des Ancorabolinae Lang. *Vie et Milieu* 15: 329–340.

New record of a phoretic flea associated with earwigs (Dermaptera, Arixeniidae) and a redescription of the bat flea *Lagaropsylla signata* (Siphonaptera, Ischnopsyllidae)

Michael W. Hastriter¹, Kelly B. Miller², Gavin J. Svenson³,
Gavin J. Martin^{3,4}, Michael F. Whiting^{1,4}

1 Monte L. Bean Life Science Museum, Brigham Young University, 290 MLBM, P.O. Box 20200, Provo, Utah 84602-0200, USA **2** Department of Biology and Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA **3** The Cleveland Museum of Natural History, 1 Wade Oval Drive, Cleveland, Ohio 44106, USA **4** Department of Biology, Brigham Young University, Provo, Utah 84606, USA

Corresponding author: Michael W. Hastriter (michaelhastriter@comcast.net)

Academic editor: P. Stoev | Received 8 November 2016 | Accepted 6 February 2017 | Published 17 February 2017

<http://zoobank.org/B18920F9-6D1B-487F-8112-93C4E1E058DC>

Citation: Hastriter MW, Miller KB, Svenson GJ, Martin GJ, Whiting MF (2017) New record of a phoretic flea associated with earwigs (Dermaptera, Arixeniidae) and a redescription of the bat flea *Lagaropsylla signata* (Siphonaptera, Ischnopsyllidae). ZooKeys 657: 67–79. <https://doi.org/10.3897/zookeys.657.11095>

Abstract

Lagaropsylla signata (Wahlgren, 1903), previously known only from the Island of Java, Indonesia is re-described and reported for the first time in Deer Cave, Gunung Mulu National Park, Sarawak, Malaysia (west coast of Borneo). Many were found clinging to the earwig *Arixenia esau* Jordan, 1909. A similar account of a phoretic flea (*Lagaropsylla turba* Smit, 1958) on the same species of cave-dwelling earwig has been reported in peninsular Malaysia in a well-documented association with the hairless naked bulldog bat, *Cheiromeles torquatus* Horsfield, 1824. The association of *L. signata* with *A. esau* is parallel to the evolution and co-existence with bats in Deer Cave just as in the case of *L. turba*, *A. esau*, and *C. torquatus*. The evidence suggests that *L. turba* and *L. signata* are obligate phoretic parasites whose survival depends on *A. esau* to access a bat host. *Arixenia esau* is reported for the first time in Deer Cave and the occurrence of *L. signata* on the island of Borneo represented a new record, previously being found only on the island of Java. Images of *L. signata* attached to *A. esau* are provided. *Xeniaria jacobsoni* (Burr, 1912), often associated with *A. esau* in other geographical areas, was not present in the material examined from Deer Cave. The natural history of the earwig genera *Arixenia* Jordan, 1909 and *Xeniaria* Maa, 1974 are discussed and summarized relative to their associations with phoretic fleas and their bat hosts.

Keywords

Arixenia esau, Deer Cave, Gunung Mulu National Park, insect phoresy, *Lagaropsylla turba*, *Xeniaria jacobsoni*

Introduction

Phoresy occurs in some insects and arachnids in which one species attaches to another species in a commensal relationship for the purpose of increasing their ability to disperse from one place to another. There is seldom a detrimental effect on the transporting host species. In some cases, attachment to another species is accidental, while others have evolved into vital components of their life history. Although there is an account of a bird flea attaching to a wasp (Rothschild & Clay, 1952), this was attributed to an accidental association of a wasp foraging on a flea-infested avian carcass. *Lagaropsylla turba* Smit, 1958 is the only documented species of flea that truly demonstrates phoretic behavior for which there was an association between a flea, another insect (*A. esau* Jordan, 1909), and a bat host (the naked bulldog bat *Cheiromeles torquatus* Horsfield, 1824). In this scenario, an earwig provides a vehicle for *L. turba* to come into contact with its only known host, *C. torquatus*. Nakata and Maa (1974) provided a summary of the commensal forms of the dermapterid suborder Arixeniina and their associated molossid bat species. Important works cited by Nakata and Maa (1974) included: Jordan (1909), Jacobson (1912), Burr and Jordan (1913), Audey (1952), Cloudsley-Thompson (1957), Medway (1958), Giles (1961), and Popham (1962). Medway (1958) and Marshall (1977) described some crucial bionomical inter-relationships relative to *L. turba*, *A. esau*, and *C. torquatus*, while Marshall (1981) discussed similar parallel behaviors of co-existing associations between bird lice, hippoboscids, and their avian hosts. We reported herein a second flea species [*Lagaropsylla signata* (Wahlgren, 1903)] that has a similar phoretic association with *A. esau*. We also redescribed *L. signata* and further documented a new locality for both *L. signata* and *A. esau*.

Materials and methods

Earwigs were collected from the floor of Deer Cave while conducting a general insect survey of Gunung Mulu National Park in October 2006 (04°01'18"N, 114°49'24"E) (KBM & GJS) and in January 2009 (04°02'32.8"N, 114°48'49.6"E) (GJS). Specimens were collected in the same location deep within the main gallery of the cave in the early evening. No earwigs were found in the entrance area and path through the first half of the cave, although mixed rock and guano piles were present. Approximately 1000 m and within sight of the "Garden of Eden doline" (a cave ceiling collapse that allows light to enter the main gallery), the path climbed an isolated, cone-shaped hill. Very few earwigs were present at the base of this hill, but numbers increased with elevation. The top of the hill, the observation path, and railings included earwigs. Thousands were present in October 2006 and only a few were present in January 2009. Individuals were observed actively walking, mating, and resting in small cracks. Males, females

and nymphs were collected using forceps to overcome their strong grip on the surface of rocks, railings, and clothing. The greatest concentrations of individuals were at the highest points on the hilltop, which suggests a negative geotactic behavior. Individuals were not observed in the area around the base of the hill and nearby cave walls.

All specimens were collected into and stored in 95% ethanol and later examined in the laboratory. During microscopic examination, many fleas were noted attached to the earwigs. One species of earwig was present and the mouthparts and genitalia were dissected to facilitate and confirm our identification. Earwigs were photographed in ethanol with the aid of a Canon 6D DSLR camera and Visionary Digital Passport II imaging system. Image stacks were montaged with Zerene Stacker v.1.04. Fleas were mounted on microscope slides in accordance with procedures outlined by Hastriter and Whiting (2003:1043) and were illustrated with the aid of an Olympus BX61 Compound Microscope and an Olympus CC12 digital camera accompanied with an Olympus Microsuite B3SV program. All images were edited in Adobe Photoshop CC 2015. Earwigs and fleas were deposited in insect collections at the Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah (three *A. esau*; MH-905: slides 7♂, 3♀ fleas, many fleas in alcohol), the Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico (99 *A. esau*; MH-905: slides 2♂, 2♀ fleas), and the Cleveland Museum of Natural History, Cleveland, Ohio (9 *A. esau*; MH-905: slides 2♂, 2♀ fleas).

Results

Siphonaptera

Ischnopsyllidae

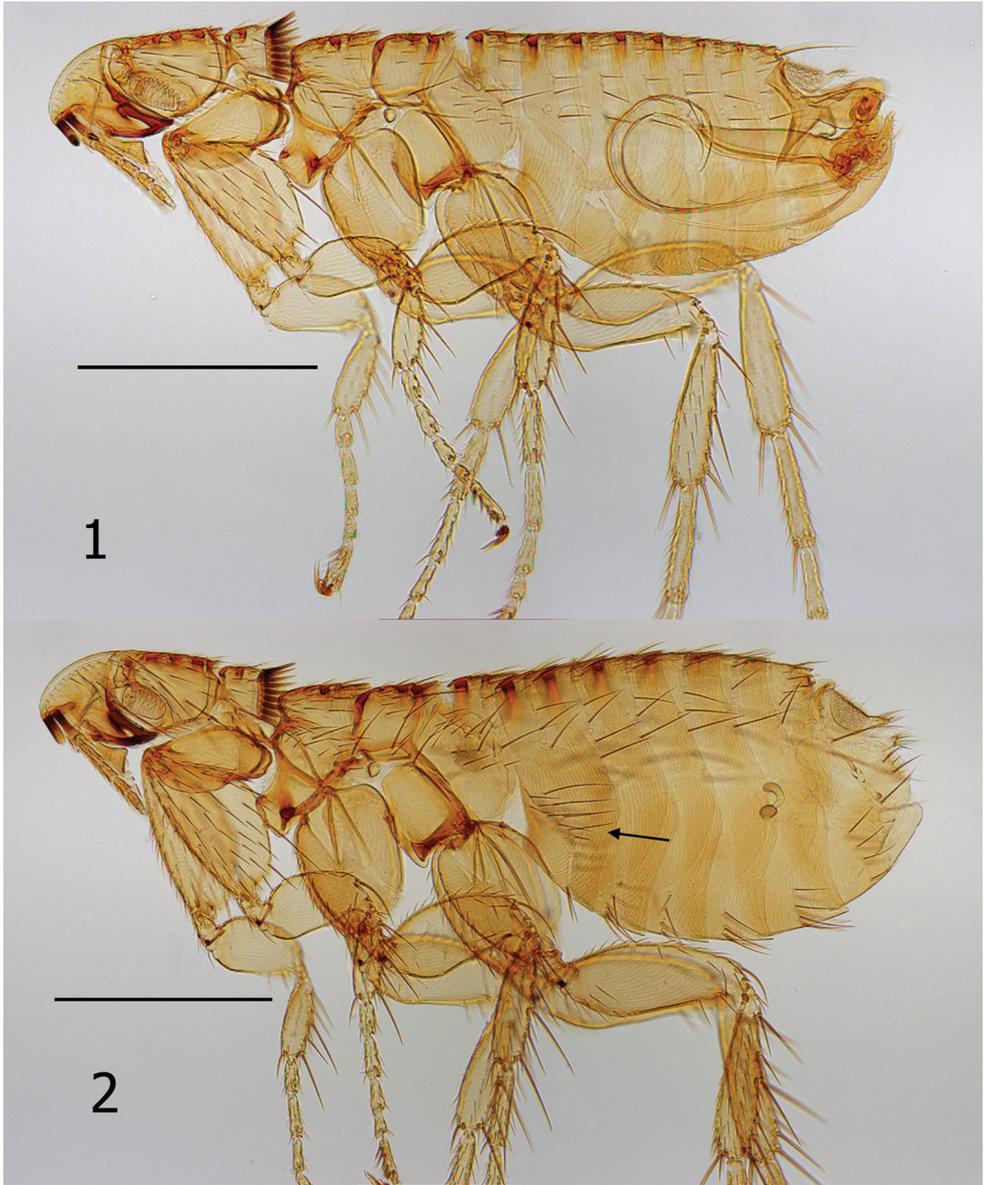
Ischnopsyllinae

Lagaropsylla signata (Walgren, 1903)

Figs 1–9

Type species. *Ceratopsylla signata* Walgren, 1903, Banjuwangi, Java, 22 V 1899, Carl Aurivillius, *Nyctinomus plicatus* [= *Chaerophon plicata* (Buchanan, 1800)] [number or sex of specimens in type series not recorded] (Swedish National Museum, Stockholm, not examined).

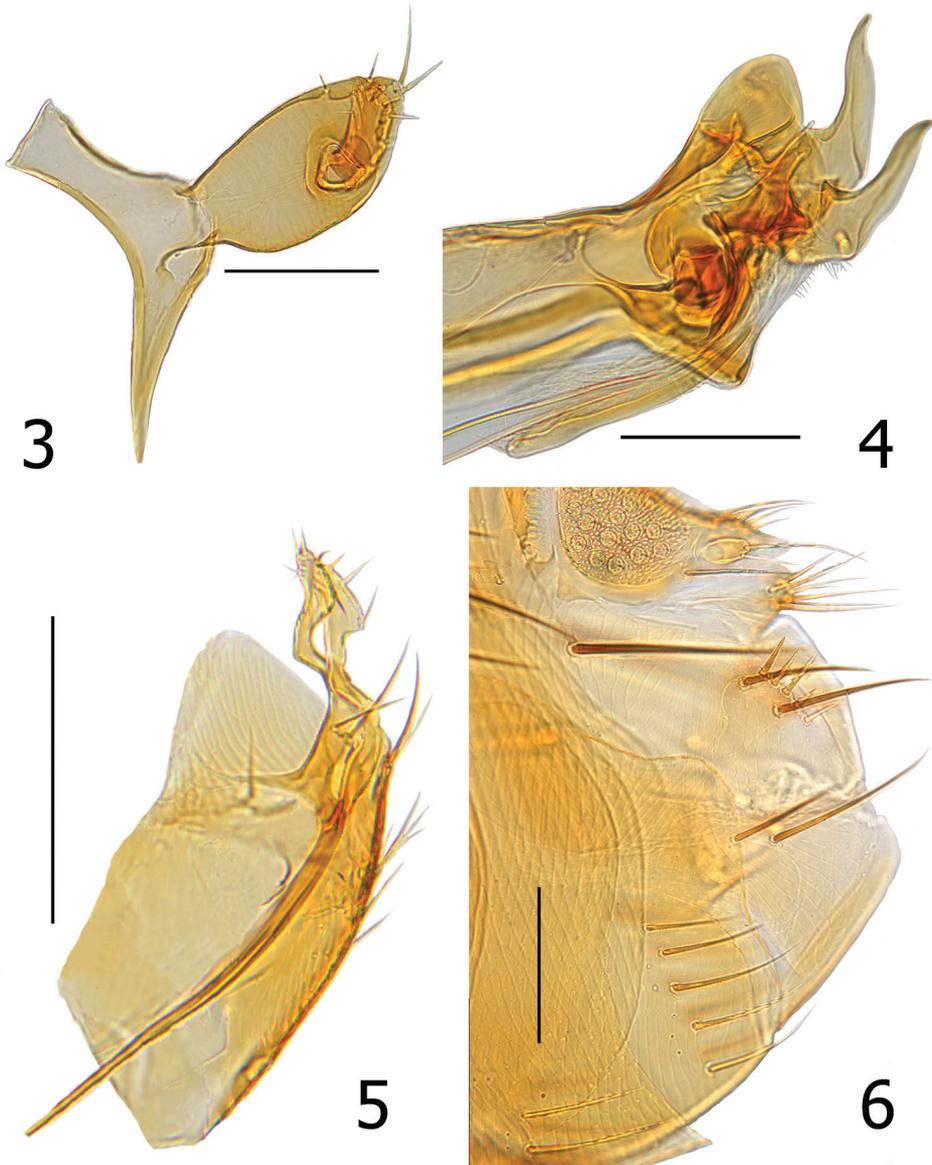
Diagnosis. Both sexes may be distinguished from all other species of *Lagaropsylla* by a narrow band separating the margin of the frons from a white area anterior to the frontal row of fine setae. This band is hardly wider than that of the marginal layer of the frons (Figs 1–2). Closely allied to *Lagaropsylla mera mera* Jordan and Rothschild, 1921, males are easily separated by the position of the acetabulum on the basimere. The acetabulum is midway on the basimere of *L. signata* (Fig. 3) and far distal to the midline of basimere in *L. m. mera*. The broad concave lobe on the caudal margin of the female S-VII (Fig. 6) differs from all other species of *Lagaropsylla* Jordan and Rothschild, 1921.



Figures 1–2. *Lagaropsylla signata* (MH-905). **1** Overview of male **2** Overview of female (arrow indicates group of setae on S-II). Scale bars: 0.2 mm.

Description. Walgren (1903) provided only a brief description of *L. signata*. A detailed description is therefore provided to include illustrations of the species. Unless otherwise specified, counts of spines and setae are for one side of the flea.

Head. Frons evenly rounded with very thin band layered between margin and a broader white area caudad; caudal margin of white zone lined with a dozen small setae



Figures 3–6. *Lagaropsylla signata* (MH-905). **3** Manubrium, basimere, and telomere, male **4** Terminal view of aedeagus, male **5** Sterna VIII and IX, male **6** Terminalia of female, illustrating T–VIII and S–VII. Scale bars: 0.1 mm (**3, 4, 6**), 0.2 mm (**5**).

from oral angle to upper antennal fossa. Second genal tooth longer than first. Pre-oral tuber short and thick, only half the length of first genal tooth. Eye fused into upper heavily sclerotized margin of genal lobe, hardly discernible as distinct eye. Labial palpus of five short segments; sub-equal to length of maxillary palpus. Occipital area with dorsal incassations (Figs 1–2).

Thorax. Length of pronotum equal to height of pronotum; 18 sharp ctenidial spines (both sides) equal to length of pronotum. One dorsal incrassation in pronotum; two dorsal incrassations in meso- and metanota. Prosternosome with antero-ventral area expanded ventrad. Pleural rod fused in center of sclerotic dome. Mesosternum and mesoepimeron fused as one; with two tuberculiform sclerotizations at juncture of sternum and epimeron. Pleural ridge feebly developed; pleural arch lacking. Lateral metanotal area dorso-ventrally flattened. Metepimeron with 12 setae in male and 21 or 22 in female (Figs 1–2).

Legs. Oblique suture of mesocoxa only indicated on ventral margin. Notch in metacoxa vestigial. All femora lacking lateral or mesal setation. Dorsal margin of all tibiae with six dorsal notches. Distitarsomeres each with five pairs of lateral plantar bristles; most proximal pair set onto plantar surface between second pair (Figs 8–9).

Unmodified abdominal segments. Abdominal terga I–VII each with a dorsal incrassation. Main rows of setae on T–I–VI interrupted; one seta below level of each spiracle. Spiracles round. One long antesensilial bristle. Sternum II of male without lateral setae. Female S-II with lateral patch of 14 setae; some short and others long and slender (Fig. 2). Both male and female with vertical parallel reticulations on S-II. Male without setae on S-III; one row of setae on S-IV–VII (2, 3, 3, 3). Female with one row of setae on S-III–VI (6, 4, 4, 4).

Modified abdominal segments, male. Saddle of T-IX and manubrium forming an obtuse angle. Basimere more convex on ventral margin than dorsal margin. Basimere with two or three small setae along dorsal margin; two moderately stout setae at apex. Acetabulum of telomere placed approximately midway between base and apex of basimere. Telomere half the length of basimere; slightly angled at ventral apical third terminating as acute angle at apex. Telomere with five or six minute setae along ventral margin; one minute seta at apex and two minute setae on dorsal margin (Fig. 3). Tergum VIII vestigial. Tendon of S-IX long; curved beyond and over apex of aedeagal apodeme (Fig. 5). Left and right halves of S-VIII fused along ventral margin; with dorsal lobe encompassing aedeagus. Two short and two longer setae at juncture of fused sclerites; lateral group of six or seven small setae (Fig. 5). Distal arm of S-IX (DA9) as in Fig. 5; apex with two setae, one slightly spiniform. Ventral margin of apical portion of DA9 with three minute marginal setae. Apex of proximal arm fused with base of aedeagal apodeme (fused area inseparable with dissection). Penis rods sub-equal in length to tendon of S-IX. Dorsal margin of aedeagal apodeme convex; apex acutely terminated. Dorsal spur present; fused with dorsal surface of T-IX. *Virga ventralis* thick but not darkly sclerotized. Crescent sclerite nearly vertical relative to longitudinal axis of aedeagal apodeme. Median dorsal lobe rounded; distal half more lightly pigmented than that of the surrounding area. Sclerotized inner tube shorter on ventral apical margin than dorsal margin; latter appearing in lateral view as fine, hair-like extension. Dorsal armature on dorsal margin of sclerotized inner tube thorn-like. Ventral surface with similar heavily sclerotized ventral armature. Crochet thin and tapering to apex; peg-like paxillus near ventral base of crochet (Fig. 4).

Modified abdominal segments, female. Tergum VIII with two small setae near spiracle VIII, three or four long lateral setae, and four or five mesal marginal setae (two stout, two



Figures 7–9. *Lagaropsylla signata*, female (MH-905). **7** Spermatheca and bursa copulatrix **8** Distitarsomere I **9** Distitarsomere III. Scale bars: 0.1 mm.

or three fine). Sternum VIII tube-like without setae. Caudal margin of S-VII with broad, truncate lobe with slight concavity at middle; with vertical row of four setae. Anal stylet twice length of width; with two minute setae at base of two slender apical setae. Ventral apical seta not much longer than anal stylet; dorsal seta twice length of stylet (Fig. 6). Bulga of spermatheca longer than wide; cribriform area extended slightly beyond margin of bulga. Hilla narrowed at juncture of bulga and enlarged towards apex; apex usually twice width of base. Bursa copulatrix long; base with “C” shaped thin sclerite merging into long and broad duct of bursa copulatrix. Duct of bursa copulatrix with small sclerotizations but no associated sclerites outside of duct. Duct of spermatheca thickened and crinkled at exit of bursa copulatrix, narrowing towards spermatheca (Fig. 7).

Dimensions. Male average length: 1.6 mm (n = 10), range: 1.4–1.8 mm. Female average length: 1.7 mm (n = 7), range: 1.5–1.9 mm.

Material examined. Malaysia, Sarawak, Deer Cave, Gunung Mulu National Park, 2♂, 3♀ attached to *A. esau*, 15♂, 35♀ from bat guano, 6 X 2006, KBM. An additional 5♂ and 12♀ were removed from the bodies of five specimens of *A. esau* collected in January 2009 by GJS.

Dermaptera

Arixeniina

Arixeniidae

Arixenia esau Jordan, 1909

Figs 10–12

Note. *Arixenia esau* is a robustly built, highly mobile earwig capable of transporting many fleas for significant distances (Fig. 10). Numerous specimens of *L. signata* were observed clinging to the hairs of *A. esau*. Two such observations were photographed



Figure 10–11. 10 *Arixenia esau*, female dorsal habitus, thought to be penultimate instar 11 Female *Lagaropsylla signata* attached to leg of female *Arixenia esau*. Scale bars: 10.0 mm (10); 1.0 mm (11).



Figure 12. Male *Lagaropsylla signata* attached to hairs of hind femur and abdomen of female *Arixenia esau* (arrow indicate five points of attachment). Scale bar: 1.0 mm.

with the fleas in situ (Figs 11–12). The flea (Fig. 12) is grasping setae with five of its six tarsal claws (arrows). It was physically difficult to dislodge the fleas from *A. esau* whose body and legs are somewhat densely covered with fine long setae (Figs 11–12).

Material examined. *Arixenia esau*, Deer Cave, 162 m, Gunung Mulu National Park, 04°01'18"N, 114°49'24"E, Sarawak, Malaysia, 16 X 2006, KBM, (20 adult ♂, 45 adult ♀, 34 nymphs). *Arixenia esau*, Headquarters, 23 m, Gunung Mulu National Park, 04°02'32.8"N, 114°48'49.6"E, Sarawak Malaysia, 14–16, 21–25 I 2009, GJS, (2 adult ♂, 2 adult ♀, 5 nymphs).

Discussion

Lagaropsylla signata was previously known only from the type series. Although the host was listed as *Ch. plicata*, no information of how the bat was collected (mist net), or its habitat (cave, open field, hollow tree, human dwelling, etc.) was provided. Banjuwangi, Java is only a general locality, since modern Banjuwangi is a sprawling metropolis at the eastern extreme of Java along the Straights of Bali. In our study, a total of 72 *L. signata* were collected from the bodies of *A. esau* (7♂, 15♀) and from bat guano on the cave floor (15♂, 35♀). *Cheiromeles torquatus* was not found in Deer Cave in our study. Evidence of its historical presence was documented by Cranbrook (2010) in Niah Cave from archaeological records from the Pleistocene (40,000 years ago). Niah cave is approximately 115 km from Deer Cave. The finding of both *L. signata* and *L. turba* on *A. esau* implies that they have both adopted the same phoretic vehicle to access a bat host. Such evidence suggests that *C. torquatus* is present in Deer Cave and may serve as the principle host for *L. signata* but the definitive bat host for *L. signata* is unknown.

Because of the association of *A. esau* (Fig. 10) and *Xeniararia jacobsoni* (Burr, 1912) (two closely related earwigs) and their potential bat hosts, a discussion of the known biology of each follows. Nakata and Maa (1974) and Marshall (1977) provided assessments of the dermapteran suborder Arixeniina and their associated molossid bats. The phylogenetic position of *A. esau* as sister group to one lineage of free-living spongiphorid earwigs in the genus *Marava* Burr, 1911 has recently been proposed (Naegele, et al. 2016). Medway (1958) documented colonies of *C. torquatus* in a “cave at Niah” (Subis Cave) in Sarawak, approximately 115 km from Deer Cave. From Subis Cave, Medway collected 23 fleas from a juvenile (non-flying) *C. torquatus* that had fallen from the roost 250 feet above the chamber floor. He noted that fleas were also widespread on the floor and were often attached to living *Arixenia*. These fleas, plus two additional males of *L. turba* that were collected in a hollow tree in Ulu Gombak, Selangor, Malaysia, comprised the type series from which Smit described *L. turba*. A bat was not associated with the Ulu Gombak collection. *Arixenia esau* was documented on the bodies of *C. torquatus* by Jordan (1909) and Medway (1958), while Cloudsley-Thompson (1957) reported “17 specimens of *A. esau* from bats harvested from a durian (*Durio* sp.) tree cavity” in addition to 168 specimens roaming free within the same tree cavity. Nineteen specimens of *X. jacobsoni* were also reported by Cloudsley-Thompson (1957) from the same durian tree cavity, but none were collected from bats. Medway and Chong (1969) documented *A. esau* on four mist-netted specimens of *Ch. plicata* in Pulau, Kelantan, and Borneo. Four specimens of *X. jacobsoni* were also

reported by Burr (1912) from a cave near the shore of Babakan, Banjoumas Residency, Java. Further historic records of *X. jacobsoni* documented by Cloudsley-Thompson (1957) included two or three specimens from *Mops mops* (de Blainville, 1840) in Kuala Lumpur, Malaysia in July 1919, seven specimens from *M. mops* in “Malaya” in 1920, an unspecified number from Mindanao, Philippines, and 35 specimens from South Java (Banjoemos) from cave near Babakan in 1933. The presence of two earwig species occurring in the same cave environments and both documented on different bat species remains an enigma. It appears that *A. esau* is associated only with *C. torquatus*, while *X. jacobsoni* has been found on *M. mops*, *Ch. plicata*, and *C. torquatus*. *Xeniaria jacobsoni* has been collected on only one occasion on the body of *C. torquatus* but is found together with *A. esau* in common environs. The discovery of multiple specimens of *L. signata* attached to *A. esau* (behavior similar to *L. turba*) with no evidence of *L. signata* on any bat species will require further identification of bats in Deer Cave (and other caves) that may prove to harbor *L. signata*. The known distribution of bat hosts of Arixeniina species and the geographic distribution of *Lagaropsylla* spp. known from these areas are summarized in Table 1. *Arixenia esau* and *X. jacobsoni* have been associated in the hollows of trees but not in caves, while *Xeniaria bicornis* Maa, 1974 and *Arixenia camura* Maa, 1974 have been found together in the hollows of trees but neither have been found in caves (Nakata and Maa, 1974). The latter two species found only in Mindanao were reported by Nakata and Maa (1974) from *Ch. plicata* and *C. torquatus*, respectively. Note: *Cheiromeles torquatus* does not occur in Mindanao, although *Cheiromeles parvidens*, a close relative, does. *Arixenia camura*, identified by Nakata and Maa (1974) would better be referred to *C. parvidens* and not *C. torquatus*.

Medway (1958) observed negative geotropic behavior of *A. esau*, many falling from associated roosting bats at heights 250 feet above, and that their principle foods were insects and glandular skin of *C. torquatus*. Medway made no mention of earwigs on the cave walls. Burr and Jordan (1913) observed *X. jacobsoni* in countless numbers on the surface of guano and everywhere on the rocky walls at Gouwa Lawa (bat-cave), at Babakan, Java. This would suggest that *X. jacobsoni* (and likely *A. esau* as well) could serve as the principle vehicle for fleas (developing in the guano on the cave floor, or in tree hollows) to ride to the roosting bats located high on the ceilings of huge caves. It is highly unlikely that adult fleas would be capable of making the journey from the cave floor to the bat hosts hundreds of feet overhead. In the massive caves that harbor *C. torquatus*, these bats never make contact with the cave floor unless they fall to the floor as juveniles (which is fatal to the pups), or adults that bump into walls and are temporarily stunned. The inappropriate behavior of bumping into things (attributed to the presence of artificial light) was observed by Kirk-Spriggs (1989). Both earwig species feed on insects but *X. jacobsoni* feeds more voraciously on insects than does *A. esau*, as their diet also includes cannibalism of their own kind, especially those that are vulnerable during molting (Burr and Jordan 1913). Popham (1962) studied the morphology of the mouthparts of both species and concluded that the mouthparts of *A. esau* are more specialized for feeding (grazing) on the peculiar hairless, glandular exudates on the skin of *C. torquatus*, whereas the mouthparts

Table 1. Records of *Arixeniina* taxa reported on bats and/or their environs by geographical localities (listed fleas apply only to localities, see footnotes).

| Localities and fleas | Dermaptera species | | | | |
|--------------------------------|------------------------|----------------------|--------------------------|---------------------------|--------------------------|
| | <i>Arixenia camura</i> | <i>Arixenia esau</i> | <i>Xeniaria bicornus</i> | <i>Xeniaria jacobsoni</i> | <i>Xeniaria truncata</i> |
| Java | | | | Cave | |
| <i>Lagaropsylla signata</i> * | | | | | |
| Malaysia, Peninsular | | Hollow Tree | | Hollow Tree | |
| <i>Lagaropsylla mira</i> ** | | <i>C. torquatus</i> | | <i>C. torquatus</i> | |
| <i>Lagaropsylla turba</i> † | | <i>Ch. plicata</i> | | | |
| Mindanao | “bats” | | <i>C. parvidens</i> | | |
| | <i>C. parvidens</i> | | <i>Ch. plicata</i> | | |
| | <i>Ch. plicata</i> ? | | | | |
| Palawan | | | | | <i>C. torquatus</i> |
| Sabah, Malaysia | | <i>C. torquatus</i> | | | |
| Sarawak, Malaysia | | Cave | | | |
| <i>Lagaropsylla signata</i> †† | | <i>C. torquatus</i> | | | |
| <i>Lagaropsylla turba</i> ‡ | | | | | |
| Sumatra | | <i>C. torquatus</i> | | | |

* *Lagaropsylla signata* reported from “*Tadarida plicatus*” = *Chaerophon plicata*.

** *Lagaropsylla mira* reported from *Chaerophon plicata* and *Mops mops*. Never phoretic.

† *Lagaropsylla turba* collected from tree hole in Selangor State, Malaysia.

†† *Lagaropsylla signata* collected from *Ariexenia esau* in Deer Cave, Gunung Mulu Nat’l Park.

‡ *Lagaropsylla turba* collected from *A. esau* and from “young non-flying individual which had fallen to the cave floor” in Niah Cave, Niah National Park.

of *X. jacobsoni* are less specialized and more suited to feeding on insects (although it has been found on *M. mops*, *Ch. plicata*, and *C. torquatus*). The differences in the recorded occurrence of *A. esau* and *X. jacobsoni* on different bat species may be a reflection of their differences in mouthpart morphology, dictating an ability to feed on the different skin (and hair) types.

Jordan (1909) made reference to *A. esau* occurring in the sack formed by the membrane of the wings of *C. torquatus*. Not to be confused with the gular pouch, Kitchener (1954) described the function of these pouches. With the wings inserted into these pouches (by manipulations of the hind legs and feet), they are neatly folded around the bat, facilitating a quadrupedal mode of mobility within their roost areas (caves, crevasses, and hollow trees). Schutt and Simmons (2001) also addressed the function of the sub-axillary pouches and their relation to quadrupedal mobility. *Arixenia esau* might have a lesser chance of falling from the highly active aerobatics of their bat hosts if they seek these protected areas during bat flight. According to Medway (1958), the gular pouch is not sufficiently large enough to accommodate *Arixenia*.

Cheiromeles torquatus is distributed in southern peninsular Thailand, Malaysia, the insular portions of Indonesia (Java, Sumatra, Borneo), and Palawan, Philippines (Schutt and Simmons, 2001). The distribution of *A. esau* likely follows that of this bat host (although the distribution of *A. esau* is less well defined than its host). Although *C. torquatus* is listed in the IUCN Red List of Threatened Species, version 3.1, as “Least Concern”, studies and assessments are particularly urgent because it is rare in some areas of its range and destruction of many habitats (hollow trees in forested areas) are being destroyed along with logging and human encroachment. The distribution of *X. jacobsoni* appears to be much broader, but may be a reflection of greater collecting activities.

Both *L. turba* and *L. signata* appear to require earwigs to transport them to a viable bat host that would otherwise be inaccessible. Such obligate phoretic behaviors requires additional studies, especially to elucidate the association of *L. signata* and its yet unknown bat host species.

Acknowledgements

We thank Michael Naegle for his phylogenetic work on *Arixenia* which directly led to the discovery of the phoretic fleas. Specimens were collected under permits from the Government of Malaysia; Economic Planning Unit of Malaysia, UPE: 40/200/19 SJ.1040 (permit ID no. 1389) and 40/200/19/1481 (permit ID no. 1933). We thank Brian Clark and Gunung Mulu National Park and staff; Fatimah Abang and the University of Malaysia Sarawak; Haji Ali Bin Yusop and Sarawak Forests Department; Lucy Chong, Sarawak Forestry Corporation and the State Government of Sarawak. We are also grateful to Glen D. Chilton and Terry D. Galloway for providing valuable suggestions to improve this paper. Portions of this project were funded with NSF Grants #DEB-1557114 (MFW), #DEB-0515924 (KBM), #DEB-0738179 (KBM), and #DEB-0845984 (KBM).

References

- Audy JR (1952) Occurrence of *Arixenia* in Malaya. Bulletin, Raffles Museum 24: 133.
- Burr M (1912) A new species of *Arixenia* (Dermaptera). Entomologist's Monthly Magazine 2(23): 105–106.
- Burr KG, Jordan K (1913) On *Arixenia* Burr, a suborder of Dermaptera. 2nd International Congress of Entomology, Oxford, August, 1912, 398–421.
- Cloudsley-Thompson JL (1957) On the habitat and growth stages of *Arixenia esau* Jordan and *A. jacobsoni* burr (Dermaptera): Arixenioidea, with descriptions of the hitherto unknown adults of the former. Proceedings of the Royal Entomological Society of London (Series A) 32: 1–12. <https://doi.org/10.1111/j.1365-3032.1957.tb00357.x>
- Cranbrook Earl of (2010) Later quaternary turnover of mammals in Borneo: the zooarchaeological record. Biodiversity and Conservation 19: 373–391. <https://doi.org/10.1007/s10531-009-9686-3>

- Giles ET (1961) Further studies on the growth stages of *Arixenia esau* Jordan and *Arixenia jacobsoni* Burr (Dermaptera: Arixeniidae), with a note on the first instar antennae of *Hemimerus talpoides* Walker (Dermaptera: Hemimeridae). Proceedings of the Royal Entomological Society of London (Series A) 36: 21–26. <https://doi.org/10.1111/j.1365-3032.1961.tb00255.x>
- Hastriter MW, Whiting MF (2003) Siphonaptera (Fleas). In: Resh VH, Carde R (Eds) Encyclopedia of Insects. Academic Press, San Diego, 1040–1044.
- Jacobson E (1912) Collections and observations at Tjilatjap, central Java. Tijdschrift zur Entomologica, Verslag 55: 7–14.
- Jordan K (1909) Description of a new kind of apterus earwig, apparently parasitic on a bat. Novitates Zoologicae 16: 313–326. <https://doi.org/10.5962/bhl.part.21965>
- Kirk-Spriggs AH (1989) Observations of the naked bat (*Cheiromeles torquatus*) and its earwig ectoparasite *Arixenia esau* In: Sabah SE, Malayan Naturalist 42(2/3): 9–11.
- Kitchener HJ (1954) A further note on the Naked Bulldog Bat. Malayan Nature Journal 9: 26–28.
- Marshall A (1977) Interrelationships between *Arixenia esau* (Dermaptera) and molossid bats and their ectoparasites in Malaysia. Ecological Entomology 2: 285–291. <https://doi.org/10.1111/j.1365-2311.1977.tb00893.x>
- Marshall A (1981) Host location and dispersal, Chapter 6.4.5, Siphonaptera. In: Marshall AG (Ed.) The ecology of ectoparasitic insects. Academic Press, New York, 459 pp.
- Medway L (1958) On the habit of *Arixenia esau* Jordan (Dermaptera). Proceedings of the Royal Entomological Society of London (Series A) 33: 191–195. <https://doi.org/10.1111/j.1365-3032.1958.tb00453.x>
- Medway L, Chong YG (1969) Parasitic earwigs (*Arixenia esau*) on the Wrinkled-lipped bat. Malayan Nature Journal 23: 33.
- Naegle MA, Mugleston JD, Bybee SM, Whiting MF (2016) Reassessing the phylogenetic position of the epizoid earwigs (Insecta: Dermaptera). Molecular Phylogenetics and Evolution 100: 382–390. <https://doi.org/10.1016/j.ympev.2016.03.012>
- Nakata S, Maa TC (1974) A review of the parasitic earwigs (Dermaptera: Arixeniina; Hemimerina). Pacific Insects 16: 307–374.
- Popham EJ (1962) The anatomy related to the feeding habits of *Arixenia* and *Hemimerus* (Dermaptera). Proceedings, Zoological Society of London 139: 429–450. <https://doi.org/10.1111/j.1469-7998.1962.tb01838.x>
- Rothschild M, Clay T (1952) Fleas, Flukes, and Cuckoos. A study of bird parasites. Collins, London, 304 pp.
- Schutt Jr WA, Simmons NB (2001) Morphological specializations of *Cheiromeles* (naked bulldog bats; Molossidae) and their possible role in quadrupedal locomotion. Acta Chiropterologica 3: 225–235.
- Smit FGAM (1958) A new bat-flea from Borneo and Malaya. Entomologische Berichten 18: 236–242.
- Walgren E (1903) Aphanipterologische notizen nebst beschreibung neuer arten. Arkiv för Zoologi 1: 181–196. [plates 7–9]

Four new species of the genus *Lathrolestes* Förster, 1869 from South Korea (Hymenoptera, Ichneumonidae, Ctenopelmatinae)

Alexey Reshchikov¹, Jin-Kyung Choi², Jong-Wook Lee²

1 College of Ecology and Evolution, Sun Yat-sen University, 135 Xingangxi St. Guangzhou, Guangdong 510275, China **2** Department of Life Sciences, Yeungnam University, Gyeongsan, South Korea

Corresponding author: Jong-Wook Lee (jwlee1@ynu.ac.kr)

Academic editor: B. Santos | Received 24 December 2016 | Accepted 5 February 2017 | Published 17 February 2017

<http://zoobank.org/F6445212-5A7E-4D2E-A67A-A06B796CF887>

Citation: Reshchikov A, Choi J-K, Lee J-W (2017) Four new species of the genus *Lathrolestes* Förster, 1869 from South Korea (Hymenoptera, Ichneumonidae, Ctenopelmatinae). ZooKeys 657: 81–92. <https://doi.org/10.3897/zookeys.657.11630>

Abstract

Four new species of the genus *Lathrolestes* Förster, 1869 are discovered from South Korea: *L. redimiculus* Reshchikov & Lee, **sp. n.**, *L. sexmaculatus* Reshchikov & Lee, **sp. n.**, *L. taebaeksanensis* Reshchikov & Lee, **sp. n.**, and *L. ungyeo* Reshchikov & Lee, **sp. n.** This is the first record of the genus from South Korea.

Keywords

Eastern Palearctic, Korea, key, Perilissini, parasitoid wasps, taxonomy

Introduction

Ctenopelmatinae Förster, 1869 comprise more than 1,390 described species within 106 genera, including 373 Eastern Palearctic species (Yu et al. 2012). The South Korean Ctenopelmatinae were reviewed by Uchida (1955), Kim (1955), Townes et al. (1965), and Townes (1970) and 14 species were reported from South Korea recently (Yu et al. 2012, Choi et al. 2016, Kasparyan et al. 2016). The current total is 38 species of Ctenopelmatinae known from South Korea. The genus *Lathrolestes* Förster, 1869 belongs to the tribe Perilissini subfamily Ctenopelmatinae (Ichneumonidae) and comprises 105 previously described distinct species (Yu et al. 2012, Reshchikov 2015a,

2015b, Lima and Kumagai 2016) including 21 species distributed in the Eastern Palearctic (Reshchikov 2012a, 2012b). In the present paper, four new species from South Korea are considered: *L. redimiculus* sp. n., *L. sexmaculatus* sp. n., *L. taebaeksanensis* sp. n., and *L. ungnyeo* sp. n. This is the first record of the genus from South Korea. It is significant that no previously known Eastern Palearctic species have been found within the rather representative material examined. In this paper, descriptions of four new species and a key to the species of South Korean *Lathrolestes* are provided.

Materials and methods

Materials used in this study were collected by sweeping (*L. redimiculus* sp. n. and *L. ungnyeo* sp. n.) and Malaise trapping (M.T.: *L. sexmaculatus* sp. n. and *L. taebaeksanensis* sp. n.). The morphological terminology follows that of Gauld (1991). Photographs were taken at the Department of Life Sciences, Yeungnam University, Gyeongsan-si, Gyeongsangbuk-do, Republic of Korea (YNU) with an AxioCam MRc5 camera attached to a stereo microscope (Zeiss SteREO Discovery. V20; Carl Zeiss, Göttingen, Germany), processed using AxioVision SE64 software (Carl Zeiss), and optimized with a Delta imaging system (i-solution, IMT I Solution Inc. Vancouver, Canada). The type specimens of the four new species are deposited in YNU. Abbreviations are used as follows: **CB**: Chungcheongbuk-do; **GB**: Gyeongsangbuk-do; **GG**: Gyeonggi-do; **GW**: Gangwon-do.

Taxonomy

Family Ichneumonidae Latreille, 1802

Subfamily Ctenopelmatinae Förster, 1869

Tribe Perilissini Thomson, 1883

Genus *Lathrolestes* Förster, 1869

Lathrolestes Förster, 1869: 135–221. Type species: *Tryphon clypeatus*.

Camporychus Förster, 1869: 135–221. Type species: *Lathrolestes marginatus*.

Ecclinops Förster, 1869: 135–221. Type species: *Tryphon orbitalis*.

Homalomma Förster, 1869: 135–221. Type species: *Homalomma caliroae*.

Lathrolestes Thomson, 1883: 873–936. Type species: *Tryphon clypeatus*.

Luphyroscopus Thomson, 1883: 873–936. Type species: *Tryphon gorskii*.

Tryphonopsis Brauns, 1898: 58–72. Type species: *Tryphonopsis ensator*.

Ritzemabosia Smits van Burgst, 1912: 263–270. Type species: *Ritzemabosia meridionalis*.

Diagnosis. Small to medium sized species, 4.0–7.5 mm. Occipital carina not intercepting hypostomal carina. Clypeus profile flat, its apical margin thick. Mandible with

lower tooth distinctly longer than upper. Areolet petiolate, oblique; vein 2m-cu of fore wing with single bulla; vein cu-a of hind wing intercepted below or at its middle. Tarsal claws pectinate, with 1 or 3 teeth or with basal lobe. Glymmae deep. Apex of subgenital plate of male not incurved on posterior margin. Tip of aedeagus somewhat decurved and swollen, its apex rounded. Ovipositor sheath 0.3 to 15 × as long as metasomal height.

Key to species of *Lathrolestes* occurring in South Korea

- 1 Apical margin of clypeus truncated. Malar space short, less than 0.5 × basal mandible width. Ovipositor straight, without notch. Hind wing with cu-a intercepted by Cu1 in the middle ***L. ungyeo* sp. n.**
- Apical margin of clypeus thick and rounded. Malar space more than 0.5 × basal mandible width. Ovipositor straight, stout, with rather shallow notch or shallow impression (except for *L. redimiculus*, female unknown). Hind wing with cu-a intercepted by Cu1 below middle **2**
- 2 First tergite more than 1.7 × as long as broad apically, without longitudinal dorsal carinae. Head not narrowed eyes posteriorly. Mesoscutum with notaulus not defined ***L. taebaeksanensis* sp. n.**
- First tergite less than 1.5 × as long as broad apically, with longitudinal dorsal carinae completely or basally. Head narrowed eyes posteriorly. Mesoscutum with notaulus shallow **3**
- 3 Clypeus distinctly separated from face. Areolet petiolate. Propodeal carinae complete ***L. sexmaculatus* sp. n.**
- Clypeus not separated from face. Areolet not petiolate. Propodeum with only apical carina complete ***L. redimiculus* sp. n.**

Lathrolestes redimiculus Reshchikov & Lee, sp. n.

<http://zoobank.org/1EB54BA7-E408-465C-A8D6-90B4AAC2F687>

Figure 1

Diagnosis. This species is similar to *L. verticalis* (Brischke, 1871) but distinguishable by combination of the following characters: clypeus not separate from face by distinct groove (Fig. 1B); mesopleuron and metasoma distinctly punctate (Fig. 1E, I); areolet of fore wing closed (Fig. 1H); area petiolaris of propodeum complete; tergites 1 and 2 entirely black; further tergites reddish (Fig. 1I).

Description. Male. Body length 7.7 mm.

Head. Matt, punctate with shallow and sparse punctures on shagreen surface (Fig. 1C). Face 1.15 × as broad as eye height, relatively flat, bulging (Fig. 1B). Clypeus not separated from face, at apex projecting anteriorly (Fig. 1B); apical margin of clypeus thick. Clypeal fovea large. Malar space 0.6 × as long as basal mandible width. Lower mandible tooth longer than upper. Occipital carina complete.

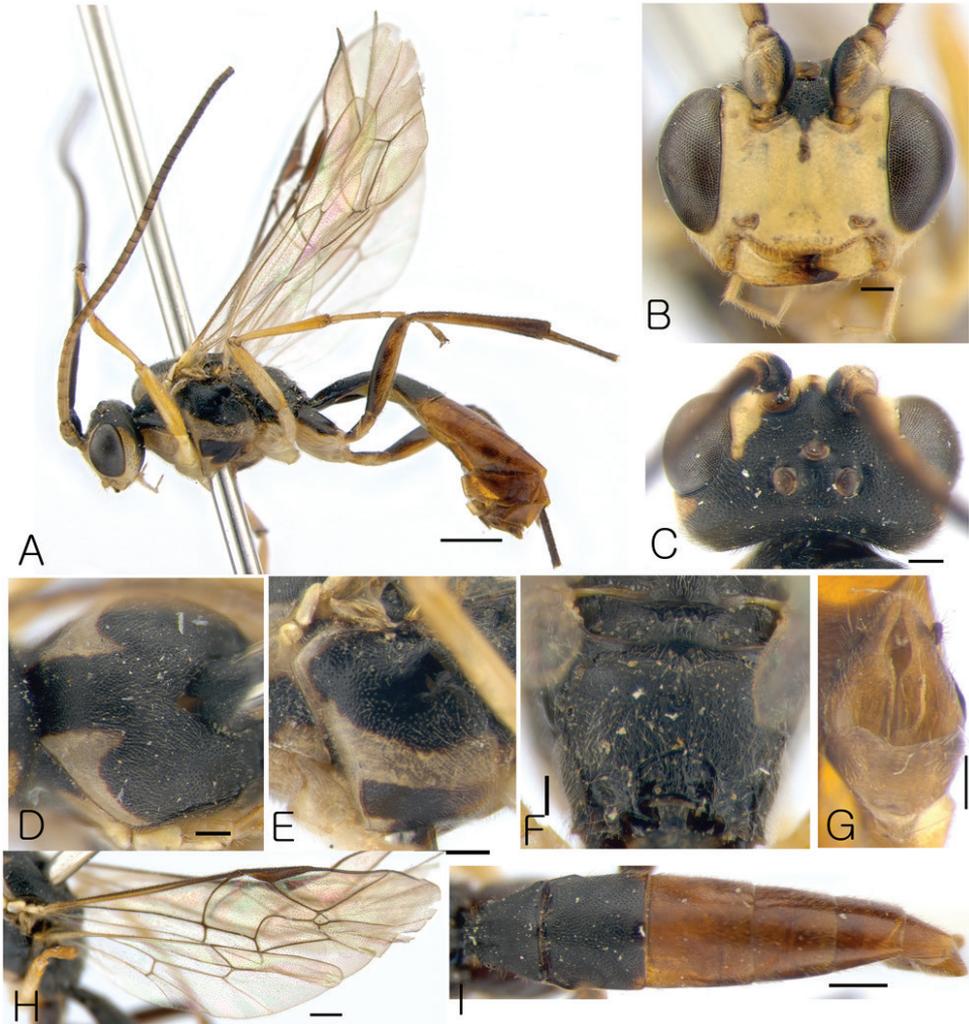


Figure 1. *Lathrolestes redimiculus*; **A** Habitus in lateral view **B** Head in anterior view **C** Head in dorsal view **D** Mesoscutum in dorsal view **E** Mesopleuron **F** Propodeum **G** Genitalia **H** Wings **I** Metasoma in dorsal view. Scale bars: 1 mm (**A**); 0.5 mm (**E**, **I**); 0.2 mm (**B–D**, **F–H**).

Mesosoma. Matt. Pronotum punctate with sparse punctures. Epomia absent. Mesoscutum punctate with shallow indistinct punctures, matt, with notaulus shallow (Fig. 1D). Mesopleuron distinctly and sparsely punctate with fine punctures, polished (Fig. 1E). Claws not pectinate. Fore wing with R intercepting pterostigma before its middle (Fig. 1H); areolet not petiolate; vein 2m-cu of fore wing with single bulla. Vein cu-a of hind wing slightly postfurcal, with cu-a intercepted by Cu1 below middle. Propodeum with only apical carina complete (Fig. 1F).

Metasoma. Matt, distinctly and densely punctate with shallow punctures (Fig. 1I). Tergite 1 1.5 × as long as broad apically, convex, with longitudinal dorsal carinae and

medial impression (Fig. 1I). Tergite 2 rectangle (Fig. 1I). Following tergites also elongate (Fig. 1I). Parameres broad at base (Fig. 1G).

Color. Body mostly black. Scape, antennal flagellum ventrally, face entirely, temple eye posteriorly, mandible except teeth, propleurum, lateral parts of mesonotum, tegula, band in the middle of mesopleurum, fore and middle legs, hind coxa ventrally yellowish. Hind femur ventrally, hind tibia basally, metasomal starting at tergite 3 reddish.

Female. Unknown.

Etymology. The name *redimiculus* refers to yellow band of the middle of mesopleurum.

Material examined. Holotype: male; type depository: YNU, GW, Hangrobong (Sweeping), 13.vi.1992, leg. S.M. Ryu. Paratypes: 3 males, GW, Hangrobong, (Sweeping), 13.vi.1992, leg. S.M. Ryu (YNU); 1 male, ditto, (Sweeping), leg. J.W. Lee (YNU).

***Lathrolestes sexmaculatus* Reshchikov & Lee, sp.n.**

<http://zoobank.org/0E01E180-5FE8-4F28-865E-0A98A0C9C105>

Figure 2

Diagnosis. This species is similar to *L. grahami* Reshchikov, 2012 and *L. tolstoyi* Reshchikov, 2012 but distinguishable by combination of the following characters: claws not pectinate, malar space $0.9 \times$ as long as basal mandible width; face in female black with small yellow macula between antennal socket and eye margin, ventral part of eye, lateral edge of clypeus, face in male mostly yellow with black bands ventral part of antennal sockets, malar space and middle apical part of clypeus.

Description. Female. Body length 7.3 mm.

Head. Matt, distinctly punctate with fine and dense punctures on shagreen surface (Fig. 2C). Face $1.3 \times$ as broad as eye height, relatively flat, bulging (Fig. 2B). Clypeus distinctly separated from face by groove, at apex projecting anteriorly (Fig. 2B); apical margin of clypeus thick. Clypeal fovea relatively small. Malar space $0.9 \times$ as long as basal mandible width. Lower mandible tooth almost equal to upper. Occipital carina complete.

Mesosoma. Matt. Pronotum distinctly punctate. Epomia absent. Mesoscutum distinctly punctate, matt, with notaulus shallow. (Fig. 2E). Mesopleuron finely and densely punctate, polished (Fig. 2D). Claws not pectinate. Fore wing with R intercepting pterostigma before its middle (Fig. 2J); areolet petiolate; vein 2m-cu of fore wing with single bulla. Vein cu-a of hind wing interstitial, with cu-a intercepted by Cu1 below middle. Propodeal carinae complete (Fig. 2H).

Metasoma. Matt, distinctly and densely punctate (Fig. 2K). Tergite 1 $1.2 \times$ as long as broad apically, convex, with longitudinal dorsal carinae distinct basally and medial impression (Fig. 2I). Tergite 2 transverse, trapezoidal (Fig. 2K). Following tergites also transverse. Ovipositor straight, stout, with rather shallow notch in the middle (Fig. 2L).

Color. Body mostly black (Figs 2A–K). Mandible except teeth, lateral edges of clypeus, maculae ventral eye and between eye and antennal sockets, fore tibia and tarsus

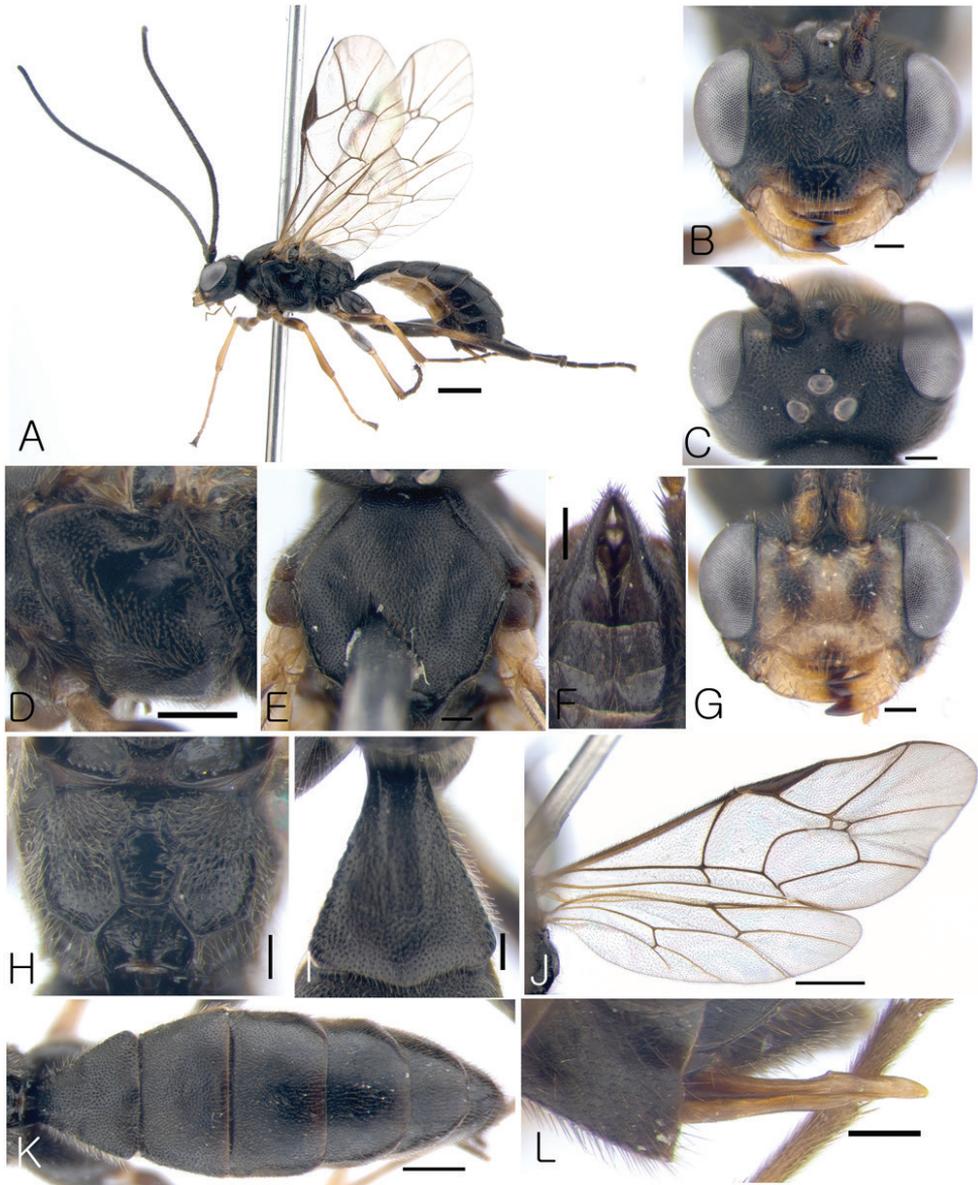


Figure 2. *Lathrolestes sexmaculatus* (Female except **F, G**); **A** Habitus in lateral view **B** Head in anterior view **C** Head in dorsal view **D** Mesopleuron **E** Mesoscutum in dorsal view **F** Genitalia of male **G** Head in anterior view **H** Propodeum **I** First tergite in dorsal view **J** Wings **K** Metasoma in dorsal view **L** Ovipositor. Scale bars: 1 mm (**A, J**); 0.5 mm (**D, K**); 0.2 mm (**B, C, E–I, L**).

except apical tarsal segment, basal half of middle tibia, and basal part of hind tibia yellowish (Fig. 2A)

Male. Body length 7 mm. Character states are mostly the same as in female. Parameres elongate (Fig. 2F).

Color. Same as in female but scape and face mostly yellow (with band ventral part of each scape, tip of clypeus and malar space black) (Fig. 2G).

Etymology. Name *sexmaculatus* refers to six yellow maculae on head in female.

Material examined. Holotype: female; type depository: YNU, CB Danyang-gun, Danyang-eup, Cheongdong-ri, 35°57'N 128°28'E (M.T.), 8.vi–6.vii.2005, leg. J.W. Lee. Paratypes: 1 female 1 male, GG Yangpyeong-gun, Yongmun-myeon, Yeonsu-ri, Mt. Yongmunsan, 37°31'49.5"N 127°34'18.8"E (M.T.), Alt. 324 m, 11–25.vi.2009, leg. J.O. Lim (YNU); 1 female, CB Danyang-gun, Danyang-eup, Cheongdong-ri, 35°57'N 128°28'E (M.T.), 8.vi–6.vii.2005, leg. J.W. Lee (YNU).

***Lathrolestes taebaeksanensis* Reshchikov & Lee, sp. n.**

<http://zoobank.org/ED651E22-89DE-48B8-832A-C65CDBA96FFC>

Figure 3

Diagnosis. This species generally is similar to *L. soperi* Reshchikov, 2010, and its ovipositor structure is similar to those of *L. breviremus* Barron, 1994, *L. erugatus* Barron, 1994, and *L. tolstoyi* Reshchikov, 2012 but distinguishable by combination of the following character states: claw simple, yellow maculae between antennal sockets and eye margin little ventral level of antennal sockets, malar space as long as basal mandible width, face 1.5 × as broad as eye, height (Fig. 3B), notaulus not defined (Fig. 3D), propodeum with carinae obliterated (Fig. 3F), tergite 1 without dorsal longitudinal carinae (Fig. 3G), ovipositor straight, stout at base, upper valve rounded and at tip with shallow impression in the middle (Fig. 3H).

Description. Female. Body length 5.3 mm.

Head. Matt, not punctate, shagreen (Fig. 3C). Face 1.5 × as broad as eye height, projecting in the middle, bulging (Fig. 3B). Clypeus distinctly separated from face by groove, at apex projecting anteriorly (Fig. 3B); apical margin of clypeus thick. Clypeal fovea small. Malar space as long as basal mandible width. Lower mandible tooth longer than upper. Occipital carina complete.

Mesosoma. Matt. Pronotum not punctate. Epomia absent. Mesoscutum finely punctate, matt, with notaulus not defined (Fig. 3D). Mesopleuron finely and sparsely punctate, shagreen (Fig. 3E). Claws not pectinate. Fore wing with R intercepting pterostigma at its middle; areolet not petiolate; vein 2m-cu of fore wing with single bulla. Vein cu-a of hind wing interstitial, with cu-a intercepted by Cu1 below middle. Propodeal carinae obliterated (Fig. 3F).

Metasoma. Matt, not punctate (Fig. 3I). First metasomal tergite 1.94 × as long as broad apically, convex, without longitudinal dorsal carinae (Fig. 3G). Second metasomal tergite transverse, trapezoidal (Fig. 3I). Following tergites also transverse. Ovipositor straight, stout at base and roundish at tip with shallow impression in the middle (Fig. 3H).

Color. Body mostly black (Fig. 3A–I). Maculae between antennal sockets and eye margin little ventral level of antennal sockets, mandible except teeth, tegula, legs except

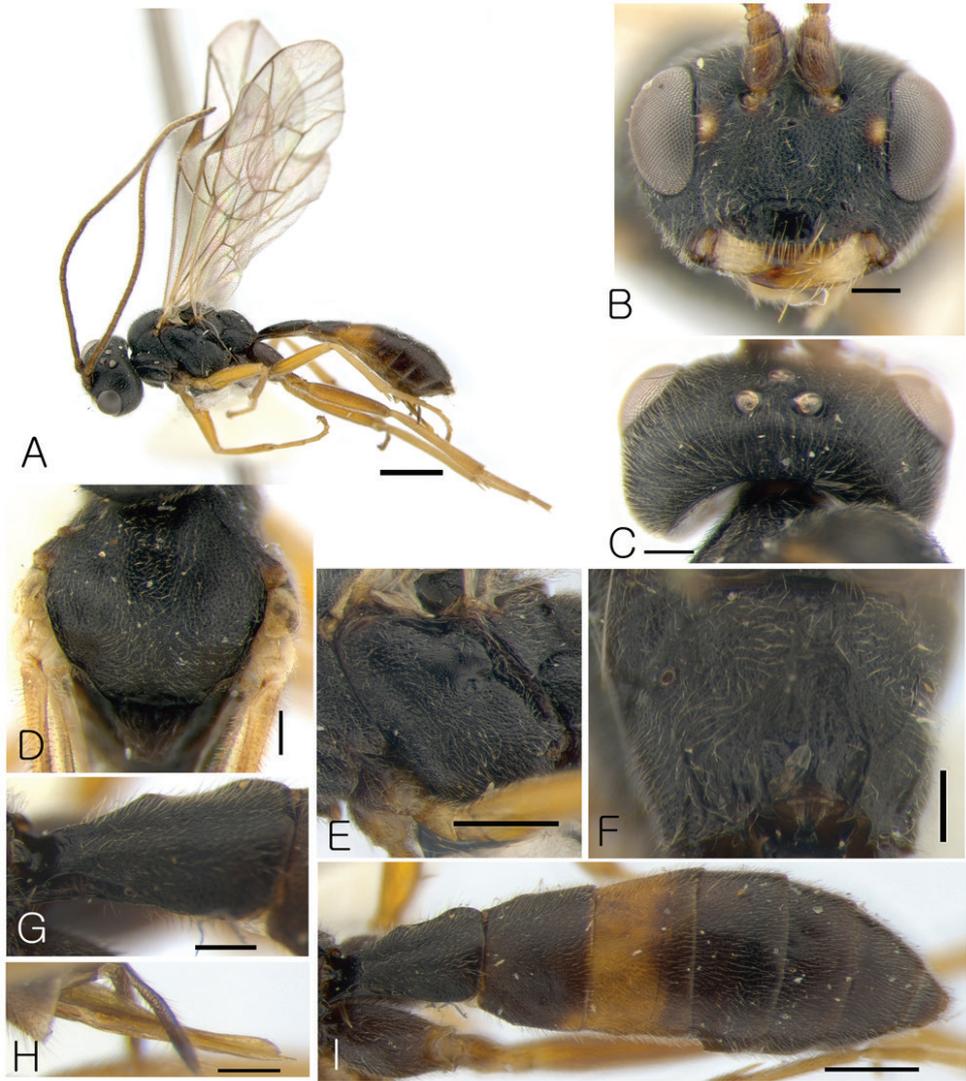


Figure 3. *Lathrolestes taebaeksanensis*: **A** Habitus in lateral view **B** Head in anterior view **C** Head in dorsal view **D** Mesoscutum in dorsal view **E** Mesopleuron **F** Propodeum **G** First tergite in dorsal view **H** Ovipositor **I** Metasoma in dorsal view. Scale bars: 1 mm (**A**); 0.5 mm (**E**, **I**); 0.2 mm (**B**–**D**, **F**–**H**).

coxae and hind femur yellowish (Fig. 3A, B, D). Antenna, hind femur and tergite 3 mostly reddish (Fig. 3I).

Male. Unknown.

Etymology. The name *taebaeksanensis* refers to Mt. Taebaeksan where the species was collected.

Material examined. Holotype: female; type depository: YNU, GW Mt. Taebaeksan National Park (M.T.), 14.v–20.vi.1999, D.S. Gu.

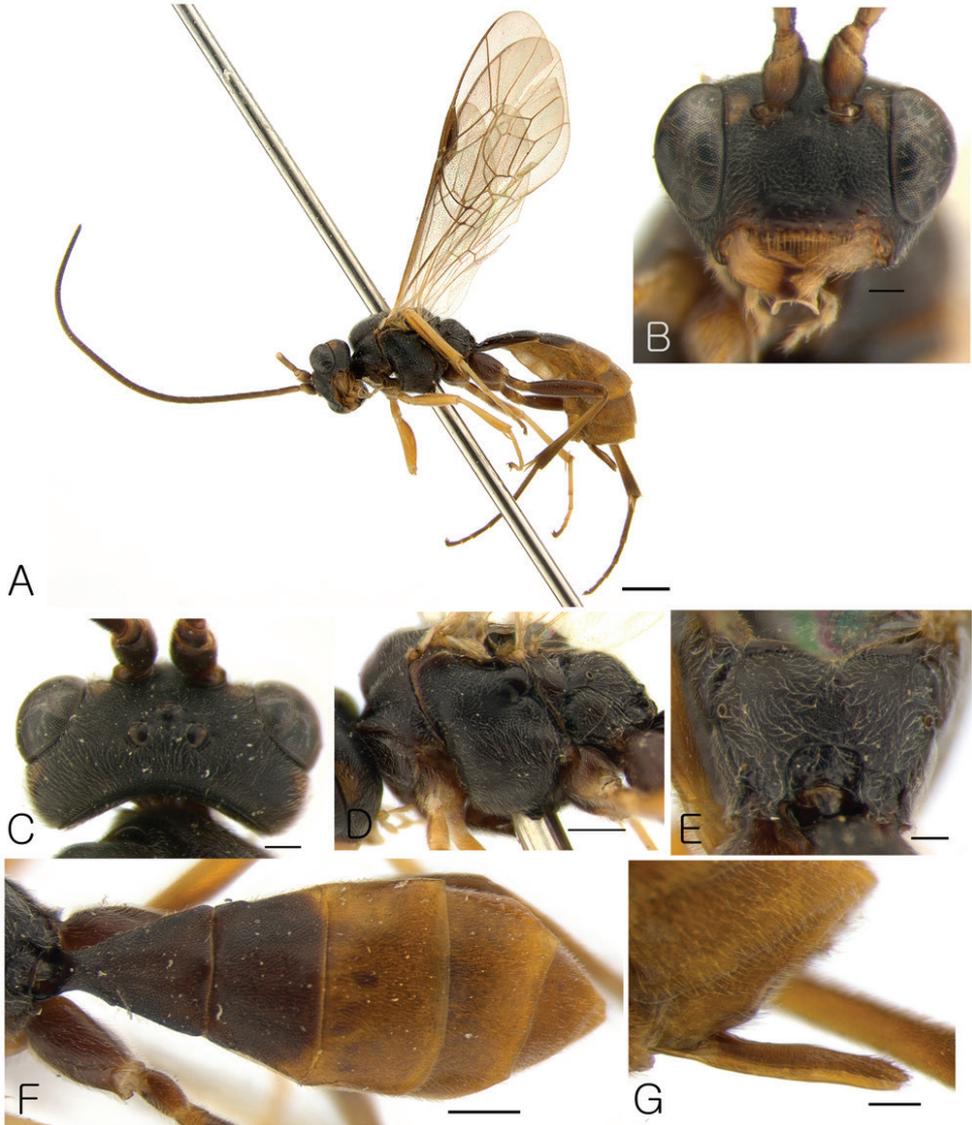


Figure 4. *Lathrolestes ungnyeo*: **A** Habitus in lateral view **B** Head in anterior view **C** Head in dorsal view **D** Mesopleuron **E** Propodeum; **F** Metasoma in dorsal view **G** Ovipositor sheath. Scale bars: 1 mm (**A**); 0.5 mm (**D, F**); 0.2 mm (**B, C, E, G**).

***Lathrolestes ungnyeo* Reshchikov & Lee, sp. n.**

<http://zoobank.org/CD54711E-BC3D-4CF0-B49D-0D117977A784>

Figure 4

Diagnosis. This species similar to *L. cruentocaudus* Reshchikov, 2012, *L. palatynus* Reshchikov, 2012, *L. redimiculus* sp. n., and *L. verticalis* (Brischke, 1871) but distin-

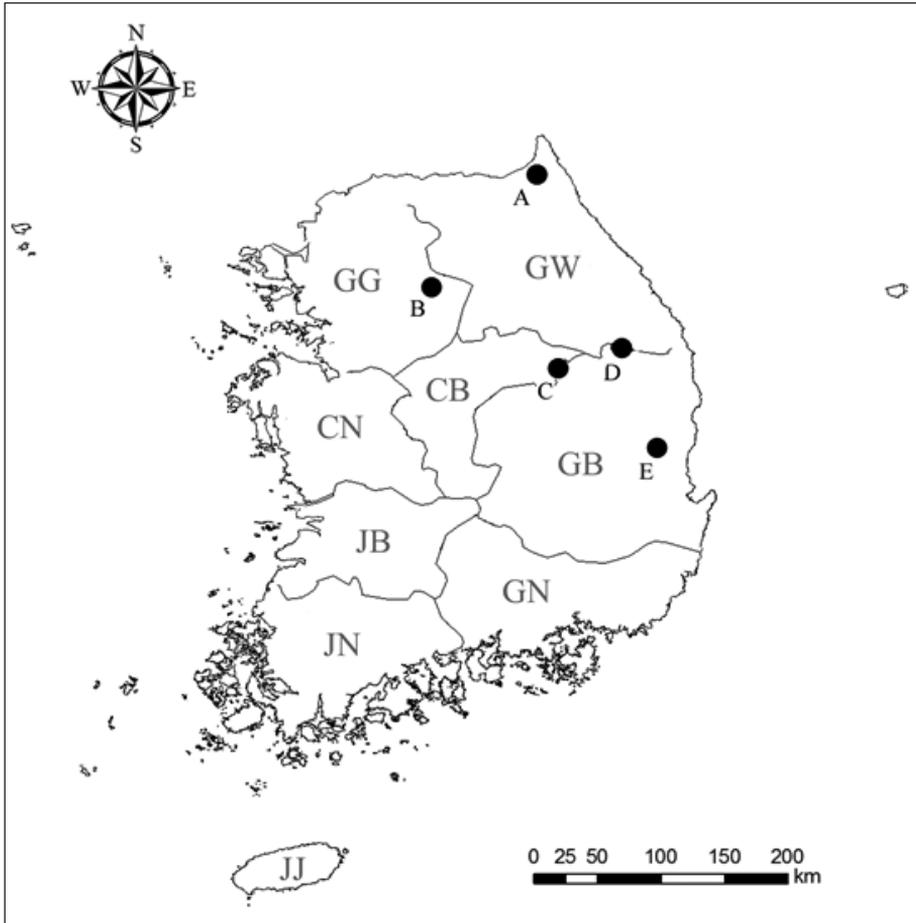


Figure 5. Distribution map of South Korean *Lathrolestes* spp.: **A** *L. redimiculus* sp. n. **B–C** *L. sexmaculatus* sp. n. **D** *L. taebaeksanensis* sp. n. **E** *L. ungyeoo* sp. n.

guishable by combination of the following character states: clypeus not separate from face, not projecting anteriorly, apically truncated (Fig. 4B), hind wing with cu-a intercepted by Cu1 in the middle (Fig. 4A), tergite 1 without longitudinal dorsal carinae, ovipositor straight, without notch (Fig. 4F).

Description. Female. Body length 7.5 mm.

Head. Matt, not punctate, shagreen (Fig. 4C). Face $1.2 \times$ as broad as eye height, flat, bulging (Fig. 4B). Clypeus not separated from face, not projecting anteriorly (Fig. 4B), apically truncated (Fig. 4B). Clypeal fovea small. Malar space $0.46 \times$ as long as basal mandible width. Lower mandible tooth almost equal to upper. Occipital carina complete.

Mesosoma. Matt. Pronotum not punctate. Epomia absent. Mesoscutum finely punctate, matt, with notaulus not defined. Mesopleuron finely and sparsely punctate, shagreen (Fig. 4D). Claws not pectinate. Fore wing with R intercepting pterostigma

before the middle (Fig. 4A); areolet not petiolate; vein 2m-cu of fore wing with single bulla. Vein cu-a of hind wing interstitial, with cu-a intercepted by Cu1 in the middle. Propodeum with only apical carina complete (Fig. 4E).

Metasoma. Matt, not punctate (Fig. 4F). Tergite 1 1.35 × as long as broad apically, convex, without longitudinal dorsal carinae (Fig. 4F). Tergite 2 transverse, trapezoidal (Fig. 4F). Following tergites also transverse. Ovipositor straight (Fig. 4G).

Color. Body mostly black (Fig. 4A–F). Maculae between antennal sockets and eye margin, ventral part of antenna, apical margin of clypeus, mandible except teeth, tegula, fore leg entirely, middle tibia and tarsus yellowish (Figs 4B,4D). Middle femur and metasoma starting at tergite 3 reddish (Fig. 4F). Hind leg brownish (Fig. 4A)

Male. Unknown.

Etymology. The name *ungnyeo* refers to the Ungnyeo, or “bear woman” which is the progenitress of Koreans in Korean mythology.

Material examined. Holotype: female; type depository: YNU, GB Cheongsong-gun, Juwangsan National Park (sweeping), 17.v.1987, S.M. Ryu.

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 201701203).

References

- Brauns S (1898) Neue Schlupfwespen aus Mecklenburg. Archiv des Vereins der Freunde der Naturgeschichte in Mecklenburg 51: 58–72.
- Choi JK, Jeong JC, Lee JW (2016) New records of thirty species of Ctenopelmatinae and Tryphoninae (Hymenoptera: Ichneumonidae) from Korea. Journal of National Park Research 7(1): 1–19.
- Förster A (1869) Synopsis der Familien und Gattungen der Ichneumonen. Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande und Westfalens 25: 135–221.
- Gauld ID (1991) The Ichneumonidae of Costa Rica, 1. Memoirs of the American Entomological Institute 47: 1–589.
- Kasparyan DR, Choi JK, Lee JW (2016) New species of *Rhorus* Förster, 1869 (Hymenoptera: Ichneumonidae: Ctenopelmatinae) from South Korea. Zootaxa 4158(4): 569–576. <https://doi.org/10.11646/zootaxa.4158.4.8>
- Kim CW (1955) A study on the Ichneumon-flies in Korea. (in Korean with German summary) Commemoration These 15th Anniv. Korea University, 423–498.
- Lima AR, Kumagai AF (2016) *Lathrolestes* Förster, 1869 (Hymenoptera, Ichneumonidae) from Brazil, with description of two new species and a key to the Neotropical species. Zootaxa 4170(3): 587–593. <https://doi.org/10.11646/zootaxa.4170.3.11>

- Reshchikov A (2012a) *Lathrolestes* (Hymenoptera, Ichneumonidae) from Central Asia, with a key to the species of the tripunctor species-group. *Zootaxa* 3175: 24–44.
- Reshchikov A (2012b) Review and key to Russian Far East *Lathrolestes* (Hymenoptera, Ichneumonidae). *Zootaxa* 3414: 1–32.
- Reshchikov A (2015a) Review of North European species of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae) with description of one new species from Öland (Sweden). *Zootaxa* 4033(1): 1–47. <https://doi.org/10.11646/zootaxa.4033.1.1>
- Reshchikov A (2015b) A new species of *Lathrolestes* Förster (Hymenoptera: Ichneumonidae) from Peruvian Amazonia. *Biodiversity Data Journal* 3.3: e4327. <http://dx.doi.org/10.3897/BDJ.3.e4327>
- Smits van Burgst CAL (1912) Tunesian Ichneumonidae. *Tijdschrift voor Entomologie* 55: 263–270.
- Thomson CG (1883) XXXII. Bidrag till kännedom om Skandinaviens Tryphoner. *Opuscula Entomologica*, Lund IX, 873–936.
- Townes HK, Momoi S, Townes M (1965) A catalogue and reclassification of the eastern Palearctic Ichneumonidae. *Memoirs of the American Entomological Institute*, No. 5, 661 pp.
- Townes HK (1970) The genera of Ichneumonidae, Part 3. *Memoirs of the American Entomological Institute*. No.13(1969), 307 pp.
- Uchida T (1955) Die von Dr.K. Tsuneki in Korea gesammelten Ichneumoniden. *Journal of the Faculty of Agriculture, Hokkaido University* 50: 95–133.
- Yu DS, Achterberg K, Horstmann K (2012) *World Ichneunoidea 2011*. Taxapad 2012, Vancouver.

Descriptions of three new *carbonaria*-group species of *Fannia* Robineau-Desvoidy from China, with a key to the *carbonaria*-group species (Diptera, Fanniidae)

Ming-fu Wang¹, Wei Li^{2,5}, Yu-wan Zhao¹, Jie Wu³, Dong Zhang⁴

1 Institute of Entomology, Shenyang Normal University, Shenyang, 110034, P.R. China **2** College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing, 100083, P.R. China **3** Shanghai Entomological Museum, Chinese Academy of Sciences, Shanghai, 200032, P.R. China **4** School of Nature Conservation, Beijing Forestry University, Beijing, 100083, P.R. China **5** Key Laboratory in Healthy Science and Technology, Division of Life Science, Graduate School at Shenzhen, Tsinghua University, Shenzhen, 518055, P.R. China

Corresponding author: Ming-fu Wang (wangmingfu403@163.com); Dong Zhang (ernest8445@163.com)

Academic editor: P. Cerretti | Received 10 May 2016 | Accepted 14 November 2016 | Published 17 February 2017

<http://zoobank.org/199B466C-0D1C-4E2D-8133-1C0D77FCBD3F>

Citation: Wang M-f, Li W, Zhao Y-w, Wu J, Zhang D (2017) Descriptions of three new *carbonaria*-group species of *Fannia* Robineau-Desvoidy from China, with a key to the *carbonaria*-group species (Diptera, Fanniidae). ZooKeys 657: 93–107. <https://doi.org/10.3897/zookeys.657.9153>

Abstract

A historical review of the *Fannia carbonaria*-group is provided and three new species are described from China: *F. fani* Wang & Wu, **sp. n.**, *F. nitidiventris* Wang & Zhang, **sp. n.** and *F. submaculata* Wang & Zhao, **sp. n.**. One species, *Fannia norvegica* Ringdahl, 1934, is recorded for the first time from China. Illustrations of male terminalia of these four species and a taxonomic key to the males of known species in the group are given. The *F. carbonaria*-group now includes 30 species distributed in the Holarctic Region and northern part of the Oriental Region.

Keywords

Fannia carbonaria-group, Fanniidae, identification key, new Chinese species

Introduction

The *Fannia carbonaria*-group is one of the species-groups of genus *Fannia*. It was established by Chillcott (1961) for 15 species arranged in two subgroups, namely the *F. carbonaria*-subgroup and the *F. minutipalpis*-subgroup. Species of this group are characterized by the following character states: mid tibia usually with two anterodorsal setae and two posterodorsal setae; hind femur without a posteroventral seta at distal part; and thorax usually with one stout prealar seta.

Since the 19th century, a number of investigators have turned their attention to what is now termed the *F. carbonaria*-group, including Meigen (1826), Verrall (1892), Stein (1895, 1920), Ringdahl (1934), D'Assis-Fonseca (1966), Nishida (1974a, 1974b, 1975a, 1975b) and Gregor and Rozkošný (1993). Studies at the end of 1900s added eight European species to the group (consisting of five species in the *F. carbonaria*-subgroup and three in the *F. minutipalpis*-subgroup; Rozkošný, Gregor & Pont, 1997), as well as five Chinese species (Xue and Wang 1998).

At the beginning of 21st century, Nishida (2002, 2003) studied the Japanese species of the *F. carbonaria*-group. Wang, Xue and Su (2004) revised eight species in the group from China. Wang et al. (2009) reviewed the cosmopolitan fauna of the *F. carbonaria*-subgroup by updating a key to males, presenting a diagnosis to each species, and describing two new species from China. Recently, study of Barták et al. (2016) found out that *F. lucida* Chillcott, 1961 is a junior synonym of *F. norvegica* Ringdahl, 1934. This brought the total number of known species of the *F. carbonaria*-group to 27, containing 17 species in the *F. carbonaria*-subgroup and ten in the *F. minutipalpis*-subgroup.

During a study of the Fanniidae fauna, three new species belonging to the *F. carbonaria*-subgroup were discovered in China and are described herein. The total number of species in the *F. carbonaria*-group is now 30. Illustrations of male terminalia are given for the species and an updated key to the identification of males is provided.

Materials and methods

Terminology follows McAlpine (1981) and Stuckenberg (1999). Absolute measurements in millimeters (mm) are given for body length. All type specimens of the new species are deposited in the Institute of Entomology, Shenyang Normal University, Shenyang, China (**IESNU**) except for those of *Fannia fani* sp. n., which are deposited in the Shanghai Entomological Museum, Chinese Academy of Science, Shanghai, China (**SHEM**). Methods for the preparation of terminalia and illustrations follow Zhang et al. (2013). Abbreviations used throughout the text are as follow:

| | | | |
|------------|---------------------|------------|----------------------|
| <i>acr</i> | acrostichal seta, | <i>ia</i> | intra-alar seta, |
| <i>ad</i> | anterodorsal seta, | <i>p</i> | posterior seta, |
| <i>av</i> | anteroventral seta, | <i>pd</i> | posterodorsal seta, |
| <i>d</i> | dorsal seta, | <i>pra</i> | prealar seta, |
| <i>dc</i> | dorsocentral seta, | <i>pv</i> | posteroventral seta. |

Taxonomic accounts

Key to the males of the *Fannia carbonaria*-group

- 1 Hind coxa bare on posterior surface; palpus normal (*Fannia carbonaria*-subgroup)..... **2**
- Hind coxa with setulae on posterior surface; palpus short (*Fannia minutipalpis*-subgroup) **21**
- 2 Hind femur swollen on posteroventral surface in distal half..... **3**
- Hind femur not swollen on posteroventral surface in distal half..... **4**
- 3 Presutural *acr* triserial; katepisternal setae 0+1; mid coxa with 3–8 *ad*; hind femur with 6 or 7 stout *av* with tips of setae curved on swollen part; cercal plate with cluster of slender setae in upper part.... ***Fannia xiaoi* Fan** [China]
- Presutural *acr* biserial; katepisternal setae 1+1; mid coxa without *ad*; hind femur with only 2 or 3 *av* with tips of setae not curved on swollen part; cercal plate without cluster of setae in upper part..... ***Fannia fani* Wang & Wu, sp. n.** [China]
- 4 Haltere dark brown or black **5**
- Haltere yellowish or brownish yellow..... **7**
- 5 *Pra* 2; hind femur with 8 or 9 *pv* in basal half..... ***Fannia subfuscitibia* Wang** [China]
- *Pra* 1; hind femur without distinct *pv*..... **6**
- 6 Presutural triserial; calypters yellowish; *pra* 3/4 to 4/5 as long as posterior notopleural seta; cercal plate without median apical process ***Fannia corvina* Verrall** [China, Japan, North America, throughout Europe]
- Presutural biserial; calypters brown; *pra* 1/2 as long as posterior notopleural seta; cercal plate with median apical process ***Fannia maculosa* Nishida** [Japan]
- 7 Hind femur without distinct *pv*..... **8**
- Hind femur with distinct *pv* **16**
- 8 Wing hyaline ***Fannia borealis* Chillcott** [Canada]
- Wing distinctly yellow or brown..... **9**
- 9 Calypters white; haltere yellowish ***Fannia fuscitibia* Stein** [Czech Republic, Great Britain, Japan, throughout North America]
- Calypters yellowish or yellow; haltere yellow or brownish yellow **10**
- 10 Lower calypter not projecting beyond upper one and smaller ***Fannia melanura* Chillcott** [Throughout North America]
- Lower calypter projecting beyond upper one and larger **11**
- 11 Abdomen without pollinosity or stripe and therefore black and shining ***Fannia nitidiventris* Wang & Zhang, sp. n.** [China]
- Abdomen with pollinosity and median linear stripe or triangular stripe **12**
- 12 Syntergite 1+2 to tergite 4 each with a median dark linear stripe **13**
- Syntergite 1+2 to tergite 4 each with a triangular stripe **15**
- 13 Presutural *acr* triserial ***Fannia dorsovittata* Wang** [China]
- Presutural *acr* biserial..... **14**

- 14 *Pra* stout, 3/4 length of posterior notopleural seta; hind tibia with 4 *ad*.....
 ***Fannia submaculata* Wang & Zhao, sp. n.** [China]
- *Pra* short, 1/2 length of posterior notopleural seta; hind tibia with 3 or 4 *ad*.....
 ***Fannia urbana* Nishida** [Japan]
- 15 Mid tibia with 2 *ad*, 1 or 2 *pd*..... ***Fannia car-***
***bonaria* Meigen** [Canada, China, Japan, throughout Europe, United States]
- Mid tibia with 3 *ad*, 3 or 4 *pd*..... ***Fannia***
***pseudonorvegica* D’Assis-Fonseca** [Czech Republic, England, Hungary]
- 16 Parafacial with setae..... ***Fannia vernalis* Nishida** [Japan]
- Parafacial bare..... **17**
- 17 Hind femur with a complete *pv* row **18**
- Hind femur with *pv* only in basal 1/2 to 3/4..... **20**
- 18 Hind femur with *pv* row becoming gradually weaker towards apex.....
 ***Fannia garretti* Chillcott** [Canada, United States]
- Hind femur with *pv* row not becoming gradually weaker towards apex..... **19**
- 19 Occipital setae present; hind tibia with 5–8 *ad*..... ***Fannia fulgida* Nishida** [Japan]
- Occipital setae absent; hind tibia with 2 or 3 *ad*..... ***Fannia norvegica***
Ringdahl [China, Czech Republic, Denmark, Great Britain, Greek, Japan,
 North Africa, Norway, Spain, Switzerland, throughout North America]
- 20 *Pra* 1, stout, 2/3 to 3/4 length of posterior notopleural seta; mid tibia with 2 *ad*;
 hind tibia with 3 *ad* and 2 *av*..... ***Fannia imperatoria* Nishida** [China, Japan]
- *Pra* 2, weak, the longest one 1/2 length of posterior notopleural seta; mid tibia
 with 1 *ad*; hind tibia with 1 *ad* and 1 *av*... ***Fannia pallidibasis* Pont** [Morocco]
- 21 Syntergite 1+2 to tergite 4 each with a distinctly median dark triangular
 stripe..... **22**
- Syntergite 1+2 to tergite 4 each with a median linear stripe or an inverted T-
 shaped linear stripe **26**
- 22 Mid tibia with 1 *ad*..... ***Fannia capricornis* Xue** [China]
- Mid tibia with 2 or 3 *ad* **23**
- 23 Occipital setae in complete row; frontal setae 7–9.....
 ***Fannia neopolychaeta* Chillcott** [North America]
- Occipital setae with gap in row in posterior 1/3; frontal setae 9–13 **24**
- 24 In ventral view, surstylus not projecting at middle on posterior margin, bacil-
 liform sclerite curved and hook-like in distal part
 ***Fannia trigonifera* Chillcott** [United States]
- In ventral view, surstylus projecting and horn-like and becoming broader at
 middle on posterior margin, bacilliform sclerite straight in distal part..... **25**
- 25 Mid femur with 6–8 long and sparse *av* in basal 2/3.....
 ***Fannia japonica japonica* Nishida** [Japan]
- Mid femur with only short *av* in basal half.....
 ***Fannia japonica amamiensis* Nishida** [Japan]
- 26 Palpus at most 1/2 length of prementum **27**
- Palpus more than 1/2 length of prementum **28**

- 27 Hind tibia with only 1 stout *ad*; palpus less than 1/2 length of prementum..... ***Fannia minutipalpis Stein*** [China, Czech Republic, Germen, North America, Slovak Republic]
 – Hind tibia with 2 stout *ad*; palpus 1/2 length of prementum.....
 ***Fannia brevipalpis Chillcott*** [United States]
 28 Parafacial at middle 2/3 as wide as width of postpedicel.....
 ***Fannia pauli Pont*** [Europe, Russia]
 – Parafacial at middle 1/3–1/2 as wide as width of postpedicel **29**
 29 Hind femur with *pv* row in basal 2/3 to basal part; postpedicel three times longer than wide..... ***Fannia tauricornis Wang, Xue & Su*** [China]
 – Hind femur with *pv* row only in basal 1/2 to basal part; postpedicel 2.5 times longer than wide..... **30**
 30 Eye with sparse and short hairs; prementum with thin gray pollinosity; *pra* 3/4 length of posterior notopleural seta..... ***Fannia polychaeta Stein*** [Austria, Czech Republic, Germany, Russia, Sweden; Bermuda]
 – Eye bare; prementum black and shining, without pollinosity; *pra* 2/3 length of posterior notopleural seta ***Fannia antilocera Wang, Xue and Su*** [China]

Descriptions of three new species from China

Fannia fani Wang & Wu, sp. n.

<http://zoobank.org/1A7E9708-AFB6-45ED-948E-EB35BAF6C084>

Fig. 1

Diagnosis. This species is characterized as follows: presutural *acr* biserial; katepisternal setae 1+1; calypters yellowish; haltere yellow; mid coxa without *ad*; mid femur without ventral spine; mid tibia with only one *d* and one *v* at apex; hind femur with only two or three stout *av* on swollen part in distal half, the longest one not longer than 1/2 of hind tibial length, all other short hair-like; cercal plate without long setae cluster in upper part.

Description. MALE. Body length 4.5–5.0 mm. Eye with sparse and short light brown hairs; upper inner facets larger than the rest; postocular setae in one row, long and fine, curved anteriorly in the upper part of head, occipital setae behind the postocular setae on vertex; fronto-orbital plate and parafacial with grayish silver pollinosity; at narrowest point frons slightly wider than the distance between outer margins of two posterior ocelli, as wide as the width of postpedicel; frontal stripe black, with thin gray pollinosity, at narrowest point slightly narrower than the width of fronto-orbital plate; frontal setae 12–15, stout, nearly reaching ocellar triangle; without orbital seta; parafacial bare, at middle as wide as the width of postpedicel; antenna black, postpedicel 1.6 times longer than wide, arista black; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; subvibrissal setulae in one row, lateral of subvibrissal setulae with some fine setae; gena

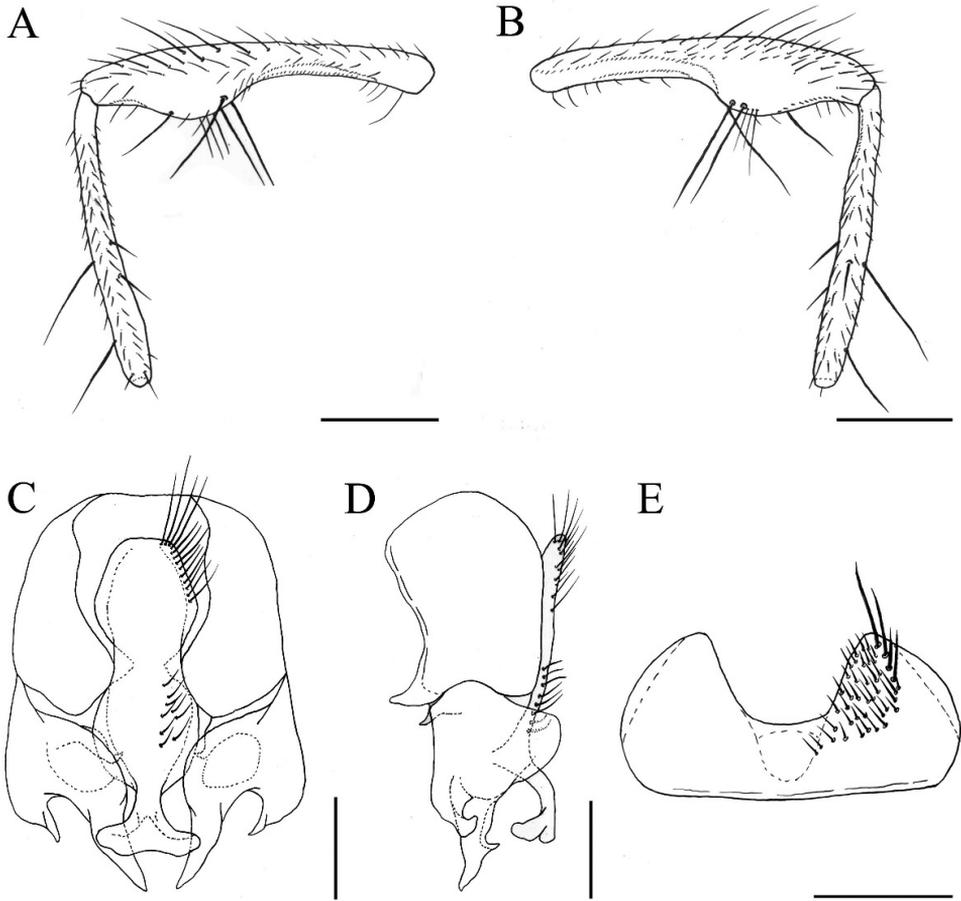


Figure 1. *Fannia fani* Wang & Wu, sp. n., male, holotype (specimen from Heilongjiang, deposited in SHEM). **A** Right hind leg, anterior view **B** Right hind leg, posterior view **C** Terminalia, ventral view **D** Terminalia, lateral view **E** Sternite 5, ventral view. Scale bars: **A–B** 0.50 mm; **C–E** 0.25 mm.

and genal dilation with black setulae, upper margin of gena with upcurved setae; prementum with thin grayish yellow pollinosity, 2.5 times longer than wide; palpus black, claviform, as long as prementum. Thorax black in ground color, notum with brownish gray pollinosity, and with four slightly wide but indistinct stripes; presutural *acr* biserial, only prescutellar pairs slightly stout, the distance between *acr* rows 1/2 of the distance between *acr* row and *dc* row, *dc* 2+3, *ia* 0+2, *pra* 1, 3/5 to 2/3 of the length of posterior notopleural seta, notopleuron without setula; proepisternal setae 2, proepimeral setae 2, around proepimeral setae with ten to 13 slender setulae; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without ventral spine; anterior spiracle yellowish, small, posterior one yellow; calypters yellowish, the lower one slightly projecting

beyond the upper one. Wing brownish; veins brown; tegula dark brown; basicosta yellowish brown; costal spine inconspicuous; node of Rs bare on ventral and dorsal surfaces; vein R_{4+5} straight, vein M_{1+2} slightly close to vein R_{4+5} distally; crossveins without obvious cloud; halter yellow but brown in distal part. Legs entirely black; fore coxa without anterior spine on ventral surface, fore femur with complete *pv* row, fore tibia without *ad* and *p*; mid coxa without a hook-like spine or spine-like seta, mid femur with long and sparse *av* in basal part, becoming gradually denser and shorter towards apex, biserial and short spine-like in preapical part, then with a gap towards apex and with four or five comb-like setae in distal part, *ad* row complete but short, *pv* row complete and stout, biserial in preapical part, behind *pv* row with a complete and stout row of setae, mid tibia slightly slender in basal 2/5 and slightly swollen in distal 3/5, with one *ad* and one or two *pd* in distal half, with one *d* and one *v* at apex, and with numerous slender setulae on ventral surface, the longest one slightly shorter than mid tibial width in distal part, mid first tarsomere without basal tooth-like spine on ventral surface; hind coxa bare on posterior surface, hind femur slightly curved and swollen in distal half, with *av* only on swollen part, 2-3 of them stout, other trichia all short hair-like, *ad* row stout (Fig. 1A), posterior to posteroventral surface bare in basal 2/3, with five *pv* in distal 1/3, only two slightly stout, with three or four slender *pv* in distal part (Fig. 1B), hind tibia with one median *av*, without *ad*, with one stout median *d*, with one *d* in distal half (Fig. 1A, B). Abdomen long and flattened, black in ground color, with gray pollinosity; syntergite 1+2 to tergite 4 each with a median triangular stripe, stripe on tergite 2 slightly broader in basal half, 1/2 as wide as the width of tergite, tergite 5 with a dark median stripe; sternite 1 with setulae, sternites 2 and 3 long and narrow, sternite 4 broad, sternite 5 strongly concave on posterior margin and straight on anterior margin, with four strong setulae above (Fig. 1E); cercal plate longish, broad in ventral view, slender in lateral view (Fig. 1C, D); surstylus broad at basal part, separated into two branches at middle, in lateral view the anterior one short and curved hook-like while the posterior one long and straight (Fig. 1C, D).

FEMALE. Unknown.

Remarks. This new species appears to be most similar to *F. xiaoi* Fan, 2000 but differs by having *acr* in two rows; katepisternal setae 1+1; calypters yellowish; haltere yellow; mid femur without ventral spine; mid tibia with only one *d* and one *v* at apex; mid first tarsomere without any special structure; hind femur with only two or three stout *av* on swollen part in distal half, the longest one not longer than 1/2 of hind tibial length, all other short hair-like (Fig. 1A); cercal plate without long setae cluster in upper part (Fig. 1C, D).

Etymology. The new species is named after Prof. Zi-de Fan in honor of his outstanding work on Calyptratae.

Type series. Holotype male: China, Heilongjiang, Wuying, 12.V.1979, Coll. J. Shen (SHEM). Paratype: 1 male, the same data as holotype.

Distribution. Known only from the type locality in Heilongjiang, China.

***Fannia nitidiventris* Wang & Zhang, sp. n.**

<http://zoobank.org/B1D49BEA-B845-4047-BF0E-396CB099CD09>

Fig. 2

Diagnosis. This species is characterized as follows: frontal setae five or six; posterior *acr* biserial; calypters yellowish; haltere yellow; fore tibia all black; hind femur with three or four *av*; abdomen black and shining, without pollinosity or stripe; sternite 5 slightly concave on posterior margin; from ventral view, cercal plate slightly rounded, the hook-like projection on its lower margin curved outward; surstylus slightly broad.

Description. MALE. Body length 4.8 mm. The whole body shining black. Eye bare and large; postocular setae in one row, short and neatly arranged, without occipital seta; fronto-orbital plate and parafacial with gray pollinosity; at narrowest point frons 2/3 of the distance between outer margins of two posterior ocelli, 2/3 of the width of postpedicel; frontal stripe linear at narrowest point; frontal setae five or six, nearly reaching 2/3 of frons, the gaps filled with numerous fine setulae, orbital setae absent; parafacial bare and narrow, at middle as wide as 2/5 of the width of postpedicel; antenna black, postpedicel 2.0 times longer than wide, arista yellow in basal half, haired, the longest hair equal to basal arista width; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; subvibrissal setulae in one row, lateral of subvibrissal setulae row with a row of short setae; gena and genal dilation with black setulae, upper margin of gena without upcurved seta; proboscis stout, labella large; prementum shining, with thin grayish yellow pollinosity, 1.2 times longer than wide; palpus black, claviform, as long as prementum. Thorax black in ground color, shining, notum with thin grayish yellow pollinosity; *acr* biserial, hair-like, only prescutellar pairs stout, the distance between *acr* rows narrower than the distance between *acr* row and *dc* row, *dc* 2+3, *ia* 0+2, *pra* 1, 2/3 of the length of posterior notopleural seta, notopleuron without setula; proepisternal setae 2, proepimeral seta 1, lower part with one slender setula; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without ventral spine, with only some fine hair; anterior spiracle yellowish and small, posterior one brownish yellow; calypters yellowish, the lower one slightly projecting beyond the upper one. Wing brownish; veins brown; wing base being similar color as rest of wing; tegula black; basicosta brownish; costal spine inconspicuous; node of Rs bare on ventral and dorsal surfaces; vein R_{4+5} straight, veins R_{4+5} and M_{1+2} parallel to each other distally; crossveins without obvious cloud; haltere yellow. Legs entirely black; fore coxa without anterior spine on ventral surface, fore femur with complete *pv* row, fore tibia without *ad* and median *p*, with only one *d* in preapical part, fore first tarsomere with several longish basal setae on ventral surface; mid coxa without a hook-like spine or spine-like seta, mid femur with complete *av* row, short and strong, at middle the longest seta shorter than the width of mid femur, without gap, comb-like in distal 1/3, with complete *pv* row, biserial in median part, the longest one is shorter than the width of mid femur, with one fine *p* row, the longest one is equal to the width of mid femur, mid tibia slightly swollen towards apex, with three *ad*, two *pd*, and with numerous slender setulae on ventral surface, the longest one 2/3 of mid tibial width in distal half, mid first tarsomere without basal

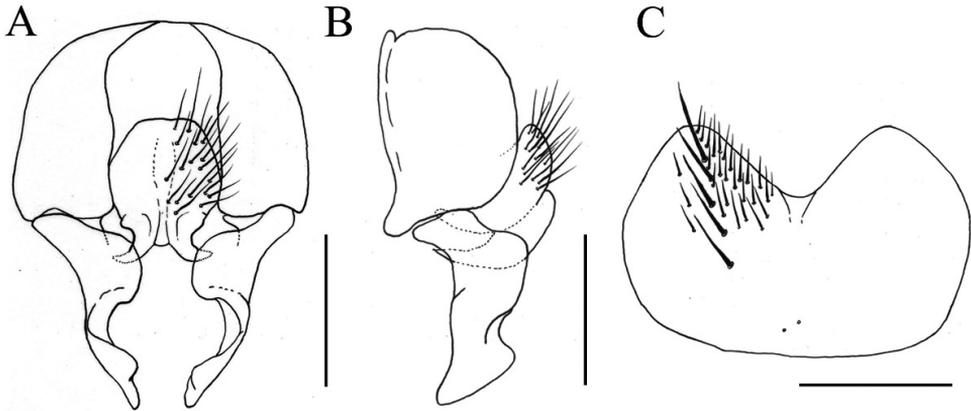


Figure 2. *Fannia nitidiventris* Wang & Zhang, sp. n., male, holotype (specimen from Ningxia, deposited in IESNU). **A** Terminalia, ventral view **B** Terminalia, lateral view **C** Sternite 5, ventral view. Scale bars 0.25 mm.

tooth-like spine on ventral surface; hind coxa bare on posterior surface, hind femur not curved, with three or four stout *av* only in distal 2/5, without *pv*, hind tibia with two *av*, two *ad* and one median *d*. Abdomen oval and flattened, black in ground color, shining, without stripe and pollinosity; sternite one bare, sternite 5 broad, concave on posterior margin (Fig. 2C); from ventral view, cercal plate slightly rounded, the hook-like projection on its lower margin curved outward (Fig. 2A); surstylus slightly broad, not separated into two branches (Fig. 2A, B).

FEMALE. Unknown.

Remarks. The new species is distinguished from its likely closest relative *Fannia fuscitibia* Stein, 1920 by having five or six frontal setae; posterior *acr* biserial; calypters yellowish; haltere yellow; fore tibia all black; hind femur with three or four *av*; abdomen black and shining, without stripe; sternite 5 slightly concave on posterior margin (Fig. 2C); from ventral view, cercal plate slightly rounded, the hook-like projection on its lower margin curved outward (Fig. 2A); surstylus slightly broad (Fig. 2B).

Etymology. This specific name refers to a characteristic of the species that the abdomen is black and shiny.

Holotype. Male: China, Ningxia, Jingyuan, Lvyuan, 1700 m, 29.V.2008, Coll. M.F. Wang (IESNU).

Distribution. Known only from the type locality in Ningxia, China.

***Fannia submaculata* Wang & Zhao, sp. n.**

<http://zoobank.org/F9EB81F9-7C6A-4FD3-90FC-411FDA09C65F>

Fig. 3

Diagnosis. This species is characterized as follows: postsutural *acr* in two rows; *pra* stout, 3/4 of the length of posterior notopleural seta; calypters and haltere in lighter

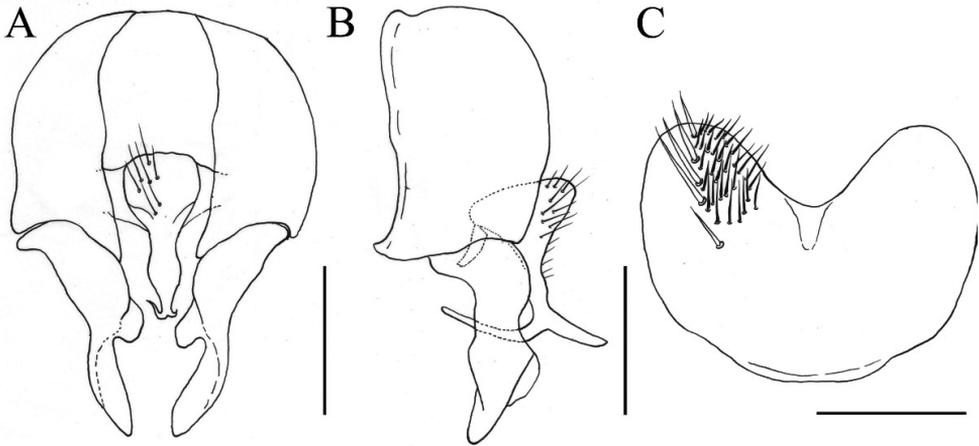


Figure 3. *Fannia submaculata* Wang & Zhao, sp. n., male, holotype (specimen from Ningxia, deposited in IESNU). **A** Terminalia, ventral view **B** Terminalia, lateral view **C** Sternite 5, ventral view. Scale bars 0.25 mm.

color; knees and base of fore tibia yellow; hind tibia with four *ad*; tergite 5 with a distinct dark median stripe; cercal plate slender in ventral view; surstylus with a distinct notch on inner margin in median part; bacilliform sclerite becoming gradually narrower towards apex.

Description. MALE. Body length 5.0 mm. Eye bare; upper inner facets larger than the rest; postocular setae in one row, irregular in length, curved anteriorly, with some occipital setae behind the postocular setae on vertex; fronto-orbital plate and parafacial with grayish white pollinosity; at narrowest point frons equal to the distance between outer margins of two posterior ocelli, equal to the width of postpedicel; frontal stripe black and narrow; frontal setae nine or ten all stout, situated on the lower 4/5 of fronto-orbital plate, nearly reaching ocellar triangle; parafacial bare, at middle 1/2 as wide as the width of postpedicel; antenna black, postpedicel 2.0 times longer than wide, arista haired and black, the longest hair equal to basal arista width; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; subvibrissal setulae in one row, gena and genal dilation with black setulae, upper margin of gena without upcurved seta; proboscis stout, labella large; prementum with grayish yellow pollinosity, 2.0 times longer than wide; palpus black, claviform, as long as prementum. Thorax black in ground color, shining, notum with thin brown pollinosity; *acr* biserial, slightly stout, prescutellar pairs stout; the distance between *acr* rows narrower than the distance between *acr* row and *dc* row, *dc* 2+3, *ia* 0+2, *pra* 1, 3/4 of the length of posterior notopleural seta, notopleuron without setula; proepisternal setae 2, proepimeral seta 1; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without ventral spine; anterior spiracle small and dark brown, posterior one dark brown; calypters yellowish, the lower one distinctly projecting beyond the upper one. Wing brownish; veins dark brown; wing base being similar color as rest of wing; tegula black; basicosta brownish; costal spine

inconspicuous; node of Rs bare on ventral and dorsal surfaces; vein R_{4+5} straight, veins R_{4+5} and M_{1+2} parallel to each other distally; crossveins without obvious cloud; haltere brown. Legs entirely black, except knees and base of fore tibia yellow; fore coxa without anterior spine on ventral surface, fore femur with complete *pv* row, fore tibia without *ad* and median *p*, fore first tarsomere with several short basal setae on ventral surface; mid coxa without any hook-like spine or spine-like seta, mid femur with complete *av* row, stout in basal half, becoming gradually shorter and denser towards apex, without gap, *pv* row complete, in one row, with one slender *p* row, mid tibia slightly swollen in distal half, with two *ad*, two *pd*, and with numerous slender setulae on ventral surface, the longest one $3/4$ as long as mid tibial width in distal part, mid first tarsomere without basal tooth-like spine on ventral surface; hind coxa bare on posterior surface, hind femur with *av* row, setula-like in basal half, with four long *av* in distal $2/5$, without *pv*, hind tibia with two *av*, four *ad* and one median *d*. Abdomen oval and flattened, black in ground color, shining, with thin gray pollinosity; syntergite 1+2 to tergite 5 each with a dark median stripe; sternite one with slender setulae, sternite 5 broad, concave on posterior margin (Fig. 3C); cercal plate slender in ventral view, separated into two branches at apex, pointed anteriorly and posteriorly, respectively, in lateral view (Fig. 3A, B); surstylus with a distinct notch on inner margin at middle in ventral view (Fig. 3A); bacilliform sclerite short, becoming gradually narrower towards apex (Fig. 3B)

FEMALE. Unknown.

Remarks. This new species is similar to *Fannia maculosa* Nishida, 2003 but can be characterised by its postsutural *acr* in two rows; *pra* long, $3/4$ of the length of posterior notopleural seta; wing base being similar color as rest of wing; calypters and all haltere in lighter color; knees and base of fore tibia yellow; hind tibia with four *ad*; tergite 5 with a distinct dark median stripe; sternite 5 broad (Fig. 3C); cercal plate slender in ventral view (Fig. 3A); surstylus with a distinct notch on inner margin in median part (Fig. 3A); bacilliform sclerite becoming gradually narrower towards apex (Fig. 3B).

Etymology. This specific name refers to the similarity between the new species and *F. maculosa* Nishida.

Holotype. Male: China, Ningxia, Jingyuan, Dongshanpo, 2200 m, 27.VI.2008, Coll. M.F. Wang (IESNU).

Distribution. Known only from the type locality in Ningxia, China.

Redescription of one newly recorded species from China

Fannia norvegica Ringdahl, 1934

Fig. 4

Diagnosis. This species is characterized as follows: parafacial bare; occipital setae absent; lower calypter distinctly projecting beyond upper one; haltere yellowish or brownish yellow; mid tibia with 2 *ad*; hind femur with a complete *pv* row, not becoming gradually weaker towards apex; hind tibia with 2 or 3 *ad*; syntergite 1+2 to tergite 4

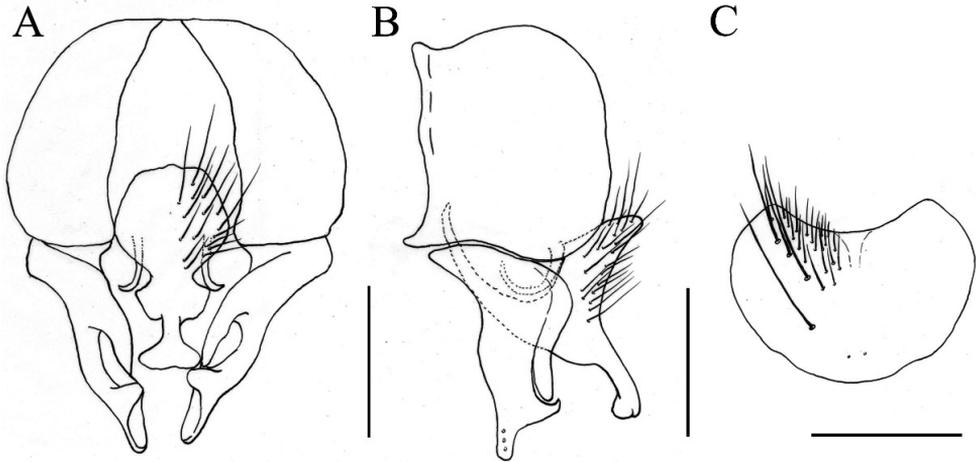


Figure 4. *Fannia norvegica* Ringdahl, 1934, male (specimen from Ningxia, deposited in IESNU). **A** Terminalia, ventral view **B** Terminalia, lateral view **C** Sternite 5, ventral view. Scale bars 0.25 mm.

each with a dark median triangular stripe, tergite 5 with a broad median stripe; sternite 5 broad and with four strong setulae; bacilliform sclerite curved.

Description. MALE. Body length 4.8–5.5 mm. Eye bare; postocular setae in one row, as long as the width of frons, neatly arranged, without occipital seta; fronto-orbital plate and parafacial with grayish silvery pollinosity; at narrowest point frons as wide as the distance between outer margins of two posterior ocelli, equal to the width of postpedicel; frontal stripe black, at narrowest point as wide as fronto-orbital plate; frontal setae ten or eleven, stout, nearly reaching ocellar triangle, orbital setae absent; parafacial bare, at middle 1/2 as wide as the width of postpedicel; antenna black, postpedicel 2.0 times longer than wide, arista black and haired, the longest hair equal to basal arista width; epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile; subvibrissal setulae in one row, lateral of subvibrissal setulae with one or two rows of fine setae; gena and genal dilation with black setulae, upper margin of gena without upcurved seta; proboscis stout, labella large; prementum with thin gray pollinosity, 1.5 times longer than wide; palpus black, claviform, as long as prementum. Thorax black in ground color, notum slightly shining, with thin brown pollinosity, without distinct stripe; *acr* biserial, slightly stout, prescutellar pairs slightly stout, the distance between *acr* rows 1/2 of the distance between *acr* row and *dc* row, *dc* 2+3, *ia* 0+2, *pra* 1, 4/5 of the length of posterior notopleural seta, notopleuron without setula; proepisternal setae 2, proepimeral seta 1, lower part with one short setula; basisternum, proepisternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+1, katepisternum without ventral spine, with only eleven long setulae on lower margin; anterior spiracle yellowish, posterior one brown; calypters yellowish, the lower one distinctly projecting beyond the upper one. Wing brownish; veins brownish; wing base being same color as rest of the wing; tegula black; basicosta yellowish; costal spine inconspicuous; node of Rs bare on ventral and dorsal surfaces;

vein R_{4+5} straight, veins R_{4+5} and M_{1+2} parallel to each other distally; crossveins without obvious cloud; haltere yellow. Legs entirely black, except joint of femur and tibia brown; fore coxa without anterior spine on ventral surface, fore femur with complete pv row, fore tibia without ad and median p , with only a stout seta in preapical part, fore first tarsomere with several longish basal setae on ventral surface; mid coxa without a hook-like spine or spine-like seta, mid femur with long and sparse av row in basal half, the longest seta equal to the width of mid femur, becoming gradually denser and shorter towards apex in distal half, comb-like in preapical part, pv row complete, slightly biserial in median part, with slender p row, mid tibia slightly swollen towards apex, with two ad and two pd , and with numerous slender setulae on ventral surface, in distal half $3/4$ as long as mid tibial width, mid first tarsomere without basal tooth-like spine on ventral surface; hind coxa bare on posterior surface, hind femur with av row, only three to five av in distal half, stout, pv row hair-like in basal half, stout and longer than tibial width in distal half, hind tibia with two av (sometimes three), three ad (sometimes two) and one median d , with several slightly erect median setae on posterior surface, hind tarsi without basal tooth-like spine. Abdomen long and flattened, black in ground color, shining, with thin gray pollinosity; syntergite 1+2 to tergite 4 each with a dark median triangular stripe, tergite 5 with a broad median stripe; sternite one with setulae, sternite 5 broad and rounded, slightly concave on posterior margin, with four strong setulae above (Fig. 4C); cercal plate with a strong curved projection pointed anteriorly in lateral view, bare in median part and at apex (Fig. 4A, B); surstylus broad, separated into two branches at middle, anterior branch broad and with three setulae at apex and posterior branch short and thin (Fig. 4A, B); bacilliform sclerite curved (Fig. 4A, B).

FEMALE. Unknown from China.

Remarks. The species *Fannia norvegica* Ringdahl, 1934 is newly recorded from China. Here, a detailed redescription is provided as it was not been adequately described previously. Illustrations of male terminalia including sternite 5 is also given.

Material examined. 1 male, China, Ningxia, Jingyuan, Mt. Heshangpu, 2000 m, 23.VI.2008, Coll. M.F. Wang (**IESNU**); 1 male, China, Ningxia, Jingyuan, Mt. Heshangpu, 2150 m, 24.VI.2008, Coll. Y.X. Wu (**IESNU**); 1 male, China, Ningxia, Jingyuan, Mt. Baiyun, 2300 m, 28.VI.2008, Coll. M.F. Wang (**IESNU**).

Distribution. China: Ningxia, Jingyuan; Czech Republic, Denmark, Great Britain, Greek, Japan, North Africa, Norway, Spain, Switzerland, throughout North America.

Acknowledgements

Sincere thanks to Dr. Ben Cleveland (School of BioSciences, the University of Melbourne), Prof. Yu-xiao Wu and Prof. Wei-bing Zhu (both of Shanghai Entomological Museum, Chinese Academy of Sciences, Shanghai, P. R. China) and Ms. Jing Wang (Institute of Entomology, Shenyang Normal University, Shenyang, P. R. China) for providing help in examining specimens. This study was funded by the Director Foundation

of the National Nature Science Foundation of China (NO. 31272347, NO. 31071957, NO. 30770252), the Director Fund of Ecology and Environment Research Centre, Shenyang Normal University (No. EERC-K-201401).

References

- Barták M, Preisler J, Kubík Š, Šuláková H, Sloup V (2016) Fanniidae (Diptera): new synonym, new records and an updated key to males of European species of *Fannia*. *ZooKeys* 593: 91–115. <https://doi.org/10.3897/zookeys.593.7735>
- Chillcott JG (1961) A revision of the Nearctic species of Fanniinae (Diptera: Muscidae). *The Canadian Entomologist. Supplement* 14(1960): 1–295.
- D’Assis-Fonseca ECM (1966) Eight undescribed species of Muscidae (Diptera) from Britain. *Entomologist’s Monthly Magazine* 101: 269–278.
- Fan ZD (2000) A new species of *Fannia* from Eastern Inner Mongolia, China (Diptera: Fanniidae). *Acta Zootaxonomica Sinica* 25(3): 345–348.
- Gregor F, Rozkošný R (1993) New synonymies in the European Fanniidae (Diptera). *European Journal of Entomology* 90: 227–234.
- McAlpine JF (1981) Morphology and terminology – adults. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Coords.), *Manual of Nearctic Diptera*. Vol. 1. Research Branch Agriculture Canada Monograph 27: 9–63.
- Meigen JW (1826) *Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten*. Vol. 5. Hamm, 412 pp.
- Nishida K (1974a) Studies on the species of Fanniinae (Diptera: Muscidae) from Japan. II. One new species from Japan and Taiwan, and four newly recorded species of genus *Fannia* from Japan. *Japanese Journal of Sanitary Zoology* 25: 205–210. <https://doi.org/10.7601/mez.25.205>
- Nishida K (1974b) On eight species of the genus *Fannia* (Diptera, Muscidae) from Japan, with the description of new species. *Kontyû* 42(2): 184–191.
- Nishida K (1975a) Studies on the species of Fanniinae (Diptera: Muscidae) from Japan. Description of *Piezura nigrigenus* sp. nov. and *Fannia japonica amamiensis* subsp. nov. and redescription of *Platycoenosia mikii* Strobl, 1894. *Japanese Journal of Sanitary Zoology* 26: 21–24. <https://doi.org/10.7601/mez.26.21>
- Nishida K (1975b) Six new and one newly recorded species of the genus *Fannia* (Diptera: Muscidae) from Taiwan, with a key to species. *Kontyû* 43(3): 364–380.
- Nishida K (2002) Two new species (Diptera: Fanniidae) collected in the Imperial Palace, Tokyo, Japan. *Medical Entomology and Zoology* 53(Supplement 2): 173–180. <https://doi.org/10.7601/mez.53.173>
- Nishida K (2003) Studies on the species of Fanniidae (Diptera) from Japan. V. A new species belonging to the *carbonaria* subgroup and three newly recorded species from Japan. *Medical Entomology and Zoology* 54(1): 97–103. <https://doi.org/10.7601/mez.54.97>
- Ringdahl O (1934) Einige neue Musciden. *Konowia* 13: 97–100.
- Rozkošný R, Gregor F, Pont AC (1997) The European Fanniidae (Diptera). *Acta Scientiarum Naturalium Academiae Scientiarum Bohemicae-Brno* 31: 1–80.

- Stein P (1895) Die Anthomyidengruppe *Homalomyia* nebst ihren Gattungen und Arten. Berliner Entomologische Zeitschrift 40: 108. <https://doi.org/10.1002/mmnd.18950400104>
- Stein P (1920) Nordamerikanische Anthomyiden. 2 Beitrag. Archiv für Naturgeschichte 84, A9: 1–106.
- Stuckenberg BR (1999) Antennal evolution in the Brachycera (Diptera), with a reassessment of terminology relating to the flagellum. Studia Dipterologica 6: 33–48.
- Verrall GH (1892) Two new English species of *Homalomyia*. Entomologist's Monthly Magazine 2 [= (2) 3]: 149.
- Wang MF, Xue WQ, Su LX (2004) Notes on *carbonaria* species group of genus *Fannia* Robineau-Desvoidy (Diptera: Fanniidae) in China. Entomologia Sinica 11: 135–142. <https://doi.org/10.1111/j.1744-7917.2004.tb00187.x>
- Wang MF, Zhang D, Zheng S, Zhang CT (2009) A review of the *carbonaria*-subgroup of *Fannia* Robineau-Desvoidy (Diptera: Fanniidae), with descriptions of two new species from China. Zootaxa 2204: 37–47.
- Xue WQ, Wang MF (1998) Fanniidae. In: Xue WQ, Chao CM (Eds) Flies of China. Vol. 1. Liaoning Science and Technology Press, 809–835. [In Chinese]
- Zhang D, Zhang M, Pape T, Gu CW, Wu W (2013) *Sarcophaga (Hoa) flexuosa* Ho (Diptera: Sarcophagidae): association of sexes using morphological and molecular approaches, and a redefinition of *Hoa* Rohdendorf. Zootaxa 3670(1): 71–79.

A review of the *Pseudobarbus afer* (Peters, 1864) species complex (Teleostei, Cyprinidae) in the eastern Cape Fold Ecoregion of South Africa

Albert Chakona¹, Paul H. Skelton¹

¹ South African Institute for Aquatic Biodiversity, Private Bag 1015, Grahamstown 6140, South Africa

Corresponding author: Albert Chakona (a.chakona@saiab.ac.za)

Academic editor: M. Bichuette | Received 5 November 2016 | Accepted 9 February 2017 | Published 20 February 2017

<http://zoobank.org/C63537A4-F302-4021-8F87-516622043976>

Citation: Chakona A, Skelton PH (2017) A review of the *Pseudobarbus afer* (Peters, 1864) species complex (Teleostei, Cyprinidae) in the eastern Cape Fold Ecoregion of South Africa. ZooKeys 657: 109–140. <https://doi.org/10.3897/zookeys.657.11076>

Abstract

The Eastern Cape redbfin, *Pseudobarbus afer*, has long been considered to be a single widespread and variable species occurring in multiple isolated river systems in the Cape Fold Ecoregion (CFE) at the southern tip of Africa. Mitochondrial cytochrome *b* and control region sequence data of individuals from populations currently assigned to *P. afer* across the species' distribution range revealed existence of four deeply divergent taxonomic units: (i) the Mandela lineage confined to the Sundays, Swartkops and Baakens river systems, (ii) the Krom lineage endemic to the Krom River system, (iii) the St Francis lineage occurring in the Gamtoos and adjacent river systems, and (iv) the Forest lineage occurring in several coastal river systems from the Tsitsikamma to the Klein Brak River system. The Forest lineage is closely related to *P. phlegethon* from the Olifants River system on the west coast of South Africa, suggesting that it does not belong to *P. afer* s.l. Herein we focus on the three lineages within the *P. afer* s.l. complex and provide new diagnosis for *P. afer* s.s (Mandela lineage), revalidate *P. senticeps* (Krom lineage) as a distinct species, and describe a new species *P. swartzi* (St Francis lineage). The three species exhibit subtle differences, which explains why they were previously considered to represent a single variable and widespread species. *Pseudobarbus senticeps* differs from both *P. afer* and *P. swartzi* by having fewer (i.e. larger) scales (25–33, mode 29 lateral line scale series; 10–12, mode 11 circumpeduncular scales) and presence of a lateral stripe which terminates in a conspicuous triangular blotch at the base of the caudal fin. Long barbels which reach or surpass the vertical through the posterior edge of the eye further separate *P. senticeps* from *P. afer* s.s. which possesses simple short barbels which do not reach the vertical through the posterior margin of the eye. *Pseudobarbus afer* s.s differs from *P. swartzi* sp. n. by

possession of fewer scale rows along the lateral line (29–35, mode 32 vs 34–37, mode 36 in *P. swartzi*), fewer scales around the caudal peduncle (12–16, mode 12 vs 13–17, mode 16 in *P. swartzi*) and a distinct mesh or net-like pigmentation pattern on latero-ventral scales.

Keywords

Cape Fold Ecoregion, endemic hotspot, single barbeled redfins, *Pseudobarbus senticeps*, *P. swartzi*

Introduction

The cyprinid genus *Pseudobarbus* currently contains nine valid species endemic to southern Africa. All species of this genus are confined to streams associated with the Cape Fold Ecoregion (CFE) at the southern tip of Africa, with the exception of *P. quathlambae* which is endemic to the headwater tributaries of the Orange River in the Lesotho Highlands (Barnard 1943; Skelton 1988; Chakona and Swartz 2013; Chakona et al. 2014). Redfins were previously assigned to the genus *Barbus* Cuvier & Cloquet, 1816 until Skelton (1988) revalidated Smith's (1841) subgenus *Pseudobarbus* and raised it to full generic status. The monophyly of *Pseudobarbus* is supported by both molecular data (mitochondrial DNA sequences) and morphological characters (Swartz et al. 2009). Species of this tetraploid genus (Naran et al. 2006) are characterised by presence of bright redfins, a soft or flexible primary dorsal spine and development of prominent nuptial tubercles in mature breeding males (Skelton 1988). Most recently Yang et al. (2015) suggested the genus *Pseudobarbus* be expanded to include all tetraploid cyprinines from southern Africa. Whilst this suggestion is accepted pending a critical evaluation of the generic status of these additional species (Skelton 2016), in this paper the traditional, more restricted lineage is intended.

Many species of *Pseudobarbus* have restricted distribution ranges (Skelton 1988; Chakona and Swartz 2013; Chakona et al. 2014). *Pseudobarbus afer* (Peters, 1864) as it is currently described was considered to be the only exception as it has the widest distribution range of all redfin species. Its distribution spans across 28 isolated river systems from the Klein Brak which discharges into Mossel Bay to the Sundays which flows into Nelson Mandela Bay (also known as Algoa Bay) near Port Elizabeth (Figure 1). As with many other southern African freshwater fishes with broad geographical ranges, *P. afer* has had a long and confused taxonomic history. The three syntype specimens (ZMB 5413) were collected by Ludwig Krebs who had settled in the Port Elizabeth-Uitenhage area in the 1820's (Ffolliot and Liversidge 1971). There are uncertainties regarding the type locality of *P. afer*, however, based on available evidence and discussions between Dr R. Liversidge and Dr R. A. Jubbs, the Swartkops River system was suggested as the likely original locality of *P. afer* (see Jubbs 1965). Smith (1936) described *P. senticeps* (as *Barbus senticeps*) based on the holotype collected from the Krom River system, near the central portion of the present distribution of *P. afer*. He identified the much longer barbels and the lower number of lateral line scales as the unique features that differentiated *P. senticeps* from *P. afer*. For a long

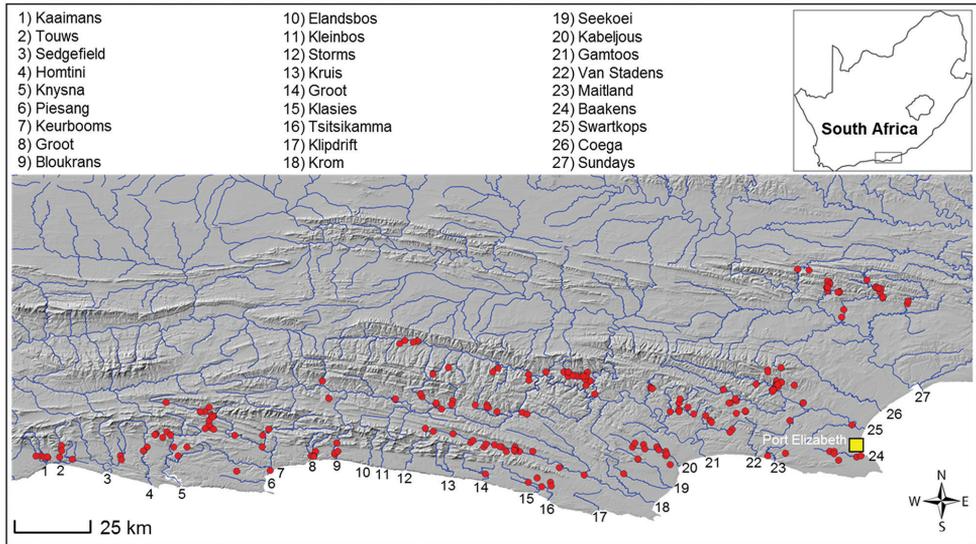


Figure 1. Distribution of the Eastern Cape redbfin, *Pseudobarbus afer*, as presently described.

period after being described, *Barbus afer* was not recognised as a ‘redfin’ species as its live colours were unknown. Jubb (1963, 1965) was the first to recognise that it was a ‘redfin’ minnow endemic to the Eastern Cape region. Jubb (1965) subsequently placed *B. senticeps* in synonymy with Peters’ *Barbus afer*. Boulenger (1911) described *P. asper* (as *B. asper*) from the Gamtoos river system based on four specimens collected from the Groot River near Steytlerville. The distinction between *P. afer* and *P. asper* was often uncertain, resulting in both Barnard (1943) and Jubb (1965) concluding that populations of *Pseudobarbus* from the southern coastal systems (Klein Brak, Kabeljous, Rondebosch and Keurbooms) belonged to *P. asper*. This decision was later revoked by Skelton (1988) who determined the southern coastal populations to be distinct from *P. asper* and he assigned them to *P. afer*. Skelton (1988) concluded that *P. asper* is restricted to the Gouritz and Gamtoos river systems.

The taxonomic integrity of the Eastern Cape redbfin, *P. afer*, however remained unclear as Skelton (1988) reported considerable meristic variability across the species’ distribution range, mainly regarding differences in scale counts (a proxy for scale size). Skelton (1988) identified four subgroupings within *P. afer* based on lateral line scale counts: the western population distributed in the Knysna, Goukamma, Keurbooms, Groot and Bloukrans (mode 35), the Krom population (mode 29), the Gamtoos population (mode 36), and the fourth group comprising the Baakens, Swartkops and Sundays populations (mode 32) (Figure 1). He commented that the variation in scale counts for *P. afer* (25 to 45 lateral line series) was not consistent with that of any redbfin species or most African cyprinids, suggesting that the Eastern Cape redbfin could potentially be a complex of morphologically very similar species.

A molecular study by Swartz et al. (2007) identified four distinct lineages within *P. afer* (which he named as the Forest, Krom, St Francis and Mandela lineages) that are

separated by deep mitochondrial cytochrome *b* (cyt *b*) genetic divergences (3.3–8.3%). The Forest lineage occurs in multiple coastal river systems from the Tsitsikamma River system to the Klein Brak River system. The Krom lineage is restricted to the Krom River system, which is the type locality of *Pseudobarbus senticeps* (Smith, 1936). The St Francis lineage occurs in the Gamtoos, Kabeljous and Swart River systems draining into St Francis Bay. The Mandela lineage is restricted to the Baakens, Swartkops and Sundays river systems draining into Algoa Bay, close to the city of Port Elizabeth (Swartz et al. 2007, 2009). The geographical distributions of these lineages were largely consistent with the subgroupings identified by Skelton (1988). The deep genetic differentiation among these lineages suggest that *P. afer* as currently recognised is a complex of two previously described species and at least two candidate taxa.

Herein we demonstrate that, in addition to their deep genetic divergence, the Krom, St Francis and Mandela lineages can be separated based on scale counts, length of oral barbels and consistent differences in body colour pattern, supporting their status as distinct species. As the specimens that were used for Peters' (1864) description were likely collected from the Swartkops River system (Jubb 1965; Skelton 1988), we consider the Mandela lineage to represent *P. afer* s.s. Here we re-describe *P. afer* s.s. and review its geographic distribution, resurrect *P. senticeps* as valid for the Krom lineage, and delineate morphological differences supporting recognition of the St Francis lineage as a new species, which is described herein as *Pseudobarbus swartzi* sp. n. The Forest lineage was not included in the present study as a phylogenetic analysis based on combined morphological and molecular data indicated that it was more closely related to *P. phlegethon* from the Olifants River system on the west coast of South Africa (Swartz et al. 2009), suggesting that phylogenetically it does not belong to the *P. afer* s.l. complex. The taxonomic status of the Forest lineage will be addressed in a further study that will incorporate the more closely related *P. phlegethon*.

Materials and methods

Sample collection

Morphometric and meristic data used for the present study were generated from both historical collections obtained from the South African Institute for Aquatic Biodiversity (SAIAB) and fresh specimens that were collected between 2010 and 2016. The samples were collected using a combination of seine nets, hand nets and electrofishing. Surveys for the freshly collected specimens followed recommended ethics guidelines of SAIAB. At each locality, a subsample of the captured fishes was anaesthetised with clove oil and a small piece of muscle tissue was dissected from the specimens and preserved in 95% ethanol. Source specimens were fixed in 10% formalin. Upon returning to the laboratory, DNA tissues were stored at -20° C and voucher specimens were transferred through 10% and 50% to 70% ethanol for long-term storage. The

specimens were deposited in the National Fish Collection at SAIAB in Grahamstown, South Africa. The type material has been deposited at SAIAB and Royal Museum of Central Africa (MRAC).

Molecular data

Fresh topotypic specimens for *P. afer* s.s. were collected from the Blindekloof River, a tributary of the Swartkops River system between 2010 and 2015. Topotypic specimens for *P. senticeps* were collected from the Assegaaibos River, a tributary of the Krom River system in 2014. Additional DNA tissue samples were taken from specimens collected from 10 localities in 2015 and 2016 to fill in sampling gaps in the Kouga and Groot sub-catchments of the Gamtoos River system. Methods for obtaining DNA sequence data and their analyses follow Swartz et al. (2007) and Chakona et al. (2013a). We sequenced the mitochondrial cytochrome *b* (*cyt b*) gene to compare with data generated from previous studies (Swartz et al. 2007; Chakona and Swartz 2013; Chakona et al. 2014). The sequences were assigned as topogenotypes (for *P. afer* and *P. senticeps*) and hologenotype and paragenotypes (for *P. swartzi* sp. n.) following Chakrabarty (2010). We included all the newly generated *cyt b* sequences ($n = 30$) to the genetic analysis done by Swartz et al. (2007) to show their phylogenetic position in relation to all known species and lineages of the single barbeled redfins in the CFE. All the new sequences were submitted to GenBank (accession numbers: KY472256–KY472285).

Morphometric and meristic measurements

Methods used to obtain meristic and morphometric data (Table 1) follow Armbruster (2012), Chakona and Swartz (2013), Chakona et al. (2014) and Skelton (1988). Measurements were taken to the nearest 0.1 mm using electronic digital or dial callipers.

We included additional raw data from Skelton (1988) to determine the degree of morphological divergence within the *P. afer* s.l. complex. Specimens were assigned to three groups based on geographical origin and genetic results: *P. afer* s.s. (specimens from the Sundays, Swartkops and Baakens River systems; $n = 68$), *P. swartzi* sp. n. (specimens from the Gamtoos River system, $n = 64$) and *P. senticeps* (specimens from the Krom River system; $n = 31$).

Principal Component Analysis (PCA) was performed on raw meristic variables and morphometric variables in percentages as well as log transformed morphometric data to explore the separation of the specimens and identify the variables that contribute the most to differences among groups. Fin and barbel erosion was observed in some specimens, particularly those that were collected from polluted waters. Such specimens were excluded from the PCA for morphometric data. Morphometric and meristic data were analysed separately using the statistical program PAST (Hammer et al. 2001).

Table 1. Morphological characters of *Pseudobarbus* species used in the present study (reproduced from Chakona et al., 2014).

| Character | Description | Acronym |
|-----------------------------------|--|---------|
| <i>Morphometric measurements</i> | | |
| Standard length | Tip of the snout to the point of flexure of the caudal fin | SL |
| Pre-dorsal length | Tip of the snout to the origin of the dorsal fin | PDL |
| Head length | Tip of the snout to the posterior bony margin of the operculum | HL |
| Snout length | Tip of the snout to the anterior bony edge of the orbit | S |
| Orbit diameter | The greatest bony diameter of the orbit | OD |
| Inter-orbit length | Straight line distance between the bony edges of the orbits | IO |
| Post-orbit length | Distance between the posterior bony edge of orbit to the posterior bony edge of operculum | PO |
| Head depth | Maximum depth measured from the nape | HD |
| Body depth | Maximum depth measured from the anterior base of the dorsal fin | BD |
| Anterior barbel length | From base to tip of anterior barbel | AB |
| Posterior barbel length | From base to tip of posterior barbel | PB |
| Dorsal fin base | Distance between anterior and posterior base of dorsal fin | DB |
| Dorsal fin height | From anterior base to tip of dorsal fin | DH |
| Pectoral fin length | From anterior base to tip of pectoral fin | PtL |
| Pelvic fin length | From anterior base to tip of pelvic fin | PvL |
| Anal fin base | Distance between anterior and posterior base of anal fin | AfB |
| Anal fin height | From anterior base to tip of anal fin | AfH |
| Caudal peduncle length | Distance from posterior base of anal fin the point of flexure of the caudal fin | CPL |
| Caudal peduncle depth | The least depth of the caudal peduncle | CPD |
| Pectoral to pelvic fin length | Distance between the posterior margins of the fin bases | PP |
| Pelvic to anal fin length | Distance between the posterior base of the pelvic fin to the anterior base of the anal fin | PA |
| Body width | The greatest width just anterior to the origin of the dorsal fin | BW |
| <i>Meristic counts</i> | | |
| Lateral line scales | Number of scale rows along the lateral line | LL |
| Lateral line to dorsal fin scales | Number of scale rows between lateral line scale row and anterior base of the dorsal fin | LD |
| Lateral line to pelvic fin scales | Number of scale rows between lateral line scale row and base of pelvic fin | LP |
| Lateral line to anal fin scales | Number of scale rows between lateral line scale row and anterior base of the anal fin | LA |
| Caudal peduncle scales | Number of scale rows around the caudal peduncle | CP |
| Predorsal scales | Number of scale rows from the edge of the nape to the anterior base of the dorsal fin | PDS |
| Unbranched dorsal fin rays | Number of unbranched primary dorsal rays | UdR |
| Branched dorsal fin rays | Number of branched dorsal rays | BdR |
| Total vertebrae | Total number of vertebrae in vertebral column (including four Weberian vertebrae and a single ural centrum) | TV |
| Pre-dorsal vertebrae | Total number of vertebrae in advance of the leading dorsal fin pterygiophore (including the four Weberian vertebrae) | PdV |
| Pre-caudal vertebrae | Total number of vertebrae in advance of the first caudal vertebrae (i.e. the vertebrae opposite the leading anal pterygiophore) plus the four Weberian vertebrae | PcV |
| Pre-anal vertebrae | Total number of vertebrae in advance of the leading anal pterygiophore (including the four Weberian vertebrae) | PaV |
| Caudal vertebrae | Total number of vertebrae before the last precaudal vertebrae (including a single ural centrum) | CV |

Results

Molecular data

Consistent with results from previous studies, Bayesian phylogenetic analysis recovered four major clades within *P. afer* (Figure 2). Samples from the Swartkops and Sundays (*P. afer* s.s.) were closely related with 0.00–1.03% sequence divergence, but were 6.75–7.81% divergent from *P. senticeps* and 4.76–6.61% divergent from *P. swartzi* sp. n. Samples from the Krom River system (*P. senticeps*) were 0.00–0.20% divergent from each other, but were separated by 3.32–4.09% divergence from *P. swartzi* sp. n. Genetic divergence within *P. swartzi* sp. n. (samples from the Gamtoos, Kabeljous and Swart River systems) ranged from 0.00–0.40%. The ranges of genetic divergence values among *P. afer* s.s., *P. senticeps* and *P. swartzi* are comparable to typical interspecific divergences found between other single barbeled redbfins in the CFE that possess a flexible primary dorsal spine (e.g. see Table 2 for divergence between *P. asper* and *P. tenuis*). *Pseudobarbus afer* s.s., *P. senticeps* and *P. swartzi* are also genetically differentiated from all currently described species or known genetic lineages of *Pseudobarbus* (Table 2), indicating that they are distinct taxonomic entities.

Morphological data

The first PCA performed on five meristics for 162 specimens of *P. afer* s.l. shows clear separation of *P. afer* s.s., *P. senticeps* and *P. swartzi* sp. n. based on scale counts (Figure 3a). The first Principal Component axis (PCI), primarily contrasting differences in the number of scale rows along the lateral line, explained 87.2% of the total variation (Table 3). The second PCA axis (PCII), primarily contrasting differences in the number of scale rows around the caudal peduncle, explained 9.0% of the total variation (Table 3). Specimens of *P. swartzi* sp. n. were associated positively with PCI (Figure 3a), describing individuals with a higher number of scales along the lateral line (mode = 36; range = 34–37; Table 4). Specimens of *P. senticeps* which are situated on the negative part of PCI have fewer scale rows along the lateral line (mode = 29; range = 25–33) compared to *P. afer* s.s. and *P. swartzi* (Table 4). Note that the holotype of *P. senticeps* is not conspecific with *P. afer* s.s. and *P. swartzi*. Scatterplots of scale counts against standard length show that *P. senticeps* and *P. swartzi* can be clearly separated based on differences in the number of scale rows along the lateral line and circumpeduncular scales (Figure 4a, b). Specimens of *P. afer* s.s. have intermediate meristic counts (lateral line scale rows and circumpeduncular scales) compared to *P. senticeps* and *P. swartzi*, with only a few individuals showing some overlap between *P. afer* and the other two species. Of the 31 specimens of *P. senticeps* examined for this study, only two had more than 30 scale rows along the lateral line, while only two of the 64 specimens of *P. swartzi* had 34 scale rows along the lateral line, and only five (out of 68) specimens of *P. afer* s.s. had 35 scale rows along the lateral line.

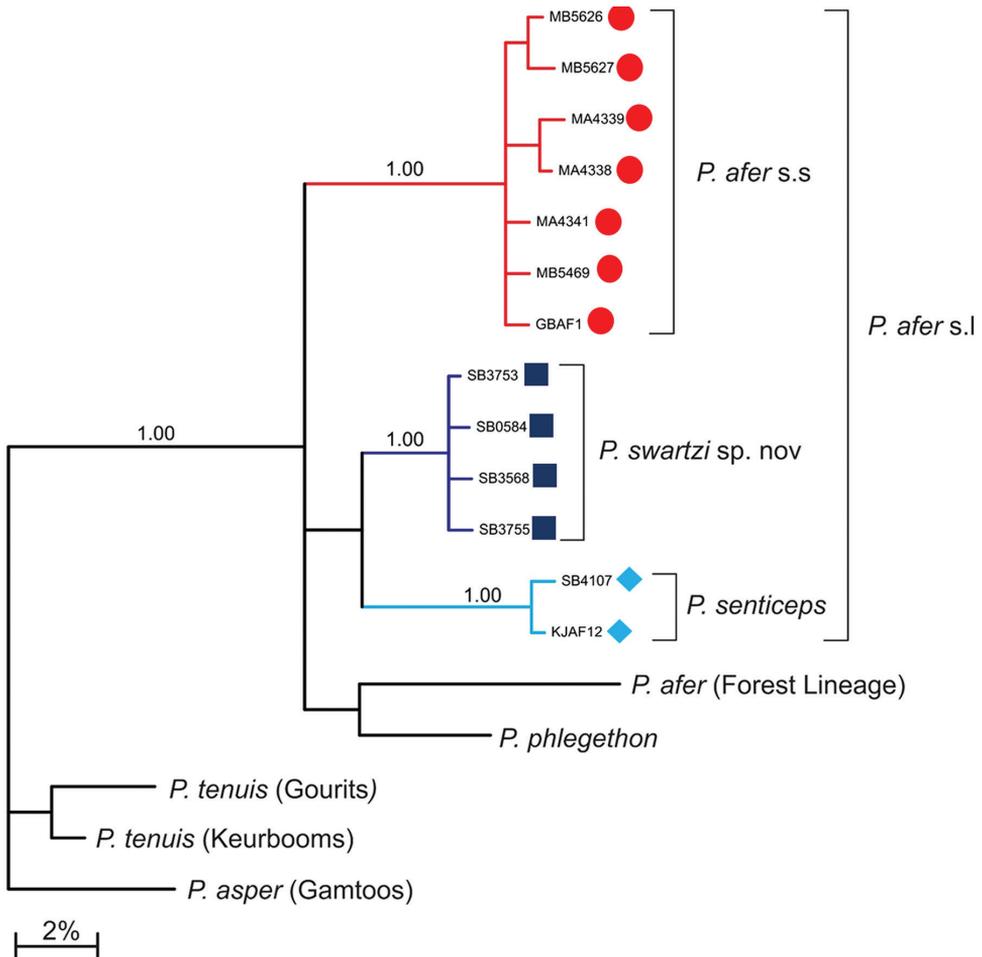


Figure 2. Bayesian phylogenetic tree showing genetic distances between *Pseudobarbus afer* s. s., *P. senticeps* and *P. swartzi* sp. n. and their relationships with the other single barbeled *Pseudobarbus* species and lineages in the Cape Fold Ecoregion of South Africa. Bayesian posterior probabilities are shown on the branches. The symbols correspond to the distribution map of the three species in Figure 7.

A PCA performed on 17 morphometric characters shows complete overlap between *P. afer* and *P. swartzi*, with marginal separation of *P. senticeps* from these two species (Figure 3b). The first four PCA axes, primarily contrasting differences in barbel length (PCI), caudal peduncle depth (PCII), snout length (PCIII) and head depth (PCIII & IV) explained 76.6% of the total variation (Table 5). Scatter plots of these four morphometric characters are presented in Figure 4c–f. *Pseudobarbus senticeps* is clearly differentiated from *P. afer* based on barbel length (Figure 4c). The three species show considerable overlap in snout length, head depth and caudal peduncle depth (Figure 4d–f), indicating that these characters are taxonomically uninformative to differentiate these species.

Table 2. Ranges of model-corrected genetic divergences (%) between species and lineages (in parenthesis) of the soft-rayed redfins of the genus *Pseudobarbus*.

| | <i>afer</i> s.s | <i>swartzii</i> | <i>seniticeps</i> | <i>asper</i> | <i>tenuis</i> | <i>phlegathon</i> | <i>afer</i> (forest) | <i>quathlambae</i> | <i>skeltoni</i> | <i>burchelli</i> (breede) | <i>burchelli</i> (heuningnes) | <i>burchelli</i> (tradou) | <i>burgii</i> | <i>velloreni</i> |
|-------------------------------|-----------------|-----------------|-------------------|--------------|---------------|-------------------|----------------------|--------------------|-----------------|---------------------------|-------------------------------|---------------------------|---------------|------------------|
| <i>afer</i> s.s | 0.00–1.03 | | | | | | | | | | | | | |
| <i>swartzii</i> | 4.76–6.61 | 0.00–0.40 | | | | | | | | | | | | |
| <i>seniticeps</i> | 6.75–7.81 | 3.32–4.09 | 0.00–0.20 | | | | | | | | | | | |
| <i>asper</i> | 8.59–10.12 | 8.25–8.89 | 9.87–10.23 | – | | | | | | | | | | |
| <i>tenuis</i> | 5.94–8.97 | 5.86–7.37 | 6.69–9.23 | 3.04–4.25 | 0.00–1.88 | | | | | | | | | |
| <i>phlegathon</i> | 5.27–5.84 | 4.34–4.89 | 6.24–6.54 | 9.17 | 7.90–9.56 | – | | | | | | | | |
| <i>afer</i> (forest) | 7.65–8.30 | 5.66–6.23 | 7.91–7.58 | 10.75 | 8.76–10.52 | 5.72 | – | | | | | | | |
| <i>quathlambae</i> | 19.43–22.06 | 19.28–21.54 | 20.20–22.48 | 21.23–22.91 | 18.71–20.48 | 20.80–21.59 | 19.75–21.59 | 0.00–2.34 | | | | | | |
| <i>skeltoni</i> | 12.84–15.03 | 10.39–11.19 | 13.00–13.02 | 17.22–17.72 | 14.96–17.38 | 13.73–14.16 | 10.98–11.38 | 25.22–27.79 | 0.00–0.20 | | | | | |
| <i>burchelli</i> (breede) | 6.80–8.48 | 6.00–6.89 | 8.08–8.72 | 9.77–10.10 | 7.86–9.82 | 8.10–8.40 | 8.36–8.68 | 16.79–17.47 | 9.92–10.65 | 0.00–0.19 | | | | |
| <i>burchelli</i> (heuningnes) | 6.51–7.87 | 5.19–5.76 | 7.80–8.12 | 10.86 | 8.23–9.91 | 7.81 | 8.11 | 19.10–19.85 | 9.98–10.35 | 1.89–2.10 | – | | | |
| <i>burchelli</i> (tradou) | 6.90–8.29 | 6.70–7.03 | 8.30–8.64 | 10.47 | 8.07–9.65 | 9.61 | 8.28 | 16.05–16.66 | 9.49–9.87 | 4.10–4.35 | – | | | |
| <i>burgii</i> | 8.95–10.03 | 7.63–7.98 | 9.31–9.67 | 9.74 | 9.86–11.55 | 8.67 | 9.92 | 22.73–23.52 | 13.01–13.43 | 7.19–7.49 | 6.32 | 7.97 | – | |
| <i>velloreni</i> | 9.84–11.44 | 8.95–9.62 | 9.22–9.91 | 11.85–12.54 | 8.71–10.97 | 12.47–12.83 | 10.26–0.97 | 19.66–23.78 | 12.40–12.83 | 6.92–8.12 | 7.27–8.19 | 6.44–7.33 | 9.50–10.19 | 0.00–0.60 |

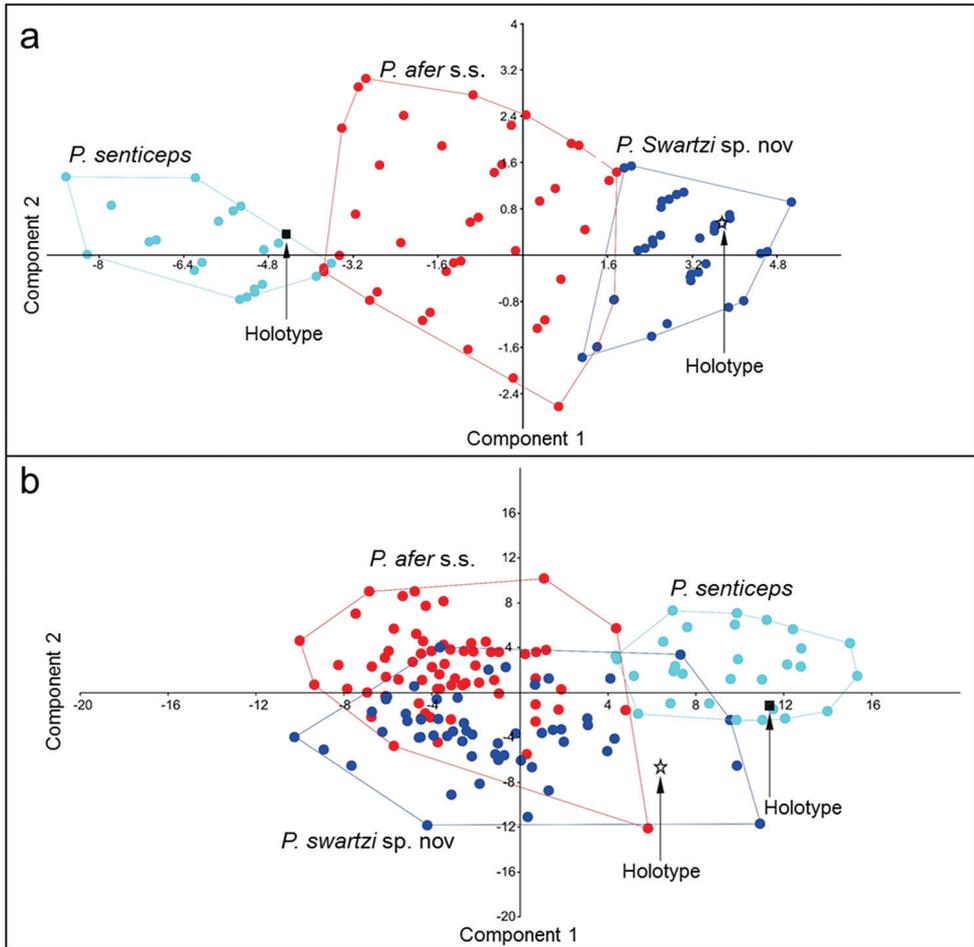


Figure 3. **a** Scatter plot of PC1 against PC2 for a PCA carried out on five raw meristic characters (scale counts) for 162 specimens of the *Pseudobarbus afer* complex **b** Scatter plot of PC1 against PC2 for a PCA carried out on 17 morphometric characters for 154 specimens of the *Pseudobarbus afer* complex. Syntypes were not included in the analyses as all three specimens are in very poor condition, with very few intact scales, flaccid bodies and damaged fins. The plots indicate that *P. senticeps*, *P. afer s.s.* and *P. swartzi* can be clearly separated based on scale counts, but the three species show considerable overlap in morphological characters.

The first three axes of a PCA performed on log transformed data explained 89.4% of the total variation in the data set, but there was no separation of the species along PCI and PCII (results not shown). *Pseudobarbus senticeps* was completely separated from both *P. afer* and *P. swartzi* along PCIII (results not shown), with barbel length loading heavily on this axis (0.012 eigenvalue, 9.3% of total variation, 0.789 factor loading). Specimens of *P. senticeps* were positively associated with PCIII, describing individuals characterised by relatively long barbels, a pat-

Table 3. Factor loadings for the first two principal component (PC) axes of a PCA carried out on five meristic characters from 162 specimens of the *Pseudobarbus afer* complex. The most important factor loadings are in bold.

| | PCI | PCII | PCIII |
|---------------------------------------|--------------|--------------|---------------|
| Eigenvalue | 12.105 | 1.251 | 0.284 |
| % Variance | 87.20 | 9.01 | 2.05 |
| Lateral line scale series | 0.857 | -0.494 | 0.131 |
| Lateral line to dorsal fin scale rows | 0.143 | 0.143 | -0.727 |
| Lateral line to pelvic fin scale rows | 0.134 | 0.034 | -0.491 |
| Lateral line to anal fin scale rows | 0.150 | 0.080 | -0.386 |
| Circumpeduncular scale rows | 0.453 | 0.853 | 0.254 |

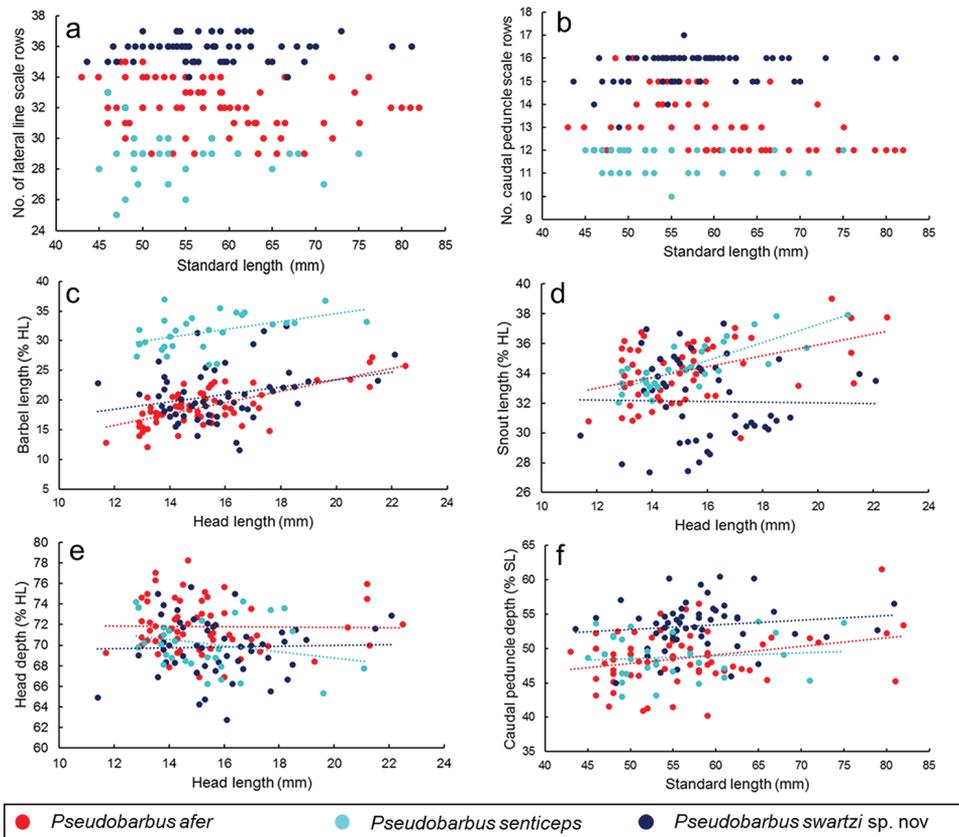


Figure 4. Scatter plots of scale counts and selected morphometric characters of *Pseudobarbus afer* s.s., *P. senticeps* and *P. swartzii* sp. n.

tern which is similar to the one revealed using percentage data described above. In this paper, we have thus only presented PCA scatter plots recovered from analysis of percentage data.

Table 4. Morphometric and meristic data for *Pseudobarbus afer* s.s., *P. senticeps* and *P. swartzii* sp. n.

| | <i>P. afer</i> | | <i>P. senticeps</i> | | <i>P. swartzii</i> sp. n. | |
|--|--------------------------------|---|---------------------|---------------------|---------------------------|---|
| | syntypes [‡] n = 3 | other specimens including topotypes n = 68 | holotype | topotypes n = 29 | holotype | other specimens including paratypes n = 63 |
| Standard length (SL) (mm) | 79.1–89.0 | 43.0–82.0 | 67.0 | 45.0–79.3 | 81.1 | 46.5–75.2 |
| Head length (HL) (mm) | 22.4–26.7 | 11.7–22.5 | 18.2 | 12.8–21.1 | 22.3 | 12.8–21.2 |
| Percentage of SL (%) | | | | | | |
| Head length | 24.1–30.0 | 25.4–28.9 | 27.2 | 26.1–29.6 | 27.5 | 26.6–30.0 |
| Predorsal length | 51.2–55.0 | 49.6–55.5 | 50.7 | 49.3–55.0 | 53.8 | 53.3–56.6 |
| Dorsal fin base | 11.4–12.4 | 11.0–14.4 | 14.9 | 11.0–14.9 | 12.7 | 10.8–13.4 |
| Dorsal fin height | – | 20.4–25.2 | 25.4 | 20.8–25.4 | 24.9 | 23.5–27.8 |
| Body depth | 24.3–27.9 | 22.5–31.6 | 28.7 | 22.9–28.7 | 26.1 | 22.6–25.9 |
| Body width | 10.8–13.2 | 11.9–20.2 | 13.9 | 13.9–19.3 | 17.8 | 14.6–17.7 |
| Caudal peduncle length | 23.4–24.9 | 23.0–27.6 | 26.0 | 22.5–26.2 | 23.4 | 22.2–25.4 |
| Percentage of HL (%) | | | | | | |
| Head depth | 61.8–70.7 | 66.5–78.2 | 73.6 | 65.3–74.3 | 72.6 | 63.7–71.6 |
| Inter-orbit | 27.7–34.8 | 25.2–33.0 | 30.8 | 27.1–33.0 | 34.5 | 25.7–31.2 |
| Snout length | 24.0–29.3 | 29.7–39.0 | 34.6 | 29.8–37.9 | 33.6 | 28.9–34.0 |
| Post orbit | 40.8–49.7 | 42.2–51.2 | 52.7 | 44.2–52.7 | 50.7 | 44.6–47.7 |
| Posterior barbel length | 16.4–23.6 | 12.1–27.2 | 33.0 | 26.0–37.0 | 27.8 | 26.7–39.9 |
| Orbit diameter | 21.7–29.5 | 21.7–30.4 | 25.8 | 23.5–29.5 | 25.6 | 23.0–27.7 |
| Percentage of caudal peduncle length (%) | | | | | | |
| Caudal peduncle depth | 43.2–50.9 | 40.3–61.5 | 52.3 | 43.0–54.5 | 57.9 | 43.6–54.3 |
| Unbranched dorsal fin rays | ii–iii | iv (iii–iv) | iii | iv (iii–iv) | iii | iii |
| Branched dorsal fin rays | 7 | 7 (6–7) | 7 | 7 | 7 | 7 |
| Unbranched anal fin rays | iii | iii | iii | iii | iii | iii |
| Branched anal fin rays | 5 | 5 | 5 | 5 | 5 | 5 |
| Pectoral fin rays | 13–14 | 15 (14–17) | 14 | 14 (13–15) | 13 | 14 (12–14) |
| Pelvic fin rays | 8 | 8 (8–9) | 8 | 8 (8–9) | 7 | 8 (7–8) |
| Lateral line scales | 29–33 | 32 (29–35) | 29 | 29 (25–30) | 36 | 36 (35–37) |
| Lateral line to dorsal fin scale rows | 4 | 5 (4–6) | 5 | 5 (4–5) | 6 | 6 (6–7) |
| Lateral line to pelvic fin scale rows | – | 4 (3–5) | 4 | 4 (3–4) | 5 | 5 (4–5) |
| Lateral line to anal fin scale rows | – | 4 (3–5) | 4 | 3 (3–4) | 5 | 5 |
| Caudal peduncle scale rows | 12–14 | 12 (12–16) | 12 | 12 (10–12) | 16 | 16 (15–16) |
| Predorsal scale rows | 13–15 | 15 (14–16) | 14 | 15 (12–15) | 16 | 17–18 (16–20) |
| Total vertebrae | | 37 (36–39) | | 37 (35–38) | 37 | 37 (37–38)* |
| Precaudal vertebrae | | 19 (18–20) | | 19 (18–19) | 19 | 20 (19–20)* |
| Caudal vertebrae | | 18 (17–19) | | 18 (16–18) | 18 | 18 (17–18)* |
| Predorsal vertebrae | | 12 (11–13) | | 12 (11–13) | 13 | 13 (12–13)* |

[‡]all three specimens are in very poor condition, with very few intact scales, flaccid bodies and damaged fins.

*counts based on radiographs of the holotype and 12 paratypes

Table 5. Factor loadings for the first two principal component (PC) axes of a PCA carried out on morphometric characters from 154 specimens of the *Pseudobarbus afer* s.l. complex. The most important factor loadings are in bold.

| | PCI | PCII | PCIII | PCIV |
|------------------------|--------------|---------------|---------------|---------------|
| Eigenvalue | 38.3 | 19.5 | 11.7 | 5.8 |
| % Variance | 38.9 | 19.9 | 11.9 | 5.9 |
| Head length | 0.053 | -0.024 | 0.132 | -0.030 |
| Predorsal length | 0.009 | -0.156 | 0.261 | 0.154 |
| Dorsal fin base | 0.008 | 0.019 | -0.160 | -0.048 |
| Dorsal fin height | 0.031 | -0.078 | 0.237 | 0.217 |
| Anal fin base | 0.020 | -0.009 | -0.106 | -0.024 |
| Body depth | -0.010 | -0.145 | -0.195 | 0.098 |
| Body width | 0.069 | -0.075 | -0.041 | -0.001 |
| Caudal peduncle length | -0.039 | 0.164 | -0.034 | 0.087 |
| Caudal peduncle depth | 0.006 | -0.927 | -0.211 | -0.106 |
| Posterior barbel | 0.986 | 0.022 | -0.018 | 0.052 |
| Pectoral to pelvic | -0.020 | 0.076 | -0.290 | -0.187 |
| Pelvic to anal | 0.000 | 0.007 | -0.073 | -0.088 |
| Head depth | -0.050 | 0.049 | -0.590 | 0.712 |
| Snout length | 0.039 | 0.201 | -0.517 | -0.496 |
| Orbit diameter | -0.046 | 0.085 | -0.019 | 0.253 |
| Post orbit | 0.089 | -0.003 | -0.015 | 0.169 |
| Inter orbit | 0.052 | -0.002 | -0.175 | -0.073 |

Taxonomic accounts

Pseudobarbus afer (Peters, 1864)

Figures 5a, 6a, 7a

Barbus (*Capoeta*) *afer* Peters, 1864; Günther 1868; non Boulenger 1911; non Gilchrist and Thompson 1913; Barnard 1938, 1943; Jubb, 1965, 1967.

Barbus anoplus (non Weber, 1897): Boulenger 1911; Gilchrist and Thompson 1917.

Barbus vulneratus (non Castelnau, 1861): Gilchrist and Thompson 1913 (in part, species from the Baakens and Swartkops Rivers).

Barbus senticeps Smith, 1936; Barnard 1938, 1943.

Barbus asper non Boulenger, 1911: Barnard 1943; Jubb 1965; Smith and Smith 1966.

Pseudobarbus afer: Skelton 1988 in part (distributed from the Baakens to the Sundays River systems).

Syntypes. ZMB 5413, 3 unsexed, 78, 89, 92 mm SL, original locality uncertain, but probably the Swartkops River system (Jubb 1965).

Topotypic specimens. SAIAB 203790, *Pseudobarbus afer*, 8 unsexed, 42.1–54.2 mm SL, Waterkloof River, -33.7149528S, 25.2783833E, Groendal Wilderness, Swartkops River system, collected by A Chakona, W Kadye and B Ellender, 4 March

2015; SAIAB 97364, 2 males, 76.2–78.7 mm SL, Groendal Wilderness, Swartkops River system, -33.7000S, 25.2800E, collected by ER Swartz and B Ellender, 20 April 2010; SAIAB 97366, *Pseudobarbus afer*, 6 unsexed (31.9–70.8 mm SL), Groendal Wilderness, Swartkops River system, -33.7000S, 25.2900E, collected by B Ellender, 13 April 2010.

Etymology. *afer* means African (citizen).

Diagnosis. *Pseudobarbus afer* differs from *Pseudobarbus burchelli*, *P. burgi*, *P. skeltoni* and *P. verloreini* by possession of a single pair of oral barbels. Possession of fewer and larger scales separates *P. afer* (29–35, mode 32 scale rows along the lateral line) from *P. quathlambae* (> 60 scale rows along the lateral line) and *P. asper* (> 35 scales along the lateral line). Lack of a mid-dorsal stripe and a relatively deeper head and body profile separates *P. afer* (mean head depth: 71.8 % HL (range: 66.5–78.2%); mean body depth: 25.3% SL (range: 22.5–31.6%)) from the more slender bodied *P. tenuis* (average head depth: 65.9 % HL (range: 61.1–71.2%); average body depth: 22.4 %SL (range: 18.8–26.8%)). Lack of prominent black spots and patches on the body distinguishes *P. afer* from *P. phlegethon*. *Pseudobarbus afer* most closely resembles *P. senticeps*, *P. swartzzi* sp. n., and *P. asper*. Barbel length and the number of scale rows along the lateral line separates *P. afer* from these three species. Short barbels which do not reach the vertical through the posterior margin of the eye and a higher number of lateral line scales (29–35, mode 32) distinguishes *P. afer* from *P. senticeps* whose barbels reach or surpass the vertical through posterior edge of eye and has fewer and larger scales (lateral line scales 25–30, mode 29; caudal peduncle scales 10–12, mode 11; Figure 4a–c). *Pseudobarbus afer* further differs from *P. senticeps* by lack of a blotch of pigment at the base of the caudal fin, while the lateral stripe in *P. senticeps* terminates in a triangular blotch at the base of the caudal fin (Figure 6a). *Pseudobarbus afer* differs from *P. swartzzi* sp. n. by possession of fewer scale rows along the lateral line (29–35, mode 32 vs 34–37, mode 36 in *P. swartzzi*; Figure 4a) and fewer scales around the caudal peduncle (12–16, mode 12 vs 13–17, mode 16 in *P. swartzzi*; Figure 4b). *Pseudobarbus afer* has a distinct mesh-like pigmentation pattern below the lateral line which further separates this species from *P. swartzzi* which lacks discernible pigmentation pattern on the latero-ventral scales (Figure 6a, d). *Pseudobarbus afer* s.s is separated from *P. asper* by possession of fewer and larger scales (lateral line scale series 29–35, mode 32 vs 35–45, mode 37–40; caudal peduncle scale rows 12–16, mode 12 vs 16–22, mode 18–20).

Description. Morphometric and meristic data summarised in Table 4 are based on three syntypes and 68 specimens (43–82 mm SL) from the Sundays, Swartkops and Baakens River systems. General body shape and colouration are shown in Figures 5a, 6a, 7a.

Body fusiform, more or less laterally compressed, with dorsal profile generally more convex than ventral profile. Body deepest around the anterior bases of the dorsal and pelvic fins. Caudal peduncle length is almost twice its depth. Head length sub-equal to body depth, snout slightly blunt, mouth sub-terminal and sickle shaped, with a single pair of simple short maxillary barbels. Barbel length shorter than orbit diameter, barbels do not surpass the vertical through posterior margin of pupil. Eyes moderately



Figure 5. Live colours of *Pseudobarbus afer* s.s (SAIAB 203790) from the Waterkloof River, Swartkops River system, *P. senticeps* (RS17AL01) from the source pool in the Upper Krom River system and *P. swartzi* sp. n. (SAIAB 203792) from a tributary of the Wabooms River, Gamtoos River system.

large, located dorsolaterally, closer to tip of snout than to the posterior margin of gill cover, orbit diameter shorter than snout length.

Tuberculation. Mature breeding males develop large conical tubercles on the snout and head dorsum. The bilateral clusters on the snout include 2–4 tubercles. Scattered tubercles on dorsal surface of head smaller than those on the snout. Minute tubercles develop in bands on the dorsal surface of pectoral fin rays and a row along the free edge of latero-dorsal scales.

Scales. 29–34 scale rows along the lateral line to end of hypural plate (point of flexure), 1–2 more scales to base of caudal fin. Of the 68 specimens examined, only 13 had

29–30 scale rows along the lateral line and only five specimens had 35 lateral line scale rows. The rest of the specimens (50) had 31–34 scale rows along the lateral line. Four to six scale rows between lateral line and anterior base of dorsal fin (dorsal fin origin), 3–5 scale rows between lateral line and pelvic fin origin, 3–5 scale rows between lateral line and anal fin origin, 14–16 pre-dorsal scale rows (from posterior edge of head to anterior base of dorsal fin), 12–16 circumpeduncular scales. Triangular naked patch between the gill covers and anterior base of pectoral fins, ventral scales between pectoral fin origin and pelvic fin origin reduced and embedded. Axillary scales of pelvic fin not prominent or elongate. Scales between posterior edge of head and dorsal fin origin embedded and smaller than flank scales.

Fins. Dorsal fin situated almost in the centre of the body (excluding caudal fin), origin slightly behind vertical through origin of pelvic fin, with 3–4 unbranched rays and 6–7 branched rays, distal margin straight to slightly concave, tip of depressed dorsal fin reaches within 1–2 scales to vertical through posterior base of anal fin. Pectoral fins fan-shaped, larger in males than females, with 14–17 rays, often reaches and surpasses base of pelvic fin in males, reaches 2 scales to base of pelvic fin in females. Pelvic fin with 8–9 rays, origin slightly in front of dorsal fin origin, tip of depressed pelvic fin does not reach anterior origin of anal fin, except in mature males. Anal fin with 3 simple rays and 5 (rarely 6) branched rays, distal margin almost straight to slightly convex, origin closer to anterior base of pelvic fin than caudal fin base. Caudal fin is obtusely forked, with a mode of 10+9 principal rays.

Osteology. Total vertebrae including Weberian apparatus 36–39 (mode 37), pre-dorsal vertebrae including Weberian apparatus 11–13 (mode 12), precaudal vertebrae including Weberian apparatus 18–20 (mode 19), caudal vertebrae including Weberian apparatus 17–19 (mode 18).

Colouration (live and fresh specimens). Refer to Figure 5a for general live colouration. Dorsum grey-black, sides and belly silvery white, base of fins bright red in adults. Vague dark mid-lateral band from behind the head to the base of the caudal fin.

Colouration (preserved). The bright red pigmentation on base of fins and silvery colouration fades in preservative (Figure 6a). Dorsum and flanks above lateral line dark greyish. Belly off-white to yellow in most specimens. Band of pigment around centre of scales, basal segment of exposed area of scales without pigment, leaving a lighter band along distal edges of scales, producing a mesh or net-like pattern which is more pronounced on dorso-lateral scales (Figure 6a).

Distribution. *Pseudobarbus afer* s.s. (referred to as the ‘Mandela lineage’ by Swartz et al. 2007) occurs in three isolated river systems (the Sundays, Swartkops and Baakens) which discharge into Algoa Bay near Port Elizabeth (Figure 7). Remnant populations of this species are highly fragmented, persisting in only a few less degraded upland tributaries that have not been invaded by alien species (Ellender et al. 2011).

Conservation status. Once common and widely distributed throughout the Sundays, Swartkops and Baakens River systems, *P. afer* suffered severe decline in distribution and abundance, mainly due to invasion by alien predators and competitors, deterioration of water quality and loss of critical habitat. Consequently, this species

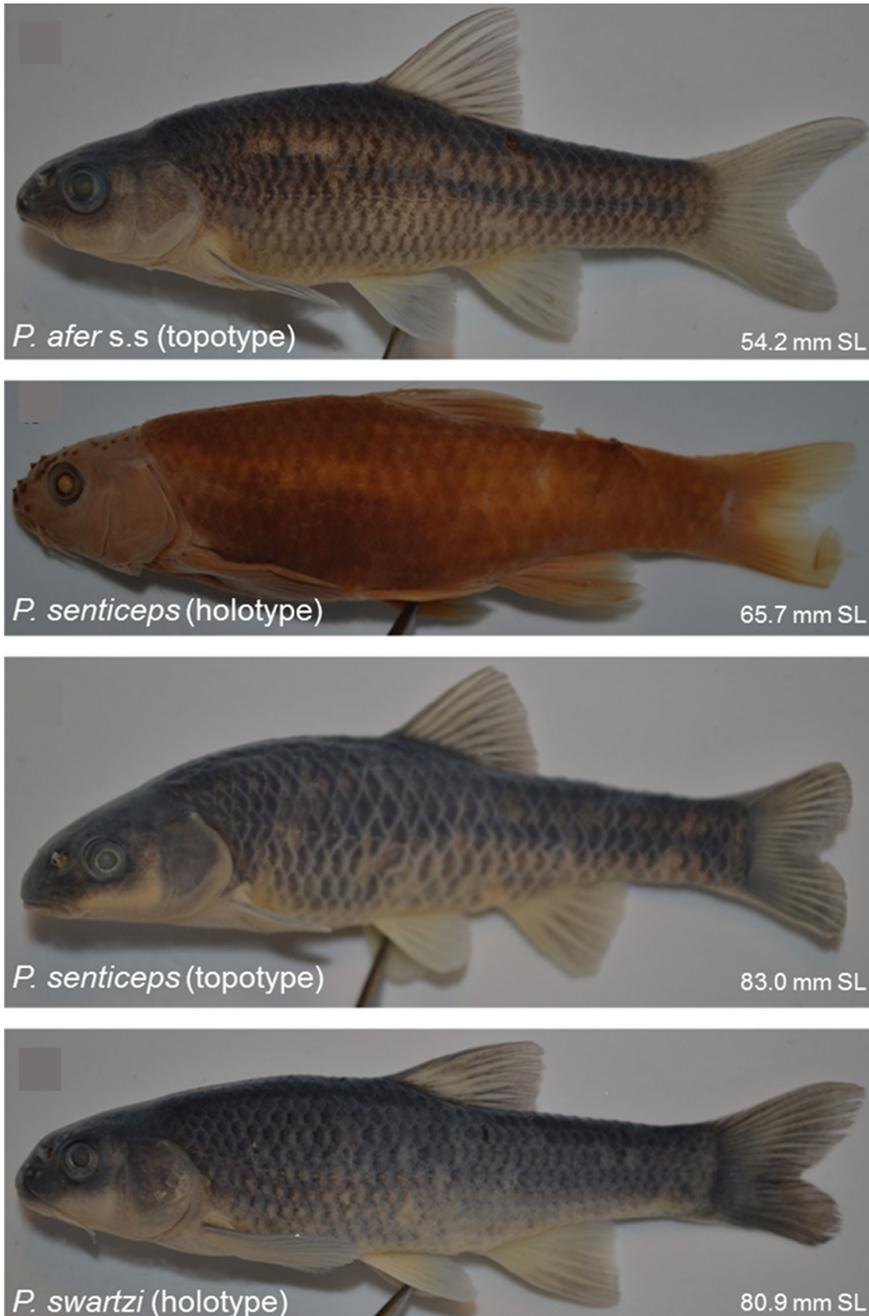


Figure 6. Preserved colours of *Pseudobarbus afer* s.s topotype (SAIAB 203790) from the Waterkloof River, Swartkops River system, *P. senticeps* holotype (SAIAB 304) from the Assegaaibosch River, Krom River system, *P. senticeps* topotype (SAIAB 200302) from the Assegaaibosch River, Krom River system, and *P. swartzi* sp. n. holotype (SAIAB 203792) from a tributary of the Wabooms River, Gamtoos River system. Note the differences in the arrangement of melanophores which produces distinct patterns on the latero-ventral scales of the three species.

(referred to as *Pseudobarbus afer* (Peters, 1864) by Tweddle et al. 2009) was listed as Endangered during the most recent IUCN assessment of the status and distribution of freshwater fishes in southern Africa (Tweddle et al. 2009). Invasion by alien fish was identified as the single most important threat to this species. Long-term persistence of this species in the Baakens River system is uncertain as the entire catchment of this system has been heavily urbanised and the river is now heavily infested with non-native species, particularly *Tilapia sparrmanii* and *Pseudocrenilabrus philander* (personal observations, March 2014). There is urgent need for comprehensive surveys to determine the status of the Baakens population and identify measures to prevent its eradication. The conservation status of *P. afer* s.s is being reassessed as part of a national program that is assessing the conservation status of all freshwater fishes of South Africa.

***Pseudobarbus senticeps* (Smith 1936)**

Figures 5b, 6b,c

Barbus senticeps Smith, 1936.

Barbus afer: Jubb 1963, 1965.

Pseudobarbus afer: Skelton 1988.

Holotype. SAIAB 304, male, 65.7 mm SL, Assegaibosch River, Krom River system.

Topotypic material. SAIAB 200302, 9 unsexed, 23–83 mm SL, Assegaibosch River, Krom River system, -33.9452778S, 24.3139167E, collected by R Bills, V Bills and D Naran, 12 August 2014. SAIAB 121815 (formerly AMG 2651), 29 unsexed, 45–75 mm SL, Assegaibosch River, -33.9413889S, 24.3188889E, Krom River system, collected by PH Skelton and J Stephenson, 20 January 1975.

Etymology. ‘*senticeps*’ refers to the thorny or prickly appearance of the head (*sentis* a thorn, bramble, and *ceps*, head) of sexually mature males due to the development of distinctly pointed tubercles on the snout, along the inner edges of the nares and orbits and head dorsum (see Figure 6b).

Diagnosis. Possession of a single pair of oral barbels separates *P. senticeps* from *P. skeltoni*, *P. verloreani*, *P. burgi* and *P. burchelli*. Possession of fewer and larger scales separates *P. senticeps* (25–30, mode 29) from *P. quathlambae* (> 60 scale rows along the lateral line), *P. asper* (35–45; mode 37–40), *P. swartzi* sp. n. (34–37, mode 36) and *P. tenuis* (32–37, mode 35–36). There is overlap (although uncommon) in lateral line scale series between *P. senticeps*, *P. afer* (29–35, mode 32) and *P. phlegethon* (29–37, mode 35). A lateral stripe which terminates in a triangular blotch at the base of the caudal fin and longer barbels (reaching or surpassing vertical through the posterior edge of the eye) further separate *P. senticeps* from *P. afer* (barbels do not surpass vertical through the centre of the eye). Body colour pattern distinguishes *P. senticeps* from *P. phlegethon*. *Pseudobarbus phlegethon* is characterised by prominent black spots and patches on the body, which are lacking in *P. senticeps*.

Description. Morphometric and meristic data summarised in Table 4 are based on the holotype and 29 topotype specimens (45–79.3 mm SL). General body shape and colouration are shown in Figures 5b, 6b, c.

A moderately laterally compressed, fusiform species. Cross-section of body between pectoral and pelvic fins ellipsoid. Dorsal profile of body, in lateral view, convex from snout tip to dorsal fin origin, straight and descending from dorsal fin origin to caudal fin insertion. Ventral profile, in lateral view, more or less straight or slightly convex from snout tip to anal fin origin, slightly concave and ascending from origin of anal fin to caudal fin insertion. Body deepest around anterior bases of dorsal and pelvic fin origins, progressively becoming narrower from anal fin origin towards the caudal fin. Caudal peduncle length almost twice as its depth, cross-section ellipsoid. Snout blunt or obtusely pointed. Mouth terminal, sickle shaped, its corner not reaching vertical through anterior margin of eye. A single pair of well developed, long and slender maxillary barbels present, barbel length longer than orbit diameter in most specimens. Eyes moderately large (23.5–29.5% HL), dorso-laterally positioned, and located closer to tip of snout than posterior margin of gill cover. Orbit diameter shorter than snout length.

Tuberculation. Mature breeding males develop prominent conical tubercles on the snout, along the nares and dorsal edges of the eyes. Bilateral clusters on snout include 2–4 tubercles in mature ripe males. Smaller, scattered tubercles develop on the head dorsum. A band of fine tubercles along dorsal surface of each of several anterior pectoral fin rays of mature breeding males.

Scales. Lateral line with 25–30 scales to end of hypural plate, 1–2 more scales to base of caudal fin. Four to five scale rows between lateral line and dorsal fin origin, 3–4 rows between lateral and pelvic fin origin, 3–4 scale rows between lateral line and anal fin origin, 12–15 pre-dorsal scale rows, 10–12 circumpeduncular scales. Breast scales reduced and embedded, giving a naked appearance to the region between the isthmus and base of pelvic fins. Elongated or triangular pelvic axillary scales absent. Scales between the nape and dorsal fin origin reduced and embedded.

Fins. Dorsal fin with 3–4 unbranched rays and 7 branched rays, origin slightly posterior to pelvic fin origin. Tip of adpressed dorsal fin reaches within 2–3 scales to vertical through posterior base of anal fin, distal margin straight. Pectoral fin with 13–15 rays, fan shaped, larger in males than females, tip of adpressed pectoral fin reaches and surpasses base of pelvic fin in males, reaches 2 scales to base of pelvic fin in females. Pelvic fin with 8–9 rays, origin slightly in front of dorsal fin origin, outer margin slightly convex, its tip reaching anterior origin of anal fin when depressed in males and reaches 2–3 scales to anal fin origin in females. Anal fin with 3 simple rays and 5 branched rays, distal margin almost straight to slightly convex or straight, origin closer to anterior base of pelvic fin than base of caudal fin. Caudal fin is forked, with 10+9 principal rays.

Osteology. Total vertebrae including Weberian apparatus 35–38 (mode 37), pre-dorsal vertebrae including Weberian apparatus 11–13 (mode 12), precaudal vertebrae including Weberian apparatus 18–19 (mode 19), caudal vertebrae including Weberian apparatus 16–18 (mode 18).

Colouration (live and fresh specimens). Refer to Figure 5 for general live colouration. Dorsum and sides dark brown, belly and underparts off-white or silvery, operculum metallic gold, base of fins bright red. Vague lateral stripe terminating in a triangular blotch at the base of the caudal fin.

Colouration (preserved). Dorsal surface of alcohol preserved specimens dark grey or black, sides and belly lighter. Distinct black lateral stripe from posterior margin of operculum terminating into a black triangular blotch of pigment at the base of the caudal peduncle. Red pigmentation at the base of fins disappears in preserved specimens (Figure 6b).

Distribution. *Pseudobarbus senticeps* (referred to as the ‘Krom lineage’ by Swartz et al. 2007, 2009) is endemic to the Krom River system which discharges into St Francis Bay (Figure 7). The species has a restricted known distribution range, surviving in a few relatively unimpacted and uninvaded tributaries of the Krom River system (Figure 7).

Habitat. *Pseudobarbus senticeps* inhabits perennial mountain streams with clear to peat stained water, cobble and pebble substrates.

Conservation status. *Pseudobarbus senticeps* (referred to as *Pseudobarbus* sp. “after Krom” by Tweddle et al. 2009) was listed as Critically Endangered during the IUCN assessment of the status and distribution of freshwater fishes in southern Africa (Tweddle et al. 2009). Invasion by alien fish (particularly *Micropterus* spp.) was identified as the major threat to this species. Further studies are required to more accurately assess the distribution, ecology and biology of this species.

***Pseudobarbus swartzi* sp. n.**

<http://zoobank.org/CCA9F17C-F36C-4B48-BAD9-C5AEA3B6D161>

Figures 5c, 6d

Proposed common name. Gamtoos redfin.

Holotype. SAIAB 203792 (Field no: AC15AL39), male, 80.9 mm SL, Tributary of the Wabooms, Gamtoos River system, -33.8639772 S, 23.8263333E, collected by A Chakona, B Motshegoa, N Mazungula, W Kadye and R Smith, 21 January 2015.

Paratypes. SAIAB 203793 (Field no: AC15AL39), 9 unsexed, 35.4–76.0 mm SL, same locality information and collectors as holotype; MRAC 2016-032-P-0001-0004 (Field no: AC16AL02), 4 unsexed, 50.2–61.4 mm SL, main tributary of the Louterwater River, -33.8333611S, 23.6373056E, Gamtoos River system, collected by A Chakona, S Reddy and R Smith, 18 January 2016.

Etymology. The species is named after Dr Ernst R. Swartz for his contribution to the biogeography and systematics of *Pseudobarbus* and the role that he played in mentoring students working on the systematics and biogeography of southern African freshwater fishes.

Diagnosis. Possession of a single pair of oral barbels separates *P. swartzi* sp. n. from *P. burchelli*, *P. burgi*, *P. skeltoni* and *P. verlorene* all with two pairs. It differs from *P. quathlambae* by having larger scales and fewer scale rows along the lateral line

(*P. swartzi*: 35–37, mode 35–36 lateral line scales; *P. quathlambae*: > 65 scales along lateral line). *Pseudobarbus swartzi* and *P. senticeps* show some overlap in barbel length (Figure 4), but are clearly separated by scale size (Figure 4). *Pseudobarbus swartzi* has a higher number of scale rows along the lateral line (34–37, mode 36) vs (25–33, mode 29), and around the caudal peduncle (13–17, mode 16) vs (10–12, mode 11) in *P. senticeps*. *Pseudobarbus swartzi* further differs from *P. senticeps* by lacking a conspicuous lateral stripe which terminates in a triangular blotch at the base of the caudal fin. While there is some overlap in scale counts between *P. swartzi* and *P. afer*, it is uncommon (see above). *Pseudobarbus swartzi* has a higher number of lateral scale series (34–37, mode 36) than *P. afer* s.s which has fewer lateral scale series (29–35, mode 32). The two species further differ in scale pigmentation pattern (see Figure 6a, 6). *Pseudobarbus afer* has a band of pigment along the centre of the scales, leaving a clear band along the distal edges of the scale, and producing a distinct mesh or net-like pattern which is more conspicuous on the latero-ventral scales. This pattern is not evident in *P. swartzi*. *Pseudobarbus swartzi* sp. n. differs from *P. asper* by possession of fewer larger scales (lateral line scale series 34–37, mode 36 vs 35–45, mode 37–40; caudal peduncle scale rows 13–17, mode 16 vs 16–22, mode 18–20). Lack of prominent black spots and blotches on the body distinguishes *P. swartzi* from *P. phlegethon*.

Description. General appearance and colouration is shown in Figs 5c, 6d. Morphometric and meristic data summarised in Table 4 are based on 64 specimens (43.6 - 81.1 mm SL) collected from 11 localities across the Gamtoos River system. A fusiform minnow with body slightly compressed laterally. Predorsal profile convex, post dorsal profile straight and descending from origin of dorsal fin to caudal fin insertion. Ventral profile more or less straight from tip of snout to pelvic fin origin. Head relatively short, length almost equal to body depth, its dorsal profile distinctly convex, particularly from its tip to interorbital area. Mouth subterminal, its corner not reaching vertical through anterior margin of eye. Barbels attached from behind the rictus of the mouth, barbel length variable, with some individuals having long barbels reaching or surpassing vertical through posterior edge of the eye, while other individuals have much shorter barbels (see Figure 4c). The eye is located closer to the tip of the snout than to posterior edge of the operculum, eye diameter shorter than snout length. Snout blunt and moderately rounded.

Tuberculation. Mature breeding males develop large conical tubercles on the snout and along the dorsal edge of the nares and eyes. Bilateral clusters on snout include 2–4 tubercles in mature ripe males. Smaller, scattered tubercles develop on the head dorsum. Bands of fine tubercles along dorsal surface of pectoral fin rays.

Scales. Scale rows along lateral line 34–37 (mode 36) ending at hypural, with 1–2 more scales to base of caudal fin; 6–7 (mode 6) scale rows between lateral line and dorsal fin origin; 4–5 (mode 5) rows between lateral line and pelvic fin origin, 5 rows between lateral line and anal fin origin, 16–20 (mode 17–18) pre-dorsal scale rows, 13–17 (mode 16) scale rows around caudal peduncle. Triangular naked patch between the gill covers and anterior base of pectoral fins, scales between pectoral fin origin and pelvic fin origin reduced and embedded. Axillary scales of pelvic fin not prominent or

elongate. Scales between posterior edge of head and dorsal fin origin embedded and smaller than flank scales.

Fins. Dorsal fin is inserted about mid-body (excluding caudal fin), origin slightly behind vertical through origin of pelvic fin, with 3–4 unbranched rays and 7 branched rays, distal margin straight to slightly, posterior tip of depressed dorsal fin does not reach vertical through posterior base of anal fin. Pectoral fins fan-shaped, larger in males than females, with 14–16 rays, tip of depressed pectoral fin almost overlapping with pelvic fin in large males, reaches 2 scales to base of pelvic fin in females. Pelvic fin with 8 rays, origin slightly in front of dorsal fin origin, tip of depressed pelvic fin not reaching anterior origin of anal fin, except in mature males. Anal fin with 3 simple rays and 5 branched rays, distal margin straight to slightly convex, origin closer to anterior base of pelvic fin than caudal fin base. In mature males, tip of depressed pelvic fin often surpass point of anal fin origin while they only reach up to the anus in females. Caudal fin is forked, with 10+9 principal rays.

Colouration (live and fresh specimens). Refer to Figure 5c for general live colouration. Body golden-tan laterally, becoming darker dorsally, and lighter to white ventrally. Base of fins bright red, operculum metallic gold.

Colouration (preserved). Alcohol preserved specimens are dark greyish above the lateral line, light grey or whitish below the lateral line and ventrally, breast of freshly preserved specimens silvery (Figure 6). Mid lateral stripe present but comparatively obscure, black blotch at the base of caudal fin inconspicuous.

Osteology. Total vertebrae including Weberian apparatus 37–38 (mode 37), predorsal vertebrae including Weberian apparatus 12–13 (mode 13), precaudal vertebrae including Weberian apparatus 19–20 (mode 20), caudal vertebrae including Weberian apparatus 17–18 (mode 18).

Distribution. *Pseudobarbus swartzi* sp. n. occurs in the Kougaberg, Baviaanskloofberg and Elandsberg tributaries of the Kouga and Groot sub-catchments of the Gamtoos River system, and the Kabeljous and Swart River systems which discharge into St Francis Bay (Figure 7). Remnant populations in the Kouga and Groot sub-catchments are highly fragmented due to invasion of the main stem sections of the rivers by alien predators in particular the African sharptooth catfish (*Clarias gariepinus*) and the North American black bass species (*Micropterus* spp.). The status and distribution of *P. swartzi* populations in the Kabeljous and Swart river systems need to be assessed through fine scale geographic surveys. There is also need for investigations to determine the taxonomic status of redfins in the Seekoei and Maitland, two river systems which also discharge into St Francis Bay (Figure 7).

Habitat. *Pseudobarbus swartzi* inhabits perennial mountain streams with clear or peat stained water as well as cobble, pebbles and boulders.

Conservation status. Remnant populations of the species are under severe threat from multiple human impacts including habitat degradation, complete water abstraction and potential invasion by alien fish predators and competitors that are now dominant in mainstem sections of the rivers (Ellender et al. 2011, 2015). *Pseudobarbus swartzi* sp. n. (referred to as *Pseudobarbus* sp. “afer Gamtoos” by Twedde et al. 2009)

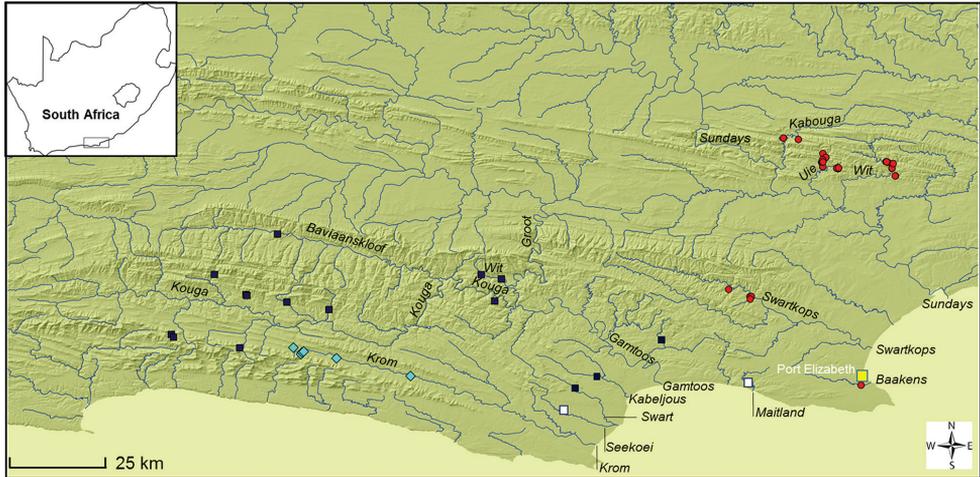


Figure 7. Map of the eastern Cape Fold Ecoregion showing confirmed distributions of *Pseudobarbus senticeps* (turquoise diamonds) restricted to the Krom River system), *Pseudobarbus swartzi* sp. n. (blue squares) (restricted to the Gamtoos River system and the Kabeljous and Seekoei Rivers) and *Pseudobarbus afer* s.s. (red circles) (Baakens, Swartkops and Sundays River systems) based on recent surveys (2000–2016). Additional surveys are required to more accurately map the distribution ranges of these species in the Krom, Gamtoos, Swart, Kabeljous, Baakens and Sundays, and determine the status of populations in the Seekoei and Maitland River systems (open squares).

was listed as Endangered following the IUCN assessment of the status and distribution of freshwater fishes in southern Africa (Tweddle et al. 2009). Invasive alien fishes were identified as the single most important threat to this species.

Other material examined

Pseudobarbus afer: SAIAB 34422, 5 males (44.9–65.5 mm SL), 5 females (59.2–74.5 mm SL), Blindekloof River, Groendal Wilderness, Swartkops River system, collected by D Boulle and PH Skelton, 11 November 1988; SAIAB 34428, 5 unsexed (60.1–75.1 mm SL), Blindekloof River, Groendal Wilderness, Swartkops River system, collected by D Boulle, 8 June 1989; SAIAB121688 (formerly AMG 2524), 24 unsexed (46.0–81.0 mm SL), Elands River, Swartkops River system, -33.7667S, 25.1278E, collected by PH Skelton and A Bok, 5 September 1974; SAIAB 119909 (formerly AMG745), 5 unsexed (46.0–61.0 mm SL), Elands River, Swartkops River system, -33.71667S, 25.1000E, collected by RA Jubbs, 15 February 1964; SAIAB 119773 (formerly AMG 609), 30 unsexed (48.5–66.5 mm SL), Wit River, Sundays River system, -33.3333333S, 25.6833333E, collected by R A Jubbs, 8 April 1959. SAIAB 119940 (formerly AMP 776), 5 unsexed (43.0–82.0 mm SL), Kragga Kamma, Baakens River system, -33.9500000S, 25.5000000E, collected by D Bicknell, 15 January 1964.

Pseudobarbus swartzi: AC16AL01 (SAIAB 203772), 10 specimens, unsexed, 25.5–57.9 mm SL, western Tributary of the Louterwater River, -33.8257500S, 23.6310000E, Gamtoos River system, collected by A Chakona, S Reddy and R Smith, 18 January 2016; AC16AL02 (SAIAB 203779), 6 specimens, unsexed, 32–64.8 mm SL, main Tributary of the Louterwater River, -33.8333611S, 23.6373056E, Gamtoos River system, same collectors and date as AC16AL01 (SAIAB 203772); AC16AL04 (SAIAB 203787), 34 specimens, unsexed, 18.2–86.7 mm SL, upper Dwars River, -33.6534444S, 23.7539722E, Gamtoos River system, same collectors and date as AC16AL01; AC16AL05 (SAIAB 203786), 17 specimens, unsexed, 34.8–64.9 mm SL, Klein River at Kouga Wilderness, -33.7112222S, 23.8440833E, Gamtoos River system, same collectors as AC16AL01 (SAIAB 203772), 19 January 2016; AC16AL06 (SAIAB 203789), 8 specimens, unsexed, 47.8–70.2 mm SL, Braam River, -33.7135278S, 23.8465833E, Gamtoos River system, same collectors as AC16AL01 (SAIAB 203772), 19 January 2016; AC16AL07 (SAIAB 203788), 13 specimens, unsexed, 17.9–63.3 mm SL, Diep River, -33.7541944S, 24.0812500E, Gamtoos River system, A Chakona and R Smith, 20 January 2016; AC16AL08 (SAIAB 203781), 45 specimens, unsexed, 14.7–53.8 mm SL, Upper Kansenkei River, -33.7296667S, 24.5545833E, Gamtoos River system, same date and collectors as AC16AL07 (SAIAB 203788); AC16BL01 (SAIAB 203774), 10 specimens unsexed, 25.5–57.9 mm SL, Wit River, -33.6538333S, 24.51605556E, Gamtoos River system, A Chakona and B Motshegoa, 7 March 2016; AC16BL02 (SAIAB 203780), 5 specimens unsexed, 24.6–58.8 mm SL, Lourie River, -33.8506944S, 25.0388194E, Gamtoos River system, A Chakona and B Motshegoa, 7 March 2016; SAIAB 120538 (formerly AMG1374), 70 unsexed, Kouga Dam, Gamtoos River system, -33.6666667S, 24.5166667E, collected by F Farquharson, 6 July 1967; SAIAB 120539, 70 unsexed, same locality and collector as SAIAB 120538.

Discussion

Comparative remarks

The three species recognised in the present study exhibit subtle morphological differences and show marginal overlap in some meristic (i.e. scale) counts. This explains why these species were previously considered to represent one widespread but variable species (Skelton 1988). Use of molecular data and careful examination of morphometric and meristic data in the present study helped to reveal consistent genetic, scale count, oral barbel length and colour pattern differences among Swartz et al.'s (2007) Mandela, St Francis and Krom lineages, supporting their recognition as distinct species. We redescribed *P. afer* s.s (Mandela lineage), resurrected *P. senticeps* (Krom lineage) and described a new species *P. swartzi* (St. Francis lineage). The three species are endemic to the streams of the eastern Cape Fold Ecoregion (CFE) at the south-eastern tip of Africa where they are allopatrically distributed.

These morphologically very similar species can be distinguished based on a combination of lateral line scale counts, circumpeduncular scale counts, body colour pattern and length of oral barbels. *Pseudobarbus senticeps* differs from both *P. afer* and *P. swartzi* by having fewer (i.e. larger) scales along the lateral line (mode 29), fewer scales around the caudal peduncle (mode 11) and a distinct lateral stripe that terminates in a triangular blotch at the base of the caudal fin. This colour pattern becomes more pronounced in preserved specimens. *Pseudobarbus senticeps* further differs from *P. afer* by having distinctly long barbels that reach or surpass vertical through the posterior edge of the eye. Barbels and other external features such as fins are however susceptible to degradation particularly in habitats affected by waste water discharge. Caution should therefore be exercised, and it is recommended that identification of the species should not be based on barbel length alone, but should be used in combination with scale counts and colour pattern. *Pseudobarbus swartzi* has smaller scales, i.e. more scales along the lateral line (mode 36) and around the caudal peduncle (mode 16) compared to *P. afer*. (mode 32 and 12, respectively). *Pseudobarbus swartzi* and *P. afer* further differ in scale pigmentation pattern. In *P. afer*, the melanophores form a semi-circular band around the centre of the scale, while the basal segment of the exposed area and the distal margin are not pigmented. This produces a distinct mesh or net-like pattern which is more pronounced on the latero-ventral scales (see Figure 6). This pigmentation pattern is not evident in *P. swartzi*.

The close morphological similarity observed among the three allopatric and genetically divergent species reported in the present study has also been reported for other riverine fishes, including the African butterfly fish, *Pantodon buchholzi*, from the Niger and Congo river systems (Lavoué et al. 2010) and the dwarf loach, *Cobitis brevifasciata* (previously *Kichulchoia brevifasciata*), from the Goheung Peninsula in South Korea (Kim et al. 2013). A combination of mechanisms, including stabilising selection (see Bickford et al. 2007), genetic variation and developmental constraints as well as ecological niche conservatism (Erwin 2007) have been proposed as possible explanations for the absence of significant accrual of species-wide morphological change over long evolutionary time scales (morphological stasis) observed in both extant taxa (e.g. Moen et al. 2013) and the fossil record (see Eldredge et al. 2005). *Pseudobarbus afer*, *P. swartzi* and *P. senticeps* inhabit mountain streams which are very similar ecologically. These streams which are characterised by clear acidic waters and rocky substrates have been classified as harsh environments by Dallas and Rivers-Moore (2014) as they experience extreme fluctuations in water temperature and flows between winter and summer months. According to Bickford et al. (2007), organisms that inhabit extreme environments may be more prone to stabilising selection which reduces or eliminates species-wide morphological change, because “there are a limited number of ways in which an organism can adapt to harsh conditions”. Thus, ecological niche conservatism (due to occurrence in similar habitats) and stabilising selection (occurrence in extreme environmental conditions) are both possible mechanisms that could explain the lack of clear morphological differentiation among *P. afer*, *P. senticeps* and *P. swartzi* sp. n., but these hypotheses require further testing with other co-distributed stream fishes. The

ongoing discovery of extreme levels of cryptic diversity within other genera of stream fishes endemic to the CFE such as *Galaxias* (Waters and Cambray 1997; Wishart et al. 2006; Chakona et al. 2013a) and *Sandelia* (Chakona et al. 2013a) suggests that morphological conservatism may be prevalent among stream fishes of the CFE. This region thus presents a particularly promising opportunity to undertake comparative studies to investigate the intrinsic and extrinsic mechanisms that are involved in maintaining morphological stasis in allopatric and genetically divergent stream fishes over varying evolutionary time scales.

Overall biogeographic patterns

The CFE experienced a complex history that left a perceptible imprint in the distribution and diversity of stream fishes in the region (see Skelton 1980; Swartz et al. 2007, 2009, 2014; Chakona et al. 2013a,b; Chakona et al. 2015). River captures and marine transgressions are likely to be the primary mechanisms that drove diversification and shaped the distribution patterns of the three redfins (*P. afer*, *P. senticeps* and *P. swartzi*) that are endemic to the eastern CFE. The geomorphology of this region (see Figure 8a) indicates that the drainages of the eastern CFE were influenced by a series of complex river captures (Haughton et al. 1937). According to Haughton et al. (1937), the Groot River historically flowed down the strike valley between the Great and Little Winterhoek mountains (arrow A in Figure 8a), probably forming a section of the headwaters of the Sundays River system. The Kouga River is thought to have formerly flowed through the strike valley between the Tsitsikamma and Baviaanskloof mountains (arrow B in Figure 8), probably forming the headwaters of the Krom River system. Both the Kouga and Groot Rivers abruptly cut across major mountain ranges to join the Baviaanskloof River (Figure 8a), suggesting that they were captured by the Gamtoos River system. These events could have had an influence on the diversification and present day distribution of redfins in the eastern CFE, but the specific role of river captures cannot be evaluated at this stage as no dating estimates exist for these events. More surveys are required to determine the extent of distribution of *P. swartzi* in the Groot catchment.

Sea-level changes offer another alternative explanation for the observed genetic and distribution patterns of redfins in the eastern CFE. Palaeoriver reconstructions for this region show that the Pliocene marine transgression resulted in fragmentation of the major river systems (the Krom, Gamtoos, Swartkops and Sundays), while smaller coastal systems (e.g. the Swart, Seekoei, Kabeljous, Van Stadens, Maitland, Baakens and Coega) were drowned and were presumably unavailable to freshwater taxa during this period (see Swartz et al. 2007; Figure 8b). Assuming the cytochrome *b* mutation rate of 2% per Myr (see Thorpe et al. 2005), age estimates for the splitting between *P. afer*, *P. senticeps* and *P. swartzi* coincide with the Pliocene sea-level transgression, suggesting that isolation of *Pseudobarbus* populations in upland refugia in the Krom, Gamtoos and the

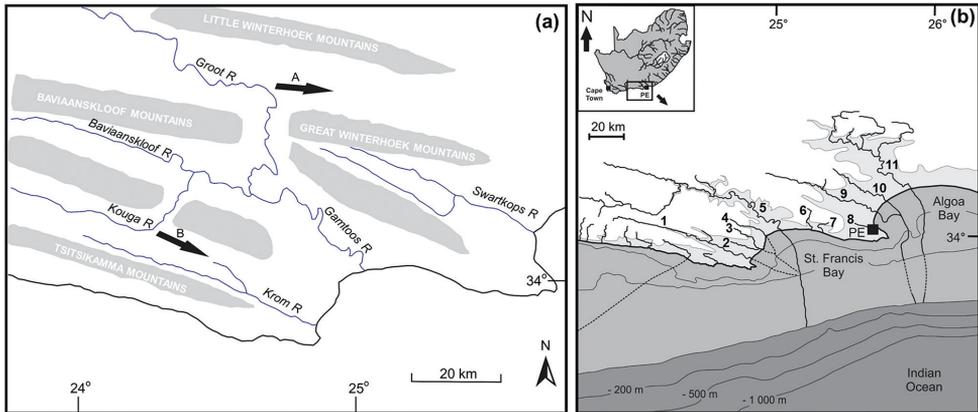


Figure 8. a An illustration of part of the Cape Fold Belt showing the drainage of the Gamtoos River system, sites of drainage capture of adjacent river systems and historical direction of flow of captured rivers (modified from Skelton, 1980) **b** Part of the eastern Cape Fold Ecoregion showing reconstructed Palaeoriver systems during the Last Glacial Maximum (modified from Swartz et al., 2007). The numerals represent present day river systems in the study area for the present study: 1, Krom; 2, Seekoei; 3, Swart; 4, Kabeljous; 5, Gamtoos; 6, Van Stadens; 7, Maitland; 8, Baakens; 9, Swartkops; 10, Coega; 11, Sundays).

Swartkops or Sundays could have played a role in promoting divergence and speciation of these redfins, as proposed for other stream fishes in the south-western CFE (Chakona et al. 2013a). Occurrence of *P. swartzi* in the currently isolated Gamtoos, Kabeljous and Swart River systems is consistent with expectations of post-speciation range expansion that is likely to have been facilitated by confluence of these rivers during the Last Glacial Maximum (LGM) low sea-levels (Swartz et al. 2007, 2009; Figure 8b). However, the lack of sharing of species between the Krom and the Gamtoos-Swart-Kabeljous is not consistent with the Palaeoriver hypothesis, as map reconstructions suggest that these four river systems would have joined before reaching the -130 m LGM sea-level (Swartz et al. 2007, 2008; Figure 8b). Presence of instream physical barriers such as waterfalls or extreme ecological barriers could have prevented post-speciation range expansion between these systems, as proposed for the Breede and Heuningnes lineages of *P. burchelli* from the south-western CFE (Swartz et al. 2014). Palaeoriver reconstructions suggest that the Swartkops-Coega-Baakens would have formed a common confluence before reaching the -130 m LGM sea-level, but it is uncertain whether this Palaeoriver system would have coalesced with the Sundays River system (see Swartz et al. 2007; Figure 8b) due to the intervening Ruy Bank (Bremner and Day 1991). If the Sundays remained isolated from the Swartkops-Coega-Baakens Palaeoriver system, a recent river capture event between the Swartkops and Sundays could provide an alternative explanation for the occurrence of *P. afer* in both systems. Presence of instream physical barriers or extreme ecological gradients could explain the absence of *P. afer* from the Coega River system which is inferred to have coalesced with the adjacent Swartkops and Baakens River systems during the LGM low sea-levels.

Conservation concerns

As with many other endemic stream fishes in the CFE, there is need for immediate intervention measures to ensure future survival of *P. afer*, *P. swartzi* and *P. senticeps*. As with elsewhere in the CFE (see Clark et al. 2009), *P. afer*, *P. swartzi* and *P. senticeps* have suffered severe range reductions mainly due to introduction of non-native predators, habitat alteration, complete water abstraction and building of weirs (Tweddle et al. 2009; Ellender et al. 2011, 2015; Ellender and Weyl 2014). Remnant populations of *P. afer*, *P. swartzi* and *P. senticeps* are highly fragmented and now only persist in upper mountain tributaries that have not been heavily degraded or invaded by alien species. There are limited or no opportunities for genetic exchange between isolated populations which can reduce the reproductive fitness and long-term evolutionary flexibility and adaptive responses of these species in the face of projected environmental changes in the region (Dallas and Rivers-Moore 2014). *Pseudobarbus afer*, *P. swartzi* and *P. senticeps* were listed under highly threatened categories of the IUCN during the previous assessment of the status of freshwater systems in southern Africa (see Tweddle et al. 2009). *Pseudobarbus afer* (identified as *P. afer* by Tweddle et al. 2009) and *P. swartzi* (identified as *Pseudobarbus* sp. “afer Gamtoos” by Tweddle et al. 2009) were both listed as Endangered, while *P. senticeps* (identified as *Pseudobarbus* sp. “afer Krom” by Tweddle et al. 2009) was listed as Critically Endangered. Ongoing decline is likely, and other populations (for example in the Baakens, Swart and Kabeljous river systems) are feared to have been extirpated or may be represented by only a few individuals as redfins were not collected from these systems during recent surveys. *Pseudobarbus senticeps* needs to be prioritised as it is represented by very few remaining populations with very small known population sizes. Eradication of non-native fishes and control of effluent discharge are the immediate conservation actions required to secure remaining populations of *P. senticeps*. Additional fine scale field surveys are required to determine the status of redfin populations in the Baakens, Van Stadens, Swart and Kabeljous river systems. Future studies should aim to examine the ecology of the three species identified in the present study and model their potential responses to present and future environmental changes that are projected to impact biotic communities of the Cape Fold Ecoregion.

Acknowledgements

This work was supported by the National Research Foundation (NRF) of South Africa under the Foundational Biodiversity Information Programme: Biodiversity surveys in priority inland areas (IBIP) grants (grant reference no. IBIP-BS13100251309). The authors acknowledge that opinions, findings and conclusions or recommendations expressed in this publication generated by the NRF supported research are that of the authors and that the NRF accepts no liability whatsoever in this regard. We thank Rodger Smith, Roger Bills, Veer Bills, Bosupeng Motshegoa, Wilbert Kadye, and Nkosinathi

Mazungula for assistance with field collections, Nkosinathi Mazungula for taking the radiographs and the Department of Economic Development, Environmental Affairs and Tourism (Eastern Cape Province) for issuing a research permit (permit numbers: CRO 88/15CR and 01/31447). Gavin Gouws, Leah Sloman, Seshnee Reddy and Sissanda Sibanga helped with laboratory work.

References

- Armbruster JW (2012) Standardized measurements, landmarks, and meristic counts for cypriniform fishes. *Zootaxa* 3586: 8–16
- Barnard KH (1938) Notes on the species of *Barbus* from the Cape Province, with descriptions of new species. *Annals and Magazine of Natural History (Series 11)* 2: 80–88.
- Barnard KH (1943) Revision of the indigenous freshwater fishes of the S.W. Cape Region. *Annals of the South African Museum* 36: 101–263.
- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22: 148–156. <https://doi.org/10.1016/j.tree.2006.11.004>
- Boulenger GA (1911) Catalogue of the Freshwater fishes of Africa in the British Museum (Natural History) (Vol. 2). British Museum (Natural History), London, 529 pp.
- Bremner JM, Day RW (1991) Acoustic stratigraphy and Late Cenozoic sediments in Algoa Bay. *Geological Survey Bulletin* 100: 123–146.
- Castelnau FL (1861) *Mémoire sur les poissons de l'Afrique australe*. Baillièere et Fils, Paris. <https://doi.org/10.5962/bhl.title.3819>
- Chakona A, Swartz ER (2013) A new redbfin species, *Pseudobarbus skeltoni* (Cyprinidae, Teleostei), from the Cape Floristic Region, South Africa. *Zootaxa* 3686: 565–577. <https://doi.org/10.11646/zootaxa.3686.5.5>
- Chakona A, Swartz ER, Gouws G (2013a) Evolutionary drivers of diversification and distribution of a southern temperate stream fish assemblage: testing the role of historical isolation and spatial range expansion. *PlosOne* 8(8): e70953: 1–13. <https://doi.org/10.1371/journal.pone.0070953>
- Chakona A, Swartz ER, Gouws G, Bloomer P (2013b) A freshwater fish defies ancient mountain ranges and drainage divides: extrinsic and intrinsic influences on the evolutionary history of a recently identified galaxiid. *Journal of Biogeography* 40: 1399–1412. <https://doi.org/10.1111/jbi.12104>
- Chakona A, Swartz ER, Skelton PH (2014) A new species of redbfin (Teleostei, Cyprinidae, *Pseudobarbus*) from the Verlorenvlei River system, South Africa. *ZooKeys* 453: 121–137. <https://doi.org/10.3897/zookeys.453.8072>
- Chakona G, Swartz ER, Chakona A (2015) Historical abiotic events or human-aided dispersal: inferring the evolutionary history of a newly discovered galaxiid fish. *Ecology and Evolution* 5(7): 1–12. <https://doi.org/10.1002/ece3.1409>
- Chakrabarty P (2010) Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. *Zootaxa* 2632: 67–68.

- Clark BM, Impson D, Rall J (2009) Present status and historical changes in the fish fauna of the Berg River, South Africa. *Transactions of the Royal Society of South Africa* 64: 142–163. <https://doi.org/10.1080/00359190909519249>
- Dallas H, Rivers-Moore N (2014) Ecological consequences of global climate change for freshwater ecosystems in South Africa. *South African Journal of Science* 110: 1–11. <https://doi.org/10.1590/sajs.2014/20130274>
- Eldredge N, Thompson JN, Brakefield PM, Gavrillets S, Jablonski D, Jackson JBC, Lenski RE, Lieberman BS, McPeck MA, Miller WIII (2005) The dynamics of evolutionary stasis. *Paleobiology* 31: 133–145. [https://doi.org/10.1666/0094-8373\(2005\)031\[0133:T-DOES\]2.0.CO;2](https://doi.org/10.1666/0094-8373(2005)031[0133:T-DOES]2.0.CO;2)
- Ellender BR, Weyl OLF (2014) A review of the current knowledge, risk and impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions* 9: 117–132. <https://doi.org/10.3391/ai.2014.9.2.01>
- Ellender BR, Weyl OLF, Swartz ER (2011) Invasion of a headwater stream by non-native fishes in the Swartkops River system, South Africa. *African Zoology* 46: 39–46. <https://doi.org/10.1080/15627020.2011.11407477>
- Ellender BR, Woodford DJ, Weyl OLF (2015) The invasibility of small headwater streams by an emerging invader, *Clarias gariepinus*. *Biological Invasions* 17: 57–61. <http://dx.doi.org/10.1007/s10530-014-0744-8>
- Erwin DH (2007) Disparity: morphological pattern and developmental context. *Palaeontology* 50: 57–73. <https://doi.org/10.1111/j.1475-4983.2006.00614.x>
- Ffolliot P, Liversidge (1971) Ludwig Krebs Cape Naturalist to the King of Prussia 1792–1844. Balkema, Cape Town.
- Gilchrist JDF, Thompson WW (1913) The freshwater fishes of South Africa. *Annals of the South African Museum* 11: 321–463. <https://doi.org/10.5962/bhl.part.5073>
- Gilchrist JDF, Thompson WW (1917) The freshwater fishes of South Africa (Continued). *Annals of the South African Museum* 11: 465–575.
- Günther A (1868) Catalogue of the Physostomi in the collection of the British Museum (Vol. 7). British Museum, London. <http://biodiversitylibrary.org/page/9365020>
- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 9 pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm
- Houghton SH, Frommurge HF, Visser DJL (1937) The geology of portion of the coastal belt near the Gamtoos Valley, Cape Province. Explanation of Sheets 151 North and 151 South (Gamtoos River), Geological Survey Union of South Africa, Department of Mines and Industries. Government Printer, South Africa, 64 pp.
- Jubb RA (1963) A revised list of the freshwater fishes of southern Africa. *Annals of the Cape Provincial Museums* 3: 5–39.
- Jubb RA (1965) Freshwater fishes of the Cape Province. *Annals of the Cape Provincial Museum* 4: 1–72.
- Jubb RA (1967) The Freshwater Fishes of Southern Africa. Balkema, Cape Town, 248 pp.
- Kim D, Conway KW, Jeon H-B, Kwon Y-S, Won Y-J (2013) High genetic diversity within the morphologically conservative dwarf loach, *Kichulchoia brevifasciata* (Teleostei: Cobitidae),

- an endangered freshwater fish from South Korea. *Conservation Genetics* 14: 757–769. <https://doi.org/10.1007/s10592-013-0462-2>
- Lavoué S, Miya M, Arnegard ME, McIntyre PB, Mamonekene V, Nishida M (2011) Remarkable morphological stasis in an extant vertebrate despite tens of millions of years of divergence. *Proceedings of Royal Society B* 278: 1003–1008. <https://doi.org/10.1098/rspb.2010.1639>
- Moen DS, Irschick DJ, Wiens JJ (2013) Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. *Proceedings of the Royal Society B* 280: 20132156. 1–9. <https://doi.org/10.1098/rspb.2013.2156>
- Naran D, Skelton PH, Villet MH (2006) Karyology of the redfin minnows, genus *Pseudobarbus* Smith, 1841 (Teleostei, Cyprinidae): one of the evolutionarily tetraploid lineages of South African barbines. *African Zoology* 41: 178–182. <https://doi.org/10.1080/15627020.2006.11407353>
- Peters WCH (1864) Über einige neue Säugethiere, Amphibien und Fische. *Monatsbericht der Königlich Preussischen Akademie der Wissenschaften zu Berlin* 1864: 381–399.
- Shelton JM, Samways MJ, Day JA (2014) Predatory impact of non-native rainbow trout on endemic fish populations in headwater streams in the Cape Floristic Region of South Africa. *Biological Invasions* 17: 365–379. <https://doi.org/10.1007/s10530-014-0735-9>
- Skelton PH (1980) Aspects of freshwater fish biogeography in the Eastern Cape. *The Eastern Cape Naturalist* 24: 16–22.
- Skelton PH (1988) A taxonomic revision of the redfin minnows (Pisces, Cyprinidae) from southern Africa. *Annals of the Cape Provincial Museum (Natural History)* 16: 201–307.
- Skelton PH (2016) Name changes and additions to the southern African freshwater fish fauna. *African Journal of Aquatic Science* 41(3): 345–351. <https://doi.org/10.2989/16085914.2016.1186004>
- Smith A (1849) [1841] Pisces. *Illustrations of the Zoology of South Africa*. Smith, Elder and Col., London.
- Smith JLB (1936) New gobioid and cyprinid fishes from South Africa. *Transactions of the Royal Society of South Africa* 24: 47–55. <https://doi.org/10.1080/00359193609518912>
- Smith JLB and Smith MM (1966) *Fishes of the Tsitsikama Coastal National Park*. National Parks Board, Pretoria, 161 pp.
- Swartz ER, Chakona A, Skelton PH, Bloomer P (2014) The genetic legacy of lower sea-levels: does the confluence of rivers during the last glacial maximum explain the contemporary distribution of a primary freshwater fish (*Pseudobarbus burchelli*, Cyprinidae) across isolated river systems? *Hydrobiologia* 726: 109–121. <https://doi.org/10.1007/s10750-013-1755-7>
- Swartz ER, Skelton PH, Bloomer P (2007) Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redfins (*Pseudobarbus afer* and *Pseudobarbus phlegethon*, Cyprinidae) across multiple river systems in South Africa. *Journal of Biogeography* 34: 2086–2099. <https://doi.org/10.1111/j.1365-2699.2007.01768.x>
- Swartz ER, Skelton PH, Bloomer P (2009) Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution* 51: 75–84. <https://doi.org/10.1016/j.ympev.2008.10.017>

- Thorpe RS, Leadbeater DL, Pook CE (2005) Molecular clocks and geological dates: cytochrome b of *Anolis extremus* substantially contradicts dating of Barbados emergence. *Molecular Ecology* 14: 2087–2096. <https://doi.org/10.1111/j.1365-294X.2005.02574.x>
- Tweddle D, Bills R, Swartz ER, Coetzer W, Da Costa L, Engelbrecht J, Cambray JA, Marshall B, Impson D, Skelton PH (2009) The status and distribution of freshwater fishes. In: Darwall WRT, Smith KG, Tweddle D, Skelton PH (Eds) *The Status and Distribution of Freshwater Biodiversity in Southern Africa*. IUCN, Gland, and SAIAB, Grahamstown, 21–37.
- Waters JM, Cambray JA (1997) Intraspecific phylogeography of the Cape galaxias from South Africa: evidence from mitochondrial DNA sequences. *Journal of Fish Biology* 50: 1329–1338. <https://doi.org/10.1111/j.1095-8649.1997.tb01656.x>
- Weber M (1897) Beiträge zur Kenntniss der Fauna von Süd-Afrika I. Zur Kenntniss der Süswasser-Fauna von Süd-Afrika. *Zoologische Jahrbücher (Abteilung Systematik, Geographie und Biologie der Tiere)* 10: 135–155.
- Wishart M, Hughes J, Stewart B, Impson D (2006) Extreme levels of intraspecific divergence among Cape Peninsula populations of the Cape galaxias, *Galaxias zebratus* Castelnau 1861, reveals a possible species complex. *African Journal of Aquatic Science* 31: 99–106. <https://doi.org/10.2989/16085910609503876>
- Yang L, Sado T, Hirt VM, Pasco-Viel E, Arunachalam M, Li J, Wang X, Freyhof J, Saitoh K, Simons AM, Miya M, He S, Mayden RL (2015) Phylogeny and polyploidy: resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution* 85: 97–116. <https://doi.org/10.1016/j.ympev.2015.01.014>

Resurrection of *Bronchocela burmana* Blanford, 1878 for the Green Crested Lizard (Squamata, Agamidae) of southern Myanmar

George R. Zug¹, Daniel G. Mulcahy², Jens V. Vindum³

1 Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, PO Box 37012, Washington, District of Columbia, 20013-7012 USA **2** Global Genome Initiative, National Museum of Natural History, Smithsonian Institution, PO Box 37012, Washington, District of Columbia, 20013-7012 USA **3** Department of Herpetology, Californian Academy of Sciences, Golden Gate Park, San Francisco, California, 94103 USA

Corresponding author: *George R. Zug* (zugg@si.edu)

Academic editor: *A. Herrel* | Received 22 December 2016 | Accepted 31 January 2017 | Published 20 February 2017

<http://zoobank.org/D96FFD28-7945-44EA-9277-8E36B835F50C>

Citation: Zug GR, Mulcahy DG, Vindum JV (2017) Resurrection of *Bronchocela burmana* Blanford, 1878 for the Green Crested Lizard (Squamata, Agamidae) of southern Myanmar. ZooKeys 657: 141–156. <https://doi.org/10.3897/zookeys.657.11600>

Abstract

Recent fieldwork in southern Tanintharyi revealed the presence of a small Green Crested Lizard in the wet evergreen forest. We generated mtDNA sequence data (ND2) that demonstrates that this population's nearest relative is *Bronchocela rayaensis* Grismer et al., 2015 of Pulau Langkawi, northwestern Peninsular Malaysia and Phuket Island. Morphologically the Burmese *Bronchocela* shares many features with *B. rayaensis*, which potentially would make this recently described Thai-Malay species a synonym of *Bronchocela burmana* Blanford, 1878; however, we interpret the genetic and morphological differences to reflect evolutionary divergence and recommend the recognition of both species.

Keywords

Reptilia, Southeast Asia, Tanintharyi Division, Thailand, Peninsular Malaysia, morphology, molecular phylogeny, synonymy, nomenclature

Introduction

Bronchocela is a light weight among agamid lizards, rivaling *Draco* in the lightness and slenderness of its body and limbs. *Draco*'s slenderness is associated with its gliding locomotion. The slenderness and extremely long tail (~ 3× body length) of *Bronchocela* seem to be an adaptation for moving on the outer edge of the branches of shrubs and trees.

The first member of the *Bronchocela* clade to be recognized was *Agama cristatella* Kuhl, 1820 (now *B. cristatella*). This species continues to be the most commonly recognized *Bronchocela* owing to its broad distribution from Peninsular Malaysia to the Philippines and into the Lesser Sunda Islands (Grismer et al. 2015). The first mention of a *Bronchocela* in Burma (Myanmar) was in 1878 by Blanford. He recognized that a Burmese specimen differed strikingly from *B. cristatella* and the other species described to that time and named this taxon *B. burmana*. One major difference was the height, shape, and number of nuchal crest scales. Boulenger (1880) did not believe that this Burmese species was unique and synonymized *B. burmana* with *Calotes cristatellus*, a name used then because Boulenger also did not consider *Bronchocela* Kaup, 1827 to be a unique group even though *Bronchocela* had more than 50 years of recognition by other major authorities in herpetology. Malkmus (1992) appears to be the first author to use *Bronchocela* in a modern publication; however, his use was limited to *B. cristatella* presence in the Mount Kinabalu (Sabah, Borneo) area. Diong and Lim's (1998) review of *B. cristatella* is the first formal use of *Bronchocela* with a full explanation of their decision to revive the generic name. Their decision was based on a confirmation of Scott Moody's data in his unpublished dissertation demonstrating that *cristatella* was not closely related to other members of the *Calotes* clade.

Bronchocela burmana has remained a forgotten name until the present, in part because of Boulenger's nomenclatural authority, but also because few *Bronchocela* have been collected in Myanmar owing to its limited occurrence to southern Tanintharyi (Fig. 1) and the failure to examine closely the few specimens from southern Burma. One of us (DGM) has been participating in rapid assessment surveys of proposed national parks in southern Tanintharyi. He first discovered two specimens of *Bronchocela* in the Lenya NP area in 2015 and one in the Lenya NP Extension in 2016. We likely would have labeled these specimens as *B. cristatella* without examining them closely if part of the survey did not also include mtDNA barcoding of all collected specimens. When additional sequence data (ND2) indicated that the Lenya specimens were not closely related to *B. cristatella*, instead to the recently described *Bronchocela rayaensis* (Grismer et al., 2015) from Pulau Langkawi, northwestern Peninsular Malaysia and Phuket Island, Thailand (Grismer et al. 2016), we decided to make a morphological comparison as well.

We present below our analysis of the molecular and morphological data for the southern Burmese *Bronchocela* specimens. As our title indicates, these data demonstrate that the Burmese population is unique.



Figure 1. Distribution of *Bronchocela burmana* (solid circles) in southern peninsular Myanmar, Taylor's (1963) two localities for *B. cristatella* (open squares) in southern Thailand, and *B. rayaensis* type locality (star) in northwestern Peninsular Malaysia and its newly reported localities (open circles) in Thailand (Grismer et al. 2016). A solid diamond denotes the type locality of *B. burmana*. The red dashed lines depict the political boundaries between Myanmar-Thailand, Cambodia-Thailand, and Malaysia-Thailand. Base map from: CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=166887>.

Materials and methods

Molecular analyses

We sequenced eight specimens of *Bronchocela*, five from the California Academy of Sciences (CAS) from the area near Kawthung, and three National Museum of Natural History-Smithsonian (USNM) specimens from the Lenya NP area (Table 1). Liver and muscle tissue samples were collected in the field during specimen preparation and pre-

Table 1. Voucher information for specimens sequenced for this study. See holding institution for additional locality information.

| Specimen | Locality | GenBank |
|------------|--|----------|
| USNM587793 | Myanmar: Tanintharyi, proposed Lenya National Park | KY366315 |
| USNM587483 | Myanmar: Tanintharyi, proposed Lenya National Park | KY366313 |
| USNM587484 | Myanmar: Tanintharyi, proposed Lenya National Park | KY366314 |
| CAS247755 | Myanmar: Tanintharyi, Khamaukgyi Township | KY366309 |
| CAS247756 | Myanmar: Tanintharyi, Khamaukgyi Township | KY366310 |
| CAS247757 | Myanmar: Tanintharyi, Khamaukgyi Township | KY366311 |
| CAS247860 | Myanmar: Tanintharyi, Khamaukgyi Township | KY366312 |
| CAS228481 | Myanmar: Tanintharyi, Pakchan Reserve Forest | KY366308 |

served separately in EtOH (CAS specimens) or a DMSO/EDTA salt-saturated buffer (USNM specimens; Mulcahy et al. 2016). Specimens were subsequently fixed in 10% formalin and transferred to 70% ethanol for long-term storage at the USNM collection, housed at the National Museum of Natural History (NMNH) and at CAS. Extractions of genomic DNA were conducted on small pieces of liver or muscle tissue and run on an Auto-Genprep 965 (2011 AutoGen, Inc.), using standard phenol manufacturer protocols. Genomic DNA was eluted in 100 μ l of AutoGen R9 re-suspension buffer. We sequenced the mtDNA ND2 gene because that is the locus used by Grismer et al. (2015) in their descriptions of two new species of *Bronchocela*. For our PCR reactions, we used the primers L4437–H5934 (Macey et al. 1997). Cycle-sequence reactions were performed in both directions, using the PCR primers using BigDye Terminator v3.1 Cycle Sequencing Kit's in 0.25 \times 10 μ l reactions run on an ABI3730 Sequencer (2011 Life Technologies) using the 950 chemistry. Raw trace files were edited in Geneious 9.1.5 (Biomatters Ltd 2005–2016), complementary strands were aligned, edited, and inspected for translation. All sequences were deposited in GenBank under the accession numbers KY366308–KY366315. Our sequences were aligned with the ND2 samples (including the outgroups) from Grismer et al. (2015), the single new individual from Phuket Island, Thailand (Grismer et al. 2016), and another specimen in GenBank (AF128497) initially identified as *B. cristatella* (Macey et al. 2000a) and AF128495, initially identified as *Aphaniotis fusca* because these specimens were reported as being switched in GenBank (Zug et al. 2006). We used Geneious and the MUSCLE Alignment with default settings and secondarily inspected for codon alignment and translation. Grismer et al. (2016) did not deposit the ND2 sequence of their new specimen (ZMKUR0017/LSUHC12347) from Phuket Island in GenBank, but did provide us with a copy. We deposited their sequence in GenBank under the sequence number KY498356. The tRNA region of the ND2 locus required some adjustments to insure the tRNA's were properly aligned based on secondary stem and loop structure (sensu Macey et al. 1997). The tRNA secondary structure was determined using ARWEN (Laslett and Canback 2008). We performed maximum-likelihood (ML) analyses using RAxML (v8.2.9, Stamatakis 2014) with the rapid bootstrap inferences (1000 replicates) and subsequent thorough ML search, with ND2 partitioned by codon and the tRNAs as a separate partition.

Morphological analyses

Hallermann began his systematic studies of *Bronchocela* in 2004 and, in a series of studies (Hallermann 2004, 2005, 2009), developed a set of characters for morphological analysis. Grismer et al. (2015) used Hallermann's characters and introduced several new ones in his molecular and morphological study of Peninsular Malaysian populations of *Bronchocela*. Our study of morphological variation used most of the previous proposed characters and includes the following:

Measurements—snout-vent length (**SVL**), distance from the tip of snout to the vent; trunk length (**TrnkL**), distance from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; tail length (**TailL**), distance from middle of vent to tip of tail; forelimb length (**ForelL**), distance (dorsal) from trunk between forelimb's insertion to tip of third finger, not including claw (Grismer et al. 2015 listed fourth toe; we suspect that mentioned digit was incorrect); forefoot length (**ForefL**), distance from base (middle) of palm to tip of third finger, not including claw; 4th finger lengths (**4FingL**), distance from the juncture of the third and fourth finger to the end of the ultimate lamellae of the fourth finger, hence excluding the claw; 4th toe lengths (**4ToeL**), distance from the juncture of the third and fourth toes to the end of the ultimate lamellae of fourth toe; hindfoot length (**HindfL**), distance from base (middle) of sole to tip of fourth toe, not including claw; hindlimb length (**HindlL**), distance (dorsal) from trunk between hindlimb's insertion to tip of fourth toe, not including claw; head length (**HeadL**), distance from posterior edge of tympanum to tip of snout; head width (**HeadW**), transverse width of the head at posterior angles of jaws; head depth (**HeadD**), distance from top of head between orbits to lower surface of jaw (dentary); orbit diameter (**OrbD**), maximum horizontal distance from anterior and posterior edges of orbit (not eyeball); tympanum diameter (**TypmD**), greatest horizontal distance regardless tympanum height; body scale size dorsally (**BSC.dors**), width of parasagittal dorsal scale at midbody; body scale size ventrally (**BSC.vntl**), width of median ventral scale at midbody; nuchal crest length (**NucCrstL**), length of nuchal crest from anterior edge of anteriormost crest scale to posterior edge of posteriormost crest scale; nuchal crest height (**NucCrstH**), height of the highest/largest nuchal crest scale.

Scalation—supralabial scales (**Suplab**), number of scales between rostral scale and angle of jaw; infralabial scales (**Inflab**) numbers of scales between mental scale and angle of the jaw; loreal scales (**Loreal** = canthal scales in Hallermann [2009]), number of scales between nasal scale and granular scales of anterior border of orbit; postmental scales (**Postm**), number of throat scales (**Throat**), number of scales between end of jaws; third finger lamellae (**3ForefLm**), number of subdigital lamellae beneath third finger from first scale/lamella at digits' insertion point to claw; fourth toe lamellae (**4HindfLm**), number of subdigital lamellae beneath fourth toe from first scale/lamella at digits' insertion point to claw; midbody scales (**Midb**), number of scales around midbody; dorsal scales 1 (**Dorsal1**) number of dorsal scale rows with keels oriented parallel to the keels of the dorsal crest scales; dorsal scales 2 (**Dorsal2**), number of dorsal scale rows with keels oriented diagonally downward; nuchal spines (**NuchalS**),

number of enlarged nuchal crest scales at least twice as large as enlarged vertebral scales; dorsal crest (**DorsCrst**), presence or absence; nuchal crest scales' shape (**NucCrstSS**), lanceolate, crescent-shaped, or triangular.

Aspects of body shape were examined by converting paired traits to proportions, such as TailL/SVL, ForefL/ForelL, ForelL/HindlL, HeadW/HeadL. Measurements, proportions, and scale counts were analyzed by basic parametric statistics, Systat 12. We used a simple Student's *t* test to examine dimorphism between adult females and males, significance $\alpha < 0.05$. Statistical analysis used SYSTAT 12.

Specimen number abbreviations are as follows: CAS (California Academy of Sciences), EHT (Edward H. Taylor), LSUHC (La Sierra University Herpetology Collection), USNM (United States National Museum, National Museum of Natural History), TNHC (Texas Natural History Collection, Univ. of Texas, Austin), ZMKU (Zoological Museum of Kasetsrat University, Bangkok, Thailand) and ZSI (Zoological Society of India).

Results

We obtained ND2 sequence data from eight individuals ranging in size from 1,260–1,369 bp that were 98.5–99.8% identical to one another (un-corrected sequence divergence). Our samples have a truncated origin of light strand replication (21 bp) between tRNA^{ASN} and tRNA^{CYS} that appears functional (folds using the DNA-Matthews 1999 Energy Model in Geneious) and tRNA^{CYS} shows a d-arm replacement loop (Macey et al. 1997) consistent with 45% of Draconinae agamids (Seligmann and Labra 2014). Our samples formed a well-supported clade (100%) with each other and were placed sister to *Bronchocela rayaensis* with 100% bootstrap support (Fig. 2). Our samples ranged from 93.4–95.0% identical (un-corrected sequence divergence) to the *B. rayaensis* specimens in GenBank (KR053115–KR053116, KY498356). Our analyses recovered the Phuket Island *B. rayaensis* sister to the Pulau Langkawi samples with 94% bootstrap support (Fig. 2). The rest of our tree was nearly identical to that recovered by Grismer et al. (2015) with the exception of the inclusion of *B. cristatella* (AF128495) and *Aphaniotis fusca* (AF128497); the latter was placed sister to *Aphaniotis fusca* (AF288228).

All measurements, proportions, and scalation were examined for differences between adult females ($n = 5$) and adult males ($n = 3$). These samples are small so the significance of our statistical results are indicative of dimorphism but not statistical proof. Among the 33 mensural traits tested, only seven suggest dimorphism (means and ranges compared). Females average smaller (SVL 80.8, 78.8–83.8 mm) than males (SVL 88.2, 84.3–92.7 mm), fourth finger shorter in females (means, 11.0 vs. 12.6 mm 4FingL), fourth toe shorter in females (16.6 vs. 18.6 mm 4ToeL), hindlimb distinctly shorter (74.3 vs. 81.2 mm HindlL), head length less (21.2 vs. 22.6 mm HeadL), length of nuchal crest less (9.6 vs. 12.3 mm NucCrL), and crest length to orbit diameter also shorter (150% vs. 176% NucCrL/OrbD). No scalation features display sexual dimorphism.

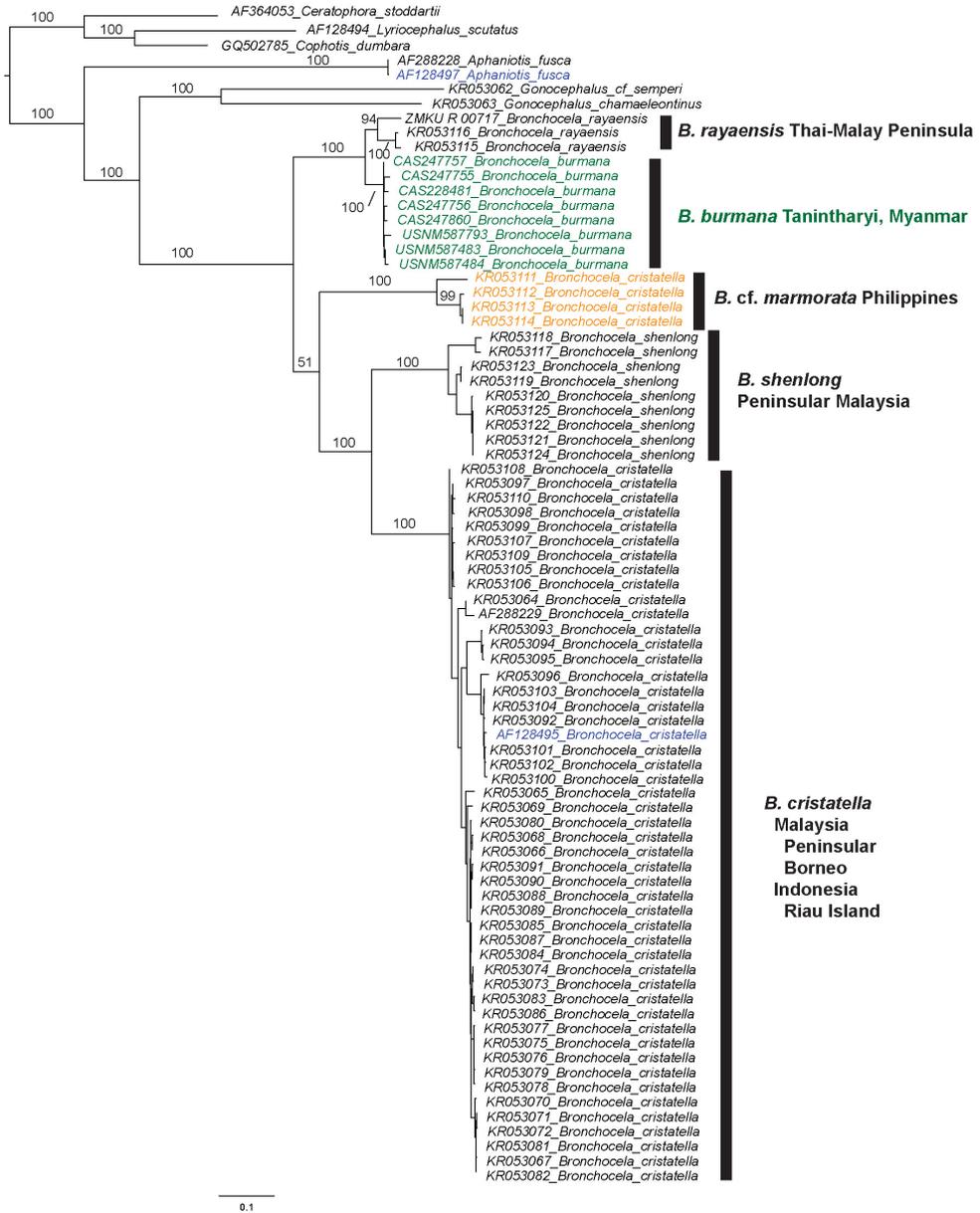


Figure 2. Phylogenetic relationships of *Bronchocela*. Maximum-likelihood phylogeny based on 1,416 bp of ND2 mtDNA, bootstrap values (based on 1,000 replicates) are shown for the major nodes relevant to this study. Terminals highlighted in blue were recently corrected in GenBank and the *B. cristatella* from the Philippines (in yellow) are now re-identified as *B. cf. marmorata*.

To summarize the statistical results for measurements and proportions (values, mean and minimum-maximum): dimorphic traits, females' SVL $x = 80.8$, 78.8–83.8 mm, 4FingL 11.0, 10.4–11.6 mm, 4ToeL 16.6, 15.1–17.9 mm, HindfL 29.5, 29.0–

30.0 mm, HeadL 21.2, 20.3–22.0 mm, NucCrsL 9.6, 9.1–10.1 mm, NucCrsL/OrbD 150, 132–168%; males' SVL $x = 88.2$, 84.3–92.7 mm, 4FingL 12.6, 11.3–13.9 mm, 4ToeL 18.6, 18.2–18.8 mm, HindfL 32.3, 30.9–33.1 mm; HeadL 22.6, 21.6–23.3 mm, NucCrsL 12.3, 10.6–13.3 mm, NucCrsL/OrbD 176, 166–184%; monomorphic traits (adults combined) TrunkL 42.8, 40.4–46.6 mm, TailL 276.3, 204–306 mm, ForeL 45.5, 42.2–51.5 mm, ForefL 16.3, 14.7–18.2 mm, HinddL 76.9, 70.6–86.6 mm, HindfL 30.6, 29.0–33.1 mm, HeadW 11.6, 9.5–14.1 mm, HeadH 10.2, 9.3–11.3 mm, OrbD 6.6, 5.9–7.5 mm, TympD 3.2, 2.4–3.5 mm, BSC.dors 0.6, 0.5–0.8 mm, BSC.vntl 1.7, 1.5–2.1 mm, NucCrsH 1.4, 0.9–3.0, TrunkL/SVL 51, 47–56%, TailL/SVL 328, 243–361%, ForeL/SVL 54, 50–58%, HinddL/SVL 92, 86–97%, HeadL/SVL 26, 25–27%, HeadW/HeadL 54, 44–62%, HeadH/HeadL 47, 45–50%, ForefL/ForeL 35, 29–40%, HindfL/HinddL 40, 38–43%, ForeL/HinddL 21, 20–23%, 4FingL/4ToeL 67, 60–74%, TympD/OrbD 48, 40–58%, DorsS/VntlS 35, 31–39%, NucCrsL/OrbD 160, 132–184%.

The results for scalation are (values, median and minimum–maximum; adults and juvenile combined): Suplab 10, 9–11, Inflab 10, 9–11, Loreal 5, 5–6, Postm 3, 3–3, Throat 26, 25–30, 3ForefLm 28, 26–32, 4HindfLm 33, 31–38, Midbody 59, 55–67, Dorsal1 7, 6–8, Dorsal2 17, 15–21, NucCrsS 8, 6–9; nuchal spines or scales are predominantly triangular, rarely broadly crescent shaped; middorsal trunk scales are large, keeled but not elevated into a crest.

We attempted to code a few coloration traits but were unsuccessful because preservation had altered life-colors and pattern. The manner of field work and genomic tissue sampling did not permit the recording of coloration in living specimens. The general impression of the Tanintharyi *Bronchocela* is green with transverse bands of white spots on trunk and alternating bands of green and white on the tail (Fig. 3). Preserved specimens have a broadly mottled pattern of dark rufous brown and dusky turquoise dorsally and laterally. These colors form broad swatches with no consistence of position on the body although the majority (eight of nine) of the specimens have the snout and interorbital (not the superciliary area) dark. The middle third of the tail (dorsally) shows a vague banding, broad bands of medium brown and dusky tan. The venter from the tip of the chin onto the tail base is light turquoise with the chin and throat lighter than chest and abdomen. The temporal area (between orbit and tympanum) is bright rufous in eight of nine specimens although the size of this rufous patch varies from a small spot to the entire area. The eye sheath is dark, the tympanum light. The loreal area also is rufous and size of rufous patch varies from a small patch to the entire area. The ventrolateral neck folds range from uniform dark rufous to uniform turquoise. In two instances of the latter, the posterior edge of the fold bears small, ill-defined rufous spots.

For comparative purposes, we extracted the equivalent measurement and scalation data from Grismer et al.'s (2015: tables 5 & 6; 2016: table 1) description of *B. rayaensis*. Both of the types were adults, holotype a male and paratype a female, and the newly discovered Thai adult female. The following is a summary for the types (see Table 2 for summary of the three known specimens): female's SVL 85.4 mm, HeadL 20.1 mm;

Table 2. Comparison of character metric of the three potentially allopatric species of *Bronchocela* in Myanmar, Thailand, and northern Peninsular Malaysia. Character abbreviations are defined in the Material and Methods section of the text. Numerical values are mean and range within parentheses.

| Characters | <i>B. burmana</i> ¹ | <i>B. rayaensis</i> ² | <i>B. cristatella</i> ³ |
|--|--|----------------------------------|------------------------------------|
| Snout–vent length, SVL (mm) | ♀ 80.8 (79–84 n=5) ♂ 88.2 (84–93 n=3) | ♀ 83.1–85.4 n=2 ♂ 82.0 n=1 | ♀ to 111 ♂ 111–119 |
| HeadW/HeadL (%) | 54 (44–62) | 57 (56–59) | 76–94 |
| HeadH/HeadL (%) | 47 (45–50) | 50 (45–56) | 48–57 |
| TympD/OrbD (%) | 48 (40–54) | 44 (38–49) | 44–60 |
| ForeL/SVL (%) | 54 (50–58) | 57 (53–59) | 50–63 |
| HindL/SVL (%) | 92 (86–97) | 92 (87–99) | 81–104 |
| Nuchal crest height | low | low | high |
| Nuchal crest scales | 8 (6–9) | 10 (8–13) | 8–11 |
| Crest on trunk | No | No | Yes |
| Upper trunk scale row orientation: Dorsal1 | 7 (6–8) | 6 (5–8) | 6–8 |
| Dorsal2 | 17 (15–21) | Not reported | 17–23 |
| 4HindfLm | 33 (31–38) | 33 (male only) | 27–34 |
| Midbody | 59 (55–67) | 71 (67–74) | 71–99 |
| NucCrsS | 8 (6–9) | 10 (8–13) | 8–10 |

male's SVL 82.0 mm, HeadL 21.8 mm; monomorphic traits (both types) TrunkL not reported, TailL 305.5, 303–308 mm, ForeL 49.1, 48.2–50.0 mm, ForefL 15.7, 15.7–15.7 mm, HindL 79, 78–81 mm, HindfL not reported, HeadW 12.2, 12.1–12.4 mm, HeadH 10.8, 10.4–11.2 mm, OrbD not reported, TympD not reported, BSC.dors not reported, BSC.vntl not reported, NucCrsH not reported, TrunkL/SVL not reported, TailL/SVL 366, 355–376%, ForeL/SVL 59, 59–59%, HindL/SVL 95, 91–99%, HeadL/SVL 25, 24–27%, HeadW/HeadL 58, 56–59%, HeadH/HeadL 52, 49–56%, ForefL/ForeL not reported, HindfL/HindL 37, 37–38%, ForeL/HindL not reported, 4FingL/4ToeL not reported, TympD/OrbD 48, 46–49%, DorsS/VntlS not reported, NucCrsL/OrbD not reported. For scalation, values are: Suplab 10, 10–11, Inflab 9, 8–10, Loreal 6, 5–7, Postm 3, 3–3, Throat not reported, 4ForefLm 32, 31–33, 4HindfLm 33 (holotype), Midbody 71 (holotype), Dorsal1 6, 5–8, Dorsal2 not reported, NucCrsS 11, 9–13; nuchal spines or scales are lanceolate; middorsal trunk scales are large, keeled but not elevated into a crest.

Discussion

The molecular (ND2) results support our Tanintharyi specimens as a discrete clade, 5–6.6% different from the closest species *Bronchocela rayaensis* available. Our samples were placed sister to *B. rayaensis*, a species known from Pulau Langkawi (island) off the west coast of Peninsular Malaysia and Phuket Island, Thailand (Fig. 2). Our phylogeny recovered the Phuket Island *B. rayaensis* sister to the Pulau Langkawi samples with 94%

bootstrap support. Grismer et al. (2016) recovered it nested among the Pulau Langkwei samples, possibly because of an inaccurate alignment of the tRNA and OL region. Grismer et al. (2016) re-identified previously published *B. cristatella* samples (KR053113–KR053114; KU326254 – KU326255, RMB8882 – RMB8883 of Grismer et al. 2015) from Polillo Island, Philippines as *B. marmorata*, but did not update the taxonomy of those sequences in GenBank. Here, we included all samples from the Philippines from Grismer et al. (2015), the two from Polillo Island and two from Luzon Island, Philippines [KR053111–KR053112, CDS2105 (KU305472) and RMB9878 (KU315805), respectively]. These samples form a well-supported clade and are 94.4–100% identical to one another for the ND2 sequences. Because there may be more than one lineage of *Bronchocela* in the Philippines (R. Brown, pers. com.), we refer to these specimens as *B. cf. marmorata* and they are sister to a clade containing *B. shenlong* from Peninsular Malaysia and *B. cristatella* from Malaysia, Borneo, and Indonesia (Pulau Natuna Besar). Though the sister relationship between *B. shenlong* and *B. cristatella* received 100% support (Fig. 2), the relationship between them and *B. cf. marmorata* received much less support, both in our study (51%, Fig. 2) and in that of Grismer et al. (2015, 72% ML bootstrap support); though with fewer individuals analyzed, this relationship received 100% support (Grismer et al. 2016).

We included all specimen in GenBank from Grismer et al. (2015), including outgroups, the *B. rayaensis* from Phuket Island (Grismer et al. 2016), and an additional *Bronchocela cristatella* sequence in GenBank (AF128495) and *Aphaniotis fusca* (AF128497). These specimens were reported as being switched (Zug et al. 2006), which our study confirms. The sequence AF128495 is 99% identical to *B. cristatella* from Selangor, Malaysia (KR053100–KR053102) and was placed in the *B. cristatella* clade sister to these specimens from the same state (Selangor) as TNHC57943, the voucher of AF128495 (Fig. 2). The identifications of these sequences were recently corrected in GenBank. The Fig. 5 of Macey et al. (2000b) appears to display the correct tRNA^{CYS} for these genera; however, our ARWEN model depicts the lower three base-pairs non-binding, extending the d-arm replacement loop to “AAAGTG.”

The tRNAs of Grismer et al. (2015) appear to be improperly annotated because virtually all tRNAs are annotated as transcribing in the light strand direction, whereas tRNA^{ALA}, tRNA^{ASN}, tRNA^{CYS}, and tRNA^{TYR} transcribe on the heavy strand in all other agamids examined (e.g. Macey et al. 1997), their sequences have “tRNA^{ASX}” (the transcribed product) in place of tRNA^{ASN}, and none of the origin of light strand (OL) sequences are identified. Secondary structure inspection of one of their specimens (KR053067) in ARWEN reveals the tRNA^{ASN} does exist, it is transcribed in the complimentary direction, and their tRNA^{ALA} as annotated does not form a tRNA, but the bases between tRNA^{TRP} and tRNA^{ASN} do form tRNA^{ALA}. Agamids, in particular, show interesting variation in the relationship between the condition of the OL and the neighboring tRNA’s d-arm replacement loop, which can be useful for the study of evolution of mtDNA replication (Seligmann and Labra 2014) and can be useful as phylogenetic information content (Macey et al. 2000b) if properly annotated.

The morphology of *Bronchocela burmana* and *B. rayaensis* are similar; however, the differences indicate adaptive divergence between the southern Burmese and Langkawi Malaysian populations. The Burmese lizards show sexual dimorphism with males larger than females with only a slight overlap. The Langkawi population seems to reverse this dimorphism with females larger than males (Table 2) although that sample includes only one adult of each sex.

The newly discovered Phuket *Bronchocela rayaensis* is a gravid female yet smaller than the paratype adult female (Table 2). As would be expected, its absolute dimensions are smaller than the typical female and surprisingly also smaller than the typical male. Also several of its body proportions (HeadH/HeadL, TympD/OrbD, ForeLL/SVL, HindLL/SVL) are smaller than those of the types. Because the Phuket sample is a single individual, we note these differences, but also note that in *B. burmana* adult size remains larger, midbody and nuchal crest scales remain fewer, thereby supporting the Phuket individual as *B. rayaensis*.

Bronchocela rayaensis possess more nuchal crest scales than *B. burmana*—with a slight overlap 6–9 vs. 9–13. Third and fourth fingers are nearly equal in *B. rayaensis*, third finger is about half a claw length longer than fourth finger, and midbody scales 71 in holotype of *B. rayaensis* vs. 59 (55–67) scales around midbody in our Tanintharyi sample

At this time, we cannot define the distribution of *B. burmana* in Thailand. The distribution map in Chan-ard et al.'s field guide (2015: p. 94) shows a Thai occurrence of *B. cristatella* in the Tenasserim range from Khanchanburi Province southward into southern Peninsular Thailand to the Malaysia border. Their morphological description appears to mix characteristics of *B. burmana* and *B. cristatella*. We assume that this characterization demonstrates that the distribution of their Thai *Bronchocela* specimens was a mix of *B. burmana* and *B. cristatella*. The Phuket and Khura Bun specimens (Grismer et al. 2016) confirm the presence of *B. rayaensis* in Thailand, at least south of the Isthmus of Kra. In an earlier review of Thai lizards, Taylor (1963) considered *cristatella* as a *Calotes*. His description was based on a specimen from Yala Province and his illustration on an individual from Sangkhla. Both of these specimens are clearly *B. cristatella*, demonstrating that *B. cristatella* occurs in southern-most peninsular Thailand. Chan-ard et al.'s (1999) photographic atlas depicts two *Bronchocela*; the one from Malaysia (left photograph, p. 92) is an adult female *B. cristatella*, the one from Khao Soi Dao, Chanthiburi is a *B. smaragdina*, thus, of no assistance in defining the distribution of *B. burmana* in Thailand or confirming its presence there. For the present, we suggest that the northern half of the distribution of *B. cristatella* as shown in the Chan-ard et al. map (2015) is *B. burmana* from the Isthmus of Kra northward, *B. rayaensis* south of the isthmus, perhaps restricted to the western side of the Thai–Malay Peninsula, and *B. cristatella* occurs southward in the eastern lowlands into Malaysia.

Bronchocela rayaensis has been recently characterized by Grismer et al. 2015 and 2016 and is not repeated here. The following is a redescription of *B. burmana* based exclusively on our recently vouchered specimens from southern Tanintharyi.

***Bronchocela burmana* Blanford, 1878**

Burmese Green Crested Lizard

Figure 3

Bronchocela burmana Blanford, 1878, Proceedings of the Asiatic Society of Bengal 1878(6): 141.

Holotype. Adult ZSI 5337 collected from ‘...near Tavoy’. We have not examined this specimen as it was not available to us.

Definition. A *Bronchocela* lizard with a short nuchal crest of six to nine erect triangular crest scales; no middorsal crest of raised scales on trunk. Snout-vent length of adults range from 80 to 94 mm with tail length 240 to 360% of snout-vent length; limbs slender, forelimbs 42–52% of SVL, hindlimbs 86–97% of SVL; digits long and slender with third finger slightly longer than fourth finger, fourth toe distinctly longer than third toe; head medium sized (25–27% of SVL); head with distinct canthal ridge, narrow triangular shaped from dorsal view, length > width \approx height and approximately 26 % of SVL; moderately large eye (OrbD/HeadL \sim 26–28%) and about twice diameter of tympanum (continuous with temporal surface).

General description. Detailed metric and scalation features are presented above in the Results section, also Table 2. *Bronchocela burmana* is a slender green lizard with long tail, usually 2.5–3.5X snout-vent length. In spite of its 80 to 94 mm body length, its slenderness and thin legs give it a delicate appearance and make it immediately recognizable among the other lizards of southern Tanintharyi.

In life, *Bronchocela burmana* appears uniformly green (Fig. 2). Preservation changed the overall coloration to light olive but highlights a light rufous vertical bar in the temporal area.

Distribution. *Bronchocela burmana* is presently confirmed for only southern Tanintharyi Division, Myanmar (Fig. 1). The type-locality is given as Tavoy, now Dawei; however, two years of surveys in both the dry and wet seasons at the Tanintharyi Nature Reserve, just north of Dawei, did not confirm the presence of this species in the former Tavoy area (approximately 350 km N of Lenya). The morphological description of *B. cristatella* in Chan-ard et al.’s field guide (2015: p. 94) appears to be a composite of *B. burmana* and *B. cristatella*. As noted in the preceding section, we interpret their distribution map also to be a composite of the two species’ occurrence in western Thailand. We suggest that the two species are allopatric with *B. burmana* in the north, likely north of the Isthmus of Kra and *B. cristatella* in the south. Taylor’s description and photograph of *B. cristatella* (1963) are definitely of that species and are plotted (Fig. 1) to confirm its occurrence in southern-most Thailand.

Etymology. Blanford did not explain his choice of *burmana*. His selection seems obvious as it was the first *Bronchocela* from British Burma and distinctly different from *B. cristatella* and the other species recognized at that time.



Figure 3. *Bronchocela burmana* Blanford, 1878 from the Lenya area (circa 11.68N 99.42E). A dorsolateral view of a living Burmese Crested Lizard, USNM 587483. Photo by DGM.

Acknowledgements

All or portions of the laboratory and/or computer work were conducted in and with the support of the Laboratories of Analytical Biology facilities of the National Museum of Natural History (NMNH) or its partner labs and lab work was funded by the Global Genome Initiative (NMNH) and lab work was assisted by L. Dickens Jr., A. Ibarra, and B. Cruz of the Youth Engagement thru Science Global Genome (YES!-GG) Program. E. Buring (NMNH HS-Intern) assisted with the tRNA secondary structure analyses.

Specimens of *Bronchocela* from Tanintharyi were collected by two survey teams. The first rediscovery of *B. burmana* were made in Kawthoung District in 2010 by a Myanmar Herpetology Survey team of rangers from the Myanmar Nature and Wildlife Conservation Division under the supervision of J. V. Vindum. The survey program was country-wide and its main support derived from the Biodiversity program of the National Sciences Foundation (DEB-9971861, DEB-0451832). The second vouchersing occurred in 2015 and 2016 as part of Fauna & Flora International's (FFI-Myanmar Program) biotic assessment of the southern Tanintharyi evergreen forest. Additional funding for the Smithsonian field team derived from the Smithsonian Myanmar Biodiversity Initiative and its support by The Leona M. and Harry B. Helmsley Charitable Trust (<http://www.helmsleytrust.org>) awarded to Melissa Songer (Smithsonian Conservation Biology Institute). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank Fauna & Flora International, Yangon office, specifically M. Grindley, F. Momberg, Nay Myo Shwe, Saw Soe Aung and their field crew (particularly Myint Kaw Thura and Thaw Zin) for in-country logistics and assistance with field surveys. The base map image of Tanintharyi and adjacent areas is the base map from <https://commons.wikimedia.org/w/index.php?curid=166887>; we appreciate and thank the publisher for the use of the image to depict the known distribution of *Bronchocela* in that area. We also wish to thank S. Gotte, K. Tighe, and A. Wynn of the USNM collections management team for their always-ready assistance. We thank L. Grismer and J. Moravec for reviewing our manuscript and L. Grismer for quickly providing us with access and consent to publish the sequence of ZMKUR0017, and J. Schulte for following up on correcting AF128495 and AF128497 sequence identifications in GenBank.

References

- Blanford WT (1878) Notes on some Reptilia from the Himalayas and Burma. *Journal of the Asiatic Society of Bengal* 3: 125–131.
- Boulenger GA (1885) *Catalogue of the Lizards in the British Museum (Natural History)*. Volume I. Geckonidae, Eublepharidae, Uroplatidae, Pygopodidae, Agamidae, 2nd edition. Trustees of the British Museum of Natural History, London, 497 pp.
- Chan-ard T, Parr JWK, Nabhitabhata J (2015) *A Field Guide to the Reptiles of Thailand*. Oxford University Press, New York, 314 pp.
- Diong CH, Lim SSL (1998) Taxonomic review and morphological description of *Bronchocela cristatella* (Kuhl, 1820) (Squamata: Agamidae) with notes on other species in the genus. *Raffles Bulletin of Zoology* 46: 345–359.
- Grismer LL (2011) *Lizards of Peninsular Malaysia, Singapore, and their Adjacent Archipelagos*. Their description, distribution, and natural history. Edition Chimaira, Frankfurt am Main, 728 pp.
- Grismer LL, Wood PL, Lee CH, Quah EVSH, Anuar S, Noadi E, Sites JW (2015) An integrative taxonomic review of the agamid genus *Bronchocela* (Kuhl, 1820) from Peninsular

- Malaysia with descriptions of new montane and insular endemics. *Zootaxa* 3948(1): 1–23. <https://doi.org/10.11646/zootaxa.3948.1.1>
- Grismer, LL, Wood PL, Aowphol A, Cota M, Murdoch ML, Aguilar C, Grismer MS (2016) Taxonomy, phylogeny, and distribution of *Bronchocela rayaensis* (Squamata: Agamidae) on the Thai–Malay Peninsula. *Zootaxa* 4092(3): 414–420. <https://doi.org/10.11646/zootaxa.4092.3.6>
- Hallermann J (2004) A new species of the genus *Bronchocela* from the tropical rain forest of southern Vietnam. *Russian Journal of Herpetology* 11(1): 30–34.
- Hallermann J (2005) A taxonomic review of the genus *Bronchocela* (Squamata: Agamidae) with description of a new species from Vietnam. *Russian Journal of Herpetology* 12(3): 167–182.
- Hallermann J (2009) A new species of *Bronchocela* (Squamata: Agamidae) from Nicobar Island. *Bonner zoologische Beiträge* 56: 279–284.
- Laslett D, Canback B (2008) ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics* 24(2): 172–175. <https://doi.org/10.1093/bioinformatics/btm573>
- Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ (1997) Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* 14(1): 91–104. <https://doi.org/10.1093/oxfordjournals.molbev.a025706>
- Macey JR, Schulte II JA, Larson A, Ananjeva NB, Wang Y, Pethiyagoda R, Rastegar-Pouyani N, Papenfuss TJ (2000a) Evaluating Trans-Tethys Migration: an example using acrodont lizard phylogenetics. *Systematic Biology* 49(2): 233–256. <https://doi.org/10.1093/sysbio/49.2.233>
- Macey JR, Schulte II JA, Larson A (2000b) Evolution and phylogenetic information content of mitochondrial genomic structural features illustrated with acrodont lizards. *Systematic Biology* 49(2): 257–277. <https://doi.org/10.1093/sysbio/49.2.257>
- Malkmus R (1992) Herpetologische Beobachtungen am Mount Kinabalu, Nord-Borneo. III. Mitteilungen aus dem Zoologischen Museum in Berlin 68(1): 101–138. <https://doi.org/10.1002/mmzn.19920680107>
- Taylor EH (1963) The lizards of Thailand. *The University of Kansas Science Bulletin* 44(14): 687–1077.
- Zug GR, Brown HHK, Schulte II JA, Vindum JV (2006) Systematics of the garden lizards, *Calotes versicolor* group (Reptilia, Squamata, Agamidae), in Myanmar: central dry zone populations. *Proceedings of the California Academy of Sciences* 57(2): 35–68.

Specimens examined

Bronchocela burmana.

Myanmar; Tanintharyi Division: CAS247755–CAS247757, CAS24786, USNM587775 – USNM587776, USNM587483 – USNM587484, USNM587793.

Bronhocela cristatella.

Malaysia; Johore Province: USNM29576, USNM29579; Selangor Province
USNM129482, USNM141704 – USNM141706.

Thailand; Songkhla Province: EHT349; Yala Province EHT134; data from Taylor
1963.

Bronhocela rayaensis.

Malaysia; Kedah Province: data from Grismer et al. 2015 and 2016.