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Notes on two closely related spider species of the *Pholcus phungiformes* species group (Araneae, Pholcidae) from Beijing, China

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Abstract

The *Pholcus phungiformes* species group is highly diverse and currently contains 53 species. In this study, *Pholcus tongyaoi* Wang & Yao, **sp. nov.** (male, female) from Huairou District, Beijing, China is described while similar congener *Pholcus lexuancanhi* Yao, Pham & Li, 2012 from neighboring Haidian District (type locality) is redescribed; the female of *P. lexuancanhi* is described for the first time. In addition, the DNA barcode COI for the two species was obtained to estimate p-distance.

Keywords

daddy-long-leg spider, DNA barcode, morphology, Pholcinae, taxonomy

Introduction

The spider family Pholcidae C.L. Koch, 1850 contains 94 genera and 1768 species (World Spider Catalog 2020). It is among the most species-rich families and has a worldwide distribution (World Spider Catalog 2020). It is composed of five subfami-

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lies: Ninetinae Simon, 1890, Arteminae Simon, 1893, Modisiminae Simon, 1893, Smeringopinae Simon, 1893, and Pholcinae C.L. Koch, 1850 based on recent morphological and molecular phylogenetic analyses (Huber 2011a; Dimitrov et al. 2013; Eberle et al. 2018). Pholcid spiders occupy a wide range of habitats in a variety of ecosystems, e.g., in buildings, under rocks, in crevices, in caves, in leaf litter, and in webs between trunks and twigs of trees (Huber 2005; Yao and Li 2012). *Pholcus* Walckenaer, 1805 is the most diverse genus in Pholcinae and Pholcidae, with 338 described species mainly distributed in the Palaearctic, Indo-Malayan, Afrotropical, and Australasian Region (Huber 2011b; Yao and Li 2012; World Spider Catalog 2020). These species belong to 21 species groups, of which the *Pholcus phungiformes* species group is highly diverse, including 53 known species definitively assigned to this species group (Huber 2011b; Peng and Zhang 2013; Kim and Ye 2015; Zhang et al. 2016; Huber et al. 2018; Zhu et al. 2018). The *P. phungiformes* species group is largely restricted to northeastern China and the Korean Peninsula; only *P. phungiformes* Oliger, 1983 occurs in Maritime Territory, Sakhalin Island, and Kurile Islands, Russia, probably as a result of human transport (Huber 2011b; World Spider Catalog 2020). This species group can often be found in caves, at cave entrances or on rock walls (Figs 1, 2), and diagnosed by the following characters: eight eyes, carapace with radiating marks, cylindrical opisthosoma, male chelicerae usually with frontal apophyses, male pedipalpal tibia with prolatero-ventral projection, procursus usually with dorsal spines, appendix

Figure 1. Distribution records of two *Pholcus* species **1** *P. tongyaoi* sp. nov. **2** *P. lexuancanhi* Yao, Pham & Li, 2012.

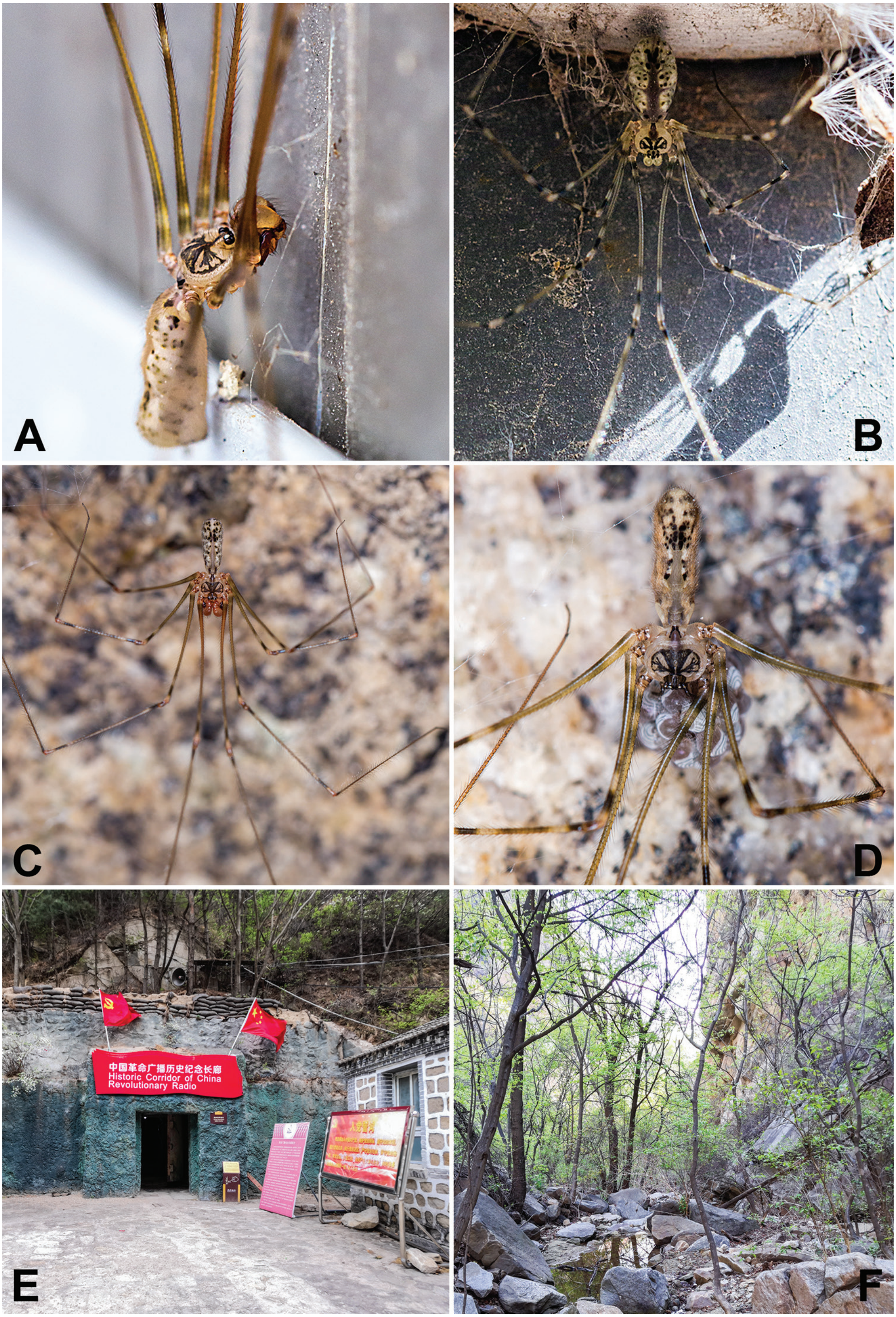

Figure 2. *Pholcus tongyaoi* sp. nov., live specimens and habitat **A, B** adult and juvenile males in old house **C, D** adult male and female with egg-sac on rock walls **E, F** habitat. Photographs by T Jiang (IZCAS).

absent, sometimes with 'pseudo-appendix', external female genitalia sclerotized, with knob (Huber 2011b; Zhu et al. 2018).

In this study, we describe one new species based on males and females from Huairou District, Beijing, China assigned to the *P. phungiformes* species group and redescribe its similar species *Pholcus lexuancanhi* Yao, Pham & Li, 2012 from a neighboring locality. The female of *P. lexuancanhi* is reported for the first time and the DNA barcode COI for the two species was obtained to estimate p-distance.

Materials and methods

Specimens were examined and measured with a Leica M205 C stereomicroscope. Left male pedipalps were illustrated. External female genitalia were photographed before dissection. Vulvae were previously treated in a 10% warm solution of potassium hydroxide (KOH) to dissolve soft tissues before illustration. Images were captured with a Canon EOS 750D wide zoom digital camera (24.2 megapixels) mounted on the stereomicroscope mentioned above, and assembled using Helicon Focus 3.10.3 image stacking software (Khmelik et al. 2005). All measurements are given in millimeters (mm). Leg measurements are shown as: total length (femur + patella + tibia + metatarsus + tarsus), missing data were coded as '–'. Leg podomeres were measured on their dorsal side. The distribution map was generated with Google Earth Pro 7.3.2 (Google Limited Liability Company). The specimens studied are preserved in 75% ethanol and deposited in the College of Life Science, Shenyang Normal University (**SYNU**) in Liaoning, China and the Institute of Zoology, Chinese Academy of Sciences (**IZCAS**) in Beijing, China.

Terminology and taxonomic descriptions follow Huber (2011b) and Yao et al. (2015). The following abbreviations are used in the descriptions:

ALE	anterior lateral eye,
AME	anterior median eye,
PME	posterior median eye,
L/d	length/diameter.

DNA barcode was obtained for estimation of p-distance between *P. tongyaoi* sp. nov. and *P. lexuancanhi*. A partial fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified and sequenced, using the following primers: forward: LCO1490-oono (5'-CWACAAAYCATARRGATATTGG-3') and reverse: C1-N-2776 (5'-GGATAATCAGAATANCGNCGAGG-3'). DNA sample is preserved in TE buffer and stored at -20 °C. The sequences are deposited in GenBank. COI p-distance is computed with MEGA 5 (Tamura et al. 2011). For additional information on extraction, amplification, and sequencing procedures, see Yao et al. (2016).

Taxonomic accounts

Family Pholcidae C.L. Koch, 1850
Subfamily Pholcinae C.L. Koch, 1850

Genus *Pholcus* Walckenaer, 1805

Type species. *Aranea phalangioides* Fuesslin, 1775.

Pholcus phungiformes species group

Diagnosis and description. See Huber (2011b).

Remarks. The 'appendix' in the original figures of four species apparently arises from the uncus: *P. papilionis* Peng & Zhang, 2011, *P. chiakensis* Seo, 2014, *P. gajiensis* Seo, 2014, and *P. palgongensis* Seo, 2014. We consider this a divided 'pseudo-appendix' and assign them to the *P. phungiformes* species group. Moreover, although the species *P. xianrendong* Liu & Tong, 2015 does not possess a prolatero-ventral projection on the male pedipalpal tibia, the bulb without appendix, the locality of this species is within the range of the *P. phungiformes* species group. Therefore, we tentatively assigned *P. xianrendong* to this species group. In total, this species group now contains 59 species. Of these, one species is newly described below.

Pholcus tongyaoi Wang & Yao, sp. nov.

http://zoobank.org/F7249E28-D367-4CA4-9D17-32977C5E5345
Figs 3, 4

Type material. ***Holotype*:** male (SYNU-Ar00016), Pool and Valley Natural Park (40°32.600'N, 116°40.687'E, elevation 574 m), Huairou District, **Beijing**, **China**, 26 April 2019, T Jiang leg. ***Paratypes*:** 2 males (SYNU-Ar00017, Ar00018, GenBank number in SYNU-Ar00017: MT843113), same data as holotype; 2 females (SYNU-Ar00019, Ar00020), same data as holotype but 23 April 2019.

Etymology. The specific name is a patronym in honor of the collector Tongyao Jiang (IZCAS) and is a noun (name) in genitive case.

Diagnosis. The species resembles *P. lexuancanhi* Yao, Pham & Li, 2012 (Figs 5, 6; Yao et al. 2012: 313, figs 1–15) with similar bulbal apophyses (Fig. 4C) and external female genitalia (Fig. 4A), but can be easily distinguished by procursus with large, semicircular, ventral membranous process (arrowed in Fig. 3A; prolateral membranous lamella in *P. lexuancanhi*, arrowed 1 in Fig. 5C), small, prolateral membranous lamella provided with sawtooth (arrowed 1 in Fig. 3C; large, dorsal membranous lamella in *P. lexuancanhi*, arrowed 2 in Fig. 5C), and small, angular ventral sclerite provided with

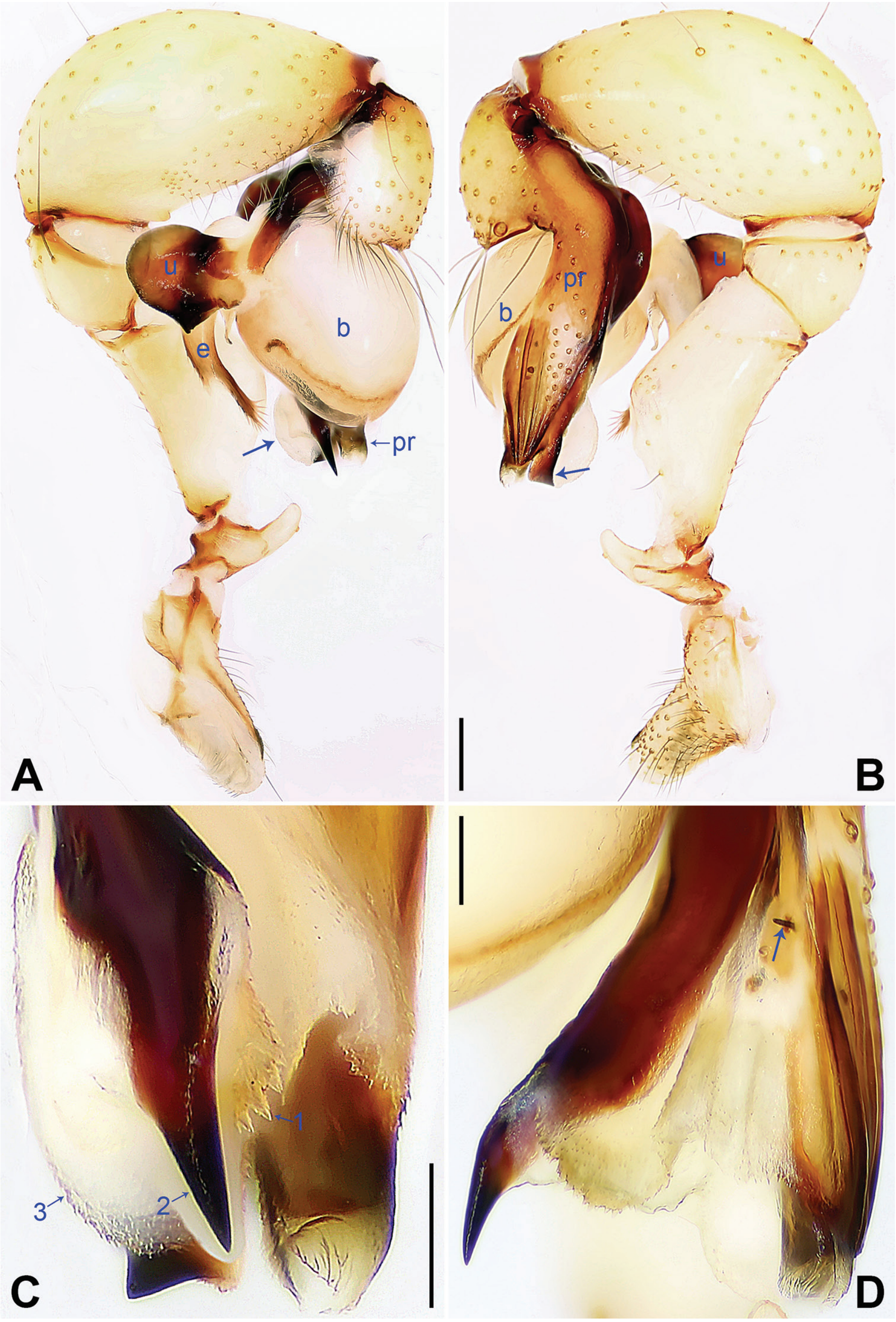

Figure 3. *Pholcus tongyaoi* sp. nov., holotype (**A, B, D**) and paratype (**C**) males **A, B** pedipalp (**A** prolateral view, arrow indicates ventral membranous process **B** retrolateral view, arrow indicates ventral sclerite) **C, D** distal part of procursus (**C** prolateral view, arrow 1 indicates prolateral membranous lamella, arrow 2 indicates spine-shaped prolateral apophysis, arrow 3 indicates ventral membranous process **D** dorsal view, arrow indicates dorsal spine). Abbreviations: b = bulb, e = embolus, pr = procursus, u = uncus. Scale bars: 0.20 (**A, B**), 0.10 (**C, D**).

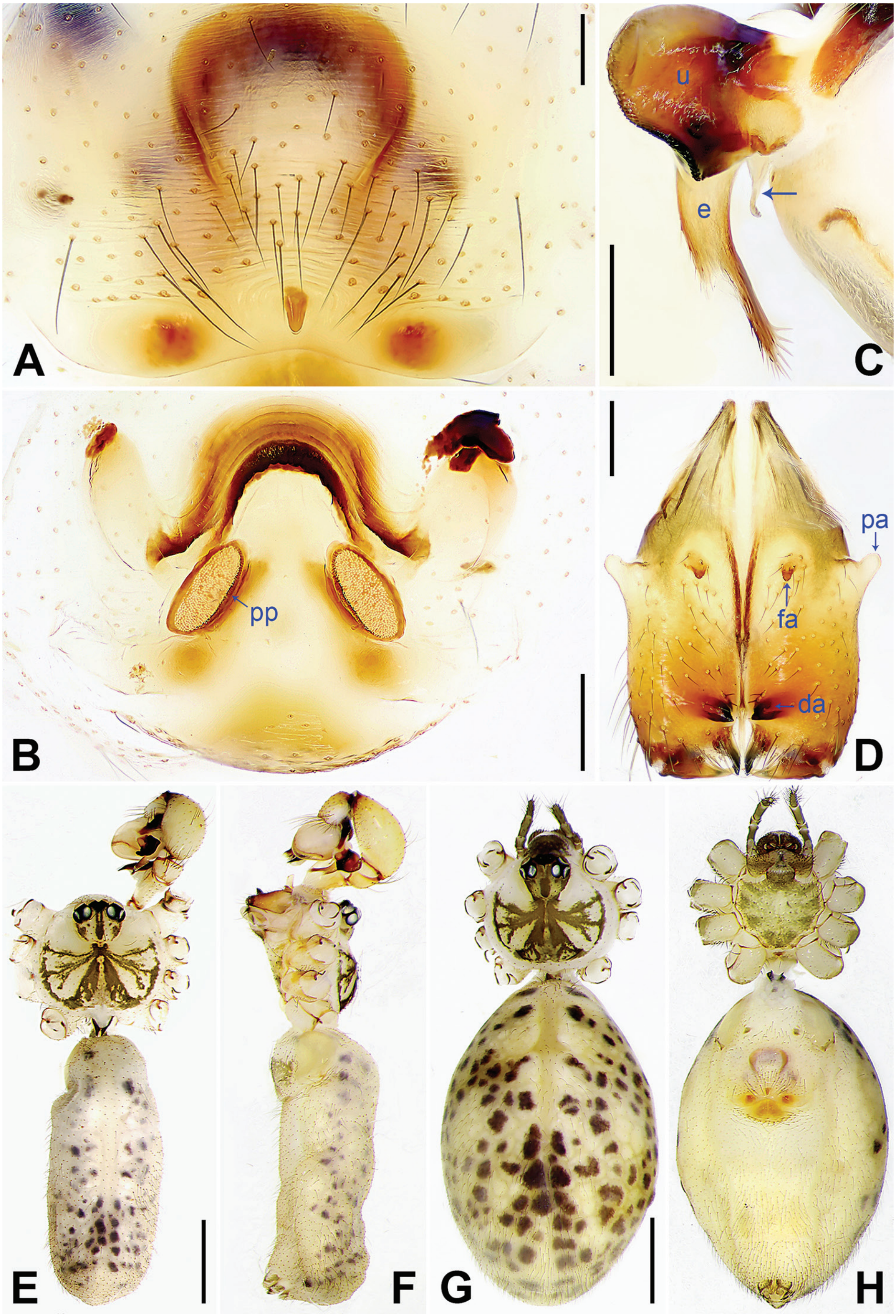

Figure 4. *Pholcus tongyaoi* sp. nov., holotype male (**C–F**) and paratype female (**A, B, G, H**) **A** external female genitalia, ventral view **B** vulva, dorsal view **C** bulbal apophyses, prolateral view, arrow indicates 'pseudo-appendix' **D** chelicerae, frontal view **E–H** habitus (**E, G** dorsal view **F** lateral view **H** ventral view). Abbreviations: da = distal apophysis, e = embolus, fa = frontal apophysis, pa = proximo-lateral apophysis, pp = pore plate, u = uncus. Scale bars: 0.20 (**A–D**), 1.00 (**E–H**).

curved tip (arrowed in Fig. 3B; large ventral sclerite and its tip not curved in *P. lexuancanhi*, arrowed in Fig. 5B), by male chelicerae with pair of frontal apophyses (arrowed fa in Fig. 4D; absent in *P. lexuancanhi*, Fig. 6D), and by vulva with n-shaped anterior arch without median sclerite (Fig. 4B; slightly curved anterior arch with median sclerite in *P. lexuancanhi*, arrowed in Fig. 6B) and elliptic pore plates (Fig. 4B; oval in *P. lexuancanhi*, Fig. 6B). This species can also be distinguished from *P. lexuancanhi* by COI p-distance 0.106 between them.

Description. Male (**holotype**, SYNU-Ar00016): Total length 4.75 (4.93 with clypeus), carapace 1.56 long, 1.75 wide, opisthosoma 3.19 long, 1.34 wide. Leg I: – (11.62 + 0.75 + – + – + –), leg II: 30.89 (8.50 + 0.55 + 7.84 + 12.75 + 1.25), leg III: 20.62 (6.40 + 0.60 + 5.12 + 7.60 + 0.90), leg IV: 28.58 (8.40 + 0.62 + 7.12 + 11.12 + 1.32). Distance PME-PME 0.20, diameter PME 0.12, distance PME-ALE 0.05, distance AME-AME 0.02, diameter AME 0.08. Sternum wider than long (1.04/0.96). Habitus as in Fig. 4E, F. Carapace yellowish, with brown radiating marks and marginal brown bands; ocular area yellowish, with median and lateral brown bands; clypeus yellowish; sternum yellowish, with marginal brown marks. Legs yellowish, but dark brown on patellae and whitish on distal parts of femora and tibiae, with darker rings on subdistal parts of femora and proximal and subdistal parts of tibiae. Opisthosoma yellowish, with dorsal and lateral spots. Ocular area elevated, without eye stalks. Thoracic furrow absent. Chelicerae (Fig. 4D) with pair of proximo-lateral apophyses, pair of distal apophyses provided with two teeth each, and pair of frontal apophyses. Pedipalps as in Fig. 3A, B; trochanter with long, retrolaterally strongly bulged ventral apophysis; femur with indistinct ventral protuberance; tibia with prolatero-ventral projection; procursus simple proximally but complex distally, with large, semicircular, ventral membranous process (arrowed in Fig. 3A), small, prolateral membranous lamella with sawtooth (arrowed 1 in Fig. 3C), small, angular ventral sclerite with curved tip (arrowed in Fig. 3B), spine-shaped prolateral apophysis (arrowed 2 in Fig. 3C), and dorsal spine (arrowed in Fig. 3D); bulb with short curved ‘pseudo-appendix’ (arrowed in Fig. 4C); uncus with scaly edge (Fig. 4C); embolus weakly sclerotized, with some transparent distal projections (Fig. 4C). Legs with short vertical setae on tibiae, metatarsi, and tarsi, without spines or curved setae.

Female (**paratype**, SYNU-Ar00019): Similar to male, habitus as in Fig. 4G, H. Total length 5.31 (5.56 with clypeus), carapace 1.43 long, 1.14 wide, opisthosoma 3.88 long, 2.43 wide; tibia I: 5.90; tibia I L/d: 54. Distance PME-PME 0.18, diameter PME 0.12, distance PME-ALE 0.04, distance AME-AME 0.03, diameter AME 0.08. Sternum wider than long (1.07/0.83). Clypeus brown. External female genitalia (Fig. 4A) curved posteriorly, with short knob. Vulva (Fig. 4B) with sclerotized, n-shaped anterior arch and pair of elliptic pore plates. Retrolateral trichobothrium of tibia I at 4% proximally; tarsus I with 22 distinct pseudosegments.

Variations. Ventral membranous process on procursus nearly crescent-shaped (arrowed 3 in Fig. 3C) in one paratype male (SYNU-Ar00017). Leg I missing in two paratype males (SYNU-Ar00017, Ar00018), total length 5.36 (5.52 with clypeus) in SYNU-Ar00017, total length 4.50 (4.60 with clypeus) in SYNU-Ar00018. Tibia I in another paratype female (SYNU-Ar00020): 5.75.

Distribution. China (Beijing, type locality; Fig. 1).

Natural history. The species was found in an old house and on rock walls.

Pholcus lexuancanhi Yao, Pham & Li, 2012

Figs 5, 6

Pholcus lexuancanhi Yao et al. 2012: 313, figs 1–15. Yao et al. 2015: 15.

Material examined. 2 males (IZCAS-Ar40901, Ar40902, GenBank number in IZCAS-Ar40901: MT843112) and 2 females (IZCAS-Ar40903, Ar40904), Beijing Botanical Garden (40°00'N, 116°12'E; type locality), Haidian District, **Beijing**, **China**, 30 July 2017, Z Yao leg.

Diagnosis. See diagnosis for *P. tongyaoi* sp. nov.

Redescription. Male (IZCAS-Ar40901): Total length 5.13 (5.38 with clypeus), carapace 1.41 long, 1.75 wide, opisthosoma 3.72 long, 1.44 wide. Leg I: 47.97 (14.47 + 0.80 + 11.41 + 18.53 + 2.76), leg II: 30.06 (8.46 + 0.78 + 7.24 + 11.79 + 1.79), leg III: 21.37 (6.35 + 0.59 + 5.13 + 8.08 + 1.22), leg IV: 28.03 (8.33 + 0.78 + 7.05 + 10.51 + 1.36); tibia I L/d: 76. Distance PME-PME 0.25, diameter PME 0.10, distance PME-ALE 0.05, distance AME-AME 0.05, diameter AME 0.10. Sternum wider than long (1.05/0.88). Habitus as in Fig. 6E, F. Carapace yellowish, with brown radiating marks and marginal brown bands; ocular area yellowish, with median and lateral brown bands; clypeus yellowish; sternum brown. Legs yellowish, but dark brown on patellae and whitish on distal parts of femora and tibiae, with darker rings on subdistal parts of femora and proximal and subdistal parts of tibiae. Opisthosoma yellowish, with dorsal and lateral spots. Ocular area elevated, without eye stalks. Thoracic furrow absent. Chelicerae (Fig. 6D) with pair of proximo-lateral apophyses and pair of distal apophyses provided with two teeth each. Pedipalps as in Fig. 5A, B; trochanter with long, retrolaterally strongly bulged ventral apophysis; femur with indistinct ventral protuberance; tibia with prolatero-ventral projection; procursus simple proximally but complex distally, with large, prolateral membranous lamella (arrowed 1 in Fig. 5C), large, dorsal membranous lamella with sawtooth (arrowed 2 in Fig. 5C), large, angular ventral sclerite (arrowed in Fig. 5B), spine-shaped prolateral apophysis (arrowed 3 in Fig. 5C), and dorsal spine (arrowed in Fig. 5D); bulb with short curved 'pseudo-appendix' (arrowed in Fig. 6C); uncus with scaly edge (Fig. 6C); embolus weakly sclerotized, with some transparent distal projections (Fig. 6C). Retrolateral trichobothrium of tibia I at 5% proximally; legs with short vertical setae on tibiae, metatarsi, and tarsi, without spines or curved setae; tarsus I with 33 distinct pseudosegments.

Female (IZCAS-Ar40903): Similar to male, habitus as in Fig. 6G, H. Total length 5.19 (5.38 with clypeus), carapace 1.36 long, 1.66 wide, opisthosoma 3.83 long, 1.68 wide; tibia I: 8.50; tibia I L/d: 54. Distance PME-PME 0.20, diameter PME 0.10, distance PME-ALE 0.05, distance AME-AME 0.05, diameter AME 0.08. Sternum wider than long (1.08/0.92). Clypeus brown. External female genitalia (Fig. 6A) curved pos-

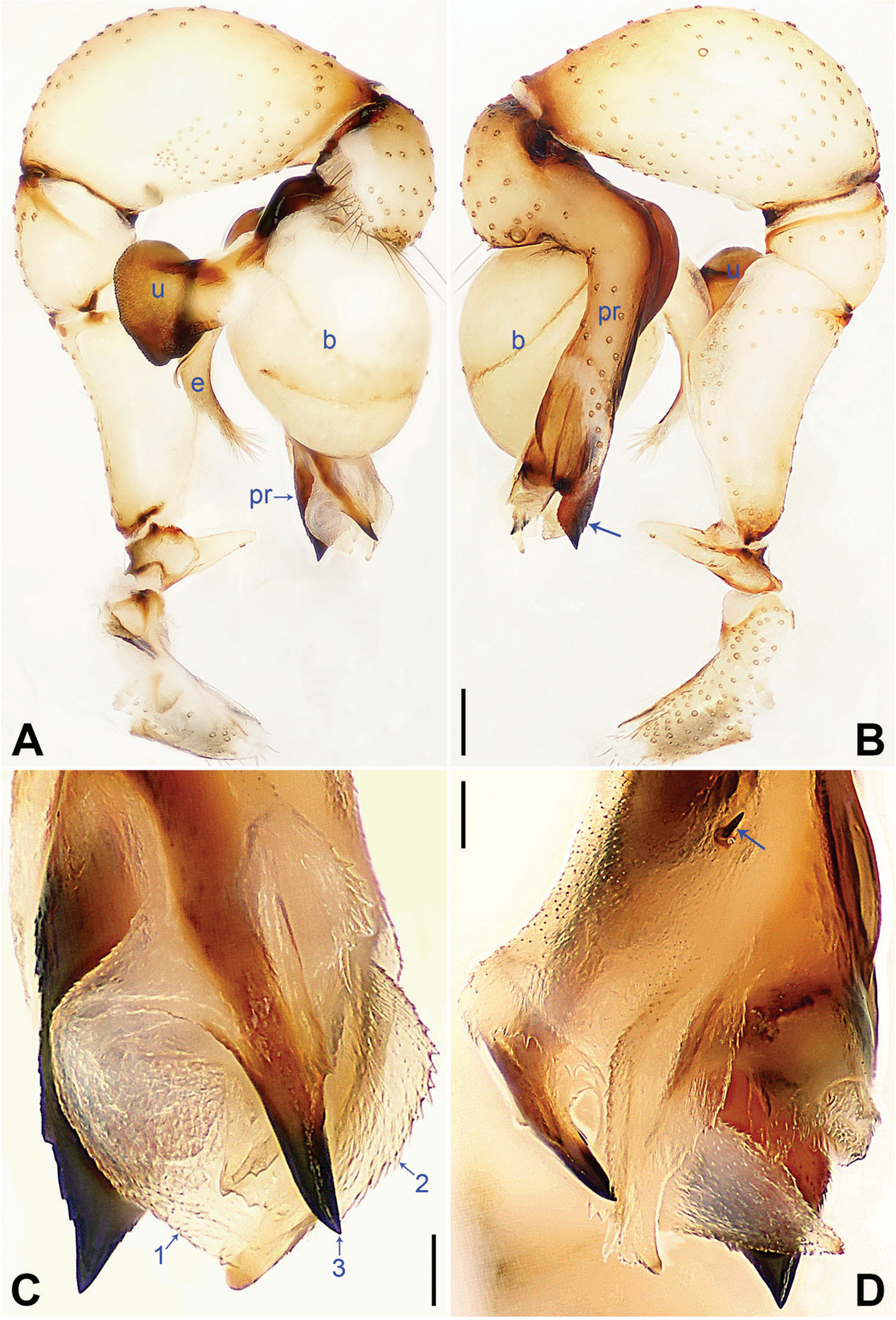

Figure 5. *Pholcus lexuancanhi* Yao, Pham & Li, 2012, male **A, B** pedipalp (**A** prolateral view **B** retrolateral view, arrow indicates ventral sclerite) **C, D** distal part of procursus (**C** prolateral view, arrows 1 and 2 indicate prolateral and dorsal membranous lamella, respectively, arrow 3 indicates spine-shaped prolateral apophysis **D** dorsal view, arrow indicates dorsal spine). Abbreviations: b = bulb, e = embolus, pr = procursus, u = uncus. Scale bars: 0.20 (**A, B**), 0.05 (**C, D**).

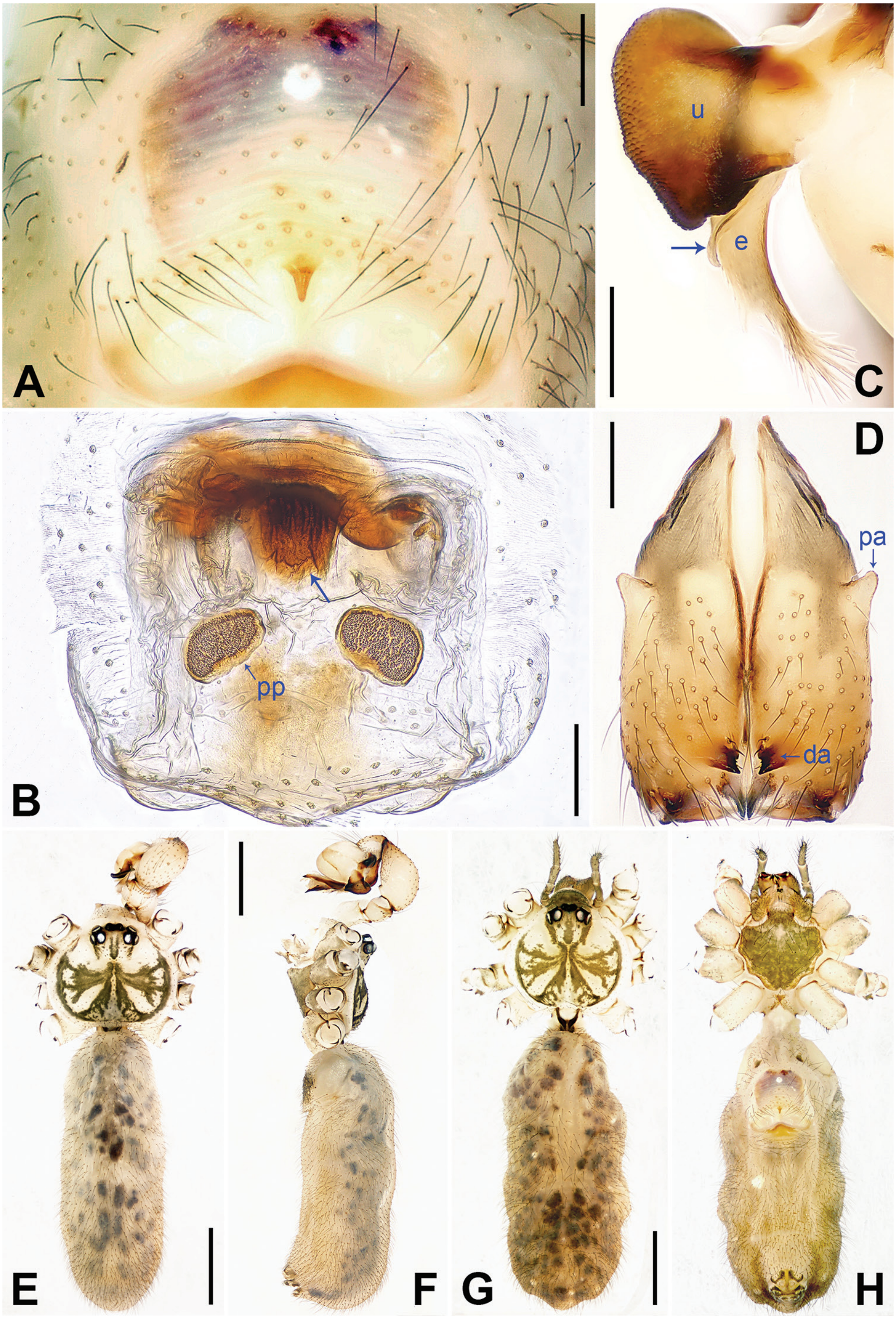

Figure 6. *Pholcus lexuancanhi* Yao, Pham & Li, 2012, male (**C–F**) and female (**A, B, G, H**) **A** external female genitalia, ventral view **B** vulva, dorsal view, arrow indicates median sclerite of anterior arch **C** bulbal apophyses, prolateral view, arrow indicates 'pseudo-appendix' **D** chelicerae, frontal view **E–H** habitus (**E, G** dorsal view **F** lateral view **H** ventral view). Abbreviations: da = distal apophysis, e = embolus, pa = proximo-lateral apophysis, pp = pore plate, u = uncus. Scale bars: 0.20 (**A–D**), 1.00 (**E–H**).

teriorly, with short knob. Vulva (Fig. 6B) with slightly curved, sclerotized anterior arch provided with median sclerite (arrowed in Fig. 6B) and pair of oval pore plates.

Variations. Tibia I in another male (IZCAS-Ar40902): 11.54. Tibia I in another female (IZCAS-Ar40904): 9.12.

Distribution. China (Beijing, type locality; Fig. 1).

Natural history. The species was found on rock walls.

Discussion

The *P. phungiformes* species group is highly diverse and currently contains 59 species including one new species in this study. These species are mainly distributed in three large mountain ranges (see the checklist below): the Mountain Taihang from southern North China (22 spp.), the Mountain Changbai (also called Mountain Paekdu in North Korea) from the border between northeastern China and North Korea (15 spp., of which *P. phungiformes* also occurs further east), and the Mountain Taebaek from Korean Peninsula (22 spp.) (Huber 2011b; Peng and Zhang 2011; Yao and Li 2012; Yao et al. 2012; Peng and Zhang 2013; Seo 2014; Kim and Ye 2015; Liu and Tong 2015; Zhang et al. 2016; Zhu et al. 2018). Nevertheless, the survey of the *P. phungiformes* species group is uneven. The highest diversity (43 spp.) concentrates in the Mountain Taihang and the southern Mountain Taebaek (South Korea). In contrast, only 15 species from the Mountain Changbai and one species from the northern Mountain Taebaek (North Korea) are recorded. Based on the high diversity of this species group from the southern Mountain Taebaek and the Mountain Taihang, as well as the similar landforms and habitats in neighboring northern Mountain Taebaek and Mountain Changbai, we strongly believe that additional species diversity likely remains undiscovered in the neighboring areas (e.g., Li 2020). Further survey in these areas is needed to fully understand the diversity that exists within this group.

A checklist of the *P. phungiformes* species group from three large mountain ranges is provided (for the complete list of references, see World Spider Catalog 2020):

The Mountain Taihang:

1. *Pholcus alloctospilus* Zhu & Gong, 1991
2. *Pholcus auricularis* Zhang, Zhang & Liu, 2016
3. *Pholcus babao* Tong & Li, 2010
4. *Pholcus beijingensis* Zhu & Song, 1999
5. *Pholcus brevis* Yao & Li, 2012
6. *Pholcus chicheng* Tong & Li, 2010
7. *Pholcus clavimaculatus* Zhu & Song, 1999
8. *Pholcus curvus* Zhang, Zhang & Liu, 2016
9. *Pholcus datan* Tong & Li, 2010
10. *Pholcus exilis* Tong & Li, 2010

11. *Pholcus jinniu* Tong & Li, 2010
12. *Pholcus lexuancanhi* Yao, Pham & Li, 2012
13. *Pholcus luya* Peng & Zhang, 2013
14. *Pholcus papilionis* Peng & Zhang, 2011
15. *Pholcus papillatus* Zhang, Zhang & Liu, 2016
16. *Pholcus pennatus* Zhang, Zhu & Song, 2005
17. *Pholcus suizhongicus* Zhu & Song, 1999
18. *Pholcus tongyaoi* sp. nov.
19. *Pholcus triangulatus* Zhang & Zhang, 2000
20. *Pholcus wangxidong* Zhang & Zhu, 2009
21. *Pholcus wuling* Tong & Li, 2010
22. *Pholcus zhuolu* Zhang & Zhu, 2009

The Mountain Changbai:

1. *Pholcus decorus* Yao & Li, 2012
2. *Pholcus fengcheng* Zhang & Zhu, 2009
3. *Pholcus foliaceus* Peng & Zhang, 2013
4. *Pholcus gaoi* Song & Ren, 1994
5. *Pholcus hamatus* Tong & Ji, 2010
6. *Pholcus jiuwei* Tong & Ji, 2010
7. *Pholcus lingulatus* Gao, Gao & Zhu, 2002
8. *Pholcus ningan* Yao & Li, 2018
9. *Pholcus phoenixus* Zhang & Zhu, 2009
10. *Pholcus phungiformes* Oliger, 1983
11. *Pholcus sublingulatus* Zhang & Zhu, 2009
12. *Pholcus tongi* Yao & Li, 2012
13. *Pholcus wangi* Yao & Li, 2012
14. *Pholcus wangtian* Tong & Ji, 2010
15. *Pholcus xianrendong* Liu & Tong, 2015

The Mountain Taebaek:

1. *Pholcus acutulus* Paik, 1978
2. *Pholcus cheongogensis* Kim & Ye, 2015
3. *Pholcus chiakensis* Seo, 2014
4. *Pholcus crassus* Paik, 1978
5. *Pholcus extumidus* Paik, 1978
6. *Pholcus gajiensis* Seo, 2014
7. *Pholcus gosuensis* Kim & Lee, 2004
8. *Pholcus joreongensis* Seo, 2004
9. *Pholcus juwangensis* Seo, 2014
10. *Pholcus kwanaksanensis* Namkung & Kim, 1990

11. *Pholcus kwangkyosanensis* Kim & Park, 2009
12. *Pholcus montanus* Paik, 1978
13. *Pholcus nodong* Huber, 2011
14. *Pholcus okgye* Huber, 2011
15. *Pholcus palgongensis* Seo, 2014
16. *Pholcus parkyeonensis* Kim & Yoo, 2009
17. *Pholcus pojeonensis* Kim & Yoo, 2008
18. *Pholcus simbok* Huber, 2011
19. *Pholcus socheunensis* Paik, 1978
20. *Pholcus sokkrisanensis* Paik, 1978
21. *Pholcus woongil* Huber, 2011
22. *Pholcus yeongwol* Huber, 2011

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On some squat lobsters from India (Decapoda, Anomura, Munididae), with description of a new species of *Paramunida* Baba, 1988

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Abstract

Squat lobster specimens belonging to the family Munididae were recently collected along the southwestern coast of the mainland of India and in the Andaman Islands. The specimens belong to two known species, *Agononida prolixa* (Alcock, 1894) and *Munida compacta* Macpherson, 1997, and a new species, *Paramunida bineeshi* **sp. nov**. We here redescribe *A. prolixa* and describe and figure the new species. *Munida compacta* is newly recorded from India, and we figure the live coloration. In addition, molecular and phylogenetic analyses of two mitochondrial markers (16S rRNA and COI) revealed the phylogenetic relationships of *M. compacta* and *P. bineeshi* **sp. nov.** with their most closely related congeners. The genetic similarity among the individuals of *M. compacta* from different locations is also addressed.

Keywords

Agononida, Indian Ocean, integrative taxonomy, molecular characters, morphology, *Munida*, new record

Introduction

Squat lobsters are a very diverse and abundant group of anomuran decapods that are distributed throughout the world (Schnabel et al. 2011). A high level of squat lobster diversity is found in the western-central Pacific Ocean, particularly in the Solomon-Vanuatu-New Caledonia region, the Coral Sea, the Indo-Malay-Philippine archipelago, and French Polynesia (Macpherson et al. 2010; Schnabel et al. 2011; Rodríguez-Flores et al. 2019a).

During the nineteenth century, several expeditions were carried out in the Indian Ocean by the Indian survey steamer 'Investigator'. The results of these expeditions – in which abundant material was gathered and many new species discovered – had been published by several workers, e.g., Wood-Mason and Alcock (1891), Alcock (1894, 1901), Alcock and Anderson (1894, 1899a, 1899b), Anderson (1896), McArdle (1901), MacGilchrist (1905), and Lloyd (1907). Of the many new species described by these authors, most were from the Bay of Bengal and the Arabian Sea. Deep-sea expeditions by the German vessel 'Valdivia' also gathered extensive material, including five new species (Doflein and Balss 1913). In addition to these expeditions, other relatively recent investigations in the Indian waters have revealed many new findings on squat lobsters, including descriptions of new species (e.g., George and Rao 1966; Rao 1974; Tirmizi and Javed 1993; Thirumilu 2011; Vaitheeswaran and Venkataramani 2012; Vaitheeswaran 2014, 2015, 2017; Komai et al. 2019). Although the seas around India are considered to be a moderately rich biogeographic region (cf. Spalding et al. 2007; Schnabel et al. 2011), the current diversity of squat lobsters from the region is not very high, and more research is needed to improve our knowledge on the group in this interesting oceanic province.

Recently, we collected specimens from India that constitute three species belonging to the genera *Agononida* Baba & de Saint Laurent, 1996, *Munida* Leach, 1820, and *Paramunida* Baba, 1988, all from the family Munididae Ahyong, Baba, Macpherson & Poore, 2010. Among our material, the lone specimen of *Paramunida* from the Andaman Islands is described herein as a new species, *Paramunida bineeshi* sp. nov. *Agononida prolixa* (Alcock, 1894) is redescribed based on a male specimen from the Andaman Islands. Several specimens of *Munida compacta* Macpherson, 1997 were collected from Kerala (southwestern coast of the Indian mainland), and the species newly recorded in Indian waters. The live colouration of *M. compacta* is described and figured here. We also conducted molecular and phylogenetic analyses using two mitochondrial markers, cytochrome oxidase subunit I (COI) and 16S rRNA (16S), for *M. compacta* and *P. bineeshi* sp. nov. to determine the phylogenetic relationships with their most closely related congeners. Furthermore, we noticed the genetic similarity among the individuals of *M. compacta* from different localities.

Material and methods

The material, including the holotype of the new species, is located in the Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram, Kerala, India (**DABFUK**). The specimens from the Andaman Islands were collected by K.K. Bineesh of the Andaman and Nicobar Regional Centre, Zoological Survey of India. The terminology and measurements follow Baba et al. (2009, 2011). The size of the specimens is indicated by the postorbital carapace length (**CL**), measured along the midline from the base of the rostrum to the posterior margin of the carapace. The rostrum was measured from its base, situated at the level of the orbit, to the distal tip. Measurements of appendages were taken in dorsal (pereopod 1), lateral (antennule, pereopods 2–4) and ventral (antenna) midlines. Abbreviations used are: **Mxp3**, maxilliped 3; **P1–4**, pereopods 1–4.

Molecular analysis

Tissue, taken from one of the pereopods, was used to extract genomic DNA with the DNeasy (Qiagen) kit following manufacturer's protocol. A prior digestion of the sample was performed during 18–24 hours, and RNase was included before the extraction. Partial sequences of the mitochondrial COI and 16S partial genes were amplified by polymerase chain reaction (PCR) using combinations of the following primers: tenuiCOIFwint/ tenuiCOIRev1int/ tenuiCOIRev2int (Rodríguez-Flores et al. 2019a), LCO1490 (Folmer et al. 1994), COI-H (Machordom et al. 2003) for COI; and 16SAR and 16SBR (Palumbi et al. 1991) for 16S markers. The amplified fragments were purified using ExoSAP-IT (Affymetrix). Sequencing of both strands was performed using BigDye Terminator in an ABI 3730 genetic analyzer in the SECUGEN service (Madrid, Spain). Forward and reverse DNA sequences were obtained for each specimen, which were checked and assembled using the program Sequencher 4.8 (Gene Code Corporation). Multiple alignment for the 16S marker was carried out using MAFFT (Katoh et al. 2002) with a posterior manual correction in AliView alignment editor (Larsson 2014).

Genetic distances between species were estimated using uncorrected divergences (*p*) calculated using PAUP v. 4.0 (build 167) (Swofford 2004). All the obtained sequences were submitted to GenBank [Accession numbers: *Munida compacta* Macpherson, 1997 (MT829201, MT827008) from Papua, (MT829202-3, MT827009-10) from Indian waters, *Paramunida bineeshi* sp. nov., holotype (MT829200, MT828867)].

Bayesian phylogenetic analyses were performed with MrBayes v. 3. 2. 1 (Huelsenbeck and Ronquist 2001) using matrices with concatenated COI and 16S for some closely related species of *Munida* and *Paramunida* separately. The sequences of the related species were obtained from GenBank, with the following accession numbers: KY230467-8 (holotype), KY230451-2 (paratype) (*Munida benguela* de Saint-Laurent & Macpherson, 1988); AY351115, AY350945 (*Munida congesta* Macpherson, 2000); AY351152, AY350985 (*Munida pagesi* Macpherson, 1994); AF283885-6, AY351160-1 (*Munida rhodonia* Macpherson, 1994); AY350994, AY351162 (*Munida rosula* Macpherson, 1994); AF283887, AY351163 (*Munida rubridigitalis* Baba, 1994); GU814914, GU814707 (*Paramunida evexa* Macpherson, 1993a); AY351032, AY351201 (*Paramunida labis* Macpherson, 1996); GU814905, GU814696, GU814911, GU814704

(*Paramunida longior* Baba, 1988); GU814886, GU814677 (*Paramunida luminata* Macpherson, 1996); HM060642-4, MT828870-2 (*Paramunida marionis* Cabezas, Macpherson & Machordom, 2010); HM173431-2, MT828868-9 (*Paramunida mozambica* Cabezas, Macpherson & Machordom, 2010); GU814952-53, GU814745-6 (*Paramunida parvispina* Cabezas, Macpherson & Machordom, 2010); EU418006, EU418009, EU417982, EU417985 (*Paramunida salai* Cabezas, Macpherson & Machordom, 2009); GU814898, GU814900, GU814689, GU814691 (*Paramunida setigera* Baba, 1988); GU814877-8, HM173483-4 (*Paramunida tenera* Cabezas, Macpherson & Machordom, 2010); GU814942, GU814735 [*Paramunida tricarinata* (Alcock, 1894)]; GU814862, GU814652, GU814893, GU814684 [*Hendersonida granulata* (Henderson, 1885)]; MT252616, MT25261, MT250542-3 (*Hendersonida parvirostris* Rodríguez-Flores, Macpherson & Machordom, 2020) (Machordom and Macpherson 2004; Cabezas et al. 2012; Rodríguez-Flores et al. 2020). The runs were realized in CIPRES portal (Miller et al. 2010). To estimate the posterior probabilities, four Markov Chains Monte Carlo (MCMC) were run for 1×10^7 generations sampling trees and parameters every 10000 generations. The initial 25 % of the generations were discarded as burn-in. The phylogenetic tree was visualized and edited in FigTree v. 1. 4. 2 (Rambaut 2014); nodes posterior probabilities from the Bayesian inference were included.

Systematic account

Family Munididae Ahyong, Baba, Macpherson & Poore, 2010
Genus *Agononida* Baba & de Saint Laurent, 1996

Agononida prolixa (Alcock, 1894)
Figure 2

Munida squamosa var. *prolixa* Alcock 1894: 322.–Alcock and Anderson 1894: 166; Alcock and Anderson 1895: pl. 13, fig. 3; Alcock 1901: 244; Doflein and Balss 1913: 142; Rao 1974: 302, fig. 1a–c; Macpherson 1993b: 425.
Agononida prolixa.–Ahyong and Poore 2004: 14; Baba 2005: 236; Baba et al. 2008: 51.

Material examined. Male (CL 16.3 mm), Andaman Islands (09°34'21"N, 92°43'94"E; depth 320 m), deepsea trawler, 10 December 2016, K.K. Bineesh leg. (DABFUK/AR-AN-118).

Description. ***Carapace***: slightly wider than long. Transverse ridges usually granular, mostly interrupted, with dense, very short, non-iridescent setae; few iridescent setae along lateral margins of carapace. Main transverse striae on posterior part of carapace interrupted in cardiac region. Two epigastric, two postcervical, and one median cardiac spine. Posterior margin with two median spines. Upper orbital margins excavated; lower orbital margins visible dorsally, mesially with spatulate, distally acute process. Lateral margins slightly convex. Anterolateral spine strong, at anterolateral angle, overreaching level of sinus between rostrum and supraocular spines. Second marginal spine anterior to cervical groove well developed. Branchial margins with three spines. Rostrum spiniform, barely half as long as remaining carapace, dorsally convex, distally directed downwards. Supraocular spines slightly thicker than rostral spine, exceeding midlength of rostrum and not reaching end of corneas, slightly divergent, dorsally convex, directed slightly upwards.

Thoracic sternum: 0.7× wider than long, sternites with numerous striae. Sternite 3 with median shallow notch, 2.7× wider than long. Sternite 4 0.3× wider than long, with anterior part narrower than sternite 3.

Pleon: tergites 2–4 with seven or eight, five, and four spines, respectively, on anterior ridge; tergite 4 with median spine on posterior ridge.

Eye: large; maximum corneal diameter 0.3–0.4 distance between bases of anterolateral spines.

Antennule: article 1 (distal spines excluded) about one-third CL, slightly overreaching cornea, with two distal spines, mesial spine clearly shorter than lateral spine; two spines on lateral margin, proximal one small, located at midlength of article, distal one long, not overreaching distolateral spine.

Antenna: article 1 with one distal spine on mesial margin, reaching end of article 2; article 2 with minute distomesial spine, distolateral angle unarmed; article 3 with long distomesial spine, slightly exceeding article 4.

Mxp3: ischium about twice length of merus measured along dorsal margin, distoventrally bearing long spine; merus with one strong median spine on flexor margin; extensor margin unarmed.

P1: long, subequal, squamous, 4× CL, with few plumose setae and few scattered iridescent setae on mesial borders of articles. Merus 1.8× CL, 2.6× as long as carpus, with few dorsal and mesial spines. Carpus 0.6× length of palm, nearly 4× as long as broad, with few spines along mesial and dorsal sides. Palm about 7× longer than broad, with few minute dorsal spines. Fingers 0.7× length of palm, unarmed.

P2–4: moderately long, slender, with numerous plumose setae and few iridescent non-plumose setae along extensor margin of articles. P2 length 3× CL. Meri slightly shorter posteriorly; P2 merus as long as CL, nearly 8× as long as broad, 1.2× longer than P2 propodus; P3 merus 8× longer than broad, 1.2× longer than P3 propodus; P4 merus 7.5× as long as broad, 1.2× longer than P4 propodus. Extensor margins of P2–4 meri with row of six to eight proximally diminishing spines; flexor margins with one strong distal spine followed proximally by several spines and eminences; lateral sides unarmed. Carpi with three or four spines on extensor margin of P2–4; lateral surface unarmed; flexor margin with distal spine. Propodi 11× as long as broad; extensor margin unarmed; flexor margin with 8–10 slender movable spines on P2–4, distal end without fixed spine. Dactyli slender, laterally with longitudinal ridge, 0.5× length of propodi; flexor margin with 12–14 movable spinules, distal half unarmed.

Remarks. *Agononida prolixa* was originally described as *Munida squamosa* var. *prolixa* by Alcock (1894) based on the specimens collected by the 'Investigator' (Station 115; 11°31'40"N, 92°46'6"E; depth, 344–406 m) from the Andaman Sea

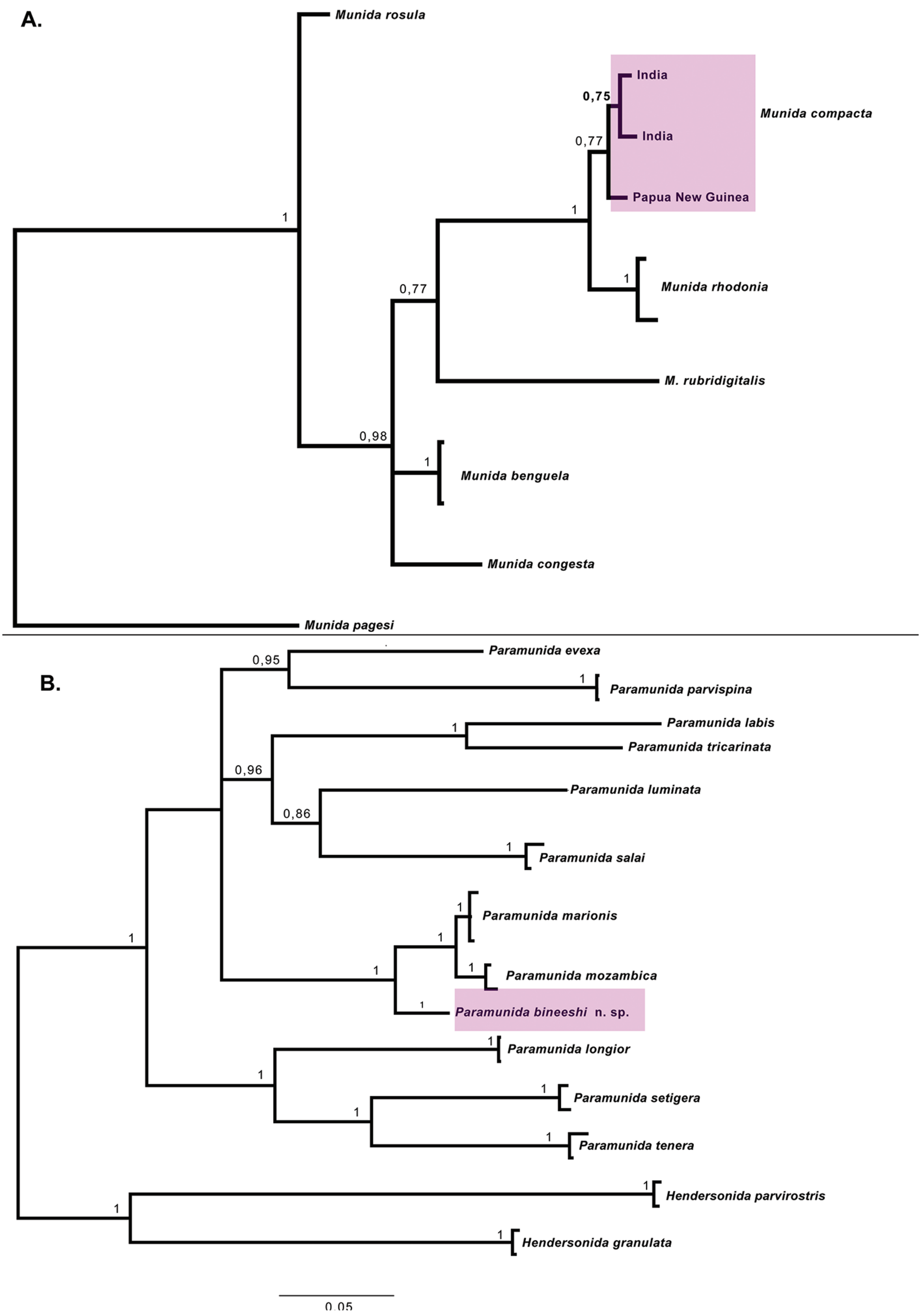

Figure 1. Bayesian phylogenetic relationship between the species found in India and their close relatives. **A** Phylogenetic hypothesis for *Munida compacta* **B** phylogenetic hypothesis for *Paramunida bineeshi* sp. nov. and related species. Bayesian support is included above the branches.

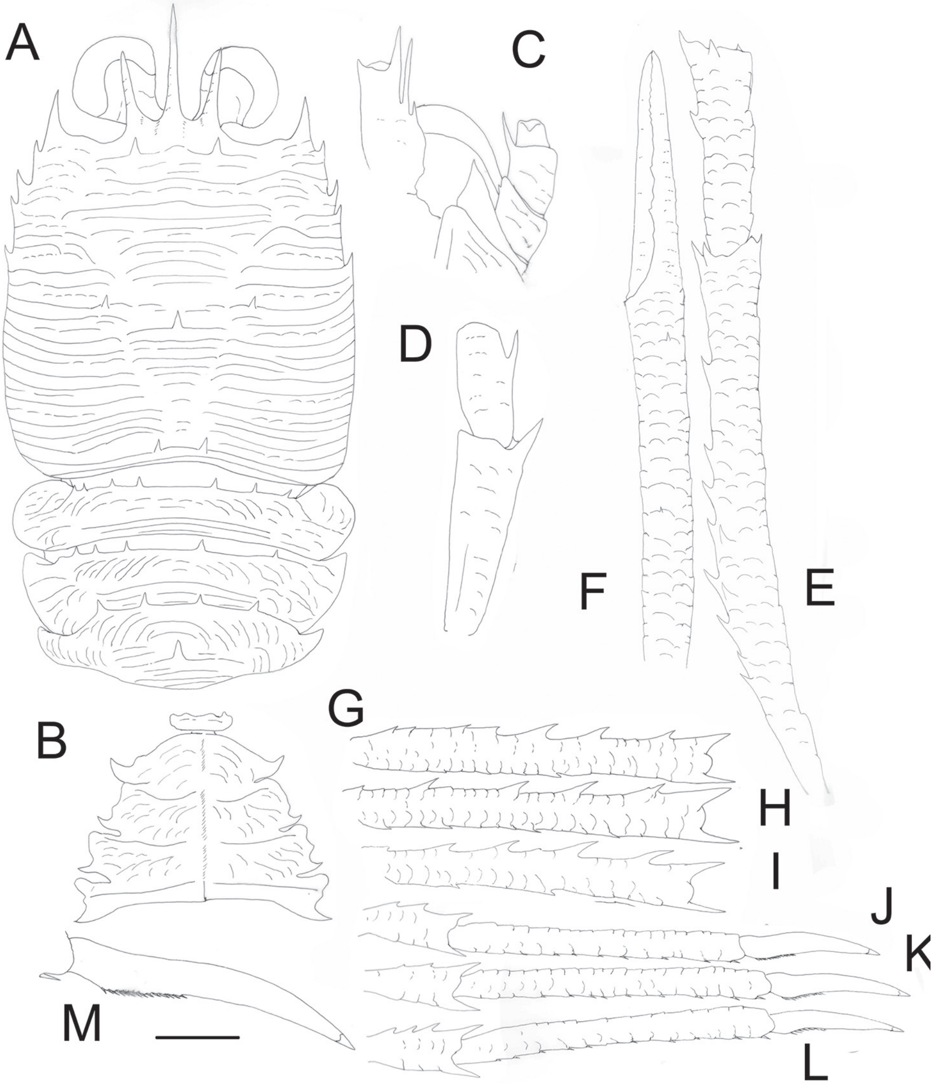

Figure 2. *Agononida prolixa* (Alcock, 1894), male (CL 16.3 mm) (DABFUK/AR-AN-118). **A** Carapace and pleonal tergites 2–4, dorsal **B** sternal plastron **C** cephalic region, showing left antennular and antennal peduncles, ventral **D** right Mxp3, lateral **E** right P1, merus and carpus, lateral **F** right P1, propodus and dactylus, lateral **G** right P2, merus, lateral **H** right P3, merus, lateral **I** right P4, merus, lateral **J** right P2, carpus to dactylus, lateral **K** right P3, carpus to dactylus, lateral **L** right P4, carpus to dactylus, lateral **M** right P2, dactylus, lateral. Scale bars: 4.0 mm (**A, B, E–L**); 2.0 mm (**C, D, M**).

(Anonymous 1914). It was subsequently found in the Gulf of Mannar, the Andaman Sea, the Arabian Sea off the coast of Sri Lanka, and south-west of Nicobar Islands (Alcock and Anderson 1894; Alcock 1901; Doflein and Balss 1913). More recently, the species was recorded near Kollam (= Quilon), south-western India at depths of

220–360 m (Rao 1974). The variety described by Alcock (1894) was raised to the rank of species by Ahyong and Poore (2004), which was subsequently followed by Baba (2005) and Baba et al. (2008). *Agononida prolixa* was figured by Alcock and Anderson (1895), and its antennule, antenna, and male pleopod by Rao (1974). We here redescribe the species and illustrate its additional body parts for clarifying its taxonomy.

Agononida prolixa belongs to the group of species that have the carapace without protogastric spines, the lateral branchial margin with three spines, the pleonal tergite 4 armed with a spine on the posterior transverse ridge, and the antennal peduncle article 1 with a moderate-sized process that does not overreach article 4. The closest relatives of *A. prolixa* are *A. isabelensis* Cabezas, Macpherson & Machordom, 2009, from the Solomon Islands and Papua New Guinea (Cabezas et al. 2009; Macpherson et al. 2020), and *A. nielbrucei* Vereshchaka, 2005, from New Zealand and the Norfolk Ridge (Vereshchaka 2005; Ahyong 2007). *Agononida prolixa* is easily distinguished from *A. isabelensis* by the presence of a pair of median spines directly anterior to the posterior margin of the carapace, whereas these spines are absent *A. isabelensis.* Differences with *A. prolixa* and *A. nielbrucei* include the following:

- In *A. nielbrucei*, the dorsal surface of the carapace has one branchiocardiac spine behind the postcervical spine on each side, and a second cardiac spine behind the median large spine. These spines are absent in *A. prolixa*.
- The distomesial spine of the antennular article 1 is larger than the distolateral spine in *A. nielbrucei* while it is shorter in *A. prolixa*.

Distribution. Indian Ocean: Arabian Sea (off Kollam, southwestern India); Gulf of Mannar (off Sri Lanka); and Andaman Sea (Andaman and Nicobar Islands). Depth: 220–752 m.

Genus *Munida* Leach, 1820

Munida compacta Macpherson, 1997
Figure 3

Munida compacta Macpherson 1997: 605, fig. 2.–Baba 2005: 261; Baba et al. 2008: 90; Macpherson et al. 2020: 44, figs 5C, 7A.

Material examined. Sakthikulangara Fishing Harbor, Kollam district, Kerala (8°55'30"N, 76°33'22"E; no depth), commercial trawler, T.-Y. Chan leg.: 14 males (CL 12.5–24.5 mm), 8 ovigerous females (CL 15.2–25.9 mm), 4 females (CL 12.9–25.7 mm), 20 March 2017 (DABFUK/AR-AN-119–121); 1 male (CL 22.1 mm), 4 March 2019 (DABFUK/AR-AN-122).

Diagnosis. Carapace with spiniform rostrum, five spines on branchial margin. Pleonal tergite 2 with more than six spines along anterior ridge, more than five transverse ridges. Thoracic sternum smooth, with few scales on sternite 4; sternite 4 narrowed, subtriangular, narrowly fitting to sternite 3. Eyes large, corneal width distinctly more than one-third distance between base of anterolateral spines. Antennular basal article with distomesial and distolateral spines subequal (or slightly different) in size. Antenna with distomesial spine of article 1 not exceeding end of article 2; distomesial spine of article 2 slightly overreaching end of article 3. Mxp3 merus unarmed on extensor distal margin. P1 fixed finger laterally with (one or two) subterminal spines only, occasionally with proximal spine; movable finger with proximal mesial spine only. P2–4 dactyli with movable spines along entire flexor margin.

Colour. Carapace, pleonal tergites 2 and 3, and appendages reddish or pinkish; pleonal tergites 4–6 and tailfan whitish. Rostrum and supraocular spines reddish. P1 fingers whitish, with reddish tips; distal portion of P2–4 propodi and proximal part of dactyli whitish.

Some specimens from Papua New Guinea have an orange carapace and whitish supraocular spines (Macpherson et al. 2020).

Genetic data. 16S and COI.

Remarks. The species was originally described from the specimens collected near the Kei Islands of Indonesia at depths 246–694 m (Macpherson 1997). It was also recently reported from Papua New Guinea at depths 220–1012 m (Macpherson et al. 2020). The specimens from India and Papua New Guinea are morphologically similar except for their colour differences (see notes on the colouration of *M. compacta*). Very low genetic distances were observed among the specimens from south-western India and Papua New Guinea (0.3–0.8% for COI and 0.0–0.6% for 16S), which support they are conspecific (Fig. 1A).

The most morphologically similar species to *M. compacta* is *M. rhodonia* from the south-western Pacific. *Munida compacta* and *M. rhodonia* can be readily distinguished from each other by the size of the second lateral marginal spine of the carapace, which is located immediately behind the anterolateral spine. The second lateral marginal spine of the carapace is well developed in *M. compacta* but very small in *M. rhodonia*. The distal part of the P2–4 propodi is distally broadened in *M. compacta*, while it is uniform in *M. rhodonia*. Furthermore, the ultimate flexor marginal spine (movable) of the P2–4 dactyli is nearly equidistant between the penultimate spine and the tip of the terminal claw in *M. compacta*, whereas it is much closer to the penultimate spine than to the tip of the terminal claw in *M. rhodonia* (Macpherson et al. 2020). A very low genetic divergence was observed between the two species (1.4% for COI and 0.3–0.5% for 16S) (Fig. 1A). In fact, the low genetic distance values may even be within the thresholds considered for intraspecific divergences. However, the morphological differences among the specimens of each species examined from Indonesia, Papua New Guinea, and India are constant (Macpherson 1997; Macpherson et al. 2020; this study), which suggests that *M. compacta* and *M. rhodonia* are separate species. A similar pattern has also been observed in other species comparisons. For instance, a low genetic distance (2.7% for COI, 0.3% for 16S), but with constant morphological differences, was observed between *Munida iris* A. Milne Edwards, 1880, from the West Atlantic and *M. speciosa* von Martens, 1878, from the East Atlantic (Rodríguez-Flores et al. 2019b).

Figure 3. *Munida compacta* Macpherson, 1997, male (CL 22.1 mm) (DABFUK/AR-AN-122), dorsal view.

In crustacean studies, a threshold to delimit species has been around 3% genetic divergence for COI (Lefébure et al. 2006; Rodríguez-Flores et al. 2019b). As such, *M. compacta* could be considered a junior subjective synonym of *M. rhodonia*. It is also possible that the sequence data is currently insufficient to differentiate these species. Nei (1987) pointed out that the speciation is based on the patterns of nucleotide substitutions, which occurs at certain rates. These two species seem to constitute lineages in an early stage of speciation. In these situations, further investigations are needed to clarify the taxonomy of *M. compacta*.

Munida andamanica Alcock, 1894, which occurs from India to the western Pacific Ocean, is also morphologically similar to *M. compacta*. These species are usually distinguished by the number of transverse ridges on the pleonal tergite 2: *M. compacta* has more than five transverse ridges while *M. andamanica* possesses only up to four transverse ridges (Baba 2005). The specimens of *M. compacta* examined by us, however, possess four to seven transverse ridges, which overlap with the count for *M. andamanica*. Therefore, this character state should be used with caution. Other characters are also variable. For instance, the P1 fixed finger has only one or two subterminal spines on the lateral margin in the specimens from Indonesia and Papua New Guinea, whereas the Indian specimens have both proximal and subterminal spines. Unfortunately, no genetic data is currently available for *M. andamanica*.

The interspecific and intraspecific variations in morphology and colour pattern seen among these three species (*M. andamanica*, *M. compacta*, and *M. rhodonia*) strongly demand a revision of the taxa involving more specimens from different localities, with both morphological and genetic data.

Distribution. Indonesia (Kei Islands); Papua New Guinea; and south-western India. Depth: 220–1012 m.

Genus *Paramunida* Baba, 1988

Paramunida bineeshi sp. nov.

http://zoobank.org/4771CE4C-5FC6-491B-8917-33D5F294F943
Figure 4

Type locality. Andaman Islands (09°34'21"N, 92°43'94"E; depth 320 m).

Type material. ***Holotype,*** ovigerous female (CL 12.2 mm), Andaman Islands (09°34'21"N, 92°43'94"E; depth 320 m), deep-sea trawler, 10 December 2016, K.K. Bineesh leg. (DABFUK/AR-AN-123).

Description of ovigerous female holotype. ***Carapace***: as long as broad. Dorsal surface covered with numerous spines and spinules, each usually on very short arcuate striae, with few uniramous setae. Epigastric region with two spines, each behind supraocular spine; with median row of spines behind rostral spine. Mesogastric region with three well-developed spines in midline, anterior two spines thicker than anterolateral spine. Cervical groove distinct. Cardiac and anterior branchial regions circumscribed. Cardiac region with median row of three well-developed spines, first thicker

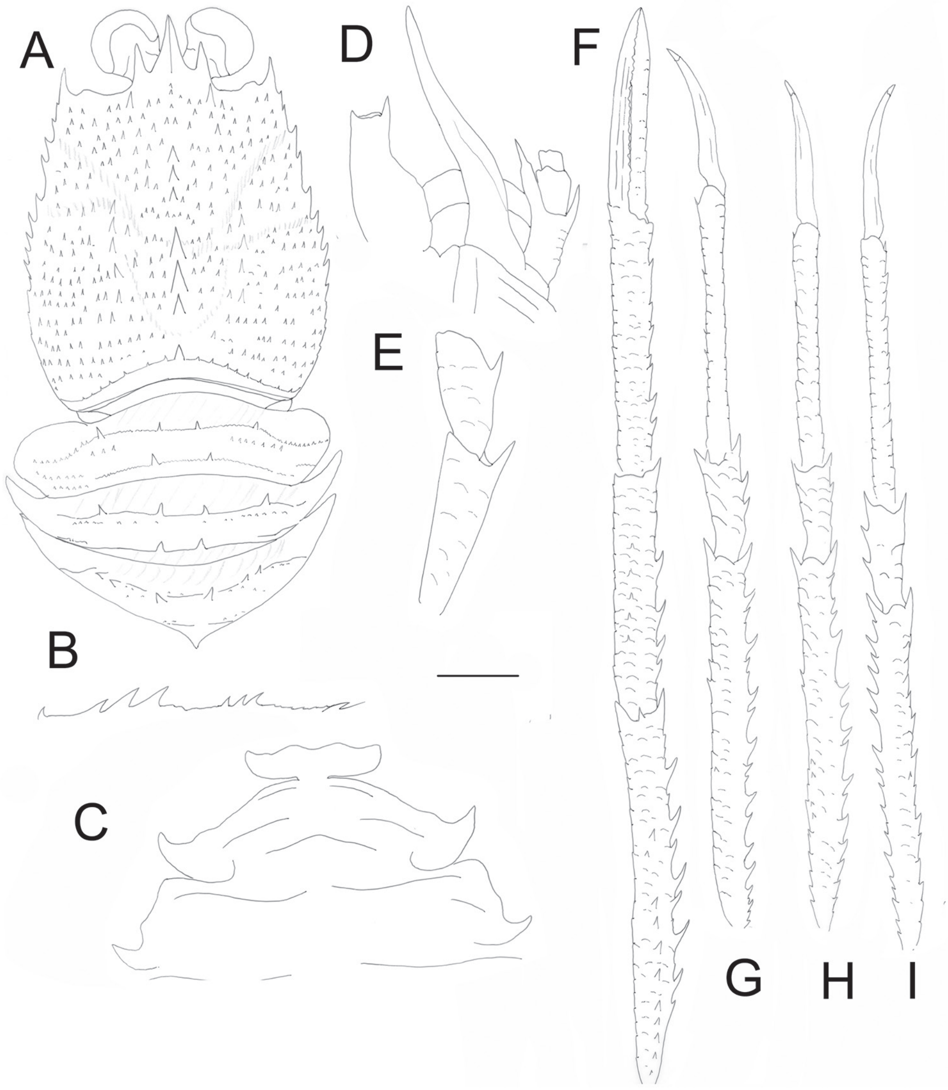

Figure 4. *Paramunida bineeshi* sp. nov., holotype, ovigerous female (CL 12.2 mm) (DABFUK/ARAN-123). **A** Carapace and pleonal tergites 2–4, dorsal **B** carapace, dorso-lateral **C** sternal plastron **D** cephalic region, showing left antennular and antennal peduncles, ventral **E** right Mxp3, lateral **F** left P1, lateral **G** left P2, lateral **H** left P3, lateral **I** right P4, lateral. Scale bars: 4.0 mm (**A, B, F–I**); 2.0 mm (**C–E**).

than others. Each branchial region with row of moderate-sized spines near cardiac region. Posterior transverse ridge with one well-developed median spine. Frontal margin slightly concave. Lateral margins convex, with small spines. Anterolateral spine reaching sinus between rostral and supraocular spines. Rostrum short, triangular, with thin dorsal longitudinal carina; supraocular spines shorter than rostrum; margin between rostral and supraocular spines straight.

Thoracic sternum: thoracic sternite 4 with few arcuate striae; sternites 5 and 6 smooth.

Pleon: tergites 2 and 3 each with four moderate-sized spines on anterior ridge, posterior ridge with two moderate-sized median spines. Tergite 4 with four to six spines on anterior ridge; posterior ridge with distinct, single median spine.

Eyes: maximum corneal diameter more than one-third distance between bases of anterolateral spines.

Antennule: article 1 exceeding cornea, with distomesial spine slightly shorter than distolateral; about twice longer than wide, with fringe of long setae along lateral margin; lateral margin with distal slender portion about half as long as proximal inflated portion.

Antenna: anterior prolongation of article 1 clearly overreaching antennular article 1 by about one-third of its length; article 2 (excluding spines) less than twice length of article 3, 1.5× as long as wide, ventral surface with small scales; distomesial spine long, slightly mucronated, slightly exceeding antennal peduncle, nearly reaching midlength of anterior prolongation of article 1, distolateral spine small, not reaching end of article 3; article 3 slightly longer than wide, unarmed.

Mxp3: ischium about twice length of merus measured along dorsal margin, distoventrally bearing 1 spine; merus with 1 strong median spine on flexor margin; extensor margin unarmed.

P1: long, subequal, squamous, 3.0× CL, with dense plumose setae and scattered iridescent setae on mesial borders of articles. Merus 1.3× CL, 1.7× as long as carpus, with dorsal and mesial spines; distal spines strong, distomesial spine not reaching proximal quarter of carpus. Carpus slightly shorter than palm, 5.5× as long as broad, with spines along mesial and dorsal sides. Palm 6× longer than broad, with spines along mesial margin. Fingers 0.8× length of palm; movable finger with one small proximal mesial spine; fixed finger unarmed.

P2–4: long and slender, with scales on lateral sides of meri, carpi, and propodi; each scale with short setae; with dense plumose setae and scattered iridescent setae on extensor borders of articles. P2 2.8× CL, merus 1.2× longer than CL, 10.5× as long as high, 1.5× as long as propodus; propodus 10× as long as high, 1.6× length of dactylus. Merus with well-developed extensor marginal spines, increasing in size distally, flexor margin with few spines and one well-developed distal spine; row of small spines along flexolateral margin. Carpus with small extensor spines, distal spine on extensor and flexor margin. Propodus with small movable flexor spines. Dactylus gently curved, with longitudinal carinae along mesial and lateral sides, ventral border unarmed. P3 with similar spination and segment proportions as in P2; merus as long as P2 merus; propodus and dactylus as long as those of P2. P4 slightly shorter than P2; merus 1.2× CL; propodus and dactylus slightly longer than those of P3; merocarpal articulation clearly exceeding end of anterior prolongation of first segment of antennal peduncle.

Etymology. The new species is named after Kinattum Kara Bineesh who collected this species and kindly passed it to us for study. The species epithet is a noun in the genitive singular.

Genetic data. 16S and COI.

Remarks. *Paramunida bineeshi* sp nov. is closely related to *P. mozambica* from the south-western Indian Ocean. The two species can be easily distinguished by the following characters:

- The two anterior spines of the cardiac region are larger than the supraocular spines in the new species, whereas they are smaller in *P. mozambica*.
- The antennal article 3 is slightly longer than wide in the new species, whereas it is nearly twice as long as wide in *P. mozambica*.
- The spines along the flexor and extensor margins of P2–4 are larger in the new species than in *P. mozambica*.
- The genetic divergence between the two species is 4.4–5.0% for COI and 1.0–1.2% for 16S (Fig. 1B).

The new species is also related to *P. marionis* from the southwestern Indian Ocean. The two species can be differentiated on the basis of the following characters:

- The spines of the median row on the gastric and cardiac regions are clearly stronger in the new species than in *P. marionis*.
- The posterior transverse ridge of the carapace has a well-developed median spine in the new species, whereas the spine is absent in *P. marionis*.
- The anterolateral spine reaches the sinus between the rostral and supraocular spines in the new species, whereas it clearly extends beyond the sinus in *P. marionis*.
- The distomesial spine of the antennal article 2 is slightly mucronated or blunt in the new species but spiniform in *P. marionis*.
- The genetic divergence between the two species is 4.3–4.5% for COI and 0.97% for 16S (Fig. 1B).

Distribution. The new species is only known from the type locality in the Andaman Sea. Depth: 320 m.

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A new species of *Nicippe* from the Bering Sea (Crustacea, Amphipoda, Pardaliscidae), with a redescription of *N. tumida*

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Abstract

A new pardaliscid amphipod, *Nicippe beringensis*, is described from the Bering Sea at depths between 520 and 536 m, and *N. tumida* Bruzelius, 1859 is redescribed based on specimens from Fredrikshald, Norway, near the species' type locality. Nucleotide sequences of mitochondrial cytochrome *c* oxidase subunit I (COI) from the holotype and a paratype of *N. beringensis* **sp. nov.** are reported. While *N. beringensis* **sp. nov.** is similar to *N. tumida*, it differs from the latter in having an asetose palp article 1 of maxilla 1, a gnathopod 1 coxa with a straight distal edge, and in the posterior margin of the basis of gnathopods 1 and 2, and pereopod 3, being heavily setose in females. A key to species of *Nicippe* is provided.

Keywords

COI, deep sea, *Nicippe beringensis*, systematics

Introduction

The genus *Nicippe* Bruzelius, 1859 has been recorded from littoral to deep-sea marine environments; it currently comprises five species: *N. tumida* Bruzelius, 1859, *N. buchi* Andres, 1975, *N. recticaudata* Matsukami, Nakano & Tomikawa, 2017, *N. rogeri* Lörz

& Schnabel, 2015, and *N. unidentata* Barnard, 1932 (Barnard and Karaman 1991; Lörz and Schnabel 2015; Matsukami et al. 2017). *Nicippe tumida*, the type species of the genus, is a widespread species occurring throughout Arctic-boreal regions (Karaman 1974; Lincoln 1979; Barnard and Karaman 1991). However, recent morphological and molecular research indicates that *N. tumida* is probably a complex of cryptic species (Matsukami et al. 2017).

Nicippe tumida was originally described from Drøbak, Norway (Bruzelius 1859). Although the original description was well written and detailed, from a modern taxonomic point of view, it is limited and the figures are restrictive. As several cryptic species may be confused with *N. tumida*, redescription of this species based on topotypic material would aid its differentiation from congeneric taxa.

During an expedition to the Bering Sea, one of us (KK) collected specimens of an evidently undescribed species of *Nicippe* from depths between approximately 500 m. These specimens are described and illustrated here. We also take this opportunity to describe specimens attributed to *N. tumida* from collections of the Swedish Museum of Natural History (SMNH); these specimens were collected from Fredrikshald, Norway, from near the type locality of *N. tumida*.

Material and methods

Samples

Specimens of an undescribed species of *Nicippe* were collected from the Bering Sea by R/V *Mirai* (JAMSTEC) in 2017 (MR17-04 Leg2) using a multiple corer or dredge attached on a deeptow camera system. Samples were fixed and preserved in 80% ethanol aboard the ship.

Eight adult females and four juveniles (sex indeterminate) of *N. tumida* from collections of the Swedish Museum of Natural History (SMNH-162979) were borrowed for comparison; one female was dissected (with permission) for detailed description.

Morphological observation

Appendages were dissected in 80% ethanol and mounted using gum chloral medium on glass slides with the aid of a stereomicroscope (Olympus SZX7). Specimens were examined by light microscopy (Nikon Eclipse Ni) and illustrated using a camera lucida. Body length was measured to the nearest 0.1 mm from the tip of the rostrum to the base of the telson along the dorsal curvature. Type materials have been deposited in the Tsukuba Collection Center of the National Museum of Nature and Science, Tokyo (NSMT).

PCR and DNA sequencing

Genomic DNA was extracted from pereopod muscle of the holotype and one paratype of the *Nicippe* specimens described herein following procedures detailed in Tomikawa et al. (2014). The primer set for the cytochrome *c* oxidase subunit I (COI) gene (LCO1490 and HCO2198; Folmer et al. 1994) was used for PCR and cycle sequencing reactions. PCR and sequencing followed methods detailed in Tomikawa et al. (2017). The DNA sequence has been deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ).

Systematics

Family Pardaliscidae Boeck, 1871
Genus *Nicippe* Bruzelius, 1859

***Nicippe beringensis* sp. nov.**
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Figures 1–6

Material examined. ***Holotype*:** ovigerous female (11.7 mm), NSMT-Cr 27383, the Bering Sea (54°28.41'N, 166°01.43'W; Sta. B), 536 m depth, multiple corer, collected by K. Kakui on 6 August 2017. Paratypes: male (10.4 mm), NSMT-Cr 27384, two females (7.8 mm, NSMT-Cr 27385; 6.8 mm, NSMT-Cr 27386), the Bering Sea (54°29.10'N, 165°57.25'W–54°29.08'N, 165°57.23'W; Sta. B), 521–520 m depth, deep tow, collected by K. Kakui on 7 August 2017.

Diagnosis. Urosomite 1 with 2 dorsal spines. Maxilla 1 with asetose palp article 1. Gnathopod 1 coxa with straight distal edge. Basis of gnathopods 1 and 2 and pereopod 3 in female with heavily setose posterior margin. Telson widely cleft with sinuous inner margin, weakly expanding proximally.

Description of female (holotype, NSMT-Cr 27383). ***Head*** (Fig. 1) with rudimentary eye, vertically deep, yellowish, only visible in living specimen (Fig. 6; paratype, NSMT-Cr 27384); rostrum short, pointed; lateral cephalic corner weakly produced. Pereonites 1–7 and pleonites 1–3 dorsally smooth (Fig. 1). Posteroventral corners of epimeral plates 1–3 pointed (Fig. 1); ventral submargins of epimeral plates 2 and 3 with 3 and 5 setae, respectively. Urosomite 1 with 2 pointed spines extending beyond posterior margin of its segment (Fig. 2A); urosomites 2 and 3 dorsally smooth.

Antenna 1 (Fig. 2B): length 0.5 times as long as body length; peduncular articles 1–3 in length ratio of 1.0 : 0.8 : 0.3; posterodistal corner of peduncular article 1 with long setae, some of which weakly plumose; accessory flagellum 6-articulate, article 1 short, length 2.5 times of width; primary flagellum 43-articulate, length of article 1 3.0 times as long as article 2.

Antenna 2 (Fig. 2C): length 0.7 times as long as antenna 1; peduncular articles 3–5 in length ratio of 1.0 : 1.5 : 1.2; anterior margin of peduncular article 3 with 2 clusters of setae; anterior margins of peduncular articles 4 and 5 with short setae, posterior margin of peduncular article 4 with long setae; flagellum 23-articulate.

Mouthparts. Upper lip (Fig. 2D) with weakly convex ventral margin, bearing fine setae. Mandibles (Fig. 2E, F): slightly asymmetric, incisor margins broad, an-

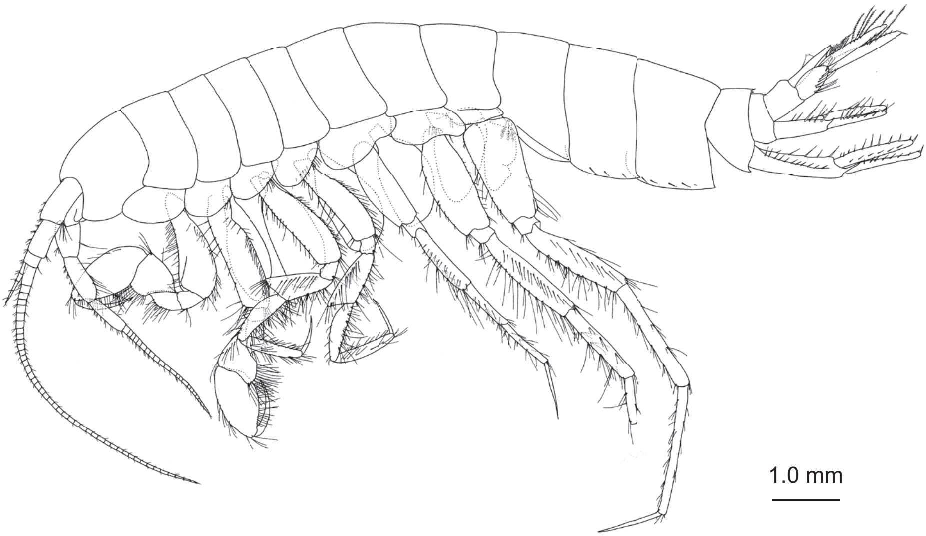

Figure 1. *Nicippe beringensis* sp. nov., holotype female (11.7 mm), NSMT-Cr 27383. Habitus, lateral view.

teroventral corner with a strong tooth; left lacinia mobilis (Fig. 2E) broad, about 0.8 times as long as incisor, multi-dentate; right incisor (Fig. 2F) with 2 teeth on proximal to anterodorsal corner; right lacinia absent; accessory setal row of left and right mandibles with 2 robust setae, respectively, and a proximal tuft of seta; molar absent; mandibular palp 3-articulate with length ratio of 1.0 : 3.3 : 2.6, article 2 with 5 marginal and 12 submarginal posterolateral setae, article 3 with 7 posterolateral and 3 apical setae. Lower lip (Fig. 2G) with broad outer lobes, inner lobes coalesced. Maxilla 1 (Fig. 2H) with inner and outer plates and palp; inner plate small with apical plumose seta; outer plate subrectangular with 8 apical robust setae, the lateral one strongest and longest; palp 2-articulate, article 1 lacking marginal setae, article 2 expanded distally with robust and slender setae. Maxilla 2 (Fig. 2I) with inner plate bearing row of 5 plumose setae on medial margin and 3 apical plumose setae; outer plate slightly longer than inner plate with 3 apical plumose setae. Maxilliped (Fig. 3A) with inner and outer plates and palp; inner plate not reaching base of palp, with long plumose apical seta and short weakly plumose subapical seta; outer plate narrow, reaching base of article 2 of palp, with setae along apical to medial margin; palp 4-articulate, long, article 2 longest with inner marginal rows of setae, article 3 with clusters of setae on dorsal and ventral faces and inner marginal setae, article 4 slender with serrate inner margin.

Gnathopod 1 (Fig. 3B): coxa short, length 1.3 times of width, distal edge straight; basis long, expanded distally, anterior margin flat, posterior margin arched with numerous setae in row; ischium short, subquadrate, 0.7 times as long as merus; posterior margin of merus with setae; carpus with rounded lobe ventrally with long setae, length 1.1 times of width; propodus oval, length 1.8 times as long as carpus, palm weakly

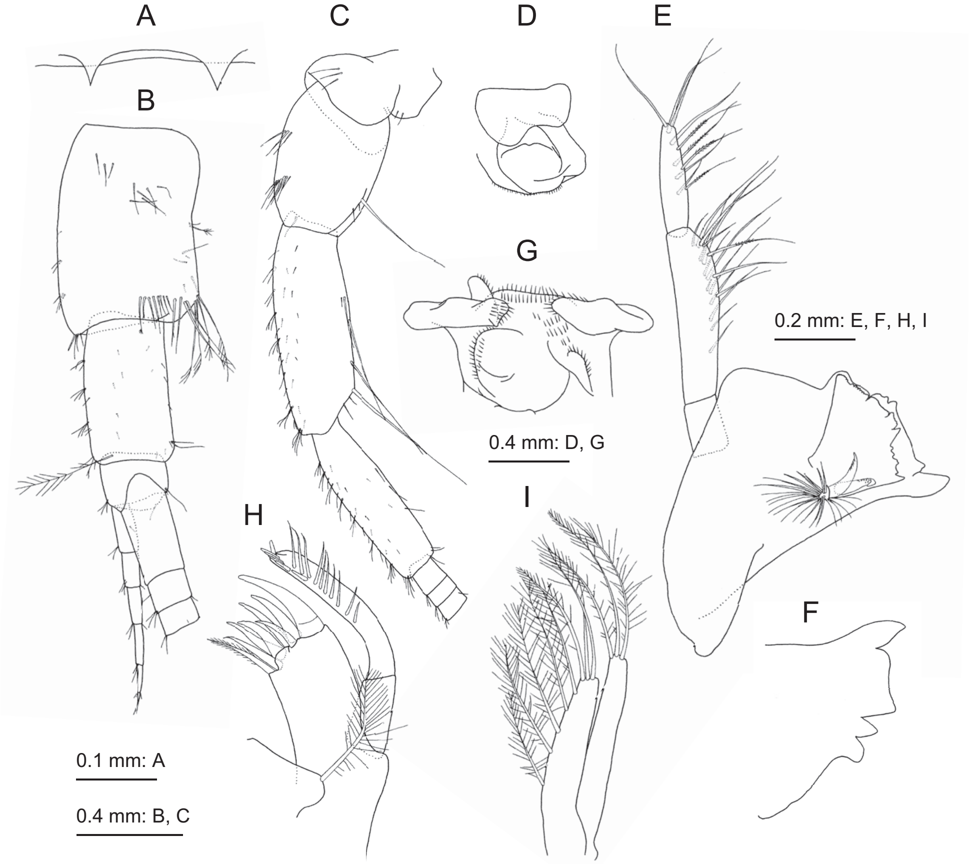

Figure 2. *Nicippe beringensis* sp. nov., holotype female (11.7 mm), NSMT-Cr 27383 **A** dorsal margin of urosomite 1, dorsal view **B** antenna 1, lateral view, some distal articles of primary flagellum omitted **C** antenna 2, lateral view, some distal articles of flagellum omitted **D** upper lip, anterior view **E** left mandible, medial view **F** right mandible, medial view, accessory setal row a proximal tuft of seta omitted **G** lower lip, ventral view **H** maxilla 1, dorsal view **I** maxilla 2, dorsal view.

convex with long setae; dactylus slender, slightly curved, posterior margin smooth with tooth near the base.

Gnathopod 2 (Fig. 3C): coxa rounded ventrally, posterior margin and medial face with setae; compared to that of gnathopod 1, basis longer and more slender; carpus ventrally more strongly produced, length 1.3 times of width; dactylus similar to that of gnathopod 1.

Pereopod 3 (Fig. 3D): coxa rounded ventrally, posterior margin and medial face with setae; basis long, posterior margin strongly setose; merus, carpus, propodus, and dactylus in length ratio of 1.0 : 1.0 : 1.0 : 0.6; merus with 9 setae on anterior submargin; carpus with 9 setae on anterior margin and submargin; propodus with 22 setae on anterior margin and submargin; dactylus slender and weakly curved.

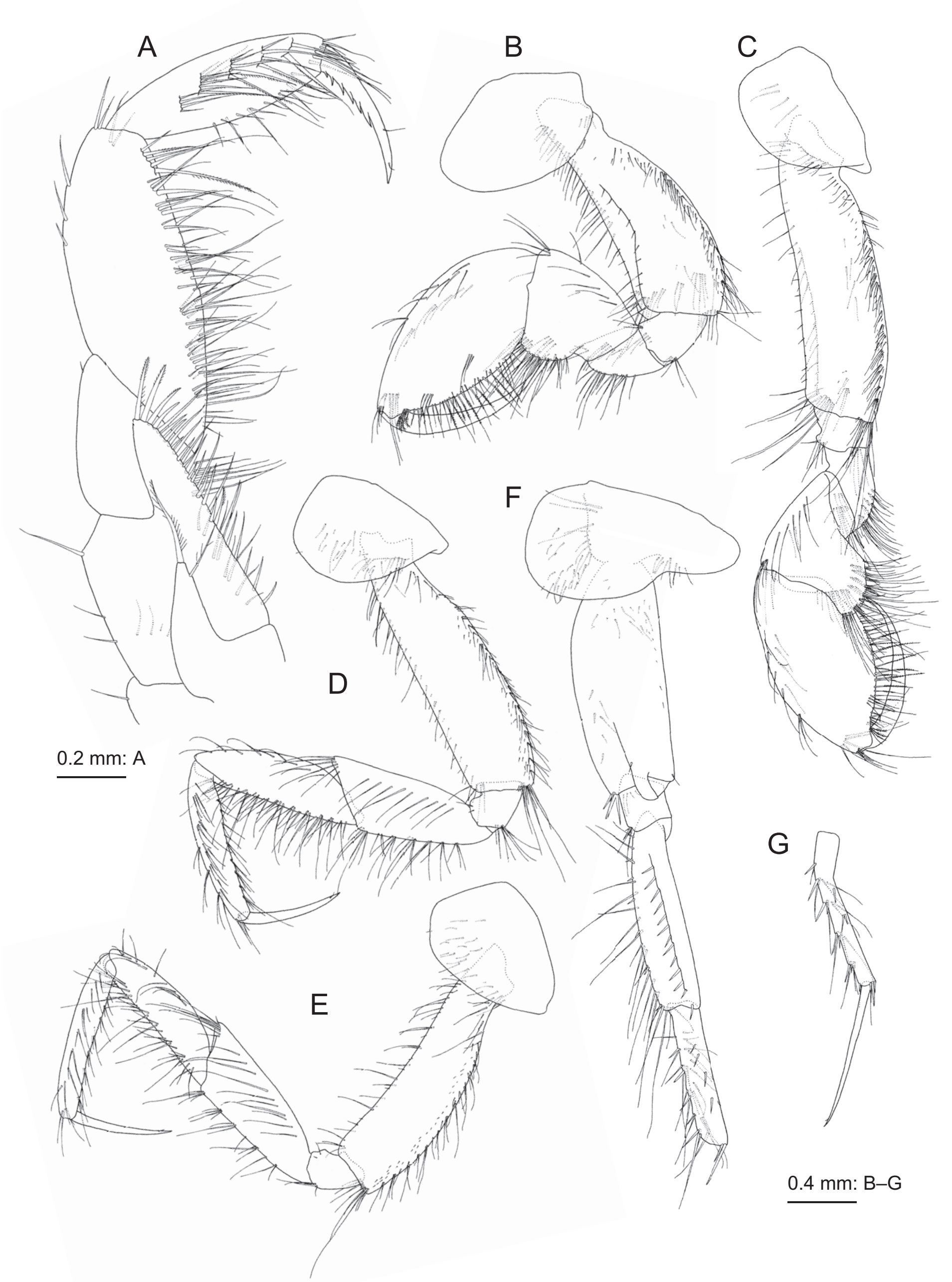

Figure 3. *Nicippe beringensis* sp. nov., holotype female (11.7 mm), NSMT-Cr 27383 **A** maxilliped, dorsal view **B** gnathopod 1, lateral view **C** gnathopod 2, lateral view **D** pereopod 3, lateral view **E** pereopod 4, lateral view **F** coxa-carpus of pereopod 5, lateral view **G** propodus and dactylus of pereopod 5, lateral view, propodus deformed due to preparation.

Pereopod 4 (Fig. 3E): similar to pereopod 3, merus, carpus, propodus, and dactylus in length ratio of 1.0 : 0.9 : 0.9 : 0.6; merus with 10 setae on anterior submargin; carpus with 10 setae on anterior margin and submargin; propodus with 13 setae on anterior submargin.

Pereopod 5 (Fig. 3F, G): coxa bilobate with facial setae medially, anterior lobe stronger and longer; basis with posterodistal lobe, anterodistal corner with robust setae; merus, carpus, propodus, and dactylus in length ratio of 1.0 : 0.7 : 0.8: 0.7; merus with 16 slender and 5 robust setae on anterior margin and 12 robust setae on posterior ridge, anterodistal corner with 5 slender and 1 robust setae; posterior ridges of carpus and propodus with 4 and 3 robust setae, respectively; dactylus slender and almost straight, longer than on pereopods 3 and 4.

Pereopod 6 (Fig. 4A, B): coxa shallowly bilobate with setae on medial face, anterior lobe slightly stronger; basis posterior margin slightly convex, anterior and posterior margins with setae, anterodistal corner with slender and robust setae, posterodistal corner subquadrate; merus, carpus, propodus, and dactylus in length ratio of 1.0 : 0.8 : 1.1: 0.6; anterior margin of merus with 20 slender and 6 robust setae, posterior margin with 2 setae, posterior ridge with 17 setae; posterior margins of carpus and propodus each with 3 clusters of robust setae; dactylus slender, slightly curved.

Pereopod 7 (Fig. 4C–E): coxa subquadrate, anterior margin and ventral submargin with setae; basis distally tapering, posteroproximal part expanded, posterior margin almost straight, anterior and posterior margins with setae, anterodistal corner with robust and slender setae, posterodistal submargin with long plumose setae (Fig. 4E), posterodistal corner rounded; merus, carpus, propodus, and dactylus in length ratio of 1.0 : 1.1 : 1.2 : 0.6; anterior margin of merus with clusters of robust setae, posterior margin with 3 slender setae, posterior ridge with 9 slender setae and trace of seta; anterior and posterior margins of carpus with 5 and 3 clusters of setae, respectively; anterior margin of propodus with 7 clusters of setae and single seta, posterior margin with 3 clusters of setae and single seta; dactylus slender, almost straight.

Pleopods 1–3 (Fig. 4F–H) each with paired retinacula (Fig. 4G) on inner distal margin of peduncle, bifid setae (clothes-pin setae) (Fig. 4H) on inner basal margin of inner ramus; inner and outer rami of each pleopod consisting of 18–19 and 23–24 articles, respectively.

Uropods. Uropod 1 (Fig. 4I): peduncle longer than rami, with 5 basofacial setae, distolateral peduncular spine very strong; inner ramus subequal to outer ramus in length, medial and lateral margins with 9 and 7 robust setae, respectively; outer ramus with 7 robust setae on medial and lateral margins, respectively; both rami with stout setae apically. Uropod 2 (Fig. 4J): peduncle slightly shorter than rami, with long stout setae on lateral margin, distolateral peduncular spine short; inner ramus slightly longer than outer ramus, with 7 and 9 robust setae on medial and lateral margins, respectively; outer ramus with 8 and 7 robust setae on medial and lateral margins, respectively; both rami each with stout seta apically. Uropod 3 (Fig. 4K): peduncle strongly setose

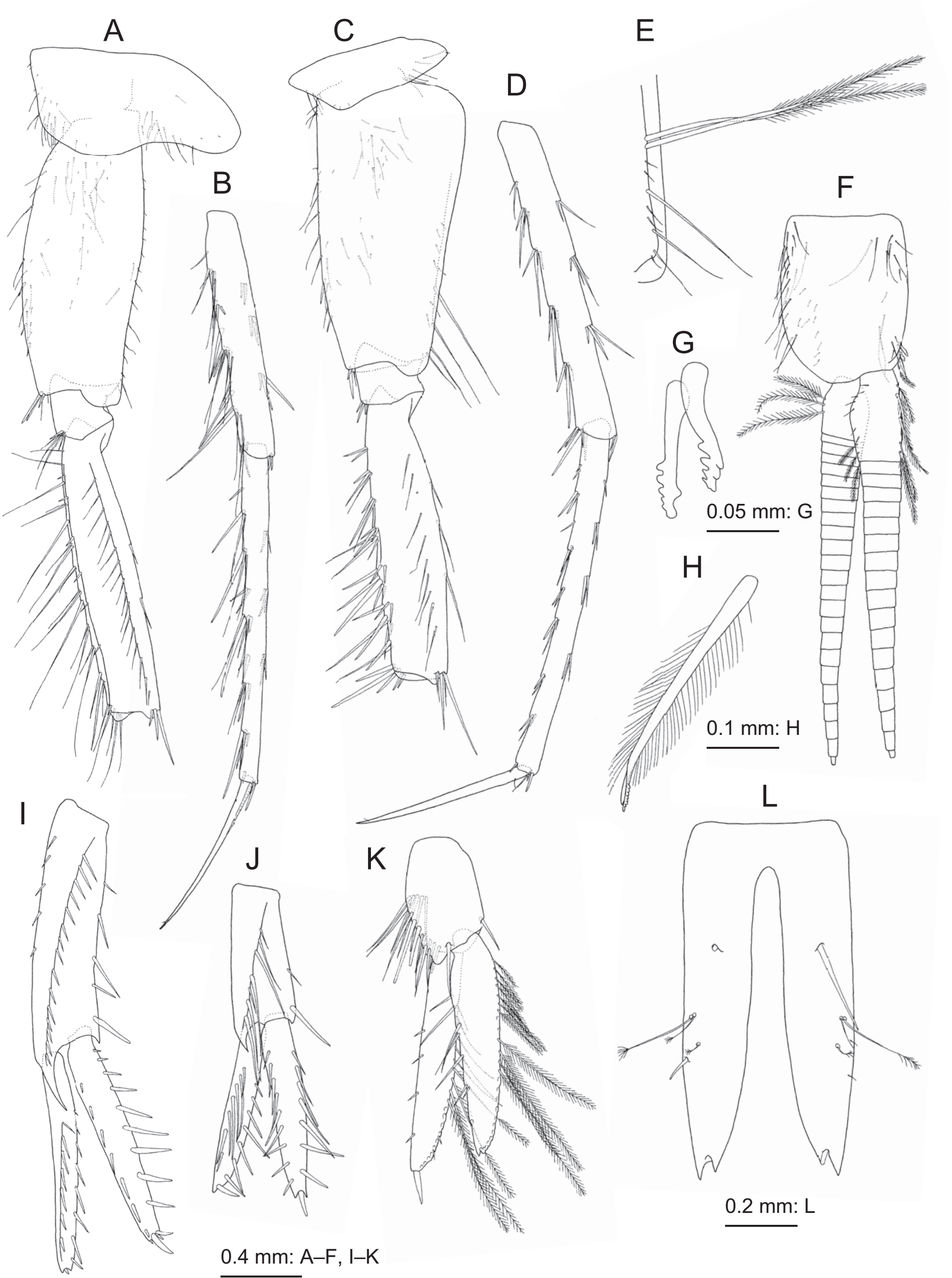

Figure 4. *Nicippe beringensis* sp. nov., holotype female (11.7 mm), NSMT-Cr 27383 **A** coxa-merus of pereopod 6, lateral view **B** carpus-dactylus of pereopod 6, medial view **C** coxa-merus of pereopod 7, lateral view **D** carpus-dactylus of pereopod 7, lateral view **E** posterodistal corner of basis of pereopod 7, medial view **F** pleopod 1, anterior view, some setae on rami omitted **G** retinacula on peduncle of pleopod 1, anterior view **H** bifid plumose seta (clothes-pin seta) on inner basal margin of inner ramus of pleopod 1, anterior view **I** left uropod 1, dorsal view **J** left uropod 2, dorsal view **K** left uropod 3, dorsal view **L** telson, dorsal view.

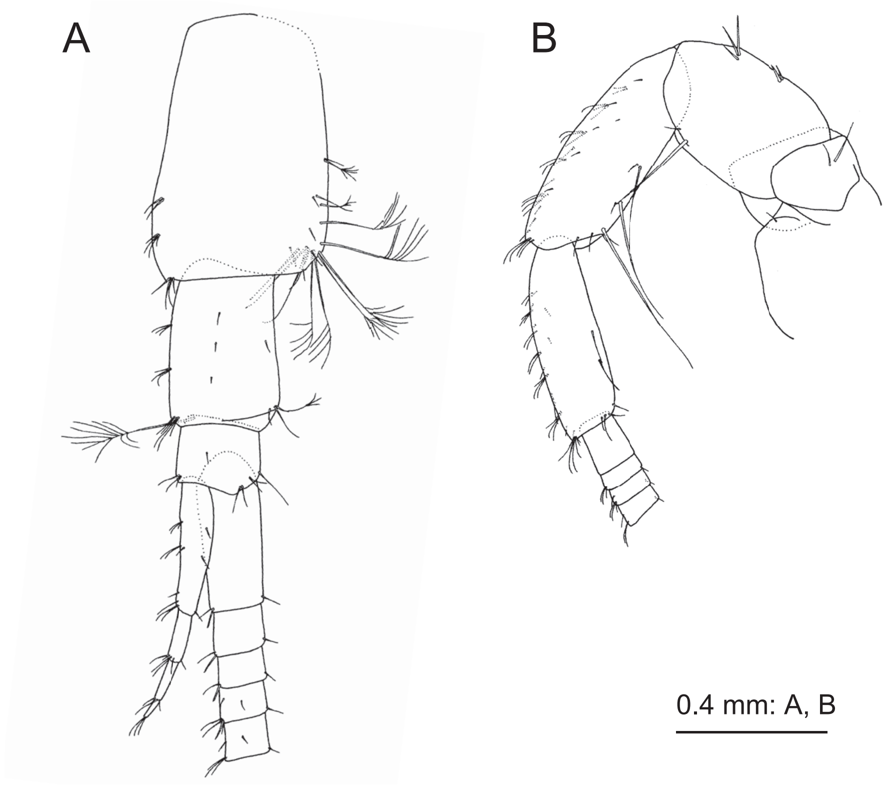

Figure 5. *Nicippe beringensis* sp. nov., paratype male (10.4 mm), NSMT-Cr 27384 **A** antenna 1, medial view, some distal articles of primary flagellum omitted **B** antenna 2, lateral view, some distal articles of flagellum omitted.

on lateral face; both rami almost equal in length, medial margins of inner and outer rami with long plumose setae.

Telson (Fig. 4L) length 2.2 times of width, cleft for 89% of length in U-shape with weakly curved margins of incision; apex of each lobe incised, lateral part of apex slightly longer than medial part, with small robust seta.

16 eggs.

Description of male (paratype, NSMT-Cr 27384). ***Antenna 1*** (Fig. 5A): peduncular articles 1–3 in length ratio of 1.0 : 0.6 : 0.3; accessory flagellum 4-articulate, article 1 long, length 4.3 times of width; primary flagellum 38-articulate, length of article 1 3.5 times as long as article 2.

Antenna 2 (Fig. 5B): peduncular articles 3–5 in length ratio of 1.0 : 1.3 : 1.1; flagellum 24-articulate.

Mandible**:** palp article 2 with 11 setae; palp article 3 with 5 posterolateral and 3 apical setae.

Gnathopods 1 and 2**:** bases with a few setae on their posterior margins.

Etymology. The specific name is an adjective derived from its type locality, the Bering Sea.

Figure 6. *Nicippe beringensis* sp. nov., paratype male (10.4 mm), NSMT-Cr 27384, in life.

Sequences and COI genetic distances. A single nucleotide sequence was obtained from each specimen of the holotype, NSMT-Cr 27383 (LC511042; 615 bp) and a paratype, NSMT-Cr 27384 (LC511043; 658 bp). Based on the 615 bp aligned sequences, the uncorrected *p*-distances between these two specimens is 0.2%.

Remarks. Though the holotype and paratypes were obtained from slightly different localities, they differ in no appreciable morphological character or state. The pairwise sequence divergence of COI between the holotype and paratype, 0.2%, is considerably less than that of interspecific distances between other amphipod taxa, 3.5–4.0% (Witt et al. 2006; Rock et al. 2007; Hou et al. 2009). Accordingly, we consider these two specimens to be conspecific.

Nicippe beringensis sp. nov. is similar to *N. tumida* in having two spines on the dorsal margin of urosomite 1, and in having a telson with a sinuous inner margin that weakly expands proximally. However, our species differs from *N. tumida* in a combination of characters and states: article 1 of the palp on the maxilla 1 is asetose in our new species, whereas it has marginal setae in *N. tumida*; the coxa of gnathopod 1 has a straight distal edge, as opposed to a curved one in *N. tumida*; and the basis of female gnathopods 1, 2 and pereopod 3 has a heavily setose posterior margin (whereas this is sparsely setose in *N. tumida*).

Nicippe tumida Bruzelius, 1859

Figures 7–10

Material examined. Eight females, 11.8–15.0 mm, 4 juveniles (sex indeterminate), 5.8–7.3 mm, SMNH-162979, Fredrikshald, Norway, collected by C. Bovallius in 1869 (the month and date unknown).

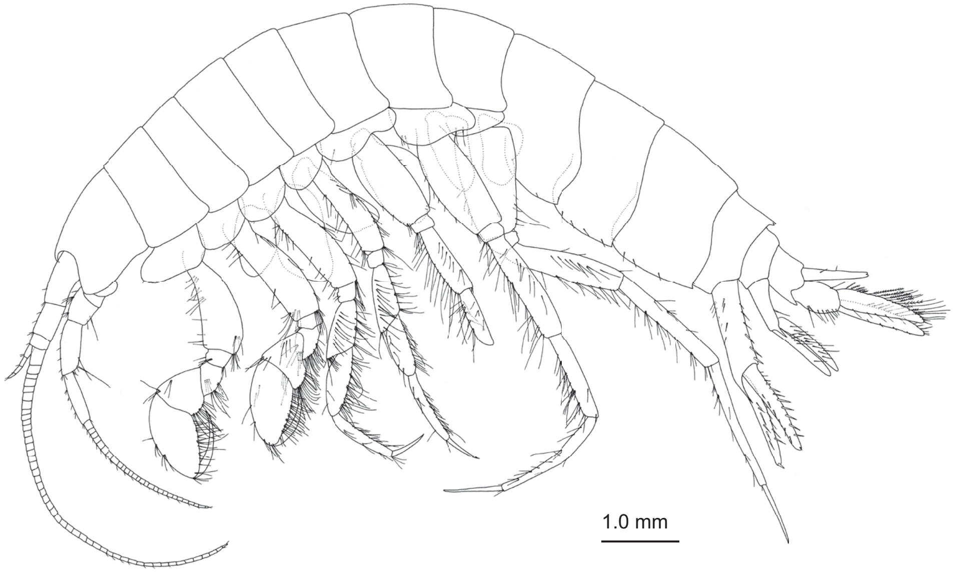

Figure 7. *Nicippe tumida* Bruzelius, 1859, female (12.1 mm), SMNH-162979. Habitus, lateral view.

Description of female (12.1 mm, SMNH-162979). Epimeral plates 1, 2, and 3 (Fig. 7) with ventral submargins bearing 1, 4, and 3 setae, respectively. Urosomite 1 with 2 pointed spines (Fig. 8A)

Antenna 1 (Fig. 8B): peduncular articles 1–3 in length ratio of 1.0 : 0.6 : 0.3; accessory flagellum 5-articulate; primary flagellum 48-articulate, length of article 1 1.9 times as long as article 2.

Antenna 2 (Fig. 8C): length 0.6 times as long as antenna 1; peduncular articles 3–5 in length ratio of 1.0 : 1.7 : 1.5; flagellum 28-articulate.

Mouthparts. Mandibles (Fig. 8E, F): left lacinia mobilis (Fig. 8E) broad, about 0.7 times as long as incisor; mandibular palp 3-articulate with length ratio of 1.0 : 2.4 : 1.7, article 2 with 20 setae, article 3 with 6 posterolateral and 3 apical setae. Maxilla 1 (Fig. 8H) with palp article 1 bearing 2 marginal setae. Maxilla 2 (Fig. 8I) with inner plate bearing 4 apical plumose setae.

Gnathopod 1 (Fig. 9B): coxa broad, length 1.8 times of width, distal edge curved; basis with posterior margin sparsely setose; ischium 0.6 times as long as merus.

Gnathopod 2 (Fig. 9C): basis with sparsely setose posterior margin.

Pereopod 3 (Fig. 9D): coxa rounded ventrally, ventral margin with seta; merus, carpus, propodus, and dactylus in length ratio of 1.0 : 1.1 : 1.1 : 0.6; carpus with 6 setae on anterior submargin; propodus with 7 setae and traces of 4 setae on anterior margin and submargin.

Pereopod 4 (Fig. 9E): merus, carpus, propodus, and dactylus in length ratio of 1.0 : 0.5 : 0.6 : 0.3; merus with 11 setae on anterior submargin; carpus with 7 setae on anterior margin and submargin; propodus with 4 setae and traces of 3 setae on anterior margin and submargin; dactylus slender and weakly curved.

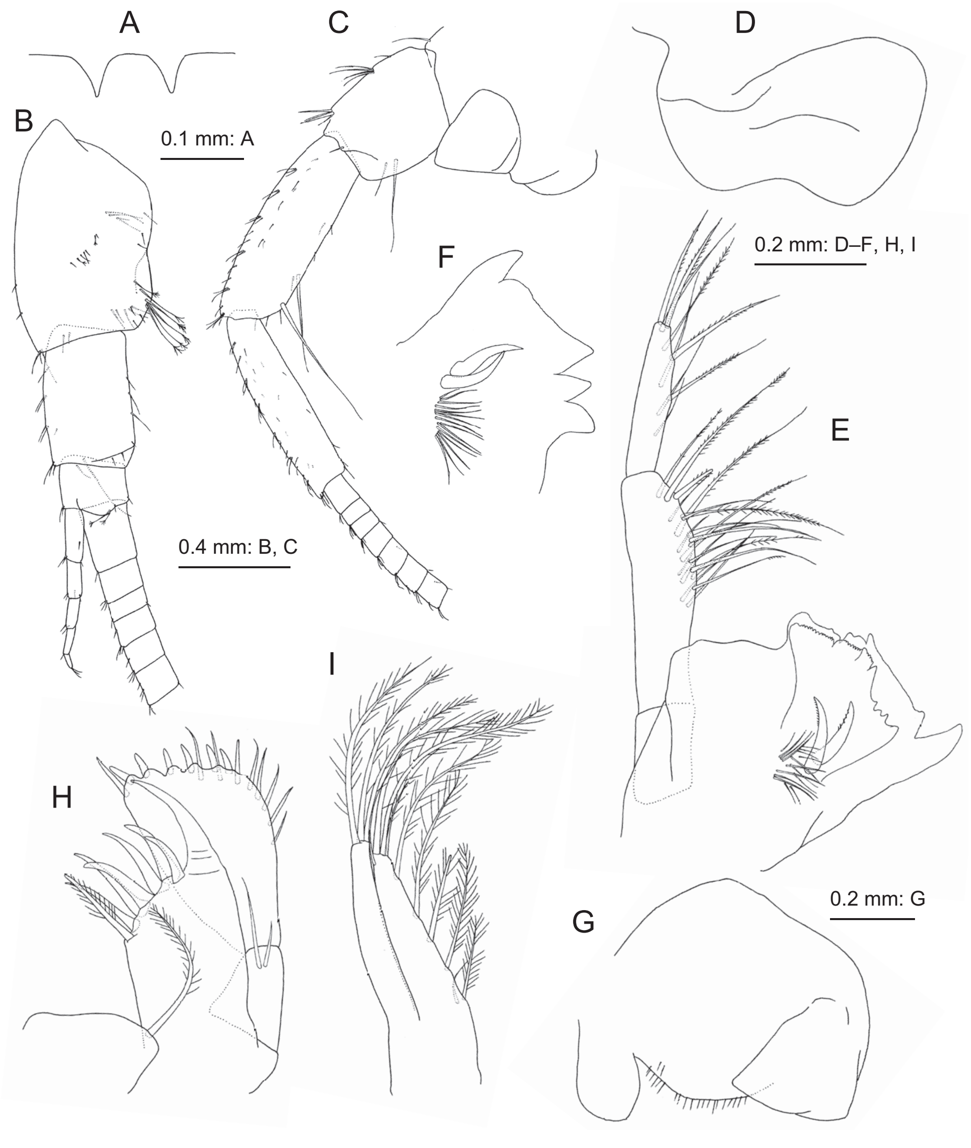

Figure 8. *Nicippe tumida* Bruzelius, 1859, female (12.1 mm), SMNH-162979 **A** dorsal margin of urosomite 1, dorsal view **B** antenna 1, medial view, some distal articles of primary flagellum omitted **C** antenna 2, medial view, some distal articles of flagellum omitted **D** upper lip (labrum broken), anterior view **E** left mandible, medial view **F** right mandible, medial view **G** lower lip, ventral view **H** maxilla 1, dorsal view **I** maxilla 2, dorsal view.

Pereopod 5 (Fig. 9F): basis with 3 and 2 setae on anterior and posterior margins, respectively, posterodistal corner of basis subquadrate; merus with 16 long and 3 short setae on anterior margin and submargin, 8 setae and trace of seta on posterior ridge, anterodistal corner with 3 setae.

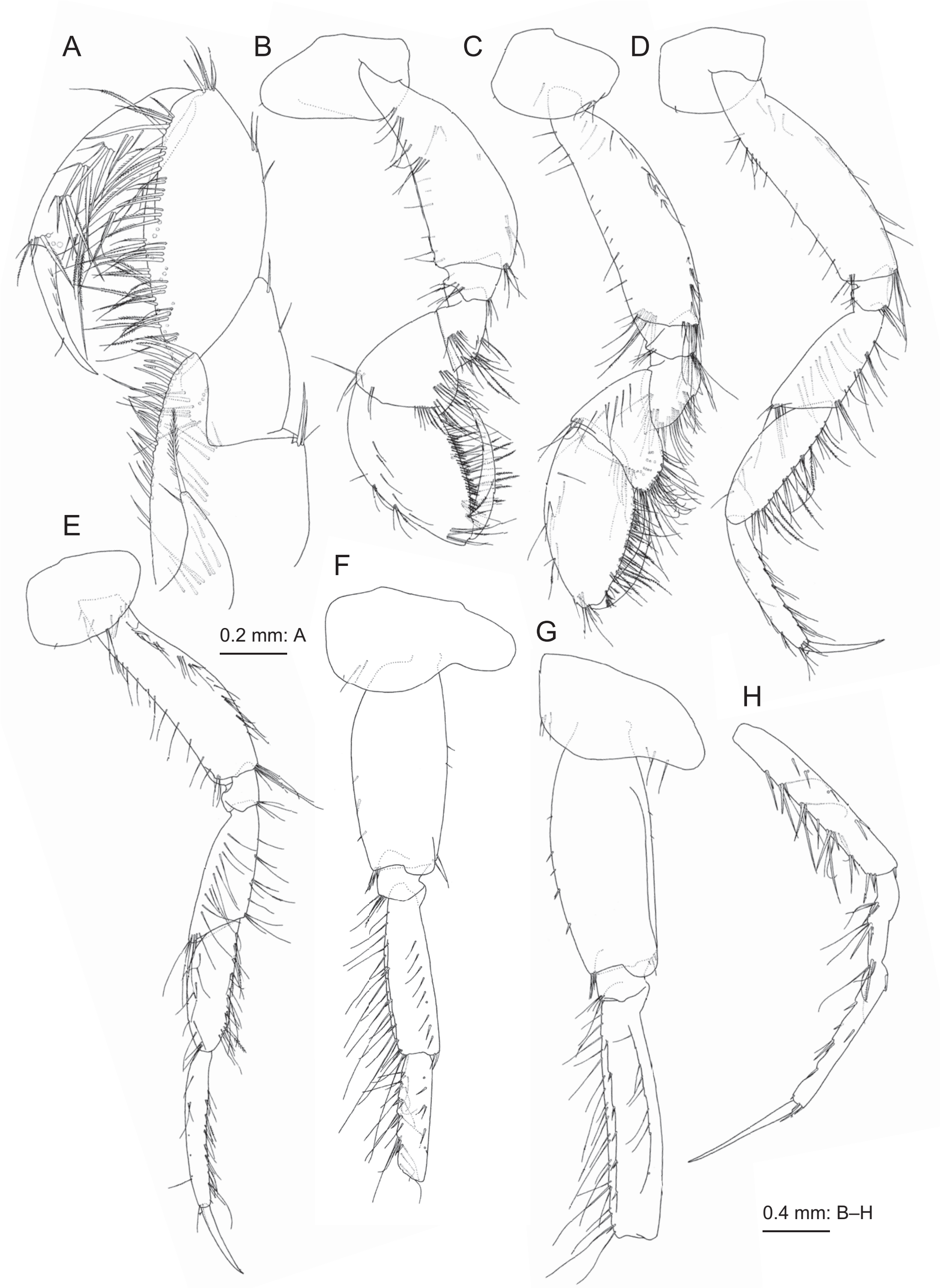

Figure 9. *Nicippe tumida* Bruzelius, 1859, female (12.1 mm), SMNH-162979 **A** maxilliped, dorsal view **B** gnathopod 1, medial view **C** gnathopod 2, lateral view **D** pereopod 3, medial view; **E** pereopod 4, lateral view **F** coxa-carpus of pereopod 5, lateral view, distal part of carpus broken **G** coxa-merus of pereopod 6, lateral view **H** carpus-dactylus of pereopod 6, lateral view, propodus deformed due to preparation.

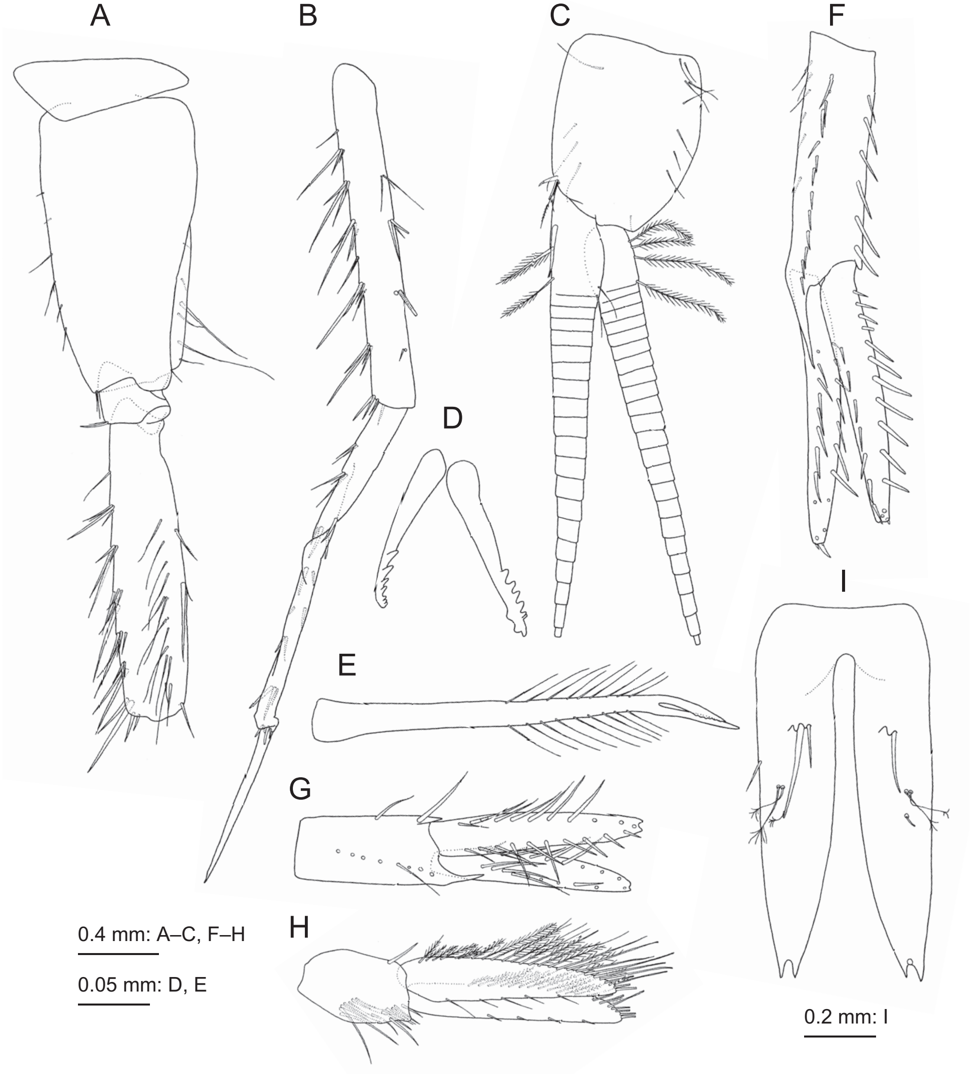

Figure 10. *Nicippe tumida* Bruzelius, 1859, female (12.1 mm), SMNH-162979 **A** coxa-merus of pereopod 7, lateral view **B** carpus-dactylus of pereopod 7, lateral view, propodus deformed due to preparation **C** pleo **E** bifid plumose seta (clothes-pin seta) on inner basal margin of inner ramus of pleopod 1, posterior view **F** left uropod 1, dorsal view **G** left uropod 2, dorsal view **H** left uropod 3, dorsal view **I** telson, dorsal view.

Pereopod 6 (Fig. 9G, H): coxa shallowly bilobate with setae on ventral margin and submargin; basis posterior margin almost straight, anterior and posterior margins with 6 and 2 setae, respectively, posterodistal corner of basis rounded; merus, carpus, propodus, and dactylus in length ratio of 1.0 : 0.8 : 1.0: 0.5; merus with 21 setae on anterior margin, 7 short setae on anterior submargin, 6 setae and traces of 4 setae on posterior ridge; posterior margins of carpus and propodus each with 5 (+ traces of 2 more setae) and 2 robust setae; dactylus slender, almost straight.

Pereopod 7 (Fig. 10A, B): merus, carpus, and propodus in length ratio of 1.0 : 1.1 : 1.1: 0.5; merus posterior ridge with 13 setae.

Pleopods 1–3 (Fig. 10C–E): inner and outer rami of each pleopod consisting of 19–20 and 22–24 articles, respectively.

Uropods. Uropod 1 (Fig. 10F): peduncle with 6 basofacial setae; inner ramus slightly shorter than outer ramus, medial and lateral margins with 11 and 7 robust setae, respectively; outer ramus with 7 robust setae and traces of setae on medial and lateral margins, respectively. Uropod 2 (Fig. 10G): inner ramus with 8 (+ traces of 2 more setae) and 10 robust setae on medial and lateral margins, respectively; outer ramus with 7 and 5 robust setae on medial and lateral margins, respectively and traces of setae.

Telson (Fig. 10I) length 2.1 times of width, cleft U-shaped, with weakly curved margins of incision.

Remarks. Though the present specimens agree with the original description of *N. tumida* (Bruzelius 1859), there are some differences between them. Pereopod 7 bears long plumose setae on the posterior margin of the basis in the original description, whereas the present specimens lack these setae. We confirmed that these plumose setae are fragile and easily broken off during handling for the observation of *N. beringensis*. Thus, these setae may have been broken off in the present specimens. The telson of the present material takes on the almost parallel shape at either inner margin. Judging from the illustration of the original description, the telson has a wide cleft distally and its inner margins are not parallel. It is a highly likely that the telson was erroneously drawn because Bruzelius' illustrations were simplified and there are some unreasonable aspects, e.g. the shape of coxae of pereopods 5–7 and epimeral plates. To confirm these dubious features as well as the telson shape, examination of the type material is needed. In this study, we could not access the type materials of *N. tumida*. Unfortunately, it is likely that the type specimens are lost.

Key to species of *Nicippe*

1 Dorsal margin of urosomite 1 smooth .... ***N. buchi***
– Dorsal margin of urosomite 1 dentate **2**
2 Dorsal margin of urosomite 1 with 1 spine **3**
– Dorsal margin of urosomite 1 with 2 spines **4**
3 Pereonites 1 and 7 the longest, pereonites 2–6 shorter, subequal; uropod 3 peduncle with unarmed distodorsal lobe .... ***N. rogeri***
– Pereonites 1–4 subequal, shorter than pereonites 5–7 with segment 5 the longest, uropod 3 peduncle with 3 pointed distodorsal processes .... ***N. unidentata***
4 Telson narrowly cleft with straight inner margin, tapering proximally .... ***N. recticaudata***
– Telson widely cleft with sinuous inner margin, weakly expanding proximally....**5**

5 Maxilla 1 with asetose palp article 1; gnathopod 1 coxa with straight distal edge; basis of female gnathopods 1 and 2, and pereopod 3, with heavily setose posterior margin .... ***N. beringensis***

– Maxilla 1 with palp article 1 bearing setae; gnathopod 1 coxa with curved distal edge; basis of female gnathopods 1 and 2, and pereopod 3, with sparsely setose posterior margin .... ***N. tumida***

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A new species in the *Tetramorium solidum*-group (Hymenoptera, Formicidae, Myrmicinae) from the Richtersveld National Park, South Africa, with an assessment of threats and conservation status

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Abstract

Eight specimens of an undescribed species of *Tetramorium* in the *T. solidum*-group were collected by pitfall trapping during an environmental impact assessment survey of the Oena Diamond Mine in the Richtersveld National Park, South Africa. The new species is most closely related to *T. grandinode* Santschi, 1913 but can be distinguished from this species by the distinctly different patterns of costulate sculpture on the mesosomal dorsum and petiole node, as well as the different forms of the petiole and postpetiole nodes, both of which in the new species are relatively narrower and longer and with no (petiole) or reduced (postpetiole) lateral extensions. *Tetramorium nama* **sp. nov.** is described here and a modification to the key presented by Mbanyana et al. (2018) to accommodate the additional species is provided. The potential threats to the species posed by alluvial diamond mining, livestock farming and climate change are discussed. A preliminary assessment of the conservation status of *Tetramorium nama* **sp. nov** is presented and suggests that, dependant on determination of the range of the species, it is likely to be classified as Vulnerable (VU) or Endangered (EN) under IUCN Red List criterion B1ab(iii).

Keywords

Afrotropical, climate change, conservation, livestock, mining, taxonomy

Introduction

A field survey for an assessment of the potential impacts of proposed extended operating hours at the Oena Diamond Mine (ODM) in the Richtersveld National Park (RNP) on terrestrial invertebrate populations, was undertaken in September 2019. Sampling of ant populations by pitfall trapping and active searching in the ODM mineral lease area and nearby sites within the RNP yielded a total of 41 species. These included at least five undescribed species: one *Camponotus*, three *Monomorium* and one *Tetramorium*, the latter being the focus of this paper. The recognition of several potential threats to the survival of invertebrate species in the ODM mineral lease area and surrounding region leads to a need for formal conservation assessments which in turn leads to a requirement for formal descriptions of the new species.

The *T. solidum*-group, to which the new *Tetramorium* species belongs, was revised by Bolton (1980). He listed 13 species, later assigning *T. rothschildi* (Forel, 1907) as the 14th species in the group upon the synonymy of *Triglyphothrix* under *Tetramorium* (Bolton 1985). The *T. solidum*-group was more recently revised by Mbanyana et al. (2018), who described five new species and presented an illustrated key to the 19 species then known. Another group-level revision in the near future is unlikely and description of a single additional species to enable identification and formal conservation assessment is thus justified. A description of *Tetramorium nama* sp. nov. and an additional couplet to be used in conjunction with the existing key in Mbanyana et al. (2018) to allow identification of the new species are therefore presented here. As this publication is intended to be used in conjunction with Mbanyana et al. (2018), the reader is referred to the latter for a full diagnosis and overview of the *T. solidum* species-group.

Members of the *T. solidum*-group are believed to be predominantly granivorous (Bolton 1980; Mbanyana et al. 2018), but no detailed studies on food preferences appear to have been done. The group is largely restricted to southern Africa and displays its greatest diversity in the arid south-western parts of this region, within which the RNP lies. Factors such as climate change, livestock grazing and habitat transformation by mining all have the potential to affect habitat and food availability within the RNP for species in this group. A preliminary assessment of the conservation status of *T. nama* sp. nov. and a discussion of potential threats to the survival of this and other ant species in the region are therefore also presented.

Materials and methods

Pitfall trap sampling was carried out between 11 and 18 September 2019 in ten transects, each with 20 traps set at 10 m intervals and run for six days; the traps were each filled with 50 ml of a 1:1 mixture of 95% ethanol and propylene glycol. Transects were placed in each of the main habitat types (riverine fringe, alluvial terrace gravels, alluvial fans, aeolian sands, Mispah soils and mountain slopes) within the ODM mineral lease area, which extends for approximately 16 km along the banks of the Orange River on the northern border of the Richtersveld National Park. Specimens were collected under permit # RNP03/19 issued by SANParks. Representatives of each morphospecies recognised in the samples were subsequently point-mounted for identification.

Measurements of mounted specimens were taken using a Leica MZ16 stereomicroscope equipped with an axial shift carrier and an ocular graticule calibrated against a stage micrometer. Specimens were photographed using a Leica DFC 425 digital camera connected to a Leica Z16APO Macroscope. Images were captured using Leica Application Systems (LAS) multifocus V4.9; montage images were generated using Helicon Focus V6.8.0 and edited with Adobe Photoshop CS3.

Terminology

Terminology relating to adult morphology largely follows Hita Garcia and Fischer (2014) as used by Mbanyana et al. (2018) with some differences as discussed below; descriptions of surface sculpture follow Harris (1979). All measurements (in millimetres) and indices are presented as minimum and maximum, with arithmetic means in parentheses. Abbreviations of the measurements taken and the indices based on them are as follows:

Measurements

EL	Eye length: maximum diameter of the compound eye measured in oblique lateral view;
HL	Head length: length of head measured in full-face view, from the midpoint of the clypeal margin to the midpoint of the occipital margin; where either of these margins is concave the measurement is taken from the midpoint of a line joining the anterior-most portions of the clypeus or the posterior-most portions of the occipital margin so that the impressions on these margins do not reduce HL;
HL2	Head length 2: maximum distance from the midpoint of the anterior clypeal margin to the midpoint of the posterior margin of head, measured in full-face view; impressions on the anterior clypeal margin and the posterior head margin reduce HL2;
HW	Head width: width of the head directly behind the eyes measured in full-face view;
PH	Pronotal height: maximum height of the pronotum measured in lateral view;
PPH	Postpetiole height: maximum height of the postpetiole measured in lateral view from the highest (median) point of the node to the ventral outline; the measuring line is placed at an orthogonal angle to the ventral outline of the node;
PPL	Postpetiole length: maximum length of the postpetiole measured in lateral view;
PPW	Postpetiole width: maximum width of the postpetiole measured in dorsal view;
PSL	Propodeal spine length: in dorsofrontal view the tip of the measured spine, its base, and the centre of the propodeal concavity between the spines must all be in focus; using a dual-axis micrometer the spine length is measured from the tip of the spine to a virtual point at its base where the spine axis meets orthogonally with a line leading to the median point of the concavity;

PTH	Petiolar node height: maximum height of the petiolar node measured in lateral view from the highest (median) point of the node to the ventral outline;
PTL	Petiolar node length: maximum length of the dorsal face of the petiolar node from the anterodorsal to the posterodorsal angle, measured in lateral view;
PTW	Petiolar node width: maximum width of the dorsal face of the petiolar node measured in dorsal view;
PW	Pronotal width: maximum width of the pronotum measured in dorsal view;
SL	Scape length: maximum scape length excluding basal condyle and neck;
WL	Weber's length: diagonal length of mesosoma in profile, from the junction of the pronotum and the cervical shield, to the posterior basal angle of the metapleuron.

Indices

CI	Cephalic index: HW / HL × 100;
CI2	Cephalic index 2: HW / HL2 × 100;
DMI	Dorsal mesosoma index: PW / WL × 100;
DPeI	Dorsal petiole index: PTW / PTL × 100;
DPpI	Dorsal postpetiole index: PPW / PPL × 100;
LMI	Lateral mesosoma index: PH / WL × 100;
LPeI	Lateral petiole index: PTL / PTH × 100;
LPpI	Lateral postpetiole index: PPL / PPH × 100;
OI	Ocular index: EL / HW × 100;
PeNI	Petiolar node index: PTW / PW × 100;
PPI	Postpetiole index: PPW / PTW × 100;
PpNI	Postpetiolar node index: PPW / PW × 100;
PSLI	Propodeal spine index: PSL / HL × 100;
PSLI2	Propodeal spine index: PSL / HL2 × 100;
SI	Scape index: SL / HW × 100.

Note that 1) HL2 and the CI2 and PSLI2 indices derived from this are included for equivalence with HL, CI and PSLI as reported in Mbanyana et al. (2018), who in following Hita Garcia and Fischer (2014) used for HL the measurement here defined as HL2, 2) petiole node length and postpetiole length are more accurately measured in profile view than in dorsal view as described in Hita Garcia and Fischer (2014), and 3) PeNI and PpNI values as defined here and in Hita Garcia and Fischer (2014) were reported in Mbanyana et al. (2018) but the definitions were omitted. Measurements and indices of all specimens are available in Suppl. material. 1: File S1: Measurements_Tetramorium_nama.xls.

Abbreviations of depositories

AFRC	AfriBugs Collection, Pretoria, South Africa
BMNH	The Natural History Museum, London, UK
CASC	California Academy of Sciences Collection, San Francisco, USA
SAMC	Iziko South African Museum Collection, Cape Town, South Africa

Additional couplet to insert in key of Mbanyana et al. (2018)

The thoroughly illustrated key provided in Mbanyana et al. (2018) contains 18 couplets to distinguish between 19 species; the couplets below are intended to be inserted at the position of couplet 8 in the existing key, with the additional couplet designated as 8a, to allow the remainder of the key to continue unaltered from couplet 9.

8 Petiole node distinctly broader than long (DPeI 145–165) **8a**

– Petiole node usually broader than long, but not distinctly so, sometimes slightly longer than broad (DPeI 90–135)... ....**continue from couplet 9 in Mbanyana et al. (2018)**

8a Mesosoma and petiole dorsally finely reticulate, postpetiole dorsum smooth to finely reticulate, with scattered piligerous foveolae, postpetiole narrower (DPpI 162–179)....***T. lerouxi*** **Mbanyana, Robertson & Hita Garcia, 2018**

– Mesosoma dorsum longitudinally costulate, petiole dorsum with roughly concentric to irregularly transverse costulae, postpetiole transversely costulate, postpetiole broader (DPpI 184–201)......................... ***T. nama*** **sp. nov.**

Tetramorium nama sp. nov.

http://zoobank.org/95780860-8777-4385-BF0F-19FE0027A692
Figures 1A–C, 2A, 3

Type material. ***Holotype.*** South Africa • **1worker**; Northern Cape Province, Namakwa, Richtersveld National Park; 28.05795S, 17.11256E ±100 m; alt. 65 ±10 m a.s.l.; P.G. Hawkes, D. Molenaar, R.N. Ungerer leg.; 12–18 Sep. 2019; Collection number: ODM2019-T8-1; Pitfall trap; Point-mounted; SAMC CASENT0818990.

Paratypes. South Africa • **6 workers**; same data as for holotype; Point-mounted; AFRC CASENT0818735, CASENT0818736, BMNH CASENT0818992, CASC CASENT0818993, CASENT0818994, SAMC CASENT0818991; • **1 worker**; same data as for holotype; 95% ethanol; AFRC CASENT0819639.

Diagnosis. Workers. *Tetramorium nama* sp. nov. is morphologically most similar to *T. grandinode* Santschi, 1913, with which it shares almost identical cephalic sculpture, but can be easily separated from this species by the following characters: in *T. nama* sp. nov., the longitudinal costulae on the dorsum of the promesonotum run parallel and separate all the way to the anterior pronotal margin (Figure 2A), whereas in *T. grandinode* the costulae become strongly arcuate anteriorly, with lines from either side of the midline usually joining medially and forming a nested set of hyperbolic curves (Figure 2B); in *T. nama* sp. nov., the petiole dorsum is much longer and has irregular concentric or whorled to irregularly transverse costulate sculpture (Figure 2A), while in *T. grandinode* the petiole node is very short and has strong transverse costulae on its dorsal surface (Figure 2B). The petiole node in *T. nama* sp. nov. is narrower (only slightly wider than the distance between the propodeal spine tips) than in *T. grandinode* (in which the petiole node is ca. 1.4–1.5 times wider than the distance between the propodeal spine tips). From *T. lerouxi* Mbanyana, Robertson & Hita Garcia, 2018, the only other glabrous species with the petiole node distinctly laterally expanded, *T. nama* sp. nov. can be readily distinguished by the sculpture of the dorsal mesosoma and petiole segments as indicated in the key. *T. nama* sp. nov. shares similar head, mesosoma, petiole and postpetiole sculpture with *T. duncani* Mbanyana, Robertson & Hita Garcia, 2018, but can be distinguished from this species as the former has substantially longer propodeal spines (PSLI2 23–25 vs. 12–17) a much shorter petiole node (LPeI 66–70 vs. 78–96), a distinct subpetiolar process (which *T. duncani* lacks) and a much less pronounced sub-postpetiolar process (very well-developed in *T. duncani*).

It should be noted that some specimens referred by Mbanyana et al. (2018) to *T. grandinode* have substantially narrower petiole and postpetiole nodes than seen in typical *T. grandinode* sensu Bolton (1980). As a result, Mbanyana et al. (2018) reported for *T. grandinode* extreme ranges of PeNI (56–83) and PpNI (68–110) which respectively overlap with the ranges they presented for 13 and eight of the 18 other *solidum*-group species they listed, as well as those of *T. nama*. However, Mbanyana et al. (2018) did not formally integrate their atypical material into *T. grandinode* and provide an updated and expanded description to accommodate these specimens. Both their key and diagnosis thus follow Bolton (1980) in emphasising the extremely broad petiole and postpetiole nodes as the main identifying characters for this species. Any atypical specimens listed by Mbanyana et al. (2018) with PpNI near or below that of *T. lerouxi* (PpNI 78–80) or *T. nama* sp. nov. (PpNI 71–76) will thus fail to key to *T. grandinode* and cannot be considered to fall within this species as defined by the most recent description (Bolton 1980) and diagnosis (Mbanyana et al. 2018). *Tetramorium nama* sp. nov. is therefore distinguished here from typical *T. grandinode* only. The placement of the atypical material referred to the latter by Mbanyana et al. (2018) requires future re-evaluation; some of this material may prove to belong to *T. nama* sp. nov, and/or may represent additional undescribed species.

Description. Workers. Measurements. ***Holotype*** CASENT0818990: HL 1.21, HL2 1.17, HW 1.10, SL 0.85, EL 0.31, PH 0.56, PW 0.74, WL 1.32, PSL 0.28, PTH 0.42, PTL 0.29, PTW 0.43, PPH 0.42, PPL 0.29, PPW 0.56. **Indices**: CI 91, CI2 94, DMI 56, DPeI 147, DPpI 191, OI 28, SI 77, LMI 42, PSLI 23, PSLI2 24, PeNI 58, LPeI 70), LPpI 69, PpNI 76, PPI 130 (all measurements in mm, indices as percentage).

Paratypes (6 measured, mean including holotype in parentheses): HL 1.14–1.22 (1.19), HL2 1.11–1.19 (1.15), HW 1.04–1.13 (1.09), SL 0.80–0.86 (0.83), EL 0.30–0.33 (0.32), PH 0.54–0.56 (0.55), PW 0.68–0.75 (0.73), WL 1.17–1.32 (1.24), PSL 0.26–0.29 (0.27), PTH 0.38–0.43 (0.41), PTL 0.27–0.29 (0.28), PTW 0.39–0.44 (0.42), PPH 0.39–0.43 (0.42), PPL 0.26–0.29 (0.28), PPW 0.49–0.56 (0.53). **Indices**: CI 90–93 (92), CI2 94–96 (95), DMI 58–60 (59), DPeI 148–158 (152), DPpI 184–201 (194), OI 28–30 (29), SI 74–77 (76), LMI 44–46 (44), PSLI 22–24 (23), PSLI2 23–25 (24), PeNI 56–60 (58), LPeI 66–70 (68), LPpI 64–69 (66), PpNI 71–75 (73), PPI 124–127 (126) (all measurements in mm, indices as percentage).

Head a little longer than wide (CI 92), posterior margin shallowly indented medially, sides slightly convex, rounding posteriorly into the broadly convex occipital corners. Clypeus with a distinct but shallow medial indentation and ca. 10–12 longitudinal costulae overlain on a very effaced shagreenate ground sculpture, the surface between the costulae almost smooth, moderately shining. Frontal carinae fading out behind the frontal lobes and merging with the remaining cephalic sculpture from the level of the anterior margin of the eyes, often broken behind this point, but occasionally fairly long and reaching to midway between the level of the posterior margin of the eyes and the occipital margin. Eyes large, situated at approximately the mid-length of the sides of the head and with 16–17 ommatidia in the longest row, ocelli absent. Mandibles weakly longitudinally rugose, with seven teeth. Psammophore well-developed, comprising a row of ca. 10–14 elongate J-shaped hairs on the ventral surface of the head behind the posterior margin of the buccal cavity, two rows of ca. five hairs each along the distal inner and outer ventral margins of each mandible and a cluster of four or five hairs proximally on the ventral mandibular margins. Antennal scapes short, stout, basally curved and distally thickened; when laid back, scapes fall short of the posterior margin of the head by ca. one-fifth of their length. Scapes with strong appressed pubescence, lacking erect setae, remaining antennal segments with sub-appressed pubescence. Scapes weakly longitudinally carinulate, the remaining antennal segments smooth and shining, unsculptured except for piligerous punctures. Dorsal surface of head with dense longitudinal costulae, ca. 22 between the frontal carinae at the level of the mid-point of the eyes; costulae approximately parallel medially but diverging posterolaterally toward the occipital corners, behind which they reflex and continue anterad on the lateral surface of the head, a few above, but most below the eyes. Spaces between the costulae shiny and almost smooth, with faint reticulate/shagreenate sculpture.

Mesosoma laterally with irregular longitudinal costulae overlaid on a weak reticulate-punctate ground sculpture, anterodorsally with one continuous and several broken transverse marginal costulae; dorsally with effaced reticulate-punctate ground sculpture overlain by fairly uniform longitudinal costulae that continue running approximately parallel all the way to the pronotal margin, where they either fade out or join approximately at right angles with one of the transverse components. Propodeal spiracles round, situated slightly above the mid-height of the sides of the propodeum. Propodeal spines long and acute (PSLI 22–24). Declivity dorsally shallowly concave in dorsal view but straight ventrally. Ground sculpture of propodeum shagreenate to reticulate-punctate, overlain by costulae which are longitudinal on the dorsal face but transverse on the declivitous face and between the bases of the propodeal spines. Propodeal lobes broadly rounded.

Petiole node in dorsal view subtrapezoidal, distinctly wider behind than in front and distinctly wider than long, in profile with a broadly rounded anterodorsal margin and an acute, slightly overhanging posterodorsal margin. Subpetiolar process a narrow lamella subtended by a short but distinct anteriorly orientated tooth. Dorsal surface of node usually with several irregular roughly concentric or whorled costulae, in some specimens the costulae are irregularly transverse.

Postpetiole node distinctly broader than the petiole node in dorsal view, with irregular, slightly recurved transverse costulae over the entire dorsal surface, the anterior face of the node slightly concave, the posterior face slightly convex.

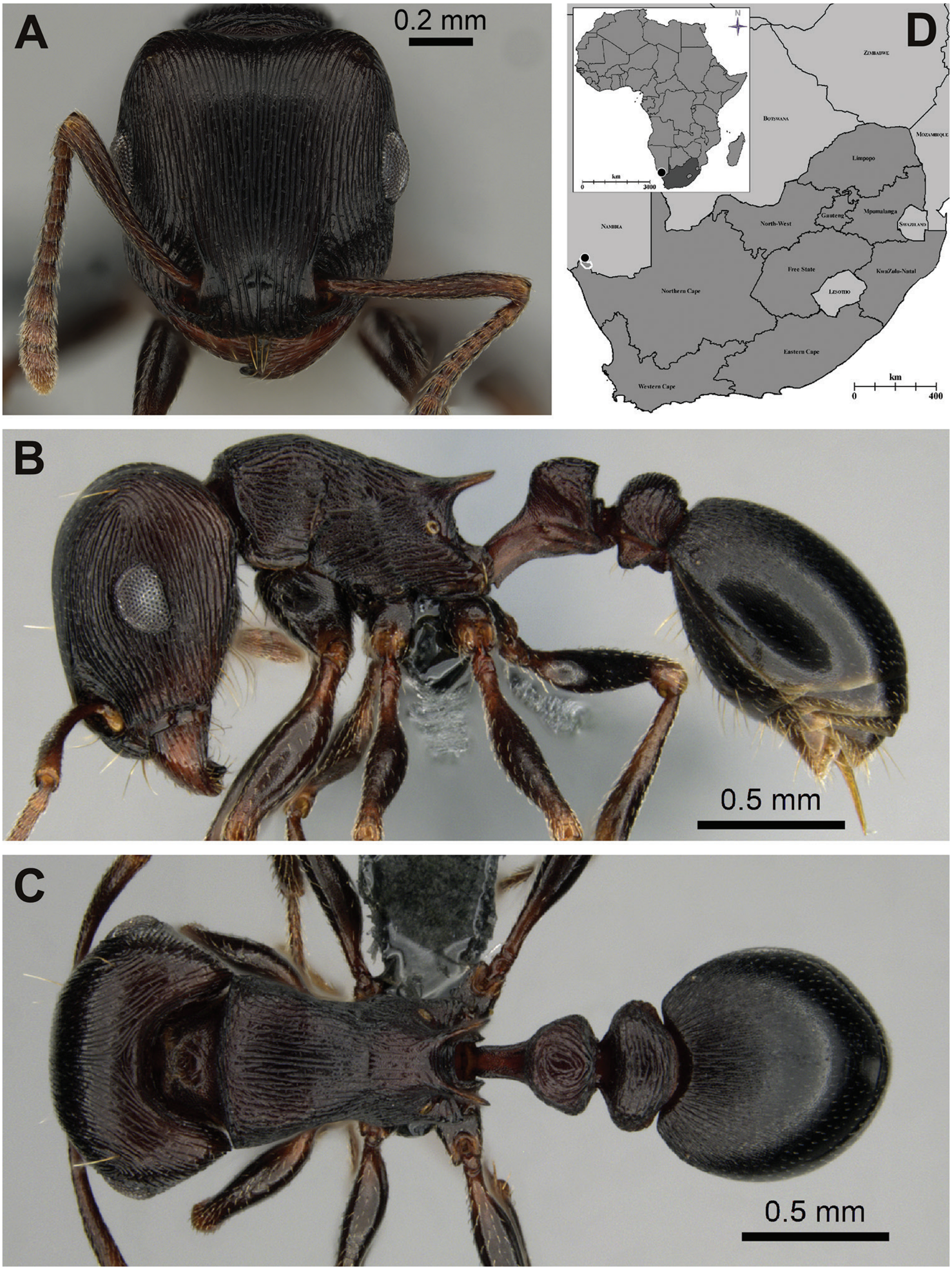

Figure 1. *Tetramorium nama* sp. nov. **A–C** holotype worker, CASENT0818990: **A** full-face view **B** lateral view **C** dorsal view **D** map of Africa (inset, with South Africa shaded dark grey) and detail of South Africa (main image), showing known distribution of *Tetramorium nama* sp. nov. (black dot), with a white outline indicating the Richtersveld National Park. (Photos by Bradley Reynolds, from www.AntWeb.org)

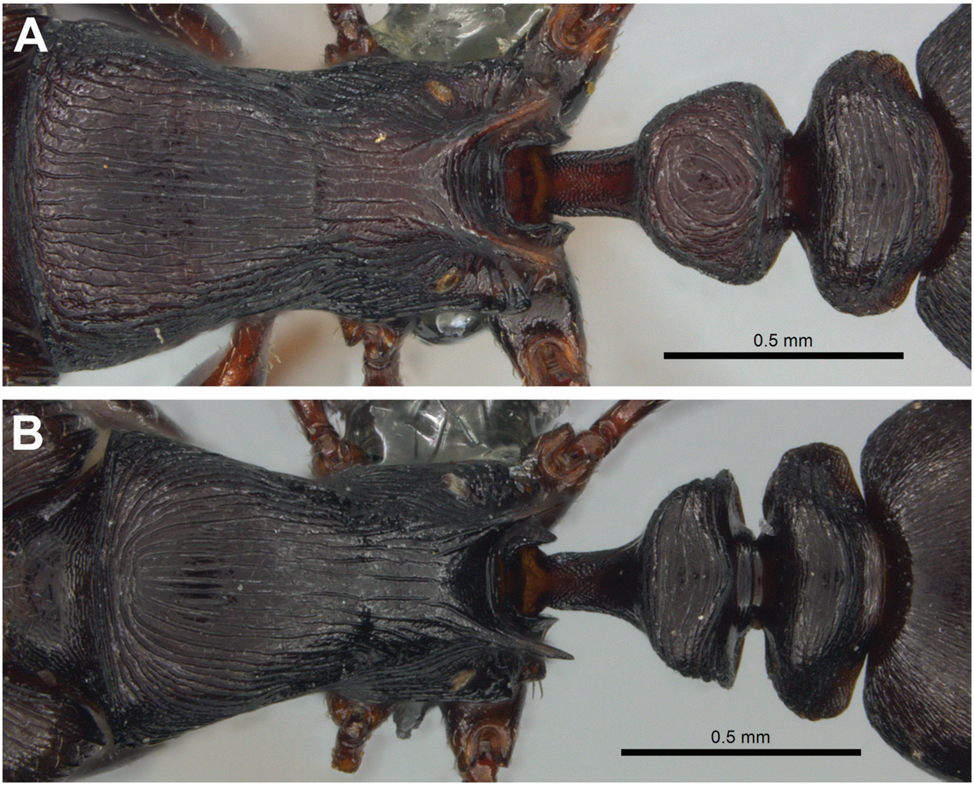

Figure 2. Dorsal view of mesosoma and petiole segments **A** *Tetramorium nama* sp. nov. holotype worker, CASENT0818990 **B** *T. grandinode*, CASENT0818846. (Photos by Peter Hawkes, from www.AntWeb.org)

Gaster with dense but fine basigastral costulae radiating over the anterior third of the first tergite, overlain on a dense shagreenate ground sculpture which weakens posteriorly. Sting present, weakly curved ventrad, with a distinct pennant-shaped lamellate appendage.

Legs with fairly uniform shagreenate sculpture throughout.

***Pilosity*:** two pairs of erect setae are present on the dorsal surface of the head behind the frontal lobes; one about level with the anterior margin of the eyes and one close to the occipital corners. Standing hairs are absent from all dorsal surfaces of the mesosoma, petiole segments, the first and usually the second gastral segment (the latter occasionally with one to two pairs of very short suberect setae). Dorsal surfaces of head, petiole, postpetiole and gaster with scattered appressed pubescence, which is very sparse, reduced in length and appearing virtually absent from the dorsal mesosoma. Femora and tibiae with appressed to sub-appressed pubescence on all surfaces. Gastral sternites with appressed pubescence and long erect setae, which on the first sternite are concentrated medially and posteriorly.

Colour mainly dark blackish brown, the mandibles a distinctly contrasting reddish brown; antennae, legs and peduncle of petiole similar to the mandibles but with the median portions of the femora and tibiae darker and similar to the body colour.

The type series is very consistent in overall appearance and colour, but there is some variation in the pattern of sculpture on the petiole node as indicated above. Worker measurements and proportions are quite consistent with measurement ranges 4–13% and index ranges 2–9% of their mean values.

Queen and male unknown.

Etymology. *nama* refers to the local Nama people who, together with SANParks, jointly manage the Richtersveld National Park. The specific epithet is a noun in apposition and is thus invariant.

Habitat and distribution. *Tetramorium nama* sp. nov. is known only from a single collection of eight workers from one pitfall trap, which was situated in an area transitional between Lower Gariep Alluvial Vegetation and Noms Mountain Desert (Mucina and Rutherford 2006). The pitfall trap transect was located in open vegetation between closed thicket on the banks of the Orange River and alluvial terrace gravels further from the river. The site is located in the extreme north of the RNP in the Namakwa district of the Northern Cape, South Africa; more extensive surveys in the surrounding region are required to determine the extent of occurrence of the species.

Discussion

Related species, habitat and distribution

Specimens representing three other species within the *Tetramorium solidum*-group were also found during the 2019 survey of the Oena Diamond Mine; these included three specimens of *T. grandinode* Santschi, 1913, 113 of *T. pogonion* Bolton, 1980 and 390 of *T. rufescens* Stitz, 1923. *Tetramorium grandinode* and *T. rufescens* are fairly widespread and are known respectively from at least 22 and 38 other sites in Namibia and South Africa (Bolton 1980; Mbanyana et al. 2018; AntWeb 2020), but the *T. pogonion* specimens represent only the second record of this species and the first record in South Africa. *Tetramorium nama* sp. nov. and *T. grandinode* were rare in the ODM project area and found in only one and two of the 200 pitfall trap samples respectively, while *T. pogonion* and *T. rufescens* were relatively common and were found in 22 and 40 samples respectively. The habitat in which the *T. nama* sp. nov. and *T. grandinode* specimens were found was open riverine fringe vegetation dominated by two indigenous tree species, *Euclea pseudebenus* E. Mey. ex A. DC (Black Ebony) and *Tamarix usneoides* E. Mey. ex Bunge (Wild Tamarisk), although a number of other tree species were present in smaller numbers. Substantial invasion by *Prosopis glandulosa* Torr. (Honey Mesquite) has occurred in the area and is the subject of an intensive eradication campaign by the Department of Environmental Affairs' Working for Water (WfW) programme; many cut and poisoned stumps, as well as piles of both dry and freshly cut growth were observed within the survey area (see Figure 3).

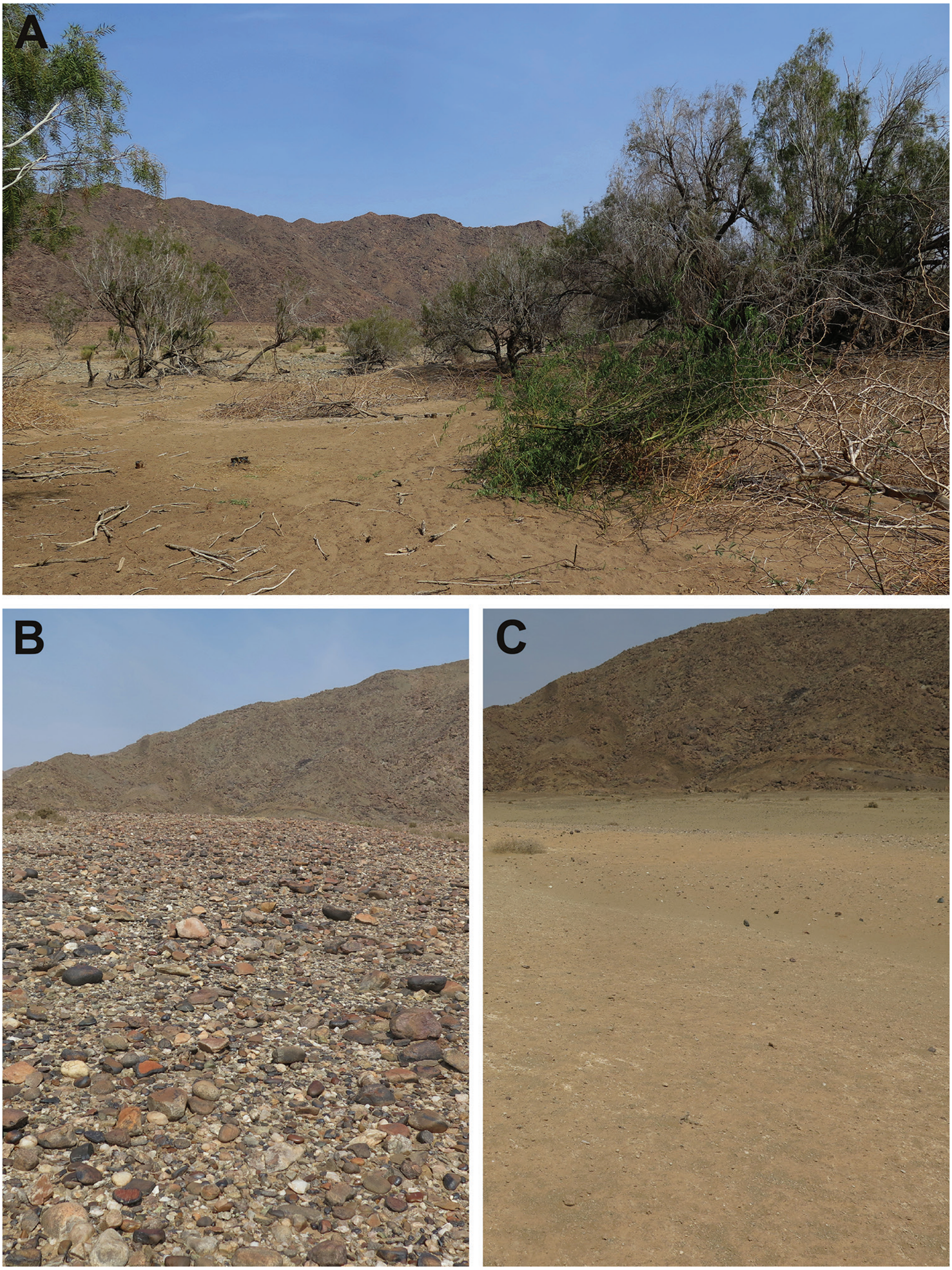

Figure 3. A riverine fringe habitat in which *Tetramorium nama* sp. nov. was collected, showing living vegetation dominated by *Euclea pseudebenus* as well as numerous cut *Prosopis glandulosa* (freshly cut and piles of dry branches) **B** alluvial terrace gravel and **C** alluvial fan habitats (in which *T. pogonion* and *T. rufescens* were collected) between the river and the rocky mountains to the southwest: the alluvial fan lies between the gravel terrace and the mountains.

Tetramorium nama sp. nov. was found only within the riverine fringe vegetation during the September 2019 ODM survey, but the single record provides insufficient information to indicate whether or not the species might inhabit other areas within the RNP. Two other seed-harvesting species, *T. pogonion* and *T. rufescens*, each occurred in five of the six habitat types sampled, together inhabiting all habitat types and suggesting the potential suitability of these habitats for *T. nama* sp. nov. as well. The absence of any live grasses and apparent dormancy of all non-tree species in Figure 3 highlights the importance of seed storage by *T. solidum*-group species in ensuring their survival through drought periods. Further, more intensive, surveys would be required to detect habitat preferences of *T. nama* sp. nov.

The distribution of *T. nama* sp. nov. beyond the type locality also cannot be determined at this stage, but this species was absent from all of the institutional collections mentioned in Mbanyana et al. (2018) and was not found during any of the targeted surveys that these authors carried out in the Northern Cape and Namibia for *T. solidum*-group species. No specimens of this species were found during a survey of 48 sites in the Central Namib Desert in 2010 (circa 500 km north of the type locality), nor in several intensive surveys on and around Gamsberg (circa 200 km SE of the type locality) in the Bushmanland Inselberg Region of the Northern Cape from 2009–2017 (unpublished data, AFRC). This suggests that *T. nama* sp. nov. has a limited distribution, or is very rare within its range, or possibly both. It is however fairly certain that the species will occur in southern Namibia, even if only in habitat similar to where it was found, on the opposite banks of the Orange River.

Conservation and threats

The future of the Richtersveld National Park

The RNP is situated in the extreme northern part of the Northern Cape Province in South Africa and forms part of the |Ai-|Ais/Richtersveld Transfrontier Park. The RNP is unusual in that human inhabitants and alluvial diamond mining operations have continued to occupy the park since its proclamation in 1991. The South African National Parks (SANParks) manages the park as a communal pasture on a contractual basis, and 26 registered farmers from the local Nama community have the right to graze the equivalent of a total of 6,600 head of small livestock units (SLU) within the park boundaries.

The initial 30-year contract park agreement that came into effect in 1991 will expire in 2021, but a new agreement has been negotiated and is expected to be signed in the near future, thereby ensuring continued protection of the park for at least another 30 years. The extreme aridity of the environment means that alternative uses are unlikely to be viable and the park is thus likely to persist in the long-term.

Climate change

The climate of the RNP is harsh; mean annual precipitation (MAP) ranges from ca. 55 mm in the north and northeast to ca. 125 mm in the southwest of the park (World-

Clim dataset, Hijmans et al. 2005), with peak temperatures at times exceeding 50 °C (SANParks 2018). In addition, rainfall is highly sporadic and patchy and some localities may receive no rain for several years; a site in the Tatasberg had no rainfall recorded from 2000–2016, during which period the average MAP for the park interior was 38 mm and one site received 163 mm MAP (SANParks 2018). While species endemic to the area clearly must be adapted to such conditions, changing climate may result in some species' tolerances being exceeded.

From 1990 to 2010 the RNP had already experienced an increase of 1.2 °C in mean maximum and 1.1 °C in mean minimum temperatures, exceeding the near-future (2035) predictions utilised by the South Africa Department of Environmental Affairs (DEA) for planning purposes, and there had been a significant increase in the number of days exceeding 35 °C (Wilgen et al. 2015). In conjunction with no change in MAP (but with a higher number of smaller rainfall events) this indicates an increase in aridity in the region from 1990–2010. A subsequent severe drought from 2016 to present, during which Sendelingsdrift (23 km west of the type locality of *T. nama* sp. nov.) has received an annual average of just over 6% of its historic MAP for four years, has resulted in large-scale vegetation loss. Even extreme arid-adapted species such as the three quiver tree species (*Aloidendron dichotomum* (Masson) Klopper & Gideon F. Sm., *A. pillansii* (L. Guthrie) Klopper & Gideon F. Sm. and *A. ramosissimum* (Pillans) Klopper & Gideon F. Sm.) present in the park have experienced extreme die-off, with an approximately 70% reduction in numbers of live trees (B. Whittington, pers. comm.). If such changes continue and the further temperature rises predicted for the coming decades occur, the RNP will become increasingly desert-like (SANParks 2018).

It is thus likely that local conditions could become unsuitable for species such as *Tetramorium nama* sp. nov. and they would have to track suitable habitat and climatic conditions in order to survive. Conditions suitable for nuptial flights often would not occur for periods of several to many years in the northern RNP and it is only by flighted dispersal that these ants could migrate far enough to track substantial climatic changes. Given the rapidity of recent climatic change it is possible that they might not be able to respond quickly enough and might therefore become locally or even globally extinct.

Mining

Alluvial diamond mining has been carried out along the banks of the Orange River in the Richtersveld since around 1900; mining at Oena started in 1992, though the mine has changed ownership since then and is currently operated by African Star Minerals (ASM). Mining operations have been carried out at three areas (Oena, Sandberg and Blokwerf) within the mineral lease area, but have at least temporarily been suspended at one (Blokwerf). Mining operations are opencast, with topsoil (where present) being stripped and stockpiled, after which the alluvial gravels are excavated, screened and processed through rotary pans to provide concentrate for extraction in the recovery plant. Approximately 80% of the coarse waste is returned to the excavated areas to be used as backfill, with the remainder deposited in waste rock dumps. The fine tailings slurry is deposited in a dam and will eventually either be covered with coarse tailings or returned to excavated areas as backfill. The backfilled pits are covered with topsoil where this is available (topsoil is absent from much of the terrace gravel areas) and the area re-contoured to a natural-looking state. However, virtually all plants and animals within the mined deposits and in areas where the processed gravels are dumped will be killed. The resulting soil structure will also probably differ very substantially from the natural alluvial deposits and rehabilitation can be expected to be extremely slow given the arid nature of the environment. All mined areas and those covered by waste rock or tailings are therefore effectively lost as habitat for indigenous species for a considerable period, which may be many decades. The area likely to be disturbed by currently planned mining activities constitutes only ca. 20% of the alluvial plains within the Oena mineral lease area, but this could be expanded (prospecting is continuing at two sections upstream of the three areas listed above) and a significant area had been disturbed by previous operations and has not yet been rehabilitated; this is to be rectified as part of the current environmental management plan (ASM 2018). The total area of habitat loss could therefore be a significant proportion of the total available.

The potential impact of artificial lighting of mining operations on *Tetramorium nama* sp. nov. cannot be determined at this stage as it is not known whether the nuptial flights of this species take place during the day or night. Impacts on nocturnal nuptial swarms could be severe, especially considering the extremely sporadic nature of suitable conditions for such events. Dust is another potentially significant factor that may reduce food availability via impacts on plants.

Livestock grazing

Members of the local Nama community run herds primarily of domestic goats, *Capra aegagrus hircus* (Linnaeus, 1758), with the total domesticated livestock population within the RNP capped at 6,600 small livestock units (a stocking rate of approximately 25 ha per SLU). However, the riverine fringe along the banks of the Orange River where *Tetramorium nama* sp. nov. was collected is probably the most intensively utilised habitat within the park, as it provides fodder during the long dry periods when the vegetation further from the river provides very little forage (SANParks 2018).

Degradation of vegetation by goat overgrazing is recognised to be more severe than that from any other ruminant livestock species, due to their ability to graze on residual biomass and woody species that would be left as vegetation cover by other livestock (Steinveld et al. 2006), as well as their ability to debark trees, range over large distances and survive without water for longer periods than other livestock (Lipson et al. 2011).

Intensive grazing even by domesticated goats would be expected to substantially reduce seed set by grasses and shrubs that provide food for *Tetramorium solidum*-group species. Continuation of the current trend of climate change is likely to reduce carrying capacity in the RNP and lead to an increased risk of overgrazing; regular review of grazing quotas should ideally be carried out as part of the park's Environmental Management Plan. Should feral populations of goats become established in the park, the cap on numbers would become very difficult to maintain and impacts could become far more severe, potentially virtually eliminating the food supply of granivorous ants and putting them at high risk of at least local extinction.

However, *T. signatum* Emery, 1895, another *T. solidum*-group species, has been observed (unpublished data) preying on live *Microhodotermes viator* (Latreille, 1804), which suggests that members of the group might not be wholly dependent on seeds as a food source. The extent to which termites and perhaps other insect prey can substitute for their normal food is unknown, but this may at least partially mitigate the impact of livestock such as goats on their food supply; further research is required.

Conservation assessment

Distribution data are, at present, too limited to allow a full IUCN Red List assessment (IUCN 2012) to be carried out and *T. nama* sp. nov. should currently be considered Data Deficient (DD). However, it is very likely that the species will prove to be restricted to the northernmost parts of the Northern Cape and southernmost parts of Namibia, given the lack of any records of the species from numerous sampling events to the north and south of this region. In light of the multiple threats discussed above (each of which is expected to contribute to continuing decline in quality and extent of habitat), even if the range proves to be as much as four times that of the entire RNP, the Extent of Occurrence (EOO) would fall within the range (< 20,000 km^2) for the species to be classified as Vulnerable (VU) under criterion B1ab(iii). The species may however be limited to a narrower region straddling the Orange River, which would lead to an Endangered (EN) classification under the same criterion if the EOO proves to be less than 5,000 km^2. Other ant species might be similarly threatened by the factors discussed here, although the impact of grazing would be expected to vary depending on their reliance on seeds or other plant-related food sources (such as honeydew from phytophagous homopterans).

Conclusions

The discovery of an undescribed *Tetramorium solidum*-group species so soon after the publication of a revision of the group, for which a substantial number of targeted surveys in the region had been carried out, suggests that there may be more short-range endemic species within the group inhabiting the ecologically complex Richtersveld region.

Tetramorium nama sp. nov. is likely to prove to be both rare and limited in range; together with the threats posed by climate change and livestock grazing, as well as current and planned mining activities in the |Ai|Ais/Richtersveld Transfrontier Park, it is thus likely that a formal assessment would indicate a categorisation of VU or higher following the IUCN criteria. Formal Red List assessments of the conservation status of range-restricted species such as *T. nama* sp. nov. may assist in enabling action to be taken to protect sufficient areas of natural habitat to ensure their continued survival and such assessments should be undertaken as a matter of urgency.

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Supplementary material 1

File S1

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Data type: measurement

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RESEARCH ARTICLE

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A new macrolepidopteran moth (Insecta, Lepidoptera, Geometridae) in Miocene Dominican amber

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Abstract

A new genus and species of fossil moth, *Miogeometrida chunjenshihi* Zhang, Shih & Shih, **gen. et sp. nov.**, assigned to Geometridae, is described from Miocene Dominican amber dating from 15–20 Mya. The new genus is characterized by the forewing without a fovea, R_1 not anastomosing with Sc, no areole formed by veins R_1 and Rs, R_1 and Rs_1 completely coincident, M_2 arising midway between M_1 and M_3, anal veins 1A and 2A fused for their entire lengths; and the hind wing with Rs running close to Sc + R_1 and M_2 absent.

Keywords

chunjenshihi, Ennominae, extinct, fossil, taxonomy

Introduction

Geometridae, the second most species-rich family of Lepidoptera, comprise approximately 24,000 described species (van Nieukerken et al. 2011; Murillo-Ramos et al. 2019). Geometridae are macrolepidopterans characterized by the presence of unique tympanal organs at the base of the abdomen, and the prolegs of their larvae reduced to two pairs causing the larvae to move by 'looping' (Minet and Scoble 1999). Geometridae were once classified into six subfamilies: Geometrinae, Ennominae, Sterrhinae, Larentiinae, Archiearinae and Oenochrominae *sensu lato* (Holloway 1994, 1996, 1997; Minet and Scoble 1999), but this classification was not fully satisfactory due to the fact that Oenochrominae *sensu lato* are a polyphyletic group (Scoble and Edwards 1990). Oenochrominae *sensu lato* were further divided into Oenochrominae *sensu stricto*, Desmobathrinae, Orthostixinae, and Alsophilinae, forming a classification system of nine subfamilies (Yamamoto and Sota 2007), but later Alsophilinae was subsumed in Ennominae (Wahlberg et al. 2010). Sihvonen et al. (2011) provided a comprehensive phylogeny of the Geometridae, and they found the previously recognized subfamilies to be monophyletic except the Oenochrominae + Desmobathrinae complex, which is a polyphyletic assemblage of taxa, and the Orthostixinae, which was positioned within the Ennominae. The systematic status of Orthostixinae remains uncertain, although Orthostixinae were synonymized with Desmobathrinae by Beljaev (2016). Systematic updates and annotated checklists of Western Palaearctic Geometridae were provided in "The Geometrid Moths of Europe" series (Hausmann 2001, 2004; Mironov 2003; Hausmann and Viidalepp 2012; Skou and Sihvonen 2015; Müller et al. 2019). Murillo-Ramos et al. (2019) established a new subfamily Epidesmiinae and transferred eight genera from Oenochrominae *sensu stricto* to Epidesmiinae.

The age of Geometroidea was calculated to trace back to 83 Mya (Wahlberg et al. 2013), and the age of Geometridae was estimated at ca 54 Mya (62–48 Mya, Yamamoto and Sota 2007). Recently, Kawahara et al. (2019) inferred a comprehensive phylogeny of Lepidoptera, and they dated the oldest members of the Lepidoptera crown group in the Late Carboniferous (ca 300 Mya), and speculated the ancestors of Geometroidea appeared in the Late Cretaceous. To date, 18 fossil records of Geometridae have been formally reported (Table 1). Harris and Raine (2002) reported a Late Cretaceous (Albian-Turonian, 113–89.8 Mya) lepidopterous genitalic fragment from New Zealand, and deemed its affinity probably lies within Geometridae, but Sohn et al. (2012) regarded the available characters insufficient to support a family-level diagnosis. The Eocene (56–33.9 Mya) species, *Eogeometer vadens* Fischer, Michalski & Hausmann, 2019, *Geometridites larentiiformis* Jarzembowski, 1980 and *Hydriomena* ? *protrita* Cockerell, 1922, respectively from the Baltic, UK, and USA, are believed to be the earliest representatives of Geometridae. However, most of the reported fossil geometrids are questionable. Evers (1907) assigned a specimen from Zanzibar Island to the extant genus *Hyperythra* and regarded this specimen as *H. lutea*, but Kozlov (1988) identified it as *Geometridites* sp. In addition, *Phalaenites proserpinae* Heer, 1861 was also considered as *Geometridites* sp. by Kozlov (1988). Lacking strong evidence, Sohn et al. (2012) disputed the Geometridae affiliation of *Problongos baudiliensis* Mérit & Mérit, 2008. Kusnezov (1941) treated *Angerona electrina* Giebel, 1862 as Macrolepidoptera *incertae sedis*. Grimaldi and Engel (2005) mentioned three specimens of Geometridae from Early Miocene Dominican amber (15–20 Mya), and provided pictures of these specimens.

Table 1. Fossil records of Geometridae.

	Subfamily	Genus	Species	Life cycle	Epoch	Locality/ Country	Reference	Note
1	Ennominae	*Angerona*	† *A. electrina*	adult	possibly Holocene	not stated	Giebel 1862; Sohn et al. 2012	Kusnezov (1941) treated this species as Macrolepidoptera *incertae sedis*.
2	Ennominae	† *Eogeometer*	† *E. vadens*	larva	Late Eocene-Early Oligocene	Baltic	Fischer et al. 2019	
3	Ennominae	*Hyperythra*	*H. lutea* ?	adult	Late Pleistocene	Tanzania	Evers 1907	Kozlov (1988) considered this specimen as *Geometridites* sp.
4	Ennominae	† *Problongos*	† *P. baudiliensis*	adult	Late Miocene	France	Mérit and Mérit 2008	Sohn et al. (2012) disputed the Geometridae affiliation of *Problongos baudiliensis*.
5	Larentiinae	*Hydriomena* ?	† *H.* ? *protrita*	adult	Late Eocene	USA	Cockerell 1922	
6	unassigned	† *Geometridites*	† *G. jordani*	adult	Late Pliocene	Germany	Kernbach 1967	
7	unassigned		† *G. repens*	larva	Late Pliocene	Germany	Kernbach 1967	
8	unassigned		† *G. larentiiformis*	adult	Late Eocene	United Kingdom	Jarzembowski 1980	
9	unassigned	† *Phalaenites*	† *P. crenatus*	adult	Early Miocene	Croatia	Heer 1849	
10	unassigned		† *P. obsoletus*	adult	Early Miocene	Croatia	Heer 1849	
11	unassigned		† *P. proserpinae*	adult	Late Oligocene-Early Miocene	France	Heer 1861	Kozlov (1988) considered this species as *Geometridites* sp.
12	unassigned	not stated	not stated	adult	Miocene	Dominican Republic	Grimaldi and Engel 2005: 568, fig. 13: 24	
13	unassigned	not stated	not stated	larva	Miocene	Dominican Republic	Grimaldi and Engel 2005: 588, fig. 13: 58	
14	unassigned	not stated	not stated	adult	Miocene	Dominican Republic	Grimaldi and Engel 2005: 588, fig. 13: 59, 60	
15	unassigned	not stated	not stated	adult	Late Cretaceous	New Zealand	Harris and Raine 2002: 461, fig. 1	Sohn et al. (2012) treated this as a questionable geometrid fossil.
16	unassigned	not stated	not stated	pupa	Late Pleistocene	Japan	FIRGNE 1990: 101, fig. 10.3.1	FIRGNE is Fossil Insect Research Group for Nojiri-ko Excavation.
17	unassigned	not stated	not stated	not stated	Pleistocene-Holocene	Benin and Guinea	Handlirsch 1908: 1133	
18	unassigned	not stated	not stated	not stated	Middle Eocene	Lutetian	Lewis 1992: 16	

†: extinct.

Here we describe a new genus and species of Geometridae based on an adult specimen preserved in Dominican amber. The age of Dominican amber-bearing deposits is the late Early Miocene through early Middle Miocene, ca 15 to 20 Mya (Iturralde-Vinent and Macphee 1996). Dominican amber, with exquisite preservation, contains a very rich Miocene biota with more than 400 described insect species (Arillo and Ortuño 2005). To date, 30 fossil records within seven superfamilies of Lepidoptera have been reported in Dominican amber (Poinar et al. 1991; Poinar and Brown 1993; Hall et al. 2004; Grimaldi and Engel 2005; Peñalver and Grimaldi 2006; Sohn et al. 2012). All these fossil records belong to the lepidopteran clade Ditrysia.

Materials and methods

The type specimen in amber described herein is housed in Laboratorio Dominicano De Ambar Y Gemas, Santo Domingo, Dominican Republic. The specimen was examined and photographed by using a Nikon SMZ 18 dissecting microscope with an attached Nikon DS-Ri2 digital camera system and a Leica M205A with an attached Leica DMC5400 digital camera system. These devices used cool white LED illuminators. Cool white transmitted light passed through the specimen from the bottom up, and cool white light, emitted from double optical fibers, irradiated the specimen from two sides simultaneously. Images were prepared for illustration using Adobe Photoshop CS6. Wing index is defined as the ratio of wing width/wing length. The body length was measured from the apex of head to the terminal end of abdomen. Family-level classification follows van Nieukerken et al. (2011). Wing venation nomenclature is based on Wootton (1979).

Systematic paleontology

Order Lepidoptera Linnaeus, 1758
Suborder Glossata Fabricius, 1775
Infraorder Heteroneura Tillyard, 1918
Superfamily Geometroidea Leach, 1815
Family Geometridae Leach, 1815
Subfamily Ennominae Duponchel, 1845

Genus *Miogeometrida* Zhang, Shih & Shih, gen. nov.

http://zoobank.org/9AB3E411-9767-4CFF-88F9-6E37C92081D1

Type species. *Miogeometrida chunjenshihi* Zhang, Shih & Shih, sp. nov.

Etymology. The generic name is a combination of the prefix "Mio-" in reference to the Miocene, and "geometrid" in reference to the family name. The gender is masculine.

Diagnosis of genus. Body length ca 5.7 mm, wingspan ca 20 mm. Antenna filiform. Forewing without fovea, R_1 not anastomosing with Sc, no areole formed by veins R_1 and Rs, R_1 and Rs_1 completely coincident, M_2 arising midway between M_1 and M_3, anal veins 1A and 2A fused for its entire length. Hind wing with Rs running close to Sc + R_1, and M_2 absent.

Remarks. The new genus can be distinguished from most extant or extinct geometrids by the absence of an areole formed by veins R_1 and Rs. As *Miogeometrida* gen. nov. lacks M_2 on the hind wing, affiliation with other subfamilies than Ennominae is excluded. *Miogeometrida* gen. nov. differs from most genera of Ennominae in its forewing without fovea and R_1 not anastomosing with Sc. *Miogeometrida* gen. nov. is similar to genera such as *Ekboarmia* (Ennominae, Boarmiini, covered in Skou et al. 2017) and *Iridopsis* (Ennominae, Boarmiini, covered in McGuffin 1977) in venation and the absence of a fovea, but the antennae of the latter are pectinated in males. Apart from this, extant *Iridopsis* are much larger than *Miogeometrida* gen. nov. on average. *Miogeometrida* gen. nov. also shows similarities with genera such as *Milocera*, *Chelotephrina*, *Tephrina*, *Isturgia* and *Macaria* (Ennominae, Macariini, covered in Krüger 2001) in the forewing with R_1 and Rs_1 completely coincident and hind wing with two anal veins, but *Miogeometrida* gen. nov. differs from them in its forewing with 1A and 2A fused for their entire lengths.

Grimaldi and Engel (2005) mentioned three specimens of Geometridae from Dominican amber and provided a photo and a line drawing of one specimen (Grimaldi and Engel 2005: 588, fig. 13: 59, 60). According to the line drawing (Grimaldi and Engel 2005: 588, fig. 13: 60), the stem of M is present on its forewing. But in *Miogeometrida* gen. nov., the loss of the stem of M results in the formation of one large discal cell. *Miogeometrida* gen. nov. differs from the Eocene species *Geometridites larentiiformis* by the absence of the areole and R_1 completely coincident with Rs_1 on the forewing. Mérit and Mérit (2008) reported Miocene *Problongos*, whose forewing length is twice as long as that of *Miogeometrida* gen. nov. (22 mm vs. 8.9 mm).

Miogeometrida chunjenshihi **Zhang, Shih & Shih, sp. nov.**

http://zoobank.org/B0B59F0C-43DB-4B48-8031-8EED7747EB43
Figures 1, 2

Material. ***Holotype***: LEP-DA-2019001, male. Mouthparts, mid- and hind legs, abdominal sternum missing.

Etymology. The specific name is dedicated to Chun Jen Shih, father of YuHong Shih, for his discovery of the type specimen and his efforts and dedication in collecting and promoting Dominican amber, especially his classification system for Dominican blue amber with the best quality known as Sky Blue Amber.

Locality and horizon. La Búcara mine, Cordilliera Septentrional, Dominican Republic. La Toca Formation; late Early Miocene to early Middle Miocene.

Diagnosis. As for the genus (see above), by monotypy.

Description. Body slender, length 5.7 mm; wingspan ca 20 mm. Forewing length 8.9 mm; hind wing length 6.2 mm.

Head densely scaled; antenna filiform, partly preserved; compound eyes oval; chaetosemata unidentifiable; ocelli absent.

Mesoscutum large, with median suture. Mesoscutellum rhomboid, smaller than mesoscutum. Metascutum triangular. A comb-like epiphysis with setae on its inner side, arising from the inner wall of the foretibia (Fig. 1D); tarsus with five tarsomeres, pretarsus with a pair of claws and a median arolium.

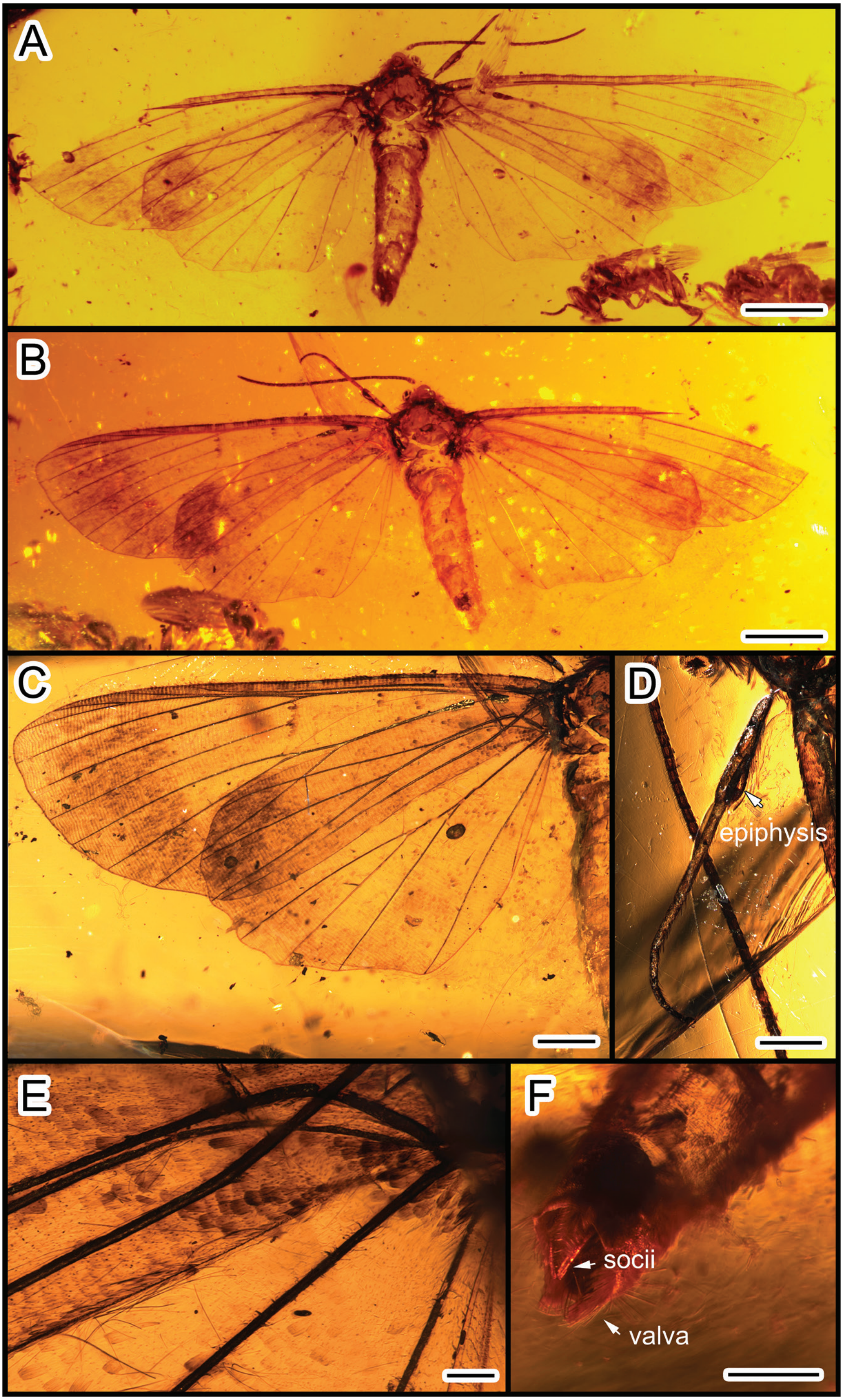

Figure 1. *Miogeometrida chunjenshihi* gen. et sp. nov., holotype, LEP-DA-2019001 **A** dorsal view **B** ventral view **C** forewing **D** foreleg **E** basal part of fore- and hind wings with scales **F** male genitalia, dorsal view. Scale bars: 2 mm (**A, B**); 1 mm (**C**); 0.5 mm (**D, F**); 0.2 mm (**E**).

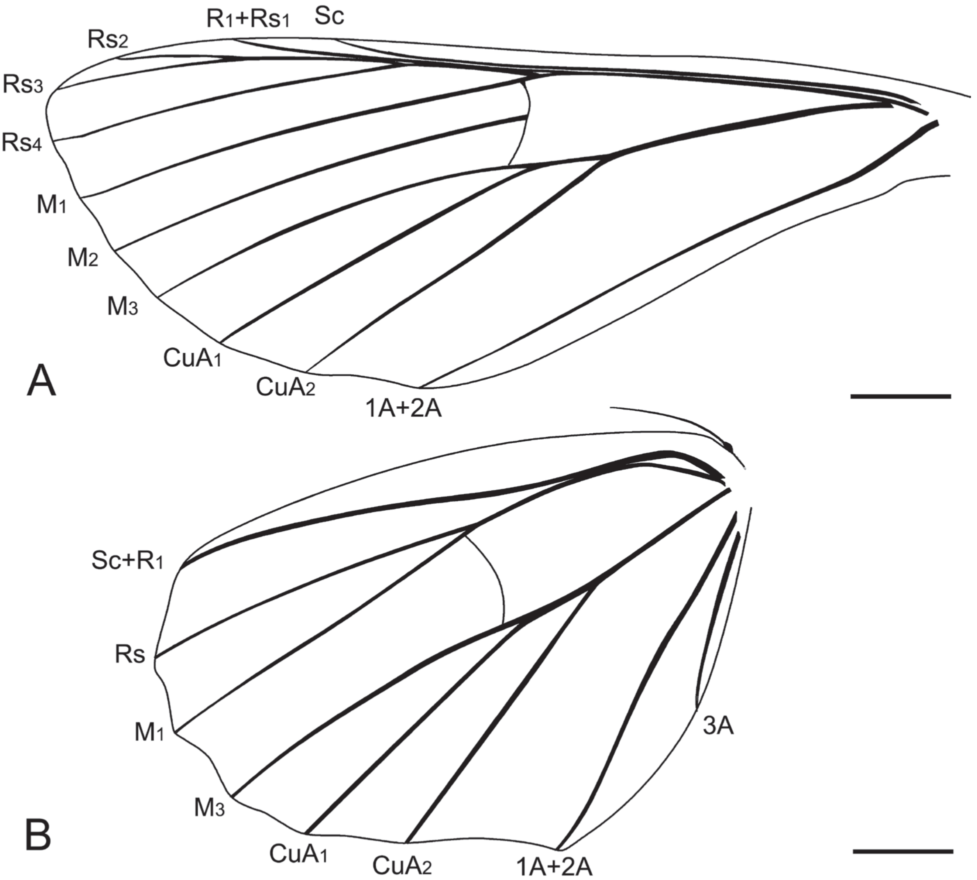

Figure 2. *Miogeometrida chunjenshihi* gen. et sp. nov., line drawings of LEP-DA-2019001 **A** forewing **B** hind wing. Scale bars: 1 mm.

Scales covering both fore- and hind wings, hair-like scales visible on the base of wings (Fig. 1C, E). Forewing elongate-triangular with the termen slightly sinuous; forewing index 0.37; fovea absent. Forewing with eleven veins (Figs 1C, 2A); discal cell approximately half as long as forewing; Sc not anastomosing with R_1; no areole formed by R_1 and Rs; R_1 and Rs_1 completely coincident; Rs_2 and Rs_3 with common stem; M 3-branched; M_1 continuous with stem of R; M_2 arising midway between M_1 and M_3; CuA bifurcating, CuA_1 originating near the end of discal cell, CuA_2 originating beyond the middle of discal cell; CuP absent; 1A and 2A fused for their entire lengths. Hind wing broad (Figs 1C, 2B), with outer margin concave between veins, apical angle rounded; hind wing index 0.66; Sc+R_1 strongly bent at its base; Rs approximated to Sc+R_1 at the base; M_2 absent; M_1 and M_3 almost parallel; CuA_1 and CuA_2 as in forewing; anal veins 1A+2A and 3A present. Wing coupling present, one strong frenular bristle on the anterior margin of the hind wing, retinaculum of the forewing indistinct.

Male genitalia (Fig. 1F) with valva simple; uncus reduced; socii long, slender, with bristles on the inner side.

Discussion

Miogeometrida gen. nov. can be assigned to Geometroidea based on forewing without spinarea (i.e., forewing-metathoracic aculeate locking device) and hind wing with basal part of the upper edge of discal cell markedly convex upwards, which are autapomorphies of the Geometroidea (Rajaei et al. 2015). In Geometroidea, the abdominal tympanal organ is an important diagnostic character, but the lateral and ventral parts of the abdomen of our specimen of *Miogeometrida* gen. nov. are damaged. It is thus impossible to determine whether a tympanal organ is present or not.

Although the essential apomorphy of Geometridae, i.e. a unique tympanal organ at the base of the abdomen, is not preserved for characterization, we chose to assign *Miogeometrida* gen. nov. to Geometridae. Based on the preserved and observable characters, *Miogeometrida* gen. nov. shows many similarities with Geometridae: (1) The size of *Miogeometrida* gen. nov. is in the common range of geometrids (wingspan ranges in most species from 20 to 45 mm; Heppner 2008a); (2) Hind wings of *Miogeometrida* gen. nov. are rounded as is the case in most species of Geometridae (Heppner 2008a); (3) *Miogeometrida* gen. nov. matches the major characters of geometrids in venation, such as forewing Rs_4 stalked with Rs_2 and Rs_3, M_2 not arising nearer to M_3 than M_1, and hind wing Sc bent strongly at its base (Minet and Scoble 1999). Although the first two similarities are also true for many other Lepidoptera, they can separate *Miogeometrida* gen. nov. from most sematurids and uraniids of Geometroidea.

We provide additional evidence to exclude three related Geometroidea families, i.e., Sematuridae, Uraniidae and Epicopeiidae. Sematuridae is a small family comprising only six extant genera and 40 species (van Nieukerken et al. 2011). An autapomorphy of Sematuridae are distally thickened antennae with swollen scape and elongate first flagellomere (Minet and Scoble 1999) – *Miogeometrida* gen. nov. does not have such an antenna. In addition, *Miogeometrida* gen. nov. with a wingspan of ca 20 mm, is obviously far smaller than sematurids whose wingspan range from 42 to 100 mm (Heppner 2008b). Moreover, *Miogeometrida* gen. nov. does not possess tails on the hind wings as found in most sematurids. In Uraniidae, the base of Rs_4 is connate or stalked with M_1, but separate from the other branches of Rs on the forewing, an apomorphy of the group (Minet and Scoble 1999). In *Miogeometrida* gen. nov., however, Rs_4 is stalked with Rs_{2+3} on the forewing, which does not conform with the state in Uraniidae. Similarly, *Miogeometrida* gen. nov. can be distinguished from Epicopeiidae whose Rs_4 is never stalked with $Rs_1 + Rs_2 + Rs_3$.

Ennominae is the largest subfamily of Geometridae, comprising ca 10,000 species worldwide, classified in approximately 1100 genera (Pitkin 2002). *Miogeometrida* gen. nov. shows many similarities with some extant taxa. We assign *Miogeometrida* gen. nov. to Ennominae based on the absence of M_2 on its hind wing that is considered as the traditionally diagnostic feature for this subfamily (Holloway 1994, Pitkin 2002). However, we cannot assign the new genus to tribe, mostly due to the poor preservation of its detailed morphological characters.

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RESEARCH ARTICLE

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'*Henicorhynchus*' *thaitui*, a new species of cavefish from Central Vietnam (Teleostei, Cyprinidae)

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Abstract

'*Henicorhynchus*' *thaitui* **sp. nov.** is described from a subterranean stream in a karst cave in Phong Nha Ke Bang National Park, Quang Binh Province, Central Vietnam. It differs from all congeners in having a pale pink body in life, smaller eyes with diameter less than the maxillary barbel length, and two pairs of barbels, the maxillary barbel being much longer than the rostral barbel.

Keywords

Cypriniformes, karst system, morphology, Southeast Asia, taxonomy

Introduction

Vietnam is rich in karst caves, and there are many magnificent caves throughout the country. The Son Dong Cave is the largest in the world (UNESCO 2016). Many caves, including Vietnam's deepest cave, were discovered in the northern part of the country, such as Lang Son, Cao Bang, Son La, and Ha Giang. Quang Binh in Central Vietnam is the largest and most important karst region of the country. The Ke Bang limestone massif, which crosses into Laos, has the longest river cave of the world in the Hang Khe Ry and the largest cave passage in the newly discovered Hang Son Doong. Many more caves are expected to be discovered in Vietnam (UNESCO 2016).

Cavefishes are adapted to hypogean aquatic ecosystems. During the past decade, many cavefishes were discovered in Vietnam as a result of ongoing cave explorations (Nguyen et al. 2018). Nevertheless, the fish fauna of those caves remains little studied. Mai (1978) described a single specimen of the family Siluridae from a cave in Cuc Phuong National Park, at the foothills of the Annamite Range in northern Vietnam, as *Silurus cucphuongensis* Mai, 1978, now in the genus *Pterocryptis* Peters (Ng and Kottelat 1998; Ng 1999). Kottelat (2004) described a new loach of the family Nemacheilidae (*Schistura spekuli* Kottelat, 2004) from a cave near Tam Duong, Lai Chau Province, Northern Vietnam. Two other new loaches, *Draconectes narinosus* Kottelat, 2012 and *Schistura mobbsi* Kottelat & Leisher, 2012, were described from Northern Vietnam. Most recently, a new species of Cyprinidae (*Speolabeo hokhanhi* Nguyen et al. 2018) was described from Central Vietnam where it is found only in the Phong Nha-Ke Bang National Park drained by the Son River of the Gianh River basin in Quang Binh Province (Nguyen et al. 2018).

The Phong Nha-Ke Bang National Park is located in the middle of the Annamite Range in the Quang Binh Province. The park, with approximately 104 km of subterranean tunnels and rivers, is among the most outstanding limestone karst ecosystems in the world (UNESCO 2016; Ho et al. 2018). To its West, it is adjacent to the Hin Namno Nature Reserve of Laos. So far, three Laotian cavefish species have been described: *Troglocyclocheilus khammouanensis* Kottelat & Bréhier, 1999, *Schistura kaysonei* Vidthayanon & Jaruthanin, 2002, and *Speolabeo musaei* (Kottelat & Steiner, 2011). More cavefish species are expected to exist in limestone karst areas of the middle Annamite Range (Kottelat and Steiner 2011; Kottelat 2017; Nguyen et al. 2018). This is evidenced by the present report of a new species of cavefish provisionally referred to the labeonine cyprinid genus *Henicorhynchus* Smith, from Phong Nha-Ke Bang National Park, Central Vietnam.

Henicorhynchus comprises species widespread in freshwater habitats throughout tropical and subtropical regions of Southeast and South Asia. This genus has a complex taxonomic history. Its included species were previously placed under various genera, such as *Cirrhinus* Oken (Roberts, 1997), and *Gymnostomus* Heckel (Kottelat, 2013). In the absence of a stable classification of parts of the Labeonini we here follow Fricke et al.'s (2020) concept of *Henicorhynchus*, where 9 species are included. The genus has four representatives in Vietnam: *H. siamensis* (Sauvage, 1881), *H. lobatus* Smith, 1945, *H. caudimaculatus* (Fowler, 1934) and *H. cryptopogon* (Fowler, 1935) (Nguyen and Ngo 2001; Serov et al. 2006; Rainboth et al. 2012; Tran et al. 2013). The new species described herein is the fifth species of *Henicorhynchus* and the sixth cavefish species recorded from Vietnam.

Material and methods

All measurements were taken point to point with a pair of dial calipers and data were recorded to the nearest 0.1 mm. Counts and measurements were made on the left side of specimens whenever possible, following the methods of Rainboth (1996) and Kottelat (2001). Predorsal, prepectoral, prepelvic and preanal lengths were measured from the snout tip to the dorsal-, pectoral-, pelvic-, and anal-fin origin, respectively. Vertebrae were counted from radiographs following the method outlined by Roberts (1989). Weberian vertebrae and the urostylar complex were included in the counts of vertebrae. The number of specimens used for a given count is indicated in brackets after the count. Values for the holotype are indicated by an asterisk. Measurements of parts of the head are given as proportions of the head length (HL). The head length and measurements of other parts of the body are given as percentages of the standard length (SL).

Abbreviations in the text include: IHB, Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan City, Hubei Province, China; IEBR, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology in Hanoi City, Vietnam; RIA1, Research Institute for Aquaculture No. 1, Bac Ninh Province, Vietnam; RIA2, Research Institute for Aquaculture No. 2, Khanh Hoa Province, Vietnam; VU, Vinh University in Nghe An Province, Vietnam; CTU, Can Tho University in Can Tho City, Vietnam; CAS, California Academy of Sciences; USNM, National Museum of Natural History, Washington, DC, USA.

‘*Henicorhynchus*’ *thaitui* sp. nov.

http://zoobank.org/0E2614B1-EEBB-4DDE-B883-EAD279245950
Figures 1–4

Holotype. IEBR 105901, 74.3 mm SL; Vietnam: Quang Binh Prov.: Phong Nha-Ke Bang National Park: Khe Lanh Cave (in Son River system in the Gianh River basin): 17°25'41"N, 106°18'31"E, altitude 185 m; collectors: A.T. Ho and N.T. Hoang, 12 August 2011 (deposited in IEBR).

Paratypes. IHB 2016105895–9, 5 specimens, 80.9–99.5 mm SL; IEBR 105900–04, 4 specimens, 68.8–75.6 mm SL; VU 5905–08, 4 specimens, 76.8–83.4 mm SL; all other data same as holotype (deposited in IHB and IEBR, VU).

Diagnosis. ‘*Henicorhynchus*’ *thaitui* sp. nov. can be distinguished from all congeners by having a whitish pink body in life (vs. white or silvery body with a humeral mark or some longitudinal stripes), smaller (vs. larger) eyes (diameter less than vs. greater than maxillary barbel length) and maxillary barbel longer (vs. shorter) than rostral barbel. ‘*Henicorhynchus*’ *thaitui*, along with *H. horai* (Bănărescu, 1986) and *H. inornatus* (Roberts, 1997), is further distinct from all other congeners in having 9 (vs. 8) branched dorsal-fin rays. Along with *H. horai*, it differs from all other congeners in the presence of rostral barbels (vs. absent) and 39–41 (vs. 34–36) lateral-line scales.

'*Henicorhynchus*' *thaitui* differs from *H. horai* and *H. inornatus* in the presence of two (vs. one) pairs of maxillary and rostral barbels (vs. only the maxillary barbel in *H. inornatus* and only the rostral barbel in *H. horai*); from *H. horai* in having fewer vertebrae (34 vs. 38–41), and a laterally compressed body (vs. cylindrical *in H. horai*); and from *H. inornatus* in having 39–40 (vs. 35 in *H. inornatus*) lateral-line pored scales, and in the absence of a humeral mark (vs. present in *H. inornatus*).

Description. Measurements and meristics of the type series are provided in Table 1. See Figs 1, 2 for general appearance, Fig. 3a for lateral and ventral views of the head, and Fig. 4 for morphology of the oromandibular structures. Body elongate and laterally compressed. Dorsal profile of body from tip of snout to dorsal-fin origin slightly convex. Predorsal profile of body convex, without distinctive hump behind head. Postdorsal profile of body slightly concave. Ventral profile of body from tip of snout to anal-fin origin convex; slightly concave from anal-fin origin to origin of ventral procurrent caudal-fin rays.

Head small, conical, longer than deep, deeper than wide. Eye relatively small, positioned laterodorsally in anterior half of head and considerably behind or above rictus, not visible when head viewed ventrally; diameter less than maxillary-barbel length. Interorbital space slightly convex. Snout slightly pointed in lateral view and broadly rounded in ventral view (Fig. 3a). Nares longitudinal and located closer to orbit than to tip of snout and covered by a flap originating from anterior end. Two pairs of long barbels; maxillary barbel much longer than rostral barbel, extending to posterior margin of orbit or beyond, and rostral barbel extending beyond nostrils but not reaching to anterior margin of orbit. Mouth inferior and slightly arched.

Rostral cap well-developed, overhanging, but covering median part of upper lip base; slightly crenulated, laterally attached to root of maxillary barbel and separated from lower lip. Upper lip well-developed, greatly enlarged, separated from upper jaw, covered with papillae; laterally continuous with lower lip around corners of mouth. Upper jaw bearing a thin, flexible horny sheath on cutting margin. Lower lip anteriorly separated from lower jaw by a deep, transversally arched groove; posteriorly confluent with mental region and covered with papillae. Post-labial groove extended anteromedially, but not meeting its counterpart at midline. Lower jaw bearing a thin horny sheath on cutting margin (Fig. 4)

Dorsal fin with 3 unbranched and 9 branched rays, last one split to base; last unbranched ray flexible, without serrations along posterior border; distal margin strongly concave; origin midway between snout tip and caudal-fin base or anterior to pelvic-fin insertion. Pectoral fin short, with 1 unbranched and 11 branched rays; tip of adpressed fin not reaching pelvic-fin insertion. Pelvic fin falcate, with 1 unbranched and 8 branched rays; inserted halfway from pectoral-fin insertion to anal-fin origin; tip of adpressed fin extending to vent. Anal fin with 3 unbranched and 5 branched rays; distal margin slightly concave; origin equidistant between pelvic-fin insertion and caudal-fin base. Caudal fin with 9/8 principal rays, deeply forked; upper and lower lobes nearly equal in length.

Table 1. Morphometric data and meristic counts for type specimens of '*Henicorhynchus*' *thaitui* sp. nov. (n = 14).

	Holotype	Paratypes (N=13)			
		Min	Max	Average	SD
SL	74.3	68.8	99.5	80.9	8.5
In percent of SL					
Head length	24.1	20.3	24.1	22.2	0.9
Predorsal length	53.3	47.8	53.3	49.9	1.6
Preanal length	74.2	72.8	77.9	74.6	1.4
Prepelvic length	49.0	49.0	54.4	50.9	1.5
Body depth at dorsal-fin origin	30.0	23.3	30.0	26.9	2.2
Body depth at anus	20.2	16.0	20.2	18.0	1.3
Depth of caudal peduncle	14.2	10.6	14.2	12.7	0.9
Length of caudal peduncle	22.1	13.6	22.1	17.8	2.4
Head depth	11.8	10.9	12.8	11.8	0.6
Head width	11.6	10.4	11.9	11.1	0.6
Snout length	8.8	6.7	9.6	8.4	0.8
Dorsal-fin length	26.3	20.1	29.6	25.7	2.7
Pectoral-fin length	21.9	18.4	25.7	22.6	1.7
Pelvic-fin length	20.6	16.9	23.7	20.8	1.9
Anal-fin length	19.1	14.0	22.7	19.1	2.2
In percent of HL					
Head depth	48.9	48.9	57.6	53.1	2.7
Head width	48.4	44.6	54.9	50.0	3.0
Snout length	36.7	32.9	42.5	38.0	3.0
Eye diameter	17.7	10.9	17.7	13.6	1.8
Interorbital width	48.4	44.6	54.9	50.0	3.0
Counts					
Lateral line scales	39	39 (11), 40 (2)			
Transverse scale rows	5/1/4	6/1/4(11), 5/1/4 (2)			
Predorsal scales	14	14 (10) –15 (3)			
Cicumpeduncular scales	16	16 (13)			
Dorsal-fin branched rays	9	9 (13)			
Pectoral-fin branched rays	11	11 (13)			
Pelvic-fin branched rays	8	8 (13)			
Anal-fin branched rays	5	5 (13)			
Caudal-fin branched rays	9+8	9+8 (13)			
Gill rakers on lower arm of 1st arch		37–39 (2)			
Vertebrae		34–35 (2)			

Scales moderately large. Lateral line complete with 39 (12*) or 40 (2) pored scales, extending along mid-lateral body from upper gill-opening extremity to middle of caudal fin. Predorsal scales 14 (10*) or 15 (3). Scales in transverse row before pelvic fin 5 (3) or 6 (12*) above lateral line and 4 (14*) below. Circumpeduncular scales 16 (14*). Gill rakers on left side of first gill arch 37–39 (2) (Fig. 3b). Pharyngeal teeth in 3 rows: 2.4.5–5.4.2 (IHB 2016105898, paratype, Fig. 3c). Air bladder bipartite (Fig. 3d). Intestines long, thin and highly coiled (Fig. 3e). Vertebrae 4+34–35=38–39 (2).

Coloration. In freshly caught-individuals, body white to pinkish or pale pink with all fins translucent (Fig. 2). In captivity, body pale pink but dorsum turning to pale

Figure 1. Lateral view of '*Henicorhynchus*' *thaitui* sp. nov.: **a** IEBR 105901, holotype, 74.3 mm SL; and **b** IHB 2016105898, paratype, 98.0 mm SL. Both specimens caught in central Vietnam: Son River system in Gianh river drainage: Khe Lanh Cave.

Figure 2. '*Henicorhynchus*' *thaitui* sp. nov., specimen not preserved, about 100 mm SL, Vietnam: Khe Lanh cave; life coloration.

brown hue in adults exposed to light, becoming gray dorsally within several hours when exposed to daylight (Fig. 1b). In alcohol-preserved specimens, body uniformly pale yellow, with all fins light gray, particularly in distal portion (Fig. 1a).

Etymology. The specific name is a noun in the genitive case, honoring Nguyen Thai Tu, ichthyologist from Vinh University, who has contributed considerably to the taxonomy of freshwater fishes in Vietnam.

Troglomorphic characters. '*Henicorhynchus*' *thaitui* sp. nov. presents a mixture of characters characterizing hypogean and epigean fish species. The absence of pigmentation, reduced eye size, and well-developed barbels are troglomorphic characters observed in '*H.*' *thaitui*. The pale pink or white to pinkish body is shared with hypogean

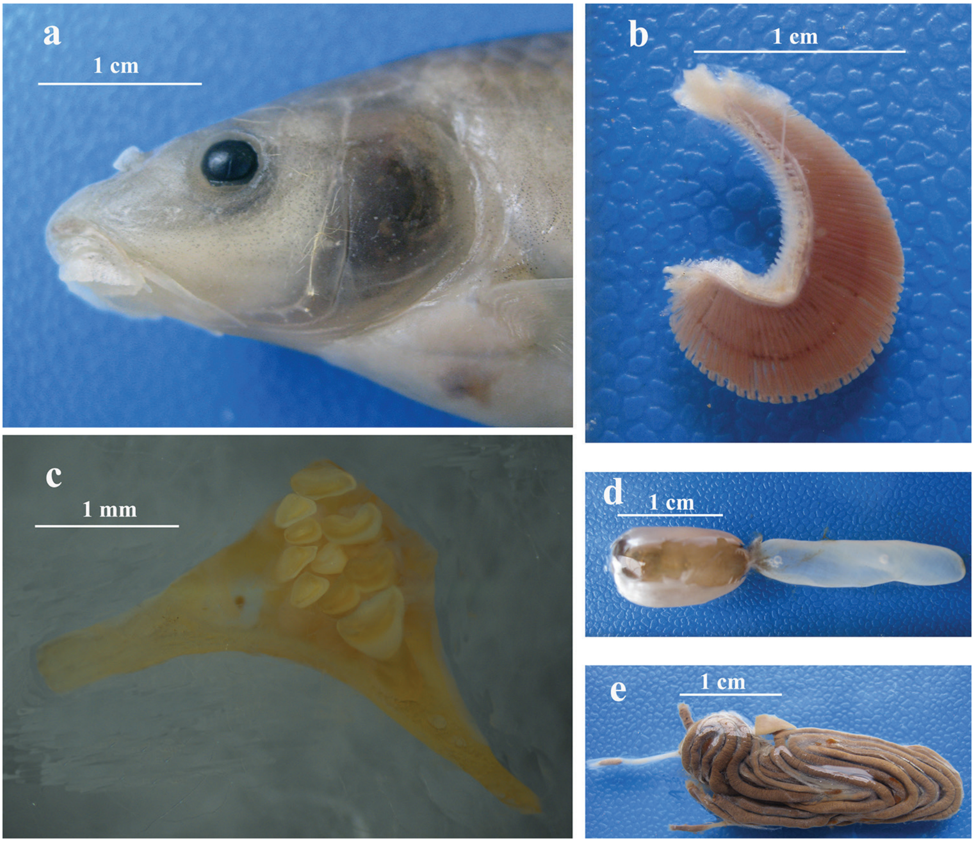

Figure 3. '*Henicorhynchus*' *thaitui* sp. nov., IHB 2016105898, paratype, 98.0 mm SL: **a** lateral view of head **b** gill rakers on lower arm of first arch **c** pharyngeal teeth **d** air bladder; and **e** intestines.

fish species. The eyes are smaller than in congeneric epigean species, but not vestigial or absent as is common in hypogean fish species. The barbels are longer and thicker compared with all congeneric epigean species, but in this regard similar to hypogean fish species.

Distribution and habitat. '*Henicorhynchus*' *thaitui* sp. nov. is known only from the Khe Lanh Cave where it inhabits shallow to deep (0.2–0.8 m) cave streams and pools about 800–1000 m from the cave entrance (Figs 5, 6). This cave is located approximately 25 km south of Phong Nha village in the Son Trach commune. It has a length of 1–2 km, completely without light, with a mixed substrate of mud and sands. The type series of '*H.*' *thaitui* was collected in August 2011, roughly 1 km from the cave entrance. At least 50 individuals of various sizes were observed in streams and pools, 14 of which were caught by hand net (Fig. 7). The fishes were swimming slowly and haphazardly, rather close to the water surface; they swam deeper when disturbed. A new shrimp species (Do and Nguyen 2014) and the labeonine fish species *Speolabeo hokhanhi* were recently discovered in the Hang Va Cave, 3–5 km away from the Khe Lanh Cave (Nguyen et al. 2018).

Table 2. Main diagnostic characters for '*Henicorhynchus*' *thaitui* sp. nov. and its congeneric species.

Characters	*H. thaitui*	*H. horai*	*H. inornatus*	*H. siamensis*	*H. lineatus*	*H. lobatus*	*H. caudiguttatus*	*H. cryptopogon*	*H. ornatipinnis*	*H. caudimaculatus*
Branched dorsal-fin rays	9	9	9	8	8	8	8	8	8	8
Rostral barbels	present	present	absent	absent	absent	absent	absent	absent	absent	absent
Maxillary barbels	present, long and well developed	absent	absent	present, very tiny	present, tiny	present, tiny	present, tiny	present, tiny	absent	absent
Lateral line scales	39–40	39–41	35	34–36	34–35	32–33	35	34–35	34–35	35–36
Transverse scales rows	5–6/1/4	6/1/4	7/1/5	6/1/4	6/1/5	5/1/4	6/1/5	6/1/5	6/1/4	6/1/5
Vertebrae	34–35	38–41	35–37	33–34	32–33	32	32	32–33	33–35	32
Dark state color distal dorsal-fin edge	absent	absent	absent	present	absent	absent	absent	present	absent	absent
Reddish-orange pectoral fins	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Longitudinal stripes on body	absent	absent	absent	absent	present	absent	absent	present	absent	absent
Humeral mark color	absent	absent	present	absent	absent	absent	absent	absent	absent	absent
Strongly projected snout	absent	absent	absent	absent	absent	present	absent	absent	absent	present
Dark state color precaudal blotch	absent	absent	absent	absent	absent	absent	present	absent	absent	present
Sources of data	this study	Vidthayanon (2008), Roberts (1997)	Roberts (1997)	Roberts (1997), Rainboth (1996), Kottelat (2001)	Roberts (1997), Kottelat (1998), Kottelat (2001)	Roberts (1997), Kottelat(2001)	Roberts (1997), Rainboth et al. (2012)	Rainboth (1996)	Roberts (1997), Kottelat (2001)	Roberts (1997), Rainboth (1996)

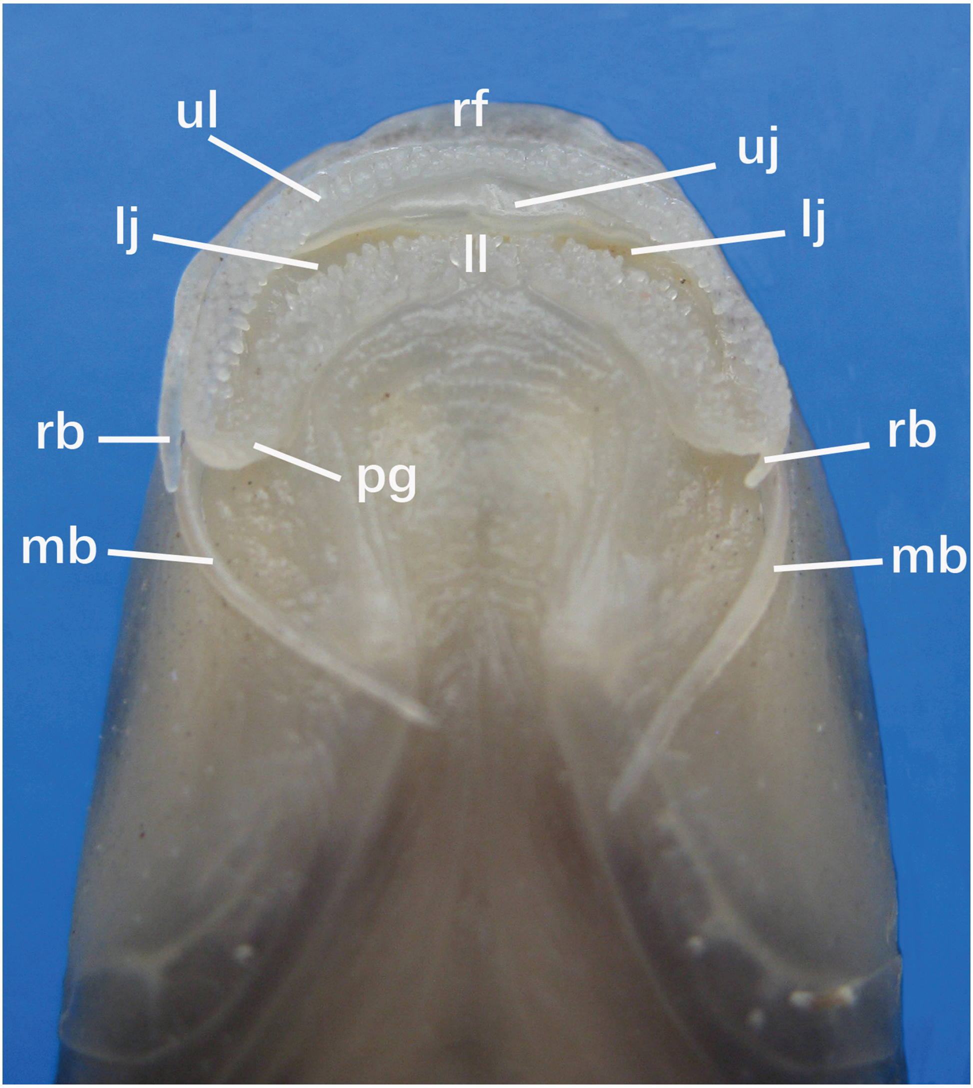

Figure 4. Ventral view of mouthpart structures in '*Henicorhynchus*' *thaitui* sp. nov., IHB 2016105898, paratype, 98.0 mm SL. lj, lower jaw; ll, lower lip; mb, maxillary barbel; pg, postlabial groove; rb, rostral barbel; rf, rostral fold; ul, upper lip; uj, upper jaw.

Discussion

'*Henicorhynchus*' *thaitui* sp. nov. is referred to the genus *Henicorhynchus* with hesitation. It can be distinguished from all other species of this genus by the troglomorphic characters, and would be the only species of *Henicorhynchus* recorded from a cave biotope.

The karst region of Central Vietnam extends into the central part of Laos from where the labeonine cavefish *Speolabeo musaei* is known. The second species of the genus, also troglobitic, is *S. hokhanhi* (Fig. 5h), recently described by Nguyen et al. (2018) from Central Vietnam. Three main characters typical for *Speolabeo* Kottelat are: 7–8 branched dorsal-fin rays, reduced smooth upper lip and postlabial groove widely interrupted at the isthmus with its counterpart (Nguyen et al. 2018). '*Henicorhynchus*' *thaitui* cannot be referred to *Speolabeo*, differing in the presence of 9 branched dorsal-fin rays, a well-developed, greatly enlarged, papillose upper lip and postlabial groove narrowly interrupted at the isthmus with its counterpart.

The Labeonini are characterized by a high degree of morphological modification of their oromandibular structures, variation in which is the basis for the diagnosis of the majority of included genera. The well-developed, greatly enlarged upper lip separated from the upper jaw in '*H.*' *thaitui* is shared only with some species of the labeonine genera *Osteochilus* Günther, *Labiobarbus* van Hasselt, *Labeo* Cuvier, *Cirrhinus* and *Henicorhynchus* (Wu et al. 1977; Zhang et al. 2000; Zhang and Chen 2006). It differs from *Osteochilus* (*sensu* Roberts, 1989) in the absence of elongate folds or plicae on the upper lip; and from *Labiobarbus* (*sensu* Rainboth, 1996) in having 9 rather than 18–30 branched rays of the dorsal fin. *Labeo*, as traditionally defined, is widespread in the tropical regions of Asia and Africa (Reid 1985). Its non-monophyly was shown in Yang et al.'s (2012) molecular phylogenetic analysis of the Labeonini; these authors therefore restricted *Labeo* s. str. to the clade sister to '*Cirrhinus*' *microlepis* Sauvage, 1878, but upholding this clade may result in multiple generic lineages. In this context, Asian *Labeo* is here confined to species like *Labeo dyocheilus* McClelland, 1839, following Rainboth (1996) and Kottelat (2013). '*Henicorhynchus*' *thaitui* is not congeneric with Asian species of *Labeo*, lacking a thickened lower lip with a deep postlabial groove narrowly interrupted at the isthmus with its counterpart. It is highly likely that '*H.*' *thaitui* is a member of *Henicorhynchus* or *Cirrhinus*, or even of a distinct genus.

'*Henicorhynchus*' *thaitui* sp. nov. can be distinguished by its troglomorphic characters from all other species of *Henicorhynchus* and closely related genera like *Cirrhinus* and *Gymnostomus*. Except for the two species of *Speolabeo* (Nguyen et al. 2018) and some species of *Garra* Hamilton in the Middle East (Khalaf-Sakerfalke von Jaffa 2009; Hamidan et al. 2014), no species with troglomorphic characters like this are found in the Labeonini.

The generic placement of '*H.*' *thaitui* is not straightforward, however. Compared with species of *Henicorhynchus* or closely related genera which have relatively simple oromandibular structures (see Roberts 1997 for details), '*H.*' *thaitui* has unique modifications in these structures, including rostral cap pendulous with a slightly crenulated distal margin, laterally attached to the root of the maxillary barbel and discontinuous with the lower lip, and papillated lips (Fig. 4). These characters might justify placing '*H.*' *thaitui* in a new genus. Such act will need a more thorough analysis, involving all labeonine genera. For the moment, the recognition of '*H.*' *thaitui* as a distinct species, regardless of genus, is justified on account of its restricted distribution and specialized ecology. Anthropogenic activities on both the global and local scales pose a potential threat to survival of all cavefishes, and their identification by a scientific name will facilitate conservation actions.

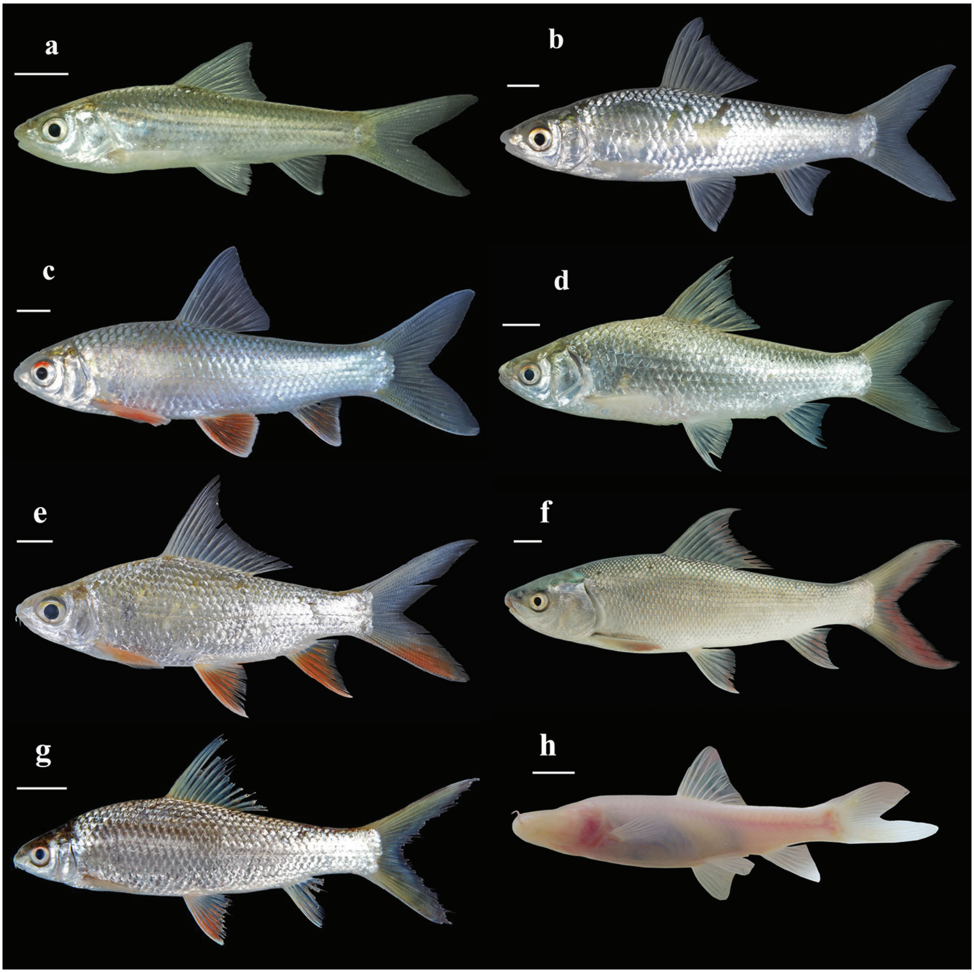

Figure 5. Species most closely related to '*Henicorhynchus*' *thaitui* sp. nov.: **a** *Henicorhynchus lineatus* **b** *H. lobatus* **c** *H. ornatipinnis* **d** *H. siamensis* **e** *Cirrhinus jullieni* **f** *C. microlepis* **g** *C. molitorella*; and **h** *Speolabeo hokhanhi*. Photos **b**, **d**, **e**, **f** and **g** from Vietnam, by D.D. Tran (Can Tho University, Vietnam); **a** and **c** from Laos, by Bounthob Praxaysombath (National University of Laos, Laos) (from Kano et al. 2013); and **h** from Vietnam, by D.T. Nguyen. Scale bars: 1 cm.

The taxonomy of *Henicorhynchus* and closely allied genera is poorly understood. Their generic delineation is not based on the morphology of the oromandibular structures, which are widely used for the diagnosis of genera of the Labeonini. The major character used to distinguish among *Henicorhynchus* and closely allied genera (*Cirrhinus* and *Gymnostomus*) is the number of branched dorsal-fin rays; their current generic definition still remains controversial, however. The focus of this controversy is on the generic assignment of species with 9 branched dorsal-fin rays. According to Kottelat (2003), *Cirrhinus* is composed of species with 10–15 branched dorsal-fin rays,

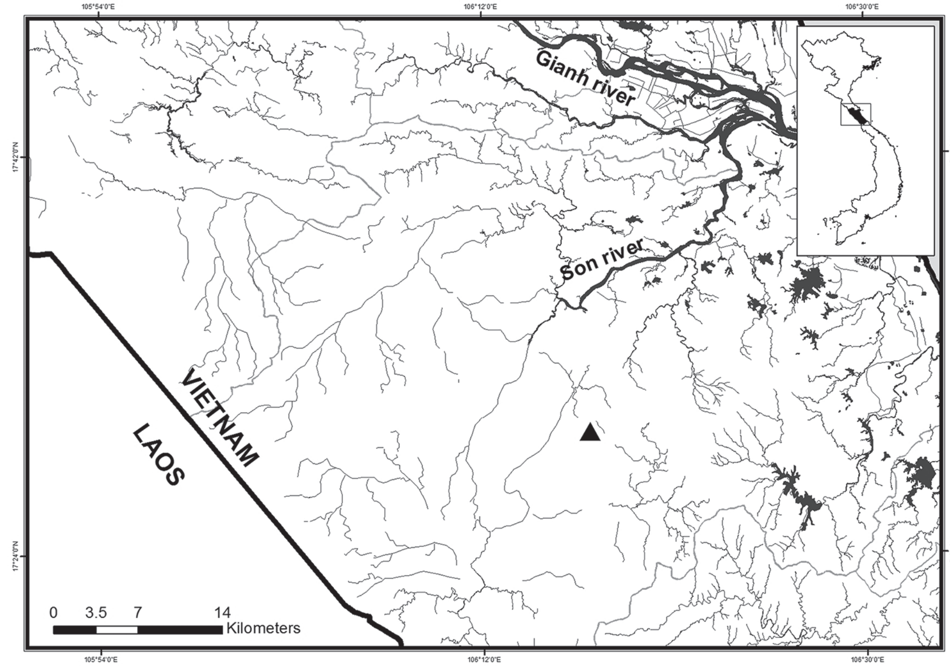

Figure 6. Map showing the distribution of '*Henicorhynchus*' *thaitui* sp. nov. (▲).

and those with 8–9 rays were ascribed to *Gymnostomus*, with which *Henicorhynchus* was synonymized. Referring to Kottelat (2013), six species were assigned to *Cirrhinus*, namely *C. cirrhosis* (Bloch, 1795), *C. jullieni* Sauvage, 1878 (Fig. 5e), *C. microlepis* (Fig. 5f), *C. molitorella* (Valenciennes in Cuvier & Valenciennes, 1844) (Fig. 5g), *C. prosemion* (Fowler, 1934), and *C. rubirostris* Roberts, 1997. Rainboth et al.'s (2012) generic definition of *Henicorhynchus* included the following seven species with 8 branched dorsal-fin rays: *H. lineatus* (Smith, 1945) (Fig. 5a), *H. lobatus* (Fig. 5b), *H. ornatipinnis* (Roberts, 1997) (Fig. 5c), *H. caudimaculatus*, *H. cryptopogon*, *H. siamensis* (Fig. 5d), and *H. caudiguttatus* (Fowler, 1934). These authors ascribed *H. inornatus*, which has 9 branched dorsal-fin rays and which was previously included in *Gymnostomus* by Kottelat (2003), to *Cirrhinus*. Earlier, Roberts (1997) lumped all species with 8–15 branched dorsal-fin rays into *Cirrhinus*. This taxonomic treatment, though, was not widely accepted by subsequent workers.

Taxonomic confusion regarding *Henicorhynchus*, *Gymnostomus*, and *Cirrhinus* was clarified in recent molecular phylogenetic analyses of the Labeonini (Yang et al. 2012; Zheng et al. 2012, 2016). It was demonstrated that sampled species of *Henicorhynchus* nested into an independent lineage, and so did that of *Gymnostomus*; *Cirrhinus* was non-monophyletic, and *C. microlepis* and *C. molitorella* were distantly related to each other or to the rest of the analyzed congeneric species. On the basis of these findings, it can be concluded that *Gymnostomus* [type species: *Cyprinus ariza* Hamilton, 1807,

Figure 7. Vietnam: Son River system in Gianh River drainage basin: Khe Lanh Cave: mouth of cave (left) and habitat of '*Henicorhynchus*' *thaitui* sp. nov. (right).

a species designated by Roberts (1997) to *Cirrhinus*] is a valid genus; *Henicorhynchus* possibly includes species with 8 branched dorsal-fin rays; and both *C. molitorella* and *C. microlepis* should be removed from *Cirrhinus*. However, insufficient sampling made it unlikely to reach a decisive conclusion about the generic placement of these three genera. Two species, namely *H. inornatus* and *H. horai*, with 9 branched dorsal-fin rays, were not sampled in Yang et al.'s (2012) analysis, rendering their generic status untested. Fricke et al. (2020) nevertheless referred these two species to *Henicorhynchus*.

Comparative material

Henicorhynchus siamensis: RIA2, uncataloged, 30, 62–184 mm SL, South Vietnam (Lower Mekong basin); CAS 91751, 109 mm, Pak Mun; CAS 91749, 4, 57.4–65.2 mm, Menam Bangpakong at Ban Khao Cha-chan, 19 km South of Sa Kaeo on highway 317.

H. caudimaculatus: CTU, uncataloged, 11, 40–80 mm SL, South of Vietnam (Lower Mekong basin); USNM 117769, 27, 45.0–78.5 mm, upper Nan River at Ban Khwang, northern Siam; USNM 119493, 2, 59. 6–60.5 mm, Chao Phraya at Bangsai, central Thailand; CAS 91781, 55.2 mm, Menam Wang, 79 km by road North of Lampang and 6 km E of highway 1035.

H. cryptopogon: RIA2, uncataloged, 1, 145 mm SL, Can Tho province.

H. inornatus: CAS 91772, 115 mm, Myanmar, Mandalay market; CAS 88903, 106 mm, Pagan market; CAS 91775, 122 mm, Myitkyina market (Irrawaddy basin); CAS 91773, 6: 94.6–116 mm, Taungoo market (Sittang River).

H. lineatus: CAS 79169, 5, 64.9–95.2 mm, mouth of Huay Ngao where it flows into Mekong River 1 km South of Ban Chaem Pong (about 30 km South of Chiang Khong); CAS 91766, 9, 54.5–106 mm, Mekong mainstream from Pak Ing to Jom Paeng (about 4–5 km downstream from Pak Ing).

H. lobatus: CAS 91769, 8, 56.9–102 mm, Menam Kok at Tha Ton and up to 5 km downstream; CAS 91767, 4, 43.4–50.5 mm, Se Khone at Stung Treng.

H. horai: CAS 81548, 24, 95.9–155 mm, Inle Lake.

H. ornatipinnis: CAS 91756, 69.7 mm, roadside ditch on highway 24 at km 150, market, 179 km by road E of Nakom Ratchasima, Thailand; CAS 91760, 9, 55.7–90.5 mm, roadside ditch 5–30 km South of Phibun Mangsahan, Thailand.

Cirrhinus molitorella: RIA1 H01930101–7, 7, 105–387 mm SL, Red river. IHB 201808027404, 1, Lam river, Nghe An province, Central Vietnam; CAS 79175, 2, 82.8–93.7 mm, Huay Sangkalia, 7 km North of Sangklaburi on road to Chedi Sam Ong.

C. microlepis: RIA1 H01930201–3, 3, 65–148 mm SL, south of Vietnam; USNM 104935, 2, 122–146 mm, Chao Phraya at Nontaburi; CAS 79173, 2: 107–113 mm, rapids in mainstream of Mekong River about 12 km South of That Phanom.

C. mrigala (Hamilton, 1822): RIA1 H01930301–5, 5, 150–258 mm SL, aquaculture. IHB 201808018201–5, 5, Lam river, Nghe An province, Central Vietnam.

C. prosemion: CTU, uncataloged, 11, 71–95 mm SL, Can Tho province.

C. jullieni: CAS 91748, 104 mm, Sawankhalok market (Menam Yom), 36 km North of Sukhothai; CAS 91610, 13: 94.7–104 mm, Phnom Penh market.

Speolabeo hokhanhi: IHB 2016092883, 76.4 mm, Hang Va cave, Phong Nha-Ke Bang National Park; IEBR 2884–5, 50.7–54.4 mm, IHB 206092886–8, 61.8–69.0 mm.

Data for *Cirrhinus reba* (Hamilton, 1822) from Vidthayanon et al. (2005) and Vidthayanon (2008); for *C. cirrhosis*, *C. rubirostris*, *Gymnostomus fulungee* (Sykes, 1839), and *G. ariza* from Roberts (1997).

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Catalogue of herpetological specimens of the Ewha Womans University Natural History Museum (EWNHM), Republic of Korea

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Abstract

The herpetology collection of the Ewha Womans University Natural History Museum (EWNHM) represents one of the oldest and largest institutional collections in the Republic of Korea. The specimens deposited in the EWNHM represent a major historical collection of the native herpetofauna, both in species diversity and time span. However, the full inventory of the herpetology collection has never been conducted and thus the collection has received little attention from researchers. Here, the first full account of the herpetology specimens held at the EWNHM is provided, with voucher information for all documented specimens to make the collection accessible for future studies.

Keywords

Amphibia, herpetology, historical collection, museum specimens, Reptilia, Republic of Korea

Introduction

Natural history collections are an invaluable repository for modern biological research. These collections have broad applications including the detection of faunal changes, species decline, biogeography, systematics, and species discovery (Shaffer et al. 1998; Graham et al. 2004; Wójcik et al. 2010; Lister et al. 2011). A series of specimens collected over time also serves to shed light on changes in genetic diversity and morphology that occurred during that time period (Babin-Fenske et al. 2008; Cornetti et al. 2018). In order to conduct such collection based research, a full inventory of specimens held at the museum is a valuable resource as it saves time locating specimens, designing studies and managing collections.

In the Republic of Korea, early efforts to collect herpetological specimens were conducted by foreign researchers. Thus, vouchered specimens collected during this time were deposited in natural history museums outside of the country (American Museum of Natural History 2020; e.g., a series of *Bombina orientalis* vouchers collected in early 1950s). Although such specimens are a valuable historical collection, they usually do not fully encompass the native herpetofauna in taxa, time span, and geographic locations (American Museum of Natural History 2020; e.g., herpetological specimens historically collected in Korea). Moreover, natural history collections within the country are poorly known to researchers outside the institution where the collection is located and thus received little attention in herpetological research.

Ewha Womans University Natural History Museum (EWNHM; Sabaj 2016) was established in 1969 as the first natural history museum for the country (EWNHM website 2014). Although small in size, the herpetology collection of EWNHM is of great historical value for herpetological research, with some specimens dating back to early 1950s being collected during the Korean War.

Despite this great value, a complete catalogue of the collection has not been available. Although a public database (Korean Natural History Research Information System; NARIS) provides information on EWNHM specimens, this database is only a partial representation of the collection. Also, this database uses a different cataloging system from EWNHM, leading to voucher inconsistencies and potential problems of locating specimens. Therefore, a full herpetological inventory of the collection at EWNHM following a consistent format is needed for future utilization of specimens. Here, we provide the first complete catalogue of the herpetological collection of EWNHM, using consistent voucher system throughout specimens. In doing so, we also changed degrading labels to prevent the loss of important information and updated the nomenclature of species if the taxon underwent taxonomic revisions between the time of initial labelling and our cataloging effort. Also, we applied a new and consistent voucher system throughout specimens to resolve confusion of conflicting voucher systems. Although this means yet another change of voucher system applied to specimens, this catalogue can serve as a reference point towards reducing confusions originating from multiple conflicting voucher systems.

Materials and methods

Description of the collection

The herpetological specimens of the museum EWNHM are located in the wet collection room. The herpetology collection was roughly divided into reptiles and amphibians. For some species of anurans, 50 to 100 individual specimens were contained per glass jar (e.g., *Bombina orientalis*, *Glandirana emeljanovi*, *Pelophylax nigromaculatus*), with specimens packaged individually in small plastic bags. These specimens were mostly collected in one location on the same date, comprising voucher series of specimens. In some cases, specimens of one anuran species collected from two different locations were held in the same glass jar. In this case, specimens from two locations were divided into two by separate plastic bags. Some amphibian species were held in smaller glass jars with fewer number of individual specimens per container (< 25 individual specimens). We also found jars that contained two or more amphibian species collected from the same location. In this case, we either separated the species into separate jars or separated them into small plastic bags containing paper labels with the appropriate species information.

Specimens of lizards were housed in small individual containers or packed into small number of specimens (< 20 individuals per container) representing voucher series. Specimens of snakes and turtles were individually contained in glass jars. All specimens were preserved in 1% formalin solution at the beginning of collection and preservation process (Jaewon Ryu, pers. comm.). The use of preservative solution has not been changed over the years.

General protocol for processing specimens

We used a table to mount a tripod and camera (Nikon D50; Nikon Corporation, Tokyo, Japan) in order to photograph the specimens. The table was covered with white synthetic fabric to provide a white background for photography. A 15-centimeter ruler was taped above the fabric to be used as a scale of reference. We used a whole-face respirator (3M Company, Minnesota, USA) to prevent potential toxic inhalation of preservative fluids. We also used industrial-grade wiper (Yuhan-Kimberly, Ltd, Seoul, Korea) to remove fluid spillage. For photography, we mounted the camera on a tripod with the lens facing vertically downwards. Each specimen was photographed in dorsal, lateral and ventral angles. Specimens of eggs and amphibian larva were not photographed to prevent potential damage caused by the handling process.

Labelling protocol

As most of the Korean herpetofauna have undergone significant nomenclatural changes over the past 50 years, and as some of the labels showed signs of degradation, it was necessary to update the labels. In doing so, we kept the original labels alongside the new labels with updated nomenclature.

For labeling, we first prepared a general label for each specimen or series of specimens. This label contained key information about the specimen(s), including scientific name, Korean common name, geographic location of collection, collection date, and collector(s). The label was printed on regular A4 papers and was 8.8 × 5.7 cm in size. All relevant information were written on the labels using a pencil. The new labels were fully immersed in preservative fluid following previous collection maintenance practice of EWNHM.

Alongside the general label, it was necessary to assign an unique voucher number to each specimen that did not currently have one, according to the nomenclature rules of the museum. The preexisting voucher system for the museum collection is such as EWNM-AR-XXXX, "XXXX" being the serial number. However, this previous system was not consistently applied throughout all of the specimens held in the collection and needed to be corrected. Moreover, the voucher system represented in NARIS is different from the preexisting voucher system of EWNHM. Thus, in order to apply a consistent voucher number system to each and all herpetological specimens, the decision was made by the museum to discard both previous EWNHM and NARIS voucher systems and to use a new protocol for the herpetology collection. The new numbering protocol is comprised of museum code EWNHM, followed by a taxon code ANIMAL, and a four-digit serial number starting from EWNHM-ANIMAL 5279. Despite the change of protocol, the previous EWNM-AR voucher labels were retained alongside the new EWNHM-ANIMAL voucher labels for the traceability of information.

Georeferencing protocol

One of the main issues concerning the use of natural history collections is determining the area of collection with reasonable accuracy (Newbold 2010). The collection labels, especially of historical collections, usually contain text-only geographic information of different extent and accuracy (Garcia-Milagros and Funk 2010). This was also the case with specimens deposited in EWNHM. Therefore, each specimen was georeferenced using the collection information available, with the locality of collection being recorded as latitude and longitude (in decimal degrees) describing the midpoint. For localities within a named town or city, the center of the settlement or named district was used as the midpoint. Google Maps was used along with the 'What's here?' tool to display the latitude and longitude once the midpoint had been found. For those specimens collected in more rural localities, the midpoint of the named location was also used as in the urban ones.

Catalogue

We organized the catalogue in Order – Family – Species order. For each species, English and Korean common names are also given alongside the current scientific name.

The nomenclature used in the original labels is also given. Location and collector information (written in either Korean or Chinese characters) are directly translated.

Terms and abbreviations used in the catalogue

Leg.	Collector
Loc.	Collection locality and collection date (see Table 1 and Table 2 for georeferenced GPS coordinates, and Fig. 1 for locations on the map)
Juv.	Juvenile
Nn.	Neonate
Lar.	Larvae
Td.	Tadpole(s)
Egg.	Egg(s)
Voucher series	A series of specimens of one species collected in the same location on the same date
-do	Korean equivalent of province
-ri	Korean equivalent of county
-myeon	Korean equivalent of village

Systematic account of vouchered specimens

Class Amphibia Linnaeus, 1758

Order Anura Duméril, 1805
Family Bombinatoridae Gray, 1825

***Bombina orientalis* (Boulenger, 1890)**
Oriental fire-bellied toad; 무당개구리; 440 specimens.

Boulenger GA (1890) A list of the reptiles and batrachians of Amoorland. Annals and Magazine of Natural History, Series 6, 5: 137–144.

EWNHM-ANIMAL 5284; Loc: Dobongsan, 10 May. 1959; Leg: no data. EWNHM-ANIMAL 5286; Loc: Mountain cabin of Chinbu-ryong (= Jinburyeong), Kangwon-do, 24 Sep. 1977; Leg: Natural History Museum. EWNHM-ANIMAL 5292; Loc: Cheoneun-sa, 7 May. 1977; Leg: Yun Seokjun. EWNHM-ANIMAL 5293; Loc: Chinbu-ryong (= Jinburyeong), 12 Aug. 1980; Leg: Yun Seokjun. EWNHM-ANIMAL 5324; Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 5325 – EWNHM-ANIMAL 5328 (total four specimens); Loc: Godongsan, Gyeonggi-do; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 5329 – EWNHM-ANIMAL 5332 (total four specimens); Loc: Gapyeong, Gyeonggi-do, 5 Jun. 1982; Leg: Noh Bunjo. EWNHM-ANIMAL 5348; Loc: Daedunsan, Jeonbuk, 3 May. 1978; Leg: Nat-

Table 1. Georeferenced collection localities of amphibian specimens deposited in the Ewha Womans University Natural History Museum (EWNHM). WGS 84 coordinate system.

Location	Latitude (°N) / Longitude (°E)
Baekryeongdo	37.9593N, 124.6654E
Baekwoondae	37.6598N, 126.9776E
Buhwangsa	37.6423N, 126.9713E
Buyong-ri, Yangsu-myeon, Yangpyeong, Gyeonggi-do	37.5551N, 127.3470E
Cheoneunsa, Jirisan	35.2728N, 127.4760E
Cheoneunsa, Jirisan	35.2728N, 127.4760E
Cheonmasan, Gyeonggi-do	37.6806N, 127.2727E
Chiaksan, Gangwon-do	37.3720N, 128.0504E
Jinburyeong	38.2652N, 128.3592E
Daeamsan, Inje, Gangwon-do	38.2118N, 128.1352E
Daedunsan, North Jeolla	36.1206N, 127.3232E
Dobongsan	37.7008N, 127.0156E
Dongducheon	37.9146N, 127.0746E
Dukcheon, Geomundo	34.0488N, 127.3184E
Gajwa-ri, Mitan-myeon, Pyeongchang	37.3109N, 128.5342E
Ganghwa	37.7134N, 126.4512E
Gapyeong, Gyeonggi-do	37.8115N, 127.4201E
Gayang Apt., Seoul	37.5639N, 126.8544E
Gayasan	35.8228N, 128.1179E
Geojedo	34.8542N, 128.6435E
Geomundo lighthouse	34.0491N, 127.3179E
Gimpo	37.6174N, 126.7088E
Godongsan, Gyeonggi-do	37.6586N, 127.4063E
Guri, Gyeonggi-do	37.5985N, 127.1282E
Gwangju, Gyeonggi-do	37.4061N, 127.3171E
Gwangneung	37.7522N, 127.1771E
Gwangneungnae	37.7462N, 127.2040E
Gyeryongsan	36.3427N, 127.2056E
Hongcheon, Gangwon-do	37.7390N, 128.0667E
Hwangyongdong 25-1, Gyeongju	35.8265N, 129.3691E
Hwayasan, Gapyeong, Gyeonggi-do	37.6716N, 127.4278E
Imjingak, Paju, Gyeonggi-do	37.8895N, 126.7401E
Jangsudae, Seoraksan	38.1196N, 128.3415E
Jeotgae, Anmyeondo	36.4229N, 126.4205E
Jingwansa	37.6381N, 126.9466E
Jirisan	35.3373N, 127.7305E
Jugeumsan, Namyangju, Gyeonggi-do	37.7845N, 127.2690E
Maesol Forest, Andong	36.5520N, 128.5901E
Mountain cabin of Chinbu-ryong, Gangwon-do	38.2652N, 128.3592E
Mugeuk, Eumseong	36.9960N, 127.5889E
Mujugucheondong	35.8775N, 127.7788E
Myeonggae-ri, Naemyeon, Hongcheon	37.8465N, 128.5373E
Myeongjisan	37.9420N, 127.4319E
Myeonmokdong, Seoul	37.5795N, 127.0972E
Namyangju, Gyeonggi-do	37.6563N, 127.2347E
Nanjido, Seoul	37.5715N, 126.8686E
Noewoon-ri, Pyeongchang, Gangwon-do	37.4142N, 128.3511E
Nogodan, Jirisan	35.2942N, 127.5326E
Noron-ri, Pyeongchang	37.3516N, 128.4317E
Odaesan	37.7986N, 128.5429E

Location	Latitude (°N) / Longitude (°E)
Outside Jahamun, Seoul	37.5925N, 126.9664E
Pyeongnae, Gyeonggi-do	37.6524N, 127.2253E
Sanghwanam, Sokrisan	36.5469N, 127.8599E
Sangwonsa, Odaesan	37.7865N, 128.5643E
Seogwipo, Jeju	33.2495N, 126.5641E
Seoraksan Checkpoint	38.1200N, 128.4659E
Sinchon	37.5671N, 126.9432E
Sinwondong, Dukyang-gu, Goyang-si, Gyeonggi-do	37.6725N, 126.8791E
Sobaeksan	36.9521N, 128.4460E
Sokrisan	36.5336N, 127.8998E
Songchu Valley	37.7125N, 126.9849E
Soyosan, Dongducheon	37.9428N, 127.0878E
Sudeoksa, South Chungcheong	36.6630N, 126.6225E
Taehadong, Ulleungdo	37.5026N, 130.8247E
Under Ewha Bridge	37.6015N, 127.0724E
Wolchulsan, Yeongam	34.7746N, 126.7104E
Woljeongsa, Odaesan	37.7270N, 128.5956E
Woraksan, North Chungcheong	36.8894N, 128.0909E
Yangsuri, Gyeonggi-do	37.5702N, 127.3395E
Yongjang-ri, Namsan, Gyeongju, North Gyeongsang	35.7682N, 129.2254E
Yongwha-ri, Cheorwon-gun, Gangwon-do	38.1316N, 127.3480E

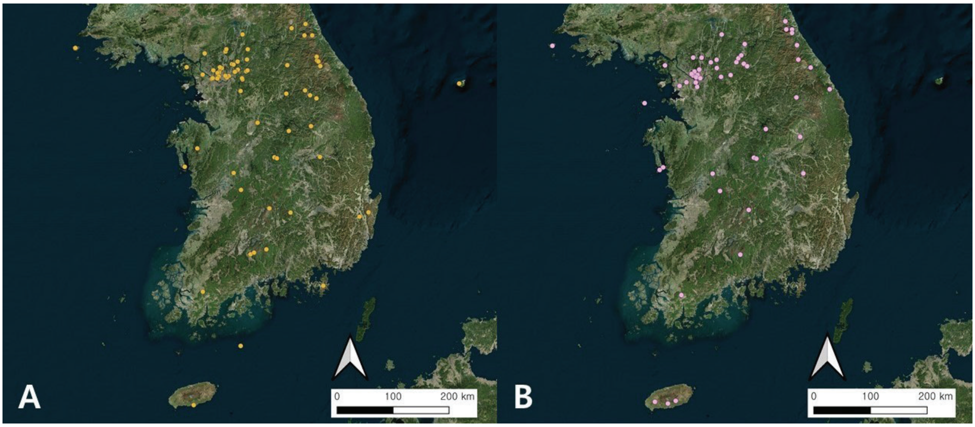

Figure 1. Collection localities of amphibian (**A**) and reptile (**B**) specimens of the Ewha Womans University Natural History Museum (EWNHM). Dubious collection localities are not shown in the map. Map generated in QGIS 3.10.0. The Bing Aerial Map was used as the base map, acquired via OpenLayers plugin implemented in QGIS 3.10.0.

ural History Museum. Voucher series EWNHM-ANIMAL 5349 – EWNHM-ANIMAL 5351 (total three specimens); Loc: Hwayasan, Gyeonggi-do, 20 Apr. 1997; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 5364 – EWNHM-ANIMAL 5367 (total four specimens); Loc: Cheonmasan, 12 May. 1968; Leg: Noh Bunjo. Voucher series EWNHM-ANIMAL 5368 – EWNHM-ANIMAL 5371 (total four specimens); Loc: Dobongsan, 16 May. 1959; Leg: Kim and Lee. Voucher series EWNHM-ANI-

Table 2. Georeferenced collection localities of reptile specimens deposited in the Ewha Womans University Natural History Museum (EWNHM). WGS 84 coordinate system.

Location	Latitude / Longitude
Anmyeondo	36.4229N, 126.4205E
Baekdamsa, Seoraksan	38.1647N, 128.3738E
Baekryeongdo	37.9593N, 124.6654E
Balang-ri, Paju, Gyeonggi-do	37.8118N, 126.8969E
Biseondae, Seoraksan	38.1636N, 128.4658E
Bucheon, Gyeonggi-do	37.5013N, 126.7903E
Bukhansan	37.6611N, 126.9932E
Byeoksoryeong, Jirisan	35.3005N, 127.6446E
Cheonmasan	37.6806N, 127.2727E
Daedunsan, North Jeolla	36.1206N, 127.3232E
Daegwanryeong	37.6875N, 128.7604E
Deokjeokdo	37.2381N, 126.1279E
Ewha Womans University Campus	37.5598N, 126.9454E
Gajamul, Susaek, Seoul	37.5861N, 126.8952E
Gajeong-ri, Nammyeon, Chuncheon-si, Gangwon-do	37.7539N, 127.5829E
Gangchon, Chunseong-gun (=Chuncheon), Gangwon-do	37.8029N, 127.6138E
Ganghwa	37.7134N, 126.4512E
Godaedo	36.3892N, 126.3655E
Gomgol, Seoraksan	38.1200N, 128.4659E
Gotan, Chunseong-gun (= Chuncheon), Gangwon-do	37.9812N, 127.7149E
Gupabal	37.6365N, 126.9191E
Gwanaksan, Gyeonggi-do	37.4431N, 126.9610E
Gwangneung	37.7522N, 127.1771E
Gwangpan-ri, Chunseong-gun (= Chuncheon), Gangwon-do	37.7339N, 127.7014E
Gyeryongsan, South Chungcheong	36.3427N, 127.2056E
Hanlim, Jeju-do	33.3723N, 126.2912E
Hwajeon, Gyeonggi-do	37.6070N, 126.8738E
Jinburyeong	38.2652N, 128.3592E
Juan	37.4558N, 126.6828E
Jugeumsan, Gyeonggi-do	37.7845N, 127.2690E
Miro, Samcheok, Gangwon-do	37.4137N, 129.0621E
Mitan, Pyeongchang, Gangwon-do	37.3109N, 128.5342E
Mujugucheondong	35.8775N, 127.7788E
Myeongseongsan, Pocheon, Gyeonggi-do	38.1012N, 127.3493E
Outside Jahamun	37.5925N, 126.9664E
Palbongsan, Hongcheon, Gangwon-do	37.7032N, 127.7538E
Samakmyeon, Chunseong-gun (= Chuncheon), Gangwon-do	37.8401N, 127.6603E
Sangdodong, Seoul	37.4988N, 126.9382E
Sanghwanam, Sokrisan	36.5469N, 127.8599E
Sangwonsa, Odaesan	37.7865N, 128.5643E
Seongpanak, Jeju	33.3851N, 126.6204E
Seorim, Yangyang, Gangwondo	37.9680N, 128.5435E
Sinchon	37.5671N, 126.9432E
Sindangdong, Seoul	37.5523N, 127.0093E
Sindang-ri, Chungju	36.9101N, 128.0492E
Sokrisan, North Chungcheong	36.5336N, 127.8998E
Wolchulsan, Yeongam	34.7746N, 126.7104E
Yangju	37.8096N, 127.0305E
Yangsuri	37.5702N, 127.3395E
Yeongju	36.8115N, 128.5941E
Yeongsil, Hanrasan, Jeju	33.3489N, 126.4969E
Yukgokcheon, Euiseong	36.3459N, 128.6438E
Yumyeongsan, Gapyeong, Gyeonggi-do	37.5927N, 127.4911E

MAL 5410 – EWNHM-ANIMAL 5441 (total 32 specimens); Loc: Chinbu-ryong (= Jinburyeong), 12 Aug. 1979; Leg: Noh Bunjo. Voucher series EWNHM-ANIMAL 5442 – EWNHM-ANIMAL 5456 (total 15 specimens); Loc: Hwayasan, Gapyeong, Gyeonggi-do, 22 Apr. 1985; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 5457 – EWNHM-ANIMAL 5486 (total 30 specimens); Loc: Chinbu-ryong (= Jinburyeong), 12 Jul. 1980; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 5487 – EWNHM-ANIMAL 5514 (total 28 specimens); Loc: Sokrisan, 17 Jul. 1961; Leg: no data. Voucher series EWNHM-ANIMAL 5561 – EWNHM-ANIMAL 5660 (total 100 specimens); Loc: Gyeryongsan, 24 Jul. 1973; Leg: "Premed collecting team". Voucher series EWNHM-ANIMAL 5661 – EWNHM-ANIMAL 5710 (total 50 specimens); Loc: Gyeryongsan, 23 Jul. 1973; Leg: Department of Biology. Voucher series EWNHM-ANIMAL 5711 – EWNHM-ANIMAL 5760 (total 50 specimens); Loc: Soyosan, Dongducheon, 11 Jun. 1972; Leg: Department of Biology. Voucher series EWNHM-ANIMAL 6060 – EWNHM-ANIMAL 6072 (total 13 specimens); Loc: Chiaksan, Gangwon, 2 Jun. 1979; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6073 – EWNHM-ANIMAL 6090 (total 18 specimens); Loc: Myeongjisan, 6 May. 1972; Leg: Jang Soonran. Voucher series EWNHM-ANIMAL 6091 – EWNHM-ANIMAL 6093 (total three specimens); Loc: Hwangyongdong 25-1, Gyeongju, 26 Apr. 2007; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6094 – EWNHM-ANIMAL 6110 (total 17 specimens); Loc: Cheonmasan, 19 May. 1979; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6111 – EWNHM-ANIMAL 6124 (total 14 specimens); Loc: Godongsan, Gyeonggi-do, 27 May. 1978; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6125 – EWNHM-ANIMAL 6128 (total four specimens); Loc: Buhwangsa, 3 May. 1964; Leg: Oh Soonja. Voucher series EWNHM-ANIMAL 6129 – EWNHM-ANIMAL 6143 (total 15 specimens); Loc: Dobongsan, Seoul, 16 May. 1959; Leg: Kim Myungae, Kim Myungsook, Lee Jongwan. EWNHM-ANIMAL 6144; Loc: Gwangneung, 11 May. 1957; Leg: Kang Jeon Il. EWNHM-ANIMAL 6355; Loc: Myeongjisan, Gyeonggi, 30 Sep. 2000; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6356 – EWNHM-ANIMAL 6358 (total three specimens); Loc: Chinbu-ryong (= Jinburyeong), 13 Aug. 1979; Leg: Noh Bunjo. EWNHM-ANIMAL 6362; Loc: Outside Jahamun, Seoul; Leg: Noh Bunjo, Yu Seongin. EWNHM-ANIMAL 6366; Loc: Gwangneung, 11 May. 1951; Leg: Kang Jeon Il. EWNHM-ANIMAL 6367; Loc: Woraksan, Chungbuk, 20 Jul. 1972; Leg: Noh Bunjo. Voucher series EWNHM-ANIMAL 6372 – EWNHM-ANIMAL 6373 (total two specimens); Loc: Jangsudae, Seoraksan, 10 Oct. 1970; Leg: "2nd grade students". EWNHM-ANIMAL 6378; Loc: Mujugucheondong, 9 May. 1979; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6391 – EWNHM-ANIMAL 6396 (total six specimens); Loc: Maebong, Odaesan, 6 Aug. 2001; Leg: Kim Byungwoo. Voucher series EWNHM-ANIMAL 6397 – EWNHM-ANIMAL 6400 (total four specimens); Loc: Gayasan, 17 May. 1963; Leg: Noh Bunjo. EWNHM-ANIMAL 6634; Loc: Woljeongsa, Odaesan, 16 Sep. 2006; Leg: Kim Byungwoo. EWNHM-ANIMAL 6661; Loc: Maebong, Odaesan, 8 Aug. 2006; Leg: no data. Voucher series EWNHM-ANIMAL 6672 – EWNHM-ANIMAL 6674 (total three specimens); Loc: Daeamsan, Inje, Gangwon-do, 18 May. 1993; Leg: Sung Gisoo.

Citation: Shin et al. (2020a).

Remarks: vouchers EWNHM-ANIMAL 5437, 5449, 5463, 5624, 5642, 5663, and 5677 had malformed limbs (Shin et al. 2020a)

Family Bufonidae Gray, 1825

Bufo gargarizans Cantor, 1842

Asiatic toad; 두꺼비; 28 specimens

Cantor T (1842) General features of Chusan, with remarks on the flora and fauna of that island. Annals and Magazine of Natural History, Series 1, 9: 481–493.

EWNHM-ANIMAL 5279; Loc: Sudeoksa, 8 Jun. 1969; Leg: Eom Jeonghui, Lee Hyeonju, Lee Okju, Choi Jeongran. EWNHM-ANIMAL 5280; Loc: Jeongneung, Gyeonggi, 8 Jun. 1967; Leg: Paik Hyangsun. EWNHM-ANIMAL 5281; Loc: Gwangneung, Gyeonggi, 17 May. 1964; Leg: Lim Jeonghye. EWNHM-ANIMAL 5282; Loc: no data; Leg: no data. EWNHM-ANIMAL 5288; Loc: Muju Gucheondong, Jul. 1972; Leg: Yun Seokjun. EWNHM-ANIMAL 5289; Loc: Chinbu-ryong (= Jinburyeong), 13 Aug. 1980; Leg: Yun Seokjun. EWNHM-ANIMAL 5290; Loc: Eoreumgol, Cheonwang-sa, Kyungpook, 23 Jul. 1986; Leg: Yun Seokjun. EWNHM-ANIMAL 5291; Loc: Sinchon, 21 Jun. 1955; Leg: Department of Biology. EWNHM-ANIMAL 5294; Loc: Dongducheon, 1 Jul. 1971; Leg: Lee Eunbok Voucher series EWNHM-ANIMAL 5299 – EWNHM-ANIMAL 5301 (total three specimens); Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 5302 – EWNHM-ANIMAL 5303 (total two specimens); Loc: no location data, 8 Aug, 1983; Leg: no data. Voucher series EWNHM-ANIMAL 5304 – EWNHM-ANIMAL 5305 (total two specimens); Loc: Mugeuk, Eumseong, Chungbuk, 31 Mar. 1994; Leg: Seo Hyeongseok. Voucher series EWNHM-ANIMAL 5306 – EWNHM-ANIMAL 5307 (total two specimens); Loc: no data; Leg: Kim Hungyu. EWNHM-ANIMAL 5308; Loc: Sanghwanam, Sokrisan, 17 Jul. 1961; Leg: no data. Voucher series EWNHM-ANIMAL 5309 – EWNHM-ANIMAL 5310 (total two specimens); Loc: Sinchon (Seoul), 5 Jul. 1955; Leg: Department of Biology. Voucher series EWNHM-ANIMAL 5311 – EWNHM-ANIMAL 5316 (total six specimens); Loc: Geojedo, 23 Jul. 1970; Leg: Kim Hungyu. EWNHM-ANIMAL 6724 (Egg.); Loc: Mugeuk, Eunseong, 31 Mar. 1994; Leg: Seo Hyeongseok.

Bufo stejnegeri Schmidt, 1931

Korean water toad; 물두꺼비; 27 specimens

Schmidt KP (1931) A new toad from Korea. Copeia 1931: 93–94.

EWNHM-ANIMAL 5285; Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 5811 – EWNHM-ANIMAL 5816 (total six specimens); Loc: Myeonggye-ri (= Myeonggae-ri), Naemyeon, Hongcheon, 21 May. 1992; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 5817 – EWNHM-ANIMAL 5826 (total ten specimens); Loc:

Seoraksan Checkpoint, 12 Oct. 1977; Leg: Donation by Prof. Yun Ilbyung. Voucher series EWNHM-ANIMAL 6369 – EWNHM-ANIMAL 6371 (total three specimens); Loc: Jangsudae, Seoraksan, 10 Oct. 1970; Leg: "2nd grade students". Voucher series EWNHM-ANIMAL 6627 – EWNHM-ANIMAL 6631 (Juv.; total five specimens); Loc: Maebong, Odaesan, 6 Sep. 2006; Leg: Kim Byungwoo. Voucher series EWNHM-ANIMAL 6678 – EWNHM-ANIMAL 6679 (total two specimens); Loc: Noewoon-ri, Pyeongchang, Gangwon-do, 7 Apr. 1995; Leg: Seo Suyeon, Yun Seokjun.

Bufo sp.

Two specimens

Voucher series EWNHM-ANIMAL 6625 – EWNHM-ANIMAL 6626 (Juv.; total two specimens); Loc: Sudeoksa, Chungnam, 3 Aug. 2006 – 1 Sep. 2006; Leg: Kim Byungwoo.

Remarks: the morphological characteristics to distinguish between *B. gargarizans* and *B. stejnegeri*, such as clearly visible tympanum, were insufficient to identify these specimens at species level.

Family Hylidae Rafinesque, 1815

Dryophytes suweonensis (Kuramoto, 1980)

Suweon treefrog; 수원청개구리; one specimen
Original label name: *Hyla suweonensis*

Kuramoto M (1980) Mating calls of treefrogs (genus *Hyla*) in the Far East, with description of a new species from Korea. Copeia 1980: 100–108.

EWNHM-ANIMAL 6377; Loc: Imjingak, Paju, Gyeonggi, 6 Jul. 2011; Leg: no data.

Remarks: updated generic assignment according to Duellman et al. (2016).

Dryophytes japonicus (Günther, 1859 (1858))

Japanese treefrog; 청개구리; 26 specimens
Original label name: *Hyla japonica* or *Hyla arborea japonica*

Günther ACLG (1859 "1858") Catalogue of the Batrachia Salientia in the Collection of the British Museum. Taylor and Francis, London, United Kingdom, xvi + 160 pp.

Voucher series EWNHM-ANIMAL 6374 – EWNHM-ANIMAL 6375 (total two specimens); Loc: Dobongsan, 16 May. 1959; Leg: Kim and Lee. EWNHM-ANIMAL 6376; Loc: Geomundo lighthouse, 16 Jul. 1977; Leg: Song Junim. EWNHM-ANIMAL 6386; Loc: Cheonmasan, Gyeonggi, 9 May. 1980; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6664 – EWNHM-ANIMAL 6665 (total two specimens); Loc: Wolchulsan, Yeongnam, 5 Oct. 2006; Leg: Kim Byungwoo. EWNHM-ANIMAL 6676; Loc: Imjingak, Paju, Gyeonggi, 6 Jul. 2011; Leg: no data. EWNHM-

ANIMAL 6677; Loc: Maesol Forest, Andong, Gyungbuk, 13 Jul. 2013; Leg: Yun Seokjun. EWNHM-ANIMAL 6680; Loc: Noewoon-ri, Pyeongchang, Gangwon-do, 7 Apr. 1995; Leg: Seo Suyeon, Yun Seokjun. EWNHM-ANIMAL 6683; Loc: Gwangneung, 11 May. 1957; Leg: Bae Yeongsoon, Moon Yeongja. EWNHM-ANIMAL 6684; Loc: no data; Leg: no data. EWNHM-ANIMAL 6685; Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 6686 – EWNHM-ANIMAL 6687 (total two specimens); Loc: Dobongsan, 17 Jul. 1959; Leg: Kim Yeonghee. EWNHM-ANIMAL 6692; Loc: Godongsan, Gyeonggi-do, 2 May. 1981; Leg: Yun Seokjun. EWNHM-ANIMAL 6693; Loc: Yangsuri, Gyeonggi, 13 Oct. 1978; Leg: Noh Bunjo, Yun Seokjun. EWNHM-ANIMAL 6694; Loc: Dukcheon, Geomundo, 15 Jul. 1977; Leg: Yun Seokjun.

Remarks: updated generic assignment as explained above for *D. suweonensis*.

Family Microhylidae Günther, 1858 (1843)

Kaloula borealis (Barbour, 1908)

Boreal digging frog; 맹꽁이; 21 specimens

Barbour T (1908) Some new reptiles and amphibians. Bulletin of the Museum of Comparative Zoology 51: 315–325.

Voucher series EWNHM-ANIMAL 5317 – EWNHM-ANIMAL 5322 (total six specimens); Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 5352 – EWNHM-ANIMAL 5363 (total 12 specimens); Loc: Gwangju, Gyeonggi-do, 14 Jul. 1982; Leg: Ko Soon Book. EWNHM-ANIMAL 6381; Loc: Gayang Apt., Seoul, 12 Jul. 1993; Leg: Yun Seokjun. EWNHM-ANIMAL 6401; Loc: Nanjido, Seoul, 24 Jul. 2000; Leg: no data. EWNHM-ANIMAL 6723; Loc: no data; Leg: no data.

Family Ranidae Batsch, 1796

Glandirana emeljanovi (Nikolsky, 1913)

Imienpo Station frog; 옴개구리; 227 specimens
Original label name: *Rana rugosa*

Nikolskii AM (1913) *Rana emeljanovi* sp. n.. Annuaire du Musée Zoologique de l'Academie Impériale des Sciences de St. Pétersbourg 18: 148–150.

EWNHM-ANIMAL 5283; Loc: Gwangneung, 11 May. 1958; Leg: no data. EWNHM-ANIMAL 5287; Loc: Gwangneung, 11 May. 1957; Leg: no data. EWNHM-ANIMAL 5295; Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 5296 – EWNHM-ANIMAL 5323 (total 28 specimens); Loc: Yongwha-ri, Cheorwon-gun, Kangwon-do, 25 Aug. 1977; Leg: Yun Seokjun. EWNHM-ANIMAL 5333; Loc: Gwangneung, 11 May. 1957; Leg: no data. EWNHM-ANIMAL 5334; Loc: Sanghwanam, Sokrisan, 17 Jul. 1961; Leg: no data. Voucher series EWNHM-ANIMAL 5335 – EWNHM-ANIMAL 5340 (total six specimens); Loc: Dobongsan, 16 May. 1959; Leg: no data. Voucher series EWNHM-ANIMAL 5341 – EWNHM-ANIMAL 5347 (total seven specimens); Loc: Gwangneung, 30 Apr. 1978; Leg: Department of Biology. Voucher series EWNHM-ANIMAL 5515 – EWNHM-ANIMAL 5560 (total 46 specimens); Loc: Gyeryongsan, 27 Oct. 1969; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 5927 – EWNHM-ANIMAL 5999 (total 73 specimens); Loc: Soyosan, Dongducheon, 19 May. 1967; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6000 – EWNHM-ANIMAL 6059 (total 60 specimens); Loc: Myeongjisan, Gapyeong, 11 May. 1969; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6145 – EWNHM-ANIMAL 6146 (total two specimens); Loc: Hwangyongdong 25-1, Gyeongju, 26 Apr. 2007; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6300 – EWNHM-ANIMAL 6301 (total two specimens); Loc: Songchu, 30 Sep. 1972; Leg: Yeom Yeonghwa. Voucher series EWNHM-ANIMAL 6302 – EWNHM-ANIMAL 6304 (total three specimens); Loc: Gwangneung, 11 May. 1958; Leg: Kang Yeongsaeng. EWNHM-ANIMAL 6368; Loc: Songchu Valley, 30 Sep. 1972; Leg: Park Myeongju (Dept. of Chemistry Education). Voucher series EWNHM-ANIMAL 6411 – EWNHM-ANIMAL 6427 (total 17 specimens); Loc: Gwangneung, 23 May. 1976; Leg: Dept. of Biology. EWNHM-ANIMAL 6632; Loc: Sinwondong, Goyang-si, Gyeonggi-do, 29 Sep.2000; Leg: Yun Seokjun.

Remarks: the labels were updated according to generic assignment by Fei et al. (1990) and species name follows the original description by Nikolskii (1913 as *Rana emeljanovi*).

***Lithobates catesbeianus* (Shaw, 1802)**
American bullfrog; 황소개구리; nine specimens
Original label name: *Rana catesbeiana*

Shaw G (1802) General zoology or systematic natural history. Volume III, Part 1. Amphibia. Thomas Davison, London, United Kingdom, 312 pp.

Voucher series EWNHM-ANIMAL 5372 – EWNHM-ANIMAL 5379 (total eight specimens); Loc: no data; Leg: no data. EWNHM-ANIMAL 6380; Loc: Gimpo, 4 Jul. 1999; Leg: Song Junim.

Remarks: the labels were updated following the original generic assignment *Lithobates* by Fitzinger (1843), resurrected by Frost et al. (2006).

***Pelophylax nigromaculatus* (Hallowell, 1861(1860))**
Black-spotted pond frog; 참개구리; 188 specimens
Original label name: *Rana nigromaculata*

Hallowell E (1861 “1860”) Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S. N. Proceedings of the Academy of Natural Sciences of Philadelphia 12: 480–510.

Voucher series EWNHM-ANIMAL 5761 – EWNHM-ANIMAL 5795 (total 35 specimens); Loc: Cheonmasan, Gyeonggi, 12 May. 1968; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 5827 – EWNHM-ANIMAL 5926 (total 100 specimens); Loc: Cheonmasan, 17 May. 1967; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6336 – EWNHM-ANIMAL 6337 (total two specimens); Loc: Gwangneung, 10 May. 1958; Leg: no data. EWNHM-ANIMAL 6338; Loc: Jeotgae, Anmyeondo, 26 Jul. 1956; Leg: Kim Hoonsoo. Voucher series EWNHM-ANIMAL 6339 – EWNHM-ANIMAL 6348 (total ten specimens); Loc: Dobongsan, 16 May. 1959; Leg: Kim and Lee. EWNHM-ANIMAL 6353; Loc: Baekryeong-do, 27 May, 1958; Leg: Kim Hoonsoo. EWNHM-ANIMAL 6354; Loc: Sanghwanam, Sokrisan, 17 Sep. 1961; Leg: no data. EWNHM-ANIMAL 6379; Loc: Gwangneung, 11 May. 1957; Leg: Ko Wha Soon. EWNHM-ANIMAL 6382; Loc: Taehadong (Ulleungdo), 11 Aug. 1958; Leg: Noh Bunjo. EWNHM-ANIMAL 6383; Loc: Gwangneung, 11 May. 1957; Leg: Ko Wha Soon (Ko Who Soon). Voucher series EWNHM-ANIMAL 6402 – EWNHM-ANIMAL 6409 (total eight specimens); Loc: no location data (label degraded), 26 Jul. 1971; Leg: no data (label degraded). EWNHM-ANIMAL 6410; Loc: Gwangneung, 23 May. 1976; Leg: Dept. of Biology. EWNHM-ANIMAL 6622; Loc: no data; Leg: no data. EWNHM-ANIMAL 6623; Loc: no data; Leg: no data. EWNHM-ANIMAL 6624; Loc: no data; Leg: no data. Voucher series EWNHM-ANIMAL 6637 – EWNHM-ANIMAL 6656 (total 20 specimens); Loc: Soyosan, Gyeonggi-do, 19 May. 1967; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6688 – EWNHM-ANIMAL 6690 (total three specimens); Loc: Hongcheon, Gangwon-do, 2 Aug. 1999; Leg: Jeong Yuhyeon.

Remarks: the labels were updated following the original generic assignment by Fitzinger (1843), considered as distinct genus by Fei et al. (1990) and supported by Frost et al. (2006).

***Pelophylax chosenicus* (Okada, 1931)**
Golden-spotted pond frog; 금개구리; 31 specimens
Original label name: *Rana plancyi*

Okada Y (1931) The tailless batrachians of the Japanese Empire. Imperial Agricultural Experiment Station, Tokyo, 215 pp.

Voucher series EWNHM-ANIMAL 5380 – EWNHM-ANIMAL 5395 (total 16 specimens); Loc: Ganghwa-do, 2 Jul. 1972; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 5396 – EWNHM-ANIMAL 5409 (total 14 specimens); Loc: Buyong-ri, Yangsu-myeon, Yangpyeong, Gyeonggi-do, 8 Jun. 1970; Leg: Lee Yonghee. EWNHM-ANIMAL 6696; Loc: Gwangneungnae, 22 May. 1971; Leg: Kim Hungyu.

Remarks: updated generic assignment as explained above for *P. nigromaculatus*.. The species name follows the original description by Okada (1931; as *Rana nigromaculata chosenica*) under new combination (Frost et al. 2006).

***Rana coreana* Okada, 1928**
Korean brown frog; 한국산개구리; 147 specimens
Original label: *Rana amurensis* or *Rana amurensis coreana*

Okada Y (1928) Frogs in Korea. Chosen Natural History Society Journal 6: 15–46.

Voucher series EWNHM-ANIMAL 6147 – EWNHM-ANIMAL 6196 (total 50 specimens); Loc: Gwangneung, 27 Jun. 1973; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6197 – EWNHM-ANIMAL 6246 (total 50 specimens); Loc: Gwangneung, 12 May. 1971; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6247 – EWNHM-ANIMAL 6285 (total 39 specimens); Loc: Pyeongnae, Gyeonggi, 12 Jun. 1959; Leg: Jang Hanwi. Voucher series EWNHM-ANIMAL 6286 – EWNHM-ANIMAL 6289 (total four specimens); Loc: Pyeongnae, Gyeonggi, 2 Jun. 1959; Leg: Jang Hanwi. Voucher series EWNHM-ANIMAL 6359 – EWNHM 6361 (total three specimens); Loc: Gwangju, Gyeonggi-do, 12 Nov. 2000; Leg: Yun Seokjun. EWNHM-ANIMAL 6390; Loc: Dobongsan, 17 Jul. 1959; Leg: Kim Yeonghee.

Remarks: *Rana coreana* was demonstrated to be distinct from *R. amurensis* by Song et al. (2006), and the labels were updated accordingly.

***Rana huanrenensis* Fei, Ye & Huang, 1990**
Huanren frog; 계곡산개구리; 13 specimens
Original label name: *Rana temporaria ornativentris*

Fei L, Ye C, Huang Y (1990) Key to Chinese amphibians. Publishing House for Scientific and Technological Literature, Chongqing, China, 364 pp.

Voucher series EWNHM-ANIMAL 6323 – EWNHM-ANIMAL 6327 (total five specimens); Loc: Sanghwanam, Sokrisan, 15 Jul. 1961; Leg: no data. Voucher series EWNHM-ANIMAL 6618 – EWNHM-ANIMAL 6621 (total four specimens); Loc: Sinwondong, Goyang-si, Gyeonggi-do, 29 Sep. 2000; Leg: Yun Seokjun. EWNHM-ANIMAL 6633; Loc: Jugeumsan, Namyangju, Gyeonggi-do, 17 Sep. 2004; Leg: Yun Seokjun. EWNHM-ANIMAL 6662; Loc: Sinwondong, Dukyang-gu, Goyang-si, Gyeonggi-do, 17 Aug. 2008 ; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6681 – EWNHM-ANIMAL 6682 (total two specimens); Loc: Noewoon-ri, Pyeongchang, Gangwon-do, 7 Apr. 1995; Leg: Seo Suyeon, Yun Seokjun.

Remarks: the labels were updated to reflect the change of species status suggested by Fei et al. (1990). The presence of this species in the Republic of Korea was confirmed by Yang et al. (2000).

***Rana uenoi* Matsui, 2014**
Prevernal frog / Ueno's brown frog; 북방산개구리; 158 specimens
Original label name: *Rana temporaria ornativentris*

Matsui M (2014) Description of a new Brown Frog from Tsushima Island, Japan (Anura: Ranidae: *Rana*). Zoological Science. Tokyo 31: 613–620.

Voucher series EWNHM-ANIMAL 5796 – EWNHM-ANIMAL 5810 (total 15 specimens); Loc: Namyangju, Gyeonggi, 1 Feb. 1970; Leg: Noh Bunjo. Voucher series EWNHM-ANIMAL 6290 – EWNHM-ANIMAL 6299 (total ten specimens); Loc: Sobaeksan, Chungbuk, 1 Oct. 1971; Leg: Kim Hungyu. Voucher series EWNHM-ANIMAL 6305 – EWNHM-ANIMAL 6310 (total six specimens); Loc: Guri, Gyeonggi, 11 May. 1970; Leg: Kim Hunkyu. Voucher series EWNHM-ANIMAL 6311 – EWNHM-ANIMAL 6315 (total five specimens); Loc: Dobongsan, 16 May. 1959; Leg: Kim Okhee. Voucher series EWNHM-ANIMAL 6316 – EWNHM-ANIMAL 6320 (total five specimens); Loc: Sokrisan, Chungbuk, 6 Apr. 1979; Leg: Noh Bunjo, Yun Seokjun. EWNHM-ANIMAL 6321; Loc: Chinbu-ryong (= Jinburyeong), 24 Sep. 1977; Leg: Natural History Museum. EWNHM-ANIMAL 6322; Loc: Songchu Valley, 30 Sep. 1972; Leg: Jeong Hyesook. Voucher series EWNHM-ANIMAL 6328 – EWNHM-ANIMAL 6330 (total three specimens); Loc: Cheoneunsa, Jirisan, 7 May. 1977; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6331 – EWNHM-ANIMAL 6335 (total five specimens); Loc: Jingwansa, 11 May. 1963; Leg: Noh Bunjo. Voucher series EWNHM-ANIMAL 6349 – EWNHM-ANIMAL 6352 (total four specimens); Loc: Sinjang-eup, Gwangju, Gyeonggi, 25 Oct. 1983; Leg: Kim Hoyeong. EWNHM-ANIMAL 6365; Loc: "Under Ewha Bridge", 11 Mar. 1964; Leg: Jeong Songgeun. Voucher series EWNHM-ANIMAL 6384 – EWNHM-ANIMAL 6385 (total two specimens); Loc: Gwangneung, 11 May. 1957; Leg: Ko Wha Soon (Ko Who Soon). Voucher series EWNHM-ANIMAL 6387 – EWNHM-ANIMAL 6388 (total two specimens); Loc: Bogwangsa, Gyeonggi, 10 May. 1980; Leg: Yun Seokjun. EWNHM-ANIMAL 6389; Loc: Dobongsan, 17 Jul. 1959; Leg: Kim Yeonghee. EWNHM-ANIMAL 6675; Loc: Seogwipo, Jeju, 30 May. 2013; Leg: no data. EWNHM-ANIMAL 6695; Loc: Gwangneungnae, 22 May. 1971; Leg: Kim Hungyu. EWNHM-ANIMAL 6722 (Egg.); Loc: Sokrisan, Chungbuk, 6 Apr. 1979; Leg: Yun Seokjun. EWNHM-ANIMAL 6729 (Egg.); Loc: under Ewha Bridge, 21 Mar. 1964; Leg: "Student". EWNHM-ANIMAL 6731 (Egg.); Loc: Munsudae, Jirisan, 8 May. 1976; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6732 – 6823 (Td.; total 92 specimens); Loc: Gwangneung, 11 May. 1957; Leg: Bae Yeongsoon, Moon Yeongja.

Remarks: the labels were updated to reflect the change of species status of *Rana* from Tsushima Island, Japan, and the Republic of Korea, demonstrated by Matsui (2014).

Rana sp.

Ten specimens

Voucher series EWNHM-ANIMAL 6363 – EWNHM-ANIMAL 6364 (total two specimens); Loc: Gwangneung, 12 May. 1959; Leg: no data. EWNHM-ANIMAL 6663; Loc: Wolchulsan, Yeongnam, 5 Oct. 2006; Leg. Kim Byungwoo. Voucher series EWNHM-ANIMAL 6666 – EWNHM-ANIMAL 6669 (total four specimens); Loc:

Sangwonsa, Odaesan, 28 Sep. 2005; Leg; Kim Byungwoo. EWNHM-ANIMAL 6701; Loc: no data; Leg: no data. EWNHM-ANIMAL 6727 (Egg.); Loc: Gwangneung, 11 May. 1957; Leg: Bae Yeongsoon, Moon Yeongja. EWNHM-ANIMAL 6728 (Td.); Loc: Nogodan, Jirisan, 8 May. 1971; Leg: Noh Bunjo.

Remarks: all of the bigger *Rana* specimens (now *R. uenoi* and *R. huanrenensis*) deposited in the EWNHM were originally labelled as "*Rana temporaria ornativentris*". Under the taxonomic framework of *Rana* used here, this name can be traced to either *Rana uenoi* or *Rana huanrenensis*. *Rana uenoi* and *R. huanrenensis* can be distinguished based on several morphometric characteristics (Yang et al. 2000); however, distinguishing between the two species based on this method was not always possible during cataloging procedure. Therefore, it is likely that some of the specimens identified as *R. uenoi* by us are actually *R. huanrenensis*, and vice versa. Moreover, there is a collection of *Rana* froglet specimens that could not be identified and could be a mix of froglets of *R. uenoi*, *R. huanrenensis*, and *R. coreana*. Also, froglets of *Pelophylax nigromaculatus* and *Glandirana emeljanovi* were mixed with these specimens but identified. The specimens of *Rana coreana* have been labelled as *Rana amurensis coreana*.

Family Rhacophoridae Hoffman, 1932 (1858)

Rhacophorus sp.

Two specimens

EWNHM-ANIMAL 6697; Loc: no data; Leg: no data. EWNHM-ANIMAL 6698; Loc: no data; Leg: no data.

Order Urodela Duméril, 1805
Family Hynobiidae Cope, 1859 (1856)

Hynobius leechii Boulenger, 1887

Korean salamander; 도롱뇽; 44 specimens

Boulenger GA (1887) Description of a new tailed batrachian from Corea. Annals and Magazine of Natural History, Series 5, 19: 1–67.

Voucher series EWNHM-ANIMAL 6428 – EWNHM-ANIMAL 6429 (total two specimens); Loc: Cheonmasan, 2 May. 1971; Leg: Noh Bunjo. EWNHM-ANIMAL 6430; Loc: Myeonmokdong, Seoul, 30 Oct. 1976; Leg: Yun Seokjun. EWNHM-ANIMAL 6431; Loc: Sokrisan, Chungbuk, 6 Apr. 1979; Leg: Noh Bunjo, Yun Seokjun. Voucher series EWNHM-ANIMAL 6432 – EWNHM-ANIMAL 6434 (total three specimens); Loc: Dobongsan (behind Bomunsa), 4 Apr. 1965; Leg: Noh Bunjo. EWNHM-ANIMAL 6435; Loc: Nogodan, Jirisan, 5 May. 1977; Leg: Kim Juwan, Hahm Taesik. EWNHM-ANIMAL 6438; Loc: "Purchased from Sejongro", 20 Apr. 1957; Leg: Kim Okju. Voucher series EWNHM-ANIMAL 6440 – EWN-

HM-ANIMAL 6453 (Lar.; total 14 specimens); Loc: Cheonmasan, 2 May. 1971; Leg: Noh Bunjo. EWNHM-ANIMAL 6657; Loc: Woljeongsa, Odaesan, 16 Sep. 2006; Leg: Kim Byungwoo. EWNHM-ANIMAL 6658; Loc: Odaesan, 9 Oct. 2006; Leg: Kim Byungwoo. EWNHM-ANIMAL 6660; Loc: Woljeongsa, 11 Oct. 2005; Leg: no data. EWNHM-ANIMAL 6671; Loc: Noron-ri, Pyeongchang, 7 Apr. 2004; Leg: Yun Seokjun, Seo Suyeon. EWNHM-ANIMAL 6702 (Egg.); Loc: Baekwoondae, 28 Mar. 1957; Leg: Noh Bunjo. Voucher series EWNHM-ANIMAL 6709 – EWNHM-ANIMAL 6721 (total 13 specimens); Loc: no data; Leg: no data. EWNHM-ANIMAL 6725 (Egg.); Loc: no data; Leg: no data. EWNHM-ANIMAL 6726 (Egg.); Loc: Munsudae, Jirisan, 8 May 1976; Leg: Noh Bunjo. EWNHM-ANIMAL 6730 (Egg.); Loc: Nogodan, Jirisan, 8 May. 1976; Leg: Yun Seokjun.

Onychodactylus koreanus Min, Poyarkov, & Vieites, 2012

Korean clawed salamander; 한국꼬리치레도롱뇽; 12 specimens
Original label name: *Onychodactylus fischeri*

Poyarkov NA, Che J, Min M-S, Kuro-o M, Yan F, Li C, Iizuka K, Vieites DR (2012) Review of the systematics, morphology and distribution of Asian Clawed Salamanders, genus *Onychodactylus* (Amphibia, Caudata: Hynobiidae), with the description of four new species. Zootaxa 3465: 1–106.

EWNHM-ANIMAL 6436; Loc: Nogodan, Jirisan, 5 May. 1977; Leg: Kim Juwan, Hahm Taesik. EWNHM-ANIMAL 6437; Loc: Jirisan, 14 Jun. 1976; Leg: Hahm Taesik. Voucher series EWNHM-ANIMAL 6635 – EWNHM-ANIMAL 6636 (total two specimens); Loc: Sangwonsa, Odaesan, 28 Sep. 2005; Leg: Kim Byungwoo. EWNHM-ANIMAL 6659; Loc: Maebong, Odaesan, 8 Aug. 2006; Leg: Kim Byungwoo. EWNHM-ANIMAL 6670 (Lar.); Loc: Gajwa-ri, Mitan-myeon, Pyeongchang, 6 Apr. 2005; Leg: Yun Seokjun, Seo Suyeon. Voucher series EWNHM-ANIMAL 6703 – EWNHM-ANIMAL 6708 (total six specimens); Loc: no data; Leg: no data.

Remarks: the labels were updated according to taxonomic revision of the genus by Poyarkov et al. (2012).

Family Plethodontidae Gray, 1850

Karsenia koreana Min, Yang, Bonnett, Vieites, Brandon, & Wake, 2005

Korean crevice salamander; 이끼도롱뇽; one specimen

Min MS, Yang SY, Bonett RM, Vieites DR, Brandon RA, Wake DB (2005) Discovery of the first Asian plethodontid salamander. Nature. London 435: 87–90.

EWNHM-ANIMAL 6439; Loc: Daedunsan, Jeonbuk, 3 May. 1978; Leg: Natural History Museum.

Citation: Shin et al. (2020c).

Remarks: this specimen was originally labelled as "*Hynobius leechii*". This particular specimen predates the formal description of the species by 27 years (Min et al. 2005; Shin et al. 2020c).

Class Reptilia Laurenti, 1768

Order Squamata Oppel, 1811
Family Colubridae Oppel, 1811

***Chrysopelea* sp.**

One specimen

EWNHM-ANIMAL 6569 (Nn.); Loc: no data; Leg: no data.

Remarks: this specimen was labelled as "a neonate of *Lycodon rufozanatus*". However, direct comparisons of head shape, tail length, and body shape with another specimen of neonate *L. rufozonatus* (EWNHM-ANIMAL 6544) suggested that the specimen is not *L. rufozonatus* but instead belongs to the genus *Chrysopelea* (Somaweera et al. 2015).

***Elaphe dione* (Pallas, 1773)**
Steppe ratsnake; 누룩뱀; 12 specimens

Pallas PS (1773) Reise durch verschiedene Provinzen des Russischen Reichs in einem ausfuehrlichen Auszuge: vol. 2. Kaiserl. Akad. Wiss., St. Petersburg, 744 pp.

EWNHM-ANIMAL 6537; Loc: no data; Leg: no data. EWNHM-ANIMAL 6539; Loc: no data; Leg: no data. EWNHM-ANIMAL 6542 (Juv.); Loc: Gajeong-ri, Nammyeon, Chuncheon-si, Gangwon-do, 21 Jun. 2009; Leg: no data. EWNHM-ANIMAL 6543 (Juv.); Loc: Ewha Womans University campus, 19 Oct. 1990; Leg: Paik Seonghoon, Lee Sanghoon, Choi Dongho, Eom Sangryeol. EWNHM-ANIMAL 6563; Loc: no data; Leg: no data. EWNHM-ANIMAL 6584; Loc: Samak-myeon, Chunseong-gun (= Chuncheon), Gangwon-do, 15 Jul. 1976; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6585; Loc: Gotan, Chunseong-gun (= Chuncheon), Gangwon-do, 7 Jun. 1978; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6586; Loc: Yangsuri, Gyeonggi, 6 Sep. 1964; Leg: Jeon Songgeun. EWNHM-ANIMAL 6591; Loc: Yangsuri, Gyeonggi, 6 Sep. 1964; Leg: Jeon Songgeun. EWNHM-ANIMAL 6592; Loc: Gajamul, Susaek, Seoul, 9 Jun. 1957; Leg: Tak Soonja. Voucher series EWNHM-ANIMAL 6601 – EWNHM-ANIMAL 6602 (total two specimens); Loc: Bukhansan, 17 Apr. 1971; Leg: Donation by Prof. Yun Ilbyung.

***Elaphe schrenckii* Strauch, 1873**
Russian ratsnake; 구렁이; five specimens

Strauch A (1873) Die Schlangen des Russischen Reichs, in systematischer und zoogeographischer Beziehung. Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg, 7 Série 21: 1–288.

EWNHM-ANIMAL 6498 (Juv.); Loc: Bukri reservoir, Deokjeokdo, 21 Sep. 2009; Leg: Yun Seokjun, Ryu Jaewon. EWNHM-ANIMAL 6540; Loc: no data; Leg: no data. EWNHM-ANIMAL 6583; Loc: Gotan, Chunseong-gun (= Chuncheon), Gangwon-do, 20 Jul. 1978; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6590; Loc: Jinburyeong, Gangwon-do, Aug. 1978; Leg: Kim Jeonggyun, Lee Jeongsoon. EWNHM-ANIMAL 6605; Loc: no region data, 21 May. 1955; Leg: Department of Biology.

Hebius vibakari ruthveni **(van Denburgh, 1923)**
Japanese keelback; 대륙유혈목이; five specimens
Original label name: *Natrix vibakari* or *Natrix vibakari ruthveni*

Van Denburgh J (1923) A new subspecies of watersnake (*Natrix vibakari ruthveni*) from eastern Asia. Proceedings of the California Academy of Sciences 13: 3–4.

EWNHM-ANIMAL 6513; Loc: no data; Leg: no data. EWNHM-ANIMAL 6514; Loc: no data; Leg: no data. EWNHM-ANIMAL 6523; Loc: Seongpanak, Jeju (1000m a.s.l), 19 Jul. 1977; Leg: Kim Sooil. EWNHM-ANIMAL 6571; Loc: Palbongsan, Hongcheon, Gangwon-do, 31 Aug. 1978; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6581; Loc: Jeju-do, 30 May. 1999; Leg: Yun Seokjun.

Remark: updated generic assignment for labels follows Thompson (1913), supported by taxonomic revision of *Amphiesma* (*sensu lato*) by Guo et al. (2014).

Lycodon rufozonatus **Cantor, 1842**
Red banded snake; 능구렁이; 11 specimens
Original label name: *Dinodon rufozonatum*

Cantor T (1842) General features of Chusan, with remarks on the flora and fauna of that island. Annals and Magazine of Natural History, Series 1, 9: 481–493.

EWNHM-ANIMAL 6499; Loc: Ganghwa, 29 Oct. 1991; Leg: Yun Seokjun. EWNHM-ANIMAL 6517; Loc: no data; Leg: no data. EWNHM-ANIMAL 6532; Loc: no data; Leg: no data. EWNHM-ANIMAL 6533; Loc: no data; Leg: no data. EWNHM-ANIMAL 6534; Loc: no data; Leg: no data. EWNHM-ANIMAL 6535; Loc: no data; Leg: no data. EWNHM-ANIMAL 6544 (Juv.); Loc: Gajeong-ri, Nam-myeon, Chuncheon-si, Gangwon-do, 27 Jun. 2009; Leg: Ryu Jaewon. EWNHM-ANIMAL 6570; Loc: no data; Leg: no data. EWNHM-ANIMAL 6587; Loc: Gangchon, Chunseong-gun (= Chuncheon), Gangwon- do, 30 May. 1977; Leg: Donation by Prof. Paik

Namgeuk. EWNHM-ANIMAL 6593; Loc: Sinchon, 15 Jun. 1955; Leg: Kim Hoonsoo. EWNHM-ANIMAL 6594; Loc: Sanghwanam, Sokrisan, 15 Jul. 1961; Leg: Chae Ingi.

Remark: updated labels reflect taxonomic revision of the group by Guo et al. (2013), who synonymized *Dinodon* with *Lycodon* based on molecular and morphological results.

***Oocatochus rufodorsatus* (Cantor, 1842)**
Frog-eating ratsnake; 무자치; 11 specimens
Original label name: *Elaphe rufodorsata*

Cantor T (1842) General features of Chusan, with remarks on the flora and fauna of that island. Annals and Magazine of Natural History, Series 1, 9: 481–493.

EWNHM-ANIMAL 6509; Loc: no data; Leg: no data. EWNHM-ANIMAL 6510; Loc: no data; Leg: no data. EWNHM-ANIMAL 6511; Loc: no data; Leg: no data. EWNHM-ANIMAL 6512; Loc: Hwajeon, Gyeonggi-do, 3 Oct. 1980; Leg: Yun Seokjun. EWNHM-ANIMAL 6538; Loc: no data; Leg: no data. EWNHM-ANIMAL 6541 (Nn.); Loc: Gwangneung, 28 Sep. 1958; Leg: no data. EWNHM-ANIMAL 6564; Loc: Bucheon, Gyeonggi-do, 28 Jun. 1974; Leg: Kim Hungyu. EWNHM-ANIMAL 6565; Loc: Sangdo-dong, Seoul, 12 Oct. 1961; Leg: Kim Bo Ok. EWNHM-ANIMAL 6566; Loc: Gwangneung; Leg: Im Okja. EWNHM-ANIMAL 6567; Loc: Gwangneung, 4 Jun. 1960; Leg: Lee Namjun. EWNHM-ANIMAL 6580; Loc: Gwangpan-ri, Chunseong-gun (= Chuncheon), Gangwon-do, 10 Aug. 1978; Leg: Donation by Prof. Paik Namgeuk.

Remark: the generic assignment for updated labels follows Helfenberger (2001), which demonstrated distinctiveness of this species in relation to other *Elaphe* species.

***Orientocoluber spinalis* (Peters, 1866)**
Slender racer; 실뱀; four specimens
Original label name: *Zamenis spinalis*

Peters WCH (1866) Mittheilung über neue Amphibien (*Amphibolurus*, *Lygosoma*, *Cyclodus*, *Masticophis*, *Crotaphopeltis*) und Fische (*Diagramma*, *Hapalogenys*) des Kgl. Zoologischen Museums. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin 1866: 86–96.

EWNHM-ANIMAL 6500; Loc: Gwanaksan, Gyeonggi-do, 18 Oct. 1958; Leg: Ko Eungwon. EWNHM-ANIMAL 6507; Loc: Biseondae, Seoraksan, Oct. 1958; Leg: Lee Dalyeong. EWNHM-ANIMAL 6508; Loc: Gotan, Chunseong-gun (= Chuncheon), Gangwon-do, 20 Jul. 1978; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6568; Loc: outside Jahamun, 14 Jul. 1961; Leg: Yun Jeongin.

Remark: the updated labels reflect genus-level revision of this species by Kharin (2011).

Rhabdophis tigrinus **(Boie, 1826)**
Tiger keelback; 유혈목이; 28 specimens
Original label name: *Natrix tigrina* or *Natrix tigrina lateralis*

Boie H (1826) Merkmale einiger japanischer Lurche. lsis von Oken 18–19: 203–216.

EWNHM-ANIMAL 6524; Loc: no data; Leg: no data. EWNHM-ANIMAL 6525; Loc: no data; Leg: no data. EWNHM-ANIMAL 6526; Loc: no data; Leg: no data. EWNHM-ANIMAL 6527; Loc: no data; Leg: no data. EWNHM-ANIMAL 6528; Loc: no data; Leg: no data. EWNHM-ANIMAL 6529; Loc: no data; Leg: no data. EWNHM-ANIMAL 6530; Loc: no data; Leg: no data. EWNHM-ANIMAL 6531 (Nn.); Loc: no data; Leg: no data. EWNHM-ANIMAL 6553; Loc: Bogwangsa, Gyeonggi-do (unknown collection date and year); Leg: no data. EWNHM-ANIMAL 6557; Loc: no data; Leg: no data. EWNHM-ANIMAL 6572; Loc: no data; Leg: no data. EWNHM-ANIMAL 6573; Loc: no data; Leg: no data. EWNHM-ANIMAL 6588; Loc: Miro, Samcheok, Gangwon-do, 16 Jul. 1978; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6589; Loc: President's residence, Ewha Womans Univ. campus, 12 May. 1958; Leg: "Workman". EWNHM-ANIMAL 6595; Loc: Gwangneung, Gyeonggi-do, 25 Jun. 1980; Leg: Kim Sooil. EWNHM-ANIMAL 6596; Loc: Yangsuri, Gyeonggi, 13 Oct. 1978; Leg: Noh Bunjo, Yun Seokjun. EWNHM-ANIMAL 6597; Loc: Baekryeong-do, 27 May. 1958; Leg: Kim Hoonsoo. EWNHM-ANIMAL 6598; Loc: Mujugucheondong, Jeonbuk, May. 1979; Leg: Yun Seokjun. EWNHM-ANIMAL 6599; Loc: Jinburyeong, Gangwon-do, Jul. 1976; Leg: Kim Jeonggyun, Lee Jeongsoon. EWNHM-ANIMAL 6600; Loc: Ewha Womans University campus, 23 May. 1966; Leg: "Workman". EWNHM-ANIMAL 6603; Loc: no data; Leg: no data. EWNHM-ANIMAL 6604; Loc: no data; Leg: no data. EWNHM-ANIMAL 6606; Loc: Ewha Womans University campus, 15 Jun. 1955; Leg: Department of Biology. EWNHM-ANIMAL 6607; Loc: Ewha Womans University campus, 21 Jun. 1956; Leg: Hahm Jongseong. Voucher series EWNHM-ANIMAL 6608 – EWNHM-ANIMAL 6609 (Nn.; total two specimens); Loc: Gyeryongsan, Chungnam, hatched in captivity, 27 Jul. 2000; Leg: no data. Voucher series EWNHM-ANIMAL 6615 – EWNHM-ANIMAL 6616 (total two specimens); Loc: dubious location name (potentially Gwanak mountain); Leg: Ko Eungwon.

Remark: the generic assignment in the updated labels follows the original description of the genus *Rhabdophis* by Fitzinger (1843), based on taxonomic revision of *Natrix* (*sensu lato*) by Malnate (1960).

Sibynophis chinensis **(Günther, 1889)**

Chinese many-toothed snake; 비바리뱀; one specimen

Günther ACLG (1889) Third contribution to our knowledge of reptiles and fishes from the Upper Yangtsze-Kiang. Annals and Magazine of Natural History 4: 218–229.

EWNHM-ANIMAL 6497; Loc: Hanlim, Jeju-do, 20 Jun. 1999; Leg: Seo Hyeongseok.

Family Viperidae Oppel, 1811

Gloydius brevicaudus (Stejneger, 1907)

Short-tailed mamushi; 살모사; ten specimens
Original label name: *Agkistrodon halys* or *Agkistrodon halys brevicaudus*

Stejneger LH (1907) Herpetology of Japan and adjacent territory. Bulletin of the United States National Museum 58: 1–577.

EWNHM-ANIMAL 6501; Loc: Yeongju, Aug. 1957; Leg: D.J. Kim. EWNHM-ANIMAL 6502; Loc: no data; Leg: no data. EWNHM-ANIMAL 6503; Loc: no data; Leg: no data. EWNHM-ANIMAL 6504; Loc: no data; Leg: Department of Biology. EWNHM-ANIMAL 6505; Loc: Gwangpan-ri, Chunseong-gun (= Chuncheon), Gangwon-do, 20 Jun. 1977; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6520; Loc: Yangju (unknown collection date and year); Leg: Kim Myeongnim. EWNHM-ANIMAL 6536; Loc: no data; Leg: no data. EWNHM-ANIMAL 6551; Loc: Godaedo, 22 Jul. 1956; Leg: Kim Hoonsoo. EWNHM-ANIMAL 6552; Loc: Yukgokcheon, Euiseong, 20 Aug. 1958; Leg: Oh Soonjo. EWNHM-ANIMAL 6562; Loc: Godaedo, 22 Jul. 1956; Leg: Kim Hoonsoo.

Remark: generic assignment in the updated labels is based on Hoge and Romano-Hoge (1981), which was supported by results demonstrated by subsequent authors (Knight et al. 1992; Parkinson, 1999). Meanwhile, species assignment in the updated labels is based on the original description by Stejneger (1907 as *Agkistrodon blomhoffii brevicaudus*) because the name *Agkistrodon* (= *Gloydius*) *halys* used in former labels is only applicable to populations of *Gloydius* in Central Asia (Wüster et al. 1997).

Gloydius intermedius (Strauch, 1868)

Rock mamushi; 까치살모사; four specimens
Original label name: *Agkistrodon saxatilis*

Strauch A (1868) Concerning poisonous snakes distributed in Russia. Trudy Perv. Siezda Russ. Yestestv. Zool., 1: 1–294.

EWNHM-ANIMAL 6454 (Nn.); Loc: no data; Leg: no data. EWNHM-ANIMAL 6506; Loc: Daegwanryeong, 3 Aug. 1977; Leg: Donation by Prof. Paik Namgeuk. Voucher series EWNHM-ANIMAL 6545 – EWNHM-ANIMAL 6546 (Nn.; total two specimens); Loc: Mitan, Pyeongchang, Gangwon-do, 3 Nov. 1999; Leg: Donation by Prof. Paik Namgeuk.

Citation: Shin et al. (2020b).

Remark: generic assignment in the updated labels as explained above for *G. brevicaudus*. Although some authors consider *G. saxatilis* as valid, here we treat that name as a synonym of *G. intermedius* following Orlov and Barabanov (1999). Voucher EWNHM-ANIMAL 6454 is the first reported specimen of *G. intermedius* with dicephalism (Shin et al. 2020b).

Gloydius ussuriensis (Emelianov, 1929)

Ussuri mamushi; 쇠살모사; 18 specimens
Original label name: *Agkistrodon halys* or *Agkistrodon caliginosus*

Emelianov AA (1929) Snakes of the Far Eastern District. Memoirs of the Vladivostok Section of the Russian State Geographical Society 3: 1–208.

EWNHM-ANIMAL 6515; Loc: no data; Leg: no data. EWNHM-ANIMAL 6516; Loc: no data; Leg: no data. EWNHM-ANIMAL 6518; Loc: Mujugucheondong, Jeonbuk, May. 1979; Leg: Yun Seokjun. EWNHM-ANIMAL 6519; Loc: no data; Leg: no data. EWNHM-ANIMAL 6521; Loc: Palbongsan, Hongcheon, Gangwon-do, 2 Jul. 1978; Leg: Donation by Prof. Paik Namgeuk. EWNHM-ANIMAL 6522; Loc: Balang-ri, Paju, Gyeonggi-do, 5 Oct. 1997; Leg: Yun Seokjun. EWNHM-ANIMAL 6547; Loc: Jinburyeong, Gangwon-do, Jul. 1976; Leg: Kim Seonggyun, Lee Jeongsoon. EWNHM-ANIMAL 6548; Loc: Hwajeon, 19 Oct. 1979; Leg: Kim Sooman. EWNHM-ANIMAL 6549; Loc: Gwangneung, Sep. 1968; Leg: Chae Ingi. EWNHM-ANIMAL 6550; Loc: Gwangneung, Gyeonggi-do, 15 May. 1981; Leg: Yun Seokjun. EWNHM-ANIMAL 6554; Loc: Yeongsil, Hanrasan, Jeju-do, 10 Jul. 1979; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6555 – EWNHM-ANIMAL 6556 (total two specimens); Loc: Mujugucheondong, Jeonbuk, 19 Jul. 1967; Leg: no data. Voucher series EWNHM-ANIMAL 6558 – ANIMAL 6559; Loc: Gupabal, 17 Sep.1962; Leg: Noh Bunjo. EWNHM-ANIMAL 6560; Loc: Gwangneung, 10 May. 1959; Leg: no data. EWNHM-ANIMAL 6561; Loc: Gwangneung, 19 May. 1955; Leg: Tak Soonae. EWNHM-ANIMAL 6574; Loc: Jinburyeong, 12 Aug. 1980; Leg: Yun Seokjun.

Remark: generic assignment in the updated labels and invalidity of the name "*Agkistrodon halys*" applied to East Asian *Gloydius* species as explained above for *G. brevicaudus*. The species name used in the updated labels follows the original species description by Emelianov (1929 as *Ancistrodon blomhoffi ussuriensis*).

Family Agamidae Gray, 1827

Draco melanopogon Boulenger, 1887

Black-bearded flying dragon; one specimen

Boulenger GA (1887) Catalogue of the lizards in the British Museum (Nat. Hist.) III. Lacertidae, Gerrhosauridae, Scincidae, Anelytropsidae, Dibamidae, Chamaeleontidae. London: 575 pp.

EWNHM-ANIMAL 6578; Loc: no data; Leg: no data.

Remarks: a female specimen with pre-existing voucher number R54,0,7.

Family Lacertidae Oppel, 1811

Eremias argus Peters, 1869

Mongolian racerunner; 표범장지뱀; 12 specimens

Peters WCH (1869) Eine Mittheilung über neue Gattungen und Arten von Eidechsen. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin. 1869: 57–66.

EWNHM-ANIMAL 6455; Loc: Yeonheedongsan, 6 Jun. (unknown collection year); Leg: Kang Yeongok. EWNHM-ANIMAL 6456; Loc: Gwangneung (sandy plain), 11 May. 1957; Leg: Kim Hoonsoo. EWNHM-ANIMAL 6457; Loc: Juan, 6 Jun. 1957; Leg: Kang Yeongsaeng. EWNHM-ANIMAL 6458; Loc: Sinchon, 28 Apr. 1959; Leg: Noh Bunjo. EWNHM-ANIMAL 6459; Loc: no location data, 25 May 1959; Leg: Kim Gihwan. EWNHM-ANIMAL 6460; Loc: Outside Jahamun, Seoul, 1 Jun. 1956; Leg: Yun Jeongin. EWNHM-ANIMAL 6461; Loc: Deokjeokdo, 8 Jul. 1956; Leg: Kim Hoonsoo. Voucher series EWNHM-ANIMAL 6462 – EWNHM-ANIMAL 6464 (total three specimens); Loc: Sindangdong, Seoul, 5 May. 1957; Leg: Yun Deokhee. EWNHM-ANIMAL 6576; Loc: no data; Leg: Kim Bongjin. EWNHM-ANIMAL 6577; Loc: no data; Leg: no data.

Takydromus amurensis Peters, 1881

Amur grass lizard; 아무르장지뱀; 20 specimens

Peters WCH (1881) Einige herpetologische Mittheilungen. 1. Uebersicht der zu den Familien der Typhlopes und Stenostomi gehörigen Gattungen oder Untergattungen. 2. Ueber eine neue Art von Tachydromus aus dem Amurlande. 3. Ueber die von Herrn Dr. finsch aus Polynesien gesandten Reptilien. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin 1881: 69–72.

EWNHM-ANIMAL 6465; Loc: Gomgol, Seoraksan, 24 Oct. 1971; Leg: no data. EWNHM-ANIMAL 6466; Loc: Bogwangsa, Gyeonggi-do, 16 Apr. 1983; Leg: Yun Seokjun. EWNHM-ANIMAL 6468; Loc: Myeongseongsan, Pocheon, Gyeonggi-do, 23 Nov. 2001; Leg: Yun Seokjun. EWNHM-ANIMAL 6470; Loc: Yumyeongsan, Gapyeong, Gyeonggi-do, 12 Jul. 1992; Leg: Yun Seokjun. EWNHM-ANIMAL 6471; Loc: Gwangneung, 11 May. 1957; Leg: Kim Hoonsoo. EWNHM-ANIMAL 6472; Loc: Daedunsan, Jeonbuk, 3 May. 1978; Leg; Natural History Museum. Voucher series EWNHM-ANIMAL 6476 – EWNHM-ANIMAL 6477 (total two specimens); Loc: Sokrisan, Chungbuk, 6 Apr. 1979; Leg: Kim Sooil. Voucher series EWNHM-ANIMAL 6478 – EWNHM-ANIMAL 6479 (total two specimens); Loc: Mujugucheondong, 8 May.1979; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6480 – EWNHM-ANIMAL 6481 (total two specimens); Loc: Gwangneung, 7 Apr. 1958;

Leg: Kim Hoonsoo. Voucher series EWNHM-ANIMAL 6485 – EWNHM-ANIMAL 6486 (total two specimens); Loc: Gwangneung (unknown collection date and year); Leg: Kim Hoonsoo. Voucher series EWNHM-ANIMAL 6487 – EWNHM 6491 (total five specimens); Loc: Baekdamsa, Seoraksan, 27 May. 1999; Leg: Yun Seokjun. EWNHM-ANIMAL 6575; Loc: Jinburyeong, 12 Aug. 1980; Leg: Yun Seokjun.

Takydromus wolteri Fischer, 1885

Mountain grass lizard; 줄장지뱀; six specimens

Fischer JG (1885) Ichthyologische und herpetologische Bemerkungen. V. Herpetologische Bemerkungen. Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten. 2: 82–121.

EWNHM-ANIMAL 6467; Loc: Cheonmasan, 19 May. 1979; Leg: Yun Seokjun. EWNHM-ANIMAL 6474; Loc: Baekryeong-do (unknown collection date and year); Leg: Kim Hoonsoo. EWNHM-ANIMAL 6475; Loc: Sindang-ri, Chungju, 5 May. 1979; Leg: Yun Seokjun. Voucher series EWNHM-ANIMAL 6482 – EWNHM-ANIMAL 6484 (total three specimens); Loc: Anmyeondo (unknown collection date or year); Leg: Kim Hoonsoo.

Takydromus sp.

Two specimens

EWNHM-ANIMAL 6469; Loc: Sangwonsa, Odaesan, 16 May. 1982; Leg: Yun Seokjun. EWNHM-ANIMAL 6473; Loc: Han River (Hangang), 27 Sep. 1959; Leg: Jeong Yeongae.

Remarks: we were unable to identify these two specimens because the number of femoral pores (a characteristic that is clearly different between *T. amurensis* and *T. wolteri*) were not clearly visible. Pholidosis characteristics alone were insufficient to make a clear diagnosis at species level.

Family Scincidae Gray, 1825

Scincella vandenburghi (Schmidt, 1927)

Tsushima smooth skink; 도마뱀; two specimens

Schmidt KP (1927) Notes on Chinese reptiles. Bulletin of the American Museum of Natural History 54: 467–551.

EWNHM-ANIMAL 6492; Loc: Jugeumsan, Gyeonggi-do, 17 Sep. 1990; Leg: Yun Seokjun. EWNHM-ANIMAL 6691; Loc: Wolchulsan, Yeongnam, 5 Oct. 2006; Leg: Kim Byungwoo.

Scincella huanrenensis Zhao & Huang, 1982

북도마뱀; four specimens

Zhao E, Huang K (1982) A survey of amphibians and reptiles in Liaoning Province. Acta Herpetologica Sinica 1: 1–23.

Voucher series EWNHM-ANIMAL 6493 – EWNHM-ANIMAL 6496 (total four specimens); Loc: Seorim, Yangyang, Gangwondo, 30 Jun. 2008; Leg: Lee Sangcheol.

Tiliqua gigas (Schneider, 1801)

Blue-tongued skink; one specimen

Schneider JG (1801) Historiae Amphibiorum naturalis et literariae. Fasciculus secundus continens Crocodilos, Scincos, Chamaesauras, Boas. Pseudoboas, Elapes, Angues. Amphisbaenas et Caecilias. Frommanni, Jena, Germany. 374 pp.

EWNHM-ANIMAL 6610; Loc: no data; Leg: no data.

Order Testudines Batsch, 1788

Family Emydidae Rafinesque, 1815

Trachemys scripta elegans (Wied, 1838)

Red-eared slider; 붉은귀거북; four specimens
Original label name: *Pseudemys scripta*

Wied M (1838) Reise in das innere Nord-America in den Jahren 1832 bis 1834, erster Band. J. Hoelscher, Coblenz, 654 pp.

EWNHM-ANIMAL 6580; Loc: no data; Leg: no data. EWNHM-ANIMAL 6617; Loc: no location data, 10 Mar. 1979; Leg: Shin Sook. EWNHM-ANIMAL 6699; Loc: no data; Leg: no data. EWNHM-ANIMAL 6700; Loc: no data; Leg: no data.

Remark. generic assignment in the updated labels is based on the original description of *Trachemys* by Agassiz (1857) following the resurrection of *Trachemys* from synonymy with *Pseudemys* by Iverson (1985). This taxonomic treatment has been supported by subsequent studies (Siedel and Smith 1986; Rhodin et al. 2017).

Family Trionychidae Fitzinger, 1826

Pelodiscus maackii (Brandt, 1858)

Northern Chinese softshell turtle; 자라; two specimens
Original label name: *Amyda maackii*

Brandt JF (1858) Observationes quaedam ad generis trionychum species duas novas spectantes. Bulletin de l'Académie Impériale des Sciences de St. Petersbourg, la classe Physico-Mathématique 16: 110–111.

EWNHM-ANIMAL 6611; Loc: Yukgokcheon, Gyeongbuk, 20 Aug. 1959; Leg: Oh Soonjo. EWNHM-ANIMAL 6614; Loc: Yangsuri, 30 Oct.1979; Leg: Yun Seokjun.

Remark: generic assignment in the updated labels reflects taxonomy used in Rhodin et al. (2017).

Pelodiscus sinensis **Wiegmann, 1835**
Chinese softshell turtle; 중국자라; one specimen
Original label name: *Amyda sinensis*

Wiegmann AFA (1834) In: Meyen FJF (Ed.) Beiträge zur zoologie gesammelt auf einer reise um die erde. siebente abhandlung. amphibien. Nova Acta Physico-Medica Academia Caesarea Leopoldino-Carolina (Halle) 17: 185–268.

EWNHM-ANIMAL 6612; Loc: "Pond", 30 Jul. 1964; Leg: Department of Biology.

Remark: generic assignment in the updated labels as explained above for *P. maackii*.

Pelodiscus **sp.**

One specimen

EWNHM-ANIMAL 6613; Loc: no data; Leg: no data.

Remark: morphological characteristics used to identify *Pelodiscus* species (Farkas et al. 2019) were insufficient to correctly identify this specimen at the species level.

Missing specimens and specimens not documented in the catalogue

Some specimens that were known to have been deposited in the EWNHM are not documented here because they were not found in the museum. For example, we failed to locate the holotype of *Karsenia koreana* (EWNHM 80314; Min et al. 2005) and the holotype, paratype series, and other associated specimens of *Onychodactylus koreanus* (holotype EWNHM 80316; paratype series EWNHM 80315, EWNHM 80317–80318; associated specimens EWNHM 80319–80320, EWNHM 80321–80322, EWNHM 80323–80325, EWNHM 80326; EWNHM 80327–80328; Poyarkov et al. 2012). Our attempts to communicate with species authors did not yield any information regarding the whereabouts of these specimens. At this point, it is uncertain whether these specimens are truly lost or on loan without traceability. However, at-

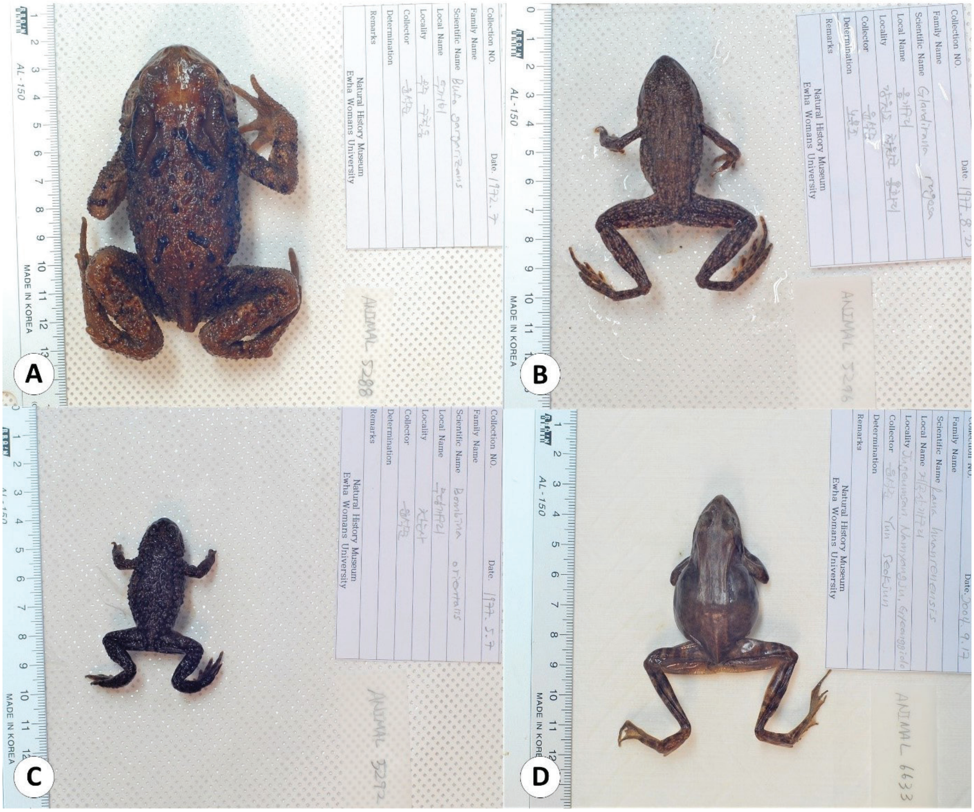

Figure 2. Some of the frog specimens deposited in the collection of the EWNHM **A** *Bufo gargarizans* (EWNHM-ANIMAL 5288) **B** *Glandirana emeljanovi* (EWNHM-ANIMAL 5296) **C** *Bombina orientalis* (EWNHM-ANIMAL 5292) **D** *Rana huanrenensis* (EWNHM-ANIMAL 6633).

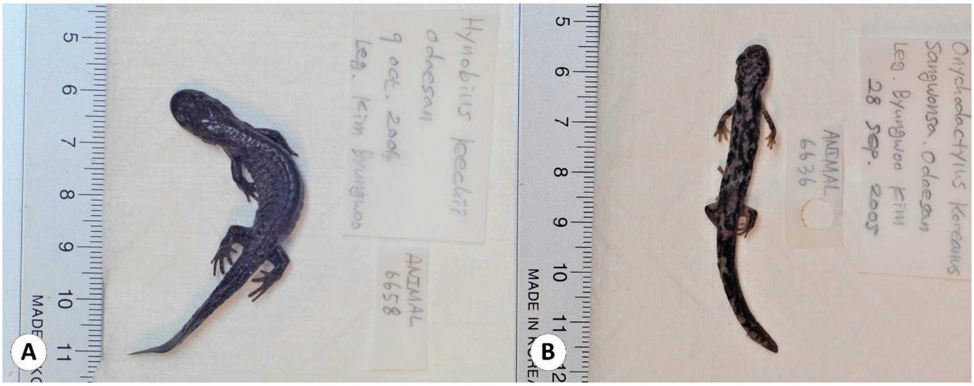

Figure 3. Some of the salamander specimens deposited in the collection of the EWNHM **A** *Hynobius leechii* (EWNHM-ANIMAL 6658) **B** *Onychodactylus koreanus* (EWNHM-ANIMAL 6636).

Figure 4. Lizard species of the Republic of Korea represented in the collection of the EWNHM **A** *Eremias argus* (EWNHM-ANIMAL 6456; dorsal view) **B** *E. argus* (EWNHM-ANIMAL 6456; ventral view) **C** *Takydromus wolteri* (EWNHM-ANIMAL 6484) **D** *Takydromus amurensis* (EWNHM-ANIMAL 6487) **E** *Scincella vandenburghi* (EWNHM-ANIMAL 6492) **F** *Scincella huanrenensis* (EWNHM-ANIMAL 6493).

Figure 5. Some of the snake specimens deposited in the collection of the EWNHM **A** *Sibynophis chinensis* (EWNHM-ANIMAL 6497) **B** *Orientocoluber spinalis* (EWNHM-ANIMAL 6500) **C** *Gloydius brevicaudus* (EWNHM-ANIMAL 6505) **D** *Oocatochus rufodorsatus* (EWNHM-ANIMAL 6564).

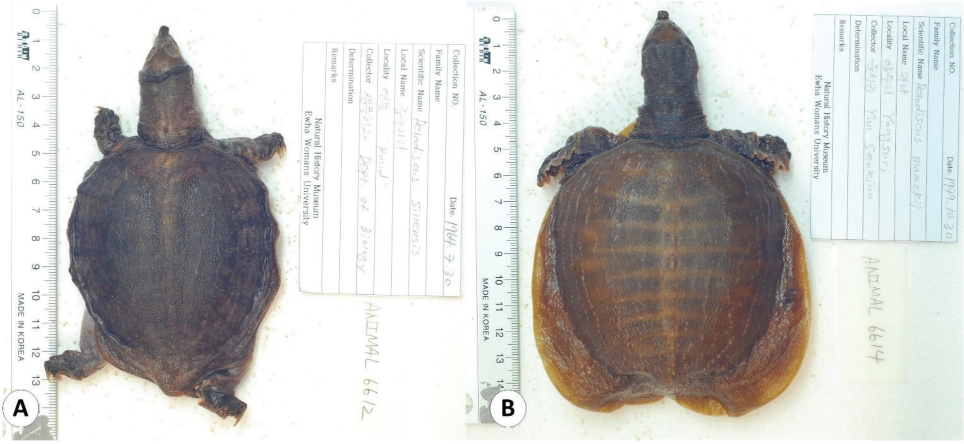

Figure 6. Some of the turtle specimens deposited in the collection of the EWNHM **A** *Pelodiscus chinensis* (EWNHM-ANIMAL 6612) **B** *Pelodiscus maackii* (EWNHM-ANIMAL 6614).

tempts to designate new type materials should be reserved until the exact whereabouts and status of these specimens are known.

In addition, we were unable to document nine specimens of *Dryophytes japonicus* because the specimen jars could not be opened. This was due to crystalized preservative fluid around the inner wall of the lids. Also, we did not document specimens of *Lithobates catesbeianus* and *Pelophylax nigromaculatus* that had been used for dissection samples. These specimens had been used in the medical school before being deposited in the museum, and are of indeterminate origins with no collection data available.

Conclusions

This catalogue is the first complete inventory of herpetology specimens deposited in the EWNHM. In total, the collection is comprised of 1554 specimens representing all native Korean terrestrial reptiles except one chelonian (*Mauremys reevesii*), all native anuran species, three of six native salamander species, and some exotic and invasive species (Figs 2–6). Some Korean herpetofauna, such as *Hynobius unisacculus* (Min et al. 2016) were not included in the collection because they were recently described and no collection have been made by the institution to acquire vouchers. The specimens of non-native species are most likely from live exhibits of the museum or from laboratory experiments (e.g., specimens of *L. catesbeianus* used in laboratory dissections). In taxonomic diversity, the herpetological collection of EWNHM contain 17 amphibian species across 12 genera and eight families and 22 reptile species across 16 genera and seven families. The specimens were collected between 1951 and 2013. Although the sampling interval is not even, the time span covered by the collection is one of the broadest among collections of Korean herpetofauna. Therefore, the EWNHM collection represents one of the most significant research collections of Korean reptiles and amphibians.

Natural history collections are a valuable resource for a number of reasons and by cataloging the collection at EWNHM, the specimens held there are now accessible to researchers for perpetuity. In recent times, natural history collections have proved valuable resources in order to trace the origins and spread of disease (Ouellet et al. 2005) as well as to reconstruct the genetic diversity in long-extinct populations (Wandeler et al. 2007). With advancing molecular methods, formalin-fixed specimens may soon be able to provide the wealth of knowledge that we can currently extract for those fixed in ethanol. As we shift towards a more holistic approach to conservation, supported by global projects, it is hoped that the above catalogue can aid in herpetofauna conservation for decades to come.

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Genetic characterisation of wild ungulates: successful isolation and analysis of DNA from widely available bones can be cheap, fast and easy

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Abstract

Genetic characterisation of wild ungulates can be a useful tool in wildlife management and in obtaining a greater understanding of their biological and ecological roles in a wider spatiotemporal context. Different ways of optimising methodologies and reducing the costs of genetic analyses using widely available bone tissues collected within regular hunting allocations were examined. Successful isolation and analysis of DNA from widely available bones can be cheap, fast and easy. In particular, this study explored the possibility of using bones for extracting high quality nuclear DNA for microsatellite analysis. The utility of applying a modified demineralisation process using two commercially available DNA isolation kits, which differ significantly in price, was evaluated. The sample sets included bones and, for comparison, muscle tissues from four wild ungulate species: chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), and Alpine ibex (*Capra ibex*). For the recent bones, these results confirmed that the DNA concentrations and microsatellite amplification were sufficiently high, even when using low-cost kits, after prior demineralisation. For old bones, prior demineralisation and use of a specially designed isolation kit led to a more successful extraction of DNA. Besides reducing kit-related costs, low-cost kits are much faster and therefore make genetic analysis more efficient.

Keywords

amplification success, bones, demineralisation, DNA extraction, population genetics, skulls, wild ungulates

Introduction

Genetic and genomic approaches can provide detailed information about past and present demographic parameters in populations, phylogenetic issues, the molecular basis for understanding genetic diseases, inbreeding, and detection of hybridisation/gene introgression (Miller et al. 2012). Genetic monitoring can also help us to understand the mechanisms that relate fitness to genetic variation, to integrate genetic and environmental methodologies into wildlife management and conservation biology, and to design advanced and fast (as well as non-invasive) monitoring protocols. Indeed, the rapid progress and decreasing costs of genetic analyses are making genetic monitoring more feasible and more widely applicable (Ouborg et al. 2010).

Nevertheless, the collection of genetic material from free-ranging animals is very challenging. At present, the ability to perform routine, large-scale genetic analyses and monitoring in a wider spatiotemporal context is quite low due to the limited availability of suitably preserved soft tissue samples (e.g. muscle tissue or internal organs) from wildlife species. The difficulty in collecting genetic material from wildlife is further exacerbated because traditional invasive techniques such as biopsy darting or blood sampling (the preferred biomaterial for genetic studies) are impractical and need appropriate preservation methods (Seutin et al. 1991). Moreover, it is often difficult to acquire a large enough set of historical samples, such as different bone structures, which are also more difficult and expensive to analyse (Hoffmann et al. 2015).

To encourage the use of genetics as a valuable management tool, we should optimise the methodology and reduce the costs of genetic analyses on widely available samples, both recent and historical, that are systematically collected by hunters as trophies (such as skulls with antlers or horns) or, in some cases, via their reporting obligations (mandibles). Indeed, in some European countries such as Slovenia, (hemi) mandibles of all harvested wild ungulates must be collected, properly labelled, linked with attributive data on the individual, and provided as part of regular hunting allocations (Pokorny and Jelenko Turinek 2018). Therefore, these mandibles represent a very useful resource and an easily accessible set of samples for both regular and genetic monitoring, such as for studying patterns in the genetic structure of species across the landscape, their hybridisation patterns and fundamental life-history traits. The same holds true for trophies, which also yield data within a given spatiotemporal context (i.e. information on the location and date of the harvest). Using regularly collected bone samples helps us to avoid the rather complex logistics involved in the sampling and storage of muscle tissue samples.

Bone structures that are equipped with attributive data on individuals enable us to study the spatiotemporal variability within and among populations and to perform retrospective studies (e.g. mandibles in different environmental studies: Kierdorf and Kierdorf 2000, 2005; Jelenko and Pokorny 2010; antlers/horns in genetic studies: Hoffmann and Griebeler 2013; Giżejewska et al. 2016; Safner et al.

2020). These bones are usually kept for decades or even centuries in well-preserved public (museums) and private collections, which may cover different regions and historical periods, and in which data on the origin of individuals, year of death and age are also often recorded.

However, bone tissue is one of the most difficult biological samples for the extraction of DNA (Wandeler et al. 2007), and working with such material is both time- and labour-consuming (Alonso et al. 2001). Luckily, improvements in the extraction of quality DNA (which used to be a limiting factor) and a significant reduction in costs related to DNA analyses of bones (Paetkau 2003; Rohland and Hofreiter 2007; Dabney et al. 2013; Huisman et al. 2016; Tsaparis et al. 2019) have opened up completely new perspectives for genetics as a wildlife management tool. This is primarily because bones, due to their structure, preserve DNA comparatively well and for a long time. Therefore, they are a useful source of genetic material but, compared with extracting DNA from muscle tissues, hair and saliva, are more difficult to handle due to their rigid structure (Kumar and Narayan 2014).

The quality and quantity of the DNA extracted from bones can be affected by several factors such as the mineralisation levels, the pre-treatment of the bone prior to the extraction step and the environmental conditions under which it was preserved. The extraction process in bone also presents two big problems: low copy number due to the fact that the majority of bone is not cells (Loreille et al. 2007), and the occurrence of incomplete or broken DNA fragments and contamination by PCR inhibitors (Chilvers et al. 2008). Therefore, isolating and purifying DNA from bones, particularly old ones, pose a huge challenge (Rohland and Hofreiter 2007; Dabney et al. 2013). The bone tissue itself is a hard, connective tissue with a high content of calcium. After death, during mineralisation and protein decay, DNA is bound with hydroxyapatite and forms a compound named bioapatite (Dorozhkin 2016). For these reasons, decalcification (softening the bones) is generally considered a necessary step for effective DNA isolation (Alers et al. 1999). However, we should take into consideration the influence of the decalcification period in old bones: Jakubowska et al. (2012) showed that for well-preserved bones decalcification length does not play an important role, while, on the contrary, degraded older bones should be decalcified for a couple of days to obtain better results from DNA isolation.

The aims of our study were to: (i) determine a low-cost and effective method for extracting high quality DNA from mandibles and skulls of harvested wild ungulates, using chemicals and tools readily available in the majority of molecular laboratories; (ii) compare the success rate of DNA extraction and amplification from bones (both recently collected and old ones) *vs.* muscle tissue samples using low-cost (wider use for all types of tissue) and high-cost isolation kits (specially made for DNA extraction from bones); (iii) rate genotyping reproducibility by genotyping seven roe deer samples shed by the same individual.

Materials and methods

Sampling and samples

We collected samples of four wild ungulate species: chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), and Alpine ibex (*Capra ibex*) (Table 1). For each species (except ibex for which muscle tissue samples were not at our disposal) we used muscle tissue and recent bone (from animals hunted in 2017), as well as old bone (from 20- to 100-years-old) samples.

Bone samples comprised hunting trophies (skulls with antlers/horns) or left hemi-mandibles and were provided by the Slovenian and Croatian hunting authorities. Considering the importance that trophies have for both owners and historical records, we were careful not to destroy their physical appearance and value. Therefore, in the case of trophies, we either sampled ethmoid bone (i.e. an exceedingly light and spongy bone that separates the nasal cavity from the brain) or the cornual process (a conical bony core which is fully overgrown by horn) (Figure 1).

All recently culled individuals of each species were hunted during regular hunting activities according to relevant yearly hunting management plans and the national legislation of both countries. We used only samples from already dead individuals, therefore no animal was shot or killed by any other means for the purposes of this research.

For microsatellite genotyping, we used sample set of 67 recent bones, 40 old bones, and 493 muscle tissues. For determining the amplification success by qPCR, we pooled them into a subset of 50 recent bones, 40 old bones, and 18 muscle tissue samples (Table 1).

Contamination prevention in old bones

To avoid possible cross-contamination, laboratory work with old bone samples was conducted in a dedicated, physically-isolated laboratory for ancient DNA. Strict pro-

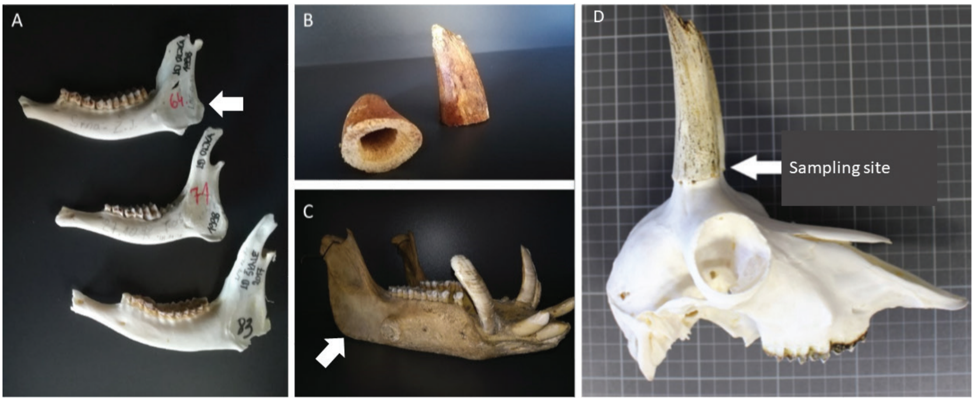

Figure 1. Micro-locations of sampling bone material for DNA isolation (see arrows) **A** roe deer mandibles **B** ethmoid bone of chamois **C** wild boar mandible **D** chamois skull.

Table 1. Summary data on samples investigated during the study (n – number of samples used for the subset in qPCR assay).

Sample ID	Species	*n*	Sample type	Organ/tissue	Year of harvest	Origin of the animal
RR1	Chamois	6	tissue	muscle	2017	Croatia
RR2		12	recent bone	skull (ethmoid bone)	2017	Croatia
RR3		13	old bone	skull (cornual process)	>80 years old	Croatia
CC1	Roe deer	7	tissue	muscle	2017	Slovenia
CC2		19	recent bone	mandible	2017	Slovenia
CC3		16	old bone	mandible	>20 years old	Slovenia
SS1	Wild boar	5	tissue	muscle	2017	Slovenia
SS2		13	recent bone	mandible	2017	Slovenia
SS3		7	old bone	mandible	>100 years old	Croatia
CI1	Alpine ibex	6	recent bone	skull (ethmoid bone)	2017	Slovenia
CI2		4	old bone	skull (ethmoid bone)	>30 years old	Slovenia

tocols for personnel hygiene were followed to minimise the amount of human DNA. In addition, personnel movements from the post-PCR environment to the extraction DNA laboratory were avoided to prevent the flow of atmospheric DNA. All surfaces in the lab were routinely double wiped with bleach and rinsed with absolute ethanol. After each analytical event, the laboratory was irradiated with ultraviolet light for at least two hours. All consumables, disposables, tools, and instruments were externally bleached and UV irradiated before entering the lab, as well as being subjected to routine cleaning before, during and after use.

DNA extraction protocol

Recent and old bone samples were carefully shredded using a sterilised sanding tool and scalpel blade. Before isolation, we proceeded with the following demineralisation protocol to obtain as much DNA as possible. Extraction from recent and old bone samples was performed using four combinations of two kits and with/without demineralisation procedures as follows: (i) PeqLab with demineralisation; (ii) PeqLab without demineralisation; (iii) Qiagen with demineralisation; and (iv) Qiagen without demineralisation.

We weighed approximately 0.3 g (± 0.05 g) of bone powder into a sterile 50 ml falcon tube, added 10 ml of 0.5 M Ethylenediaminetetraacetic acid (EDTA) and vortexed thoroughly. We incubated overnight at 37 °C with gentle agitation. Another 50 ml falcon tube was used for isolation of the blind control, which consisted of the same set of reagents but without bone powder.

We then centrifuged samples at 1300×g for 15 min; a pellet of residual powder was typically seen at this point. The supernatant was discarded. In the extraction for the negative control, we left approximately 100 µL of supernatant. We added 10 ml of sterile bi-distilled water and vortexed at high speed for 10 sec, and centrifuged again at 1300×g for 15 min.

After demineralisation of the samples, we proceeded with isolation of DNA by adding 100 µL of lysis buffer (ATL buffer -Qiagen or Lysis buffer T -PeqLab), 60 µL of Proteinase K and 20 µL of Dithiothreitol (DTT) to the pellet, and then we incubated the samples at 56 °C for 2–3 hrs. After this step, we followed an isolation protocol using the QIAamp DNA Micro Kit (Qiagen; thereafter: QIAamp) or Tissue DNA Mini Kit–S line (VWR International, Leuven, Belgium; thereafter: PeqLab), according to the manufactures' instructions. DNA was eluted in the respective kit elution buffer, then sample concentrations were normalised and dilutions were stored in a refrigerator at 4 °C to reduce thaw-freeze cycles.

We used muscle tissue as a control for each studied species (except Alpine ibex), since muscle is one of the preferred sample types for genetic studies: 2×2 mm of tissue samples, air-dried under sterile conditions in order to remove the ethanol, were used for DNA extraction using the Tissue DNA Mini Kit–S line (VWR International, Leuven, Belgium; PeqLab), according to manufacturer's instructions.

DNA concentration and quantification

The concentration and purity of the obtained DNA in the final elution volume was measured using a Qubit dsDNA BR Assay Kit (Invitrogen) on a 3.0 Qubit Fluorimeter (Life Technologies). Additionally, the spectral curve was measured on an Epoch Microplate Spectrophotometer (BioTeck) using Gene5 v.1 software to check for potential impurities. We expressed the obtained amount of DNA in ng per µL of DNA in final elution volume. The quality of DNA obtained from samples was assessed using Sybergreen chemistry. All qPCR reactions were performed on a Roche LightCycler 96 System (Roche Diagnostic GmbH, Germany) under the conditions described in Table 1 (Suppl. material 1). Degradation of DNA samples was determined by calculating the amplification success of the 100 bp and 200 bp products given by quantification cycle value (Cq value), which were generated directly at a specific threshold. The MIQE guidelines (Bustin et al. 2009) suggested that $Cq < 40$ has to be set as a threshold for positive amplification. The Cq is defined as the number of cycles needed for the fluorescence signal to reach a specific threshold. A predefined threshold is set within the exponential amplification phase, when doubling of the product can be detected above background fluorescence, and the number of cycles needed to reach this threshold is used to estimate the amount of template DNA present. By comparing the Cq values between two samples we can compare the amount of DNA fragments in one sample relative to the other. Lower Cq values mean higher initial copy numbers of the target.

We selected the fragment lengths of 100 bp and 200 bp according to: (i) the recommendation for qPCR that optimal amplicon length should be less than 200 bp (Nielsen et al. 2017); (ii) the fact that the majority of selected microsatellite alleles were around 200 bp or less in length. For quantification, all samples were analysed in triplicate. Negative controls were included alongside every batch of samples processed. The blank sample did not produce any measurable amplicons.

Microsatellite analysis

The multilocus genotyping of microsatellites was used: (i) to quantify the success rate of amplification using a larger sample set (see Table 2), and (ii) to evaluate the reproducibility of genotyping using recent bone and muscle tissue from the same roe deer. The second analysis was possible because bone and tissue samples from the same individual were available. In this individual, we performed a microsatellite analysis in seven DNA isolation replicates to confirm the multilocus genotype, and to estimate the possible proportion of mismatches between the replicated analyses.

PCR reaction and genotyping of microsatellites

The DNA extracted from bones and muscle tissue was treated identically. Microsatellites were amplified using Alltaq PCR Core kit (Qiagen), using 3 µL of template DNA under the following conditions: initial PCR activation for 2 min at 95 °C followed by 40 cycles with denaturation for 10 s at 95 °C, annealing for 30 s at 55 °C, extension for 20 sec at 72 °C, and final extension for 10 min at 72 °C. Fragment analysis was performed on a SeqStudio sequencer (Thermofischer scientific) using a GeneScan LIZ500 (-250) standard (Applied Biosystems). Results were validated using GeneMapper v.5.0 software (Applied Biosystems).

To quantify the amplification success rate, all samples were genotyped at three microsatellite loci (approximately 100 bp and 200 bp long) with species-specific microsatellites primers as follows: roe deer (Kuehn et al. 2007), chamois and Alpine ibex (Zemanová et al. 2011), and wild boar (Robic et al. 1995) (Suppl. material 1: Table S2).

To rate genotyping reproducibility, we amplified 14 microsatellite loci in 4 multiplex sets, containing 4, 2, 3 and 4 microsatellites, although locus MAF70 was carried out separately using the protocol described in Kuehn et al. (2007), which is commonly applied in population genetic studies utilising DNA from the muscle tissue of roe deer. Following the same procedure, we amplified 20 microsatellites in 4 multiplex sets (Zemanová et al. 2011) for chamois bones samples (Suppl. material 1: Tables S3, S4). We compared microsatellite amplifications of DNA extracted from recent bones and muscle tissue by genotyping seven samples shed by the same individual (roe deer).

Statistical analyses

All statistical analyses were performed using SPSS software Version 25 (SPSS Inc., Chicago IL). We used the Shapiro-Wilk's test to check the normality of: (i) DNA concentrations (yield), and (ii) quantification cycle data: Cq100 and Cq200 values. Since neither set of data was normally distributed, we used nonparametric statistics in all subsequent analyses. In the following sections, all continuous data are presented with means and standard deviations, while categorical data are presented as numbers and percentages.

The differences in DNA yields were assessed by comparing measured concentrations of DNA isolated from muscle tissue *vs*. recent bones *vs*. old bones by different isolation methodologies. We used the Kruskal-Wallis test for testing the significance of differences in DNA yield between different isolation methodologies. The effect of the demineralisation protocol on bones' DNA yield was assessed by the Mann-Whitney U test.

We assessed the quality of isolated DNA by comparing the quantification cycle data: Cq values measured for 100 bp (short) and 200 bp (long) fragments in qPCR assays. The DNA quality was assessed by: (i) the ratio between Cq100/Cq200 value as was described previously by Nielsen et al. (2017), and (ii) the amplification success expressed in percentages for Cq200, which correlate with a sufficient amount of 200 bp DNA product to be detected.

For testing the significance of differences in the ratio Cq100/Cq200, we used the Kruskal-Wallis test. The χ^2-test was used to compare Cq200 amplification success for all isolation methods, where we set the success threshold of $Cq < 40$ as described in the MIQE guidelines (Bustin et al. 2009), and then coded with "1" each result with $Cq < 40$ as successfully amplified, and unsuccessfully amplified PCR with the code "0" ($Cq > 40$).

To assess the pairwise comparison of Cq200 amplification success we used the χ^2-posthoc test for adjusted residuals with a Bonferroni correction due to multiple comparisons.

All statistical analyses for recent and old bones were performed separately, and the level of statistical significance was set as $p < 0.01$ in all analyses.

Results

DNA yield

All extraction methods used resulted in different DNA concentrations (between 0.23 ng/μL and 82.4 ng/μL; mean = 16.4 ± 20.0 ng/μL). Regardless of the species, the amounts of DNA extracted from muscle tissues were 18.8–52.7 ng/μL (mean 36.3 ± 13.9 ng/μL), from recent bones 1.40–82.4 ng/μL (mean 27.1 ± 21.9 ng/μL), and from old bones 0.23–50.3 ng/μL (mean 5.41 ± 10.7 ng/μL), respectively. DNA concentration in the extracted blank sample was not detected (see Table 2).

There were no statistical differences in recent bones' DNA concentrations isolated by different methods in comparison to tissue DNA concentration ($H = 9.028$; $p = 0.060$), but we did find significant differences when samples were prepared either with demineralisation or when this step was excluded ($H = 11.916$; $p = 0.008$). Interestingly, the methodology that includes demineralisation and the low-cost PeqLab kit isolation gave the highest DNA yield (45.3 ± 20.6 ng/μL), but DNA isolation without prior preparation of samples led to significantly lower DNA concentrations ($H = 25.048$; $p = 0.010$).

On the other hand, we found significant differences among old bones' DNA concentrations when comparing different isolation methodologies ($H = 22.938$; $p < 0.001$). There was a clear tendency for old bone samples to yield significantly less DNA compared with either recent bones ($H = 30.141$; $p < 0.001$) or tissue samples ($H = 38.797$; $p < 0.001$). The highest and significant differences ($H = 28.900$; $p < 0.001$) in DNA concentrations between old bones and muscle tissues were found when using the QIAamp kit isolation without demineralisation. However, this DNA concentration was significantly lower than the concentrations obtained either with the PeqLab kit with previous demineralisation ($H = 15.004$; $p = 0.025$) or with QIAamp kit with previous demineralisation ($H = 12.976$; $p = 0.038$).

DNA quality

Recent bones

All samples were amplified successfully using qPCR. Cycle quantification Cq100 scores for recent bones were comparable between different methods, but slightly lower for the QIAamp kit isolation without demineralisation (24.29 ± 2.46). The same pattern was also observed for Cq200 (26.94 ± 1.41). The Cq100/Cq200 ratios were above 70% for all recent bone samples, with the highest value observed for QIAamp isolation with demineralisation (0.90 ± 0.11).

Short fragments (Cq100) were successfully amplified regardless of the method used (χ^2-posthoc test: $p = 0.482$). The amplification success for Cq200 differed between methods ($p = 0.015$). The proportion of successfully amplificated Cq200 fragments for recent bones using a demineralisation protocol or muscle tissues was > 90%. Significantly, the least successful Cq200 amplification was found in DNA fragments isolated with the PeqLab kit without demineralisation (61.1%; $p < 0.001$).

Old bones

Cycle quantification scores for short fragments (Cq100) were comparable between all methods used ($p = 0.103$). However, the amplification success of long fragments differed between methods ($p = 0.003$), and was, again, significantly lower for PeqLab isolation without demineralisation ($p < 0.001$). The proportion of successfully amplifi-

Table 2. Overview of the results of different DNA extraction methods used for different samples. Number of samples (n) used in qPCR assays is summarized for all species. Average of DNA yield (concentration) achieved with different methods (means ± SD). DNA quality for different extraction methods/samples is given by quantification cycle scores Cq100, Cq200 and as ratio Cq100/Cq200 (means ± SD), which also indicates DNA degradation. The amplification success is given in percentages for Cq200 values. The microsatellite genotyping for muscle tissue, recent bones (PeqLab D and WD) and old bones (QIAamp D) success is calculated on larger samples sets (67 recent bones, 40 old bones, and 493 muscle tissues, respectively).

Sample	Isolation method	*n*	Concentration (ng/μL)	Cq100	Cq200	Ratio *(Cq100/ Cq200)*	Amplification success Cq200 (%)	*p* – value (χ^2- posthoc test)	Microsatellite success rate (%)
Muscle tissues	PeqLab	18	36.30 ± 13.91	26.51 ± 6.27	25.15 ± 4.32	1.04 ± 0.19	94.7	0.19	96%
Recent bones	PeqLab WD	18	25.43 ± 26.89	28.54 ± 2.71	31.30 ± 2.56	0.79 ± 0.05	61.1	**0.00**	90%
Recent bones	PeqLab D	12	45.31 ± 20.59	28.01 ± 2.17	37.03 ± 1.19	0.75 ± 0.05	91.7	0.84	97%
Recent bones	QIAamp WD	8	16.15 ± 19.05	27.80 ± 1.22	36.08 ± 2.52	0.77 ± 0.03	87.5	0.48	89%
Recent bones	QIAamp D	12	25.29 ± 15.39	24.29 ± 2.46	26.94 ± 1.41	0.90 ± 0.11	100	0.11	95%
Old bones	PeqLab WD	8	1.90 ± 1.69	29.44 ± 3.41	35.56 ± 0.94	0.80 ± 0.10	25.0	**0.01**	60%
Old bones	PeqLab D	8	17.04 ± 19.84	28.81 ± 7.56	37.45 ± 5.08	0.74 ± 0.17	37.5	0.09	72%
Old bones	QIAamp WD	12	1.05 ± 0.76	27.27 ± 5.58	35.25 ± 4.03	0.74 ± 0.09	58.3	0.62	68%
Old bones	QIAamp D	12	5.11 ± 5.61	26.79 ± 3.31	31.22 ± 5.72	0.93 ± 0.13	66.7	0.84	78%

Notes: WD = without demineralisation, D = with demineralisation. To determine differences between individual pairs of variables (amplification success) for χ^2-posthoc tests we used Bonferroni correction due to multiple comparisons, therefore p-value of statistical significance was set as $p < 0.01$.

cated Cq200 fragments was only 25.0% for the PeqLab kit without demineralisation and 37.5% for the PeqLab kit with demineralisation. The proportion of successfully amplificated Cq200 fragments was higher for the QIAamp kit: 58.3% without demineralisation and 66.7% with the demineralisation protocol. Short fragments amplified more effectively due to the lower concentration of longer fragments in degraded DNA from old bones.

Microsatellites

Microsatellite analysis showed that muscle tissue samples and recent bones isolated with and without demineralisation protocol (using both kits) produced amplicons with the correct microsatellite loci positions (see Suppl. material 2) for short (80–100 bp), medium (approximately 150 bp), and longer fragments (200–250 bp). Amplification produced identical fragment lengths per locus (80–300 bp), similar allele numbers, as well as comparable signal strength and allele scoring. The amplification success rate for microsatellite genotyping is shown in Table 2, which provides an overview of the results of the different DNA extraction methods used for different samples.

Quality parameters such as the height of peaks and the presence of non-specific peaks were comparable between the two kits, with fewer non-specific peaks observed when using demineralisation protocols. For old bones, the highest microsatellite success (> 78%) and higher-quality parameters were observed for DNA isolated using the QIAamp kit with prior demineralisation. However, the amplification of DNA samples was more successful when the demineralisation protocol was used (> 95%). Without prior preparation of the bones, the amplification success decreased to 90%, and in some cases longer alleles were not amplified in the multiplex (in 15% of the analyses).

The replicated DNA of both muscle tissue and bone samples of the same roe deer individual showed the same multilocus genotype.

Discussion

Despite many improvements, genetic analysis has not seen widespread use as a tool in wildlife management, in part due to the perception that genetic analyses are expensive and that sample collection is difficult (Holderegger et al. 2019). This study shows how to reduce the costs of genetic analyses on widely available samples, i.e. bony structures that are systematically collected by hunters as trophies (skulls with antlers or horns) or under reporting obligations (mandibles). Such bony structures are very favourable for genetic analyses due to the high level of DNA preservation as a result of careful storage and pretreatment of the material immediately after harvesting. Thus, when an animal is culled, the head is removed immediately and cooked to remove all soft tissues from the cranium. The dry and constant environmental conditions in which trophies and mandibles are usually stored are ideal for the preservation of high-quality DNA over a longer timescale.

The recent bones used in this study showed DNA content and DNA amplification success rates equal to that of muscle tissue samples. DNA concentrations obtained using our low-cost extraction methods were sufficient for microsatellite analysis (Table 2, Suppl. material 2). There was no evidence for a scoring error due to stuttering, allelic dropouts or null alleles for any of the microsatellite loci for the recent bones' DNA. In the case of old bones, DNA isolated with more expensive kits (specially designed for bones) and using a demineralisation protocol showed fewer genotype errors and better amplification success.

The finding that the DNA isolated from recent bones using low-cost methods showed amplification success rates equal to DNA isolated from muscle tissue is astounding. We also revealed that a modified isolation protocol, i.e. using the kit after prior demineralisation of samples instead of the classical phenol/chloroform isolation method, reduced the time needed for the procedure by at least twofold and that costs could be reduced by at least 50% due to the possibility of a using widely available low-cost kit.

We have demonstrated that bones, even old ones (> 20 years old), are a good source of DNA even if they have been previously treated with aggressive agents (i.e. hydrogen peroxide), as was the case for almost all of our samples. Where such samples and sample sets are available, even for large-scale studies in different spatiotemporal contexts, there is no need to collect additional soft tissues or non-invasive samples for genetic studies. Indeed, both low- and high-cost commercial kits showed high recovery of DNA after previous bone demineralisation (all concentrations were much higher than 16 ng/μL), even in the case of very old bones (> 100 years old). Past studies have shown the importance of developing and optimising DNA isolation methods from analytically difficult samples such as bones (Loreille et al. 2007; Rohland and Hofreiter 2007; Seo et al. 2009; Nielsen et al. 2017). For example, Loreille et al. (2007) proposed a complete demineralisation protocol, which results in full physical dissolution of the bone samples, to maximise DNA yield.

It has been previously suggested that geneticists should always use demineralisation of bones for successful DNA isolation (Jakubowska et al. 2012). However, we have shown that high DNA yields can be recovered from well-preserved bone samples even without demineralisation, presumably because free DNA was not washed away. According to our results, well-preserved bones do not need to undergo a demineralisation process, which adds more handling and pipetting steps, thereby increasing the extraction time and costs, and also increasing the possibility of secondary contamination and errors. Nevertheless, we have to stress that the success rate was also better in recent bones when using demineralisation pre-treatment of samples.

A high variability in DNA concentrations in the subset of recent bones could be a consequence of differences in bones density (Pinhasi et al. 2015), interspecies variability, the sampling micro-location at the skull (especially when using antlers or the cornual process in horns), different distribution and variable post-mortem preservation of DNA across/within individual bone. However, in spite of this variability, the lower extracted concentrations of DNA were sufficiently high for genotyping and were comparable to the concentrations of DNA isolated from muscle tissues. It is very important for successful genotyping that the extracted DNA contain an adequate quantity of longer fragments (> 250 bp), particularly in recent bone samples.

In the case of old bones, DNA concentrations varied even more, probably due to the low quantity of endogenous DNA caused by poor preservation and the high likelihood of DNA degradation. Diagenesis has variable effects in old bones and can cause localised differences in bone structure, for example, the bone is less compact and more porous at sites of muscle attachment than in the surrounding bone (Hawkey and Merbs 1995; Mann et al. 2013). Therefore, this site may be more susceptible to diagenetic processes, which increase as bones age. Differences in diageneses processes influence the variation in sampling (Rohland and Hofreiter 2007) and, consequently, DNA concentration. The demineralisation process increases DNA yield, because EDTA demineralises the bones and inactivates DNA by chelating bivalent cations such as magnesium and calcium ions (Loreille et al. 2007). In our study, the demineralisation process significantly increased the DNA yield for both isolation kits, but only the high-cost commercial kit showed adequate amplification success for long fragments (Cq200), while the low-cost kit was adequate for microsatellite genotyping. Nevertheless, our results revealed that even when using (very) old bones, using a demineralisation process allows for more successful extraction of DNA.

Conclusion

Our findings suggest that genetic population studies can be easily conducted using well-preserved bones even without any prior preparation of the bones for DNA isolation. This means that genetic studies, such as genetic identification of individuals or paternity analyses, can be carried out using readily available bony structures (trophies and particularly mandibles) in an easy, fast and inexpensive way. Moreover, the fact that mandibles are collected for both sexes and for both juveniles and adults allows the acquisition of large sample sets without sex- or demographically-biased data. This approach enables further genetic insights into various ecological and management issues for all wild ungulates for which such bony structures are available, whether on the national, regional or larger scale.

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Supplementary material 1

Tables S1–S4

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Data type: Microsoft Word 97 - 2003 Document (.doc)

Explanation note: **Table S1.** Conditions in qPCR assays. **Table S2.** Selected microsatellite loci amplified in qPCR assays or microsatellite analysis. **Table S3.** Microsatellite loci analyzed in fragmentation analysis of *R. rupicapra*. **Table S4.** Microsatellite loci analyzed in fragmentation analysis of *C. capreolus*.

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Supplementary material 2

Microsatellite loci for muscle tissue samples and recent bone samples

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Data type: Microsoft Word 97 - 2003 Document (.doc)

Explanation note: **Figure S1.** Microsatellite loci for muscle tissue samples (upper) and recent bone samples (bottom) of *R. rupicapra* isolated using the cost-efficient PeqLab kit. **Figure S2.** Microsatellite loci for muscle tissue samples (bottom) and recent bone samples (upper) of *C. capreolus* isolated with cost efficient PeqLab kit.

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