

The second known stygomorphic freshwater crab from China, *Phasmon typhlops* gen. nov. et sp. nov. (Crustacea, Decapoda, Potamidae), diverged at the beginning of the Late Miocene

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Abstract

A new genus and new species of blind freshwater cave crab are described from Chongzuo City, Guangxi Zhuang Autonomous Region, China based on morphology and mitochondrial 16S rDNA sequences. The new genus, *Phasmon* gen. nov., is established for *P. typhlops* sp. nov., which is only the second blind cave crab known from China and East Asia. The combination of a very wide carapace, overall depigmentation, reduced orbits and vestigial unpigmented eyes of *Phasmon* immediately separates it from all known potamid genera. Molecular divergence estimates based on 16S rDNA suggest that the lineage to which the new genus belongs diverged from other potamids at the beginning of the Late Miocene (10.8 million years ago), much earlier than other Chinese cave crabs.

Keywords

16S rDNA, blind crab, cave crab, new genus, new species, Guangxi, subterranean

Introduction

The Guangxi Zhuang Autonomous Region is located in southern China within the Huanan freshwater zoogeographic province (Huang et al. 2020a) and borders Vietnam. Its complex physical geography and subtropical climate make it one of the richest regions for freshwater crabs in China (Shih and Ng 2011; Chu et al. 2018). With 14 genera recorded to date (Bottapotamon Türkay & Dai, 1997, Chinapotamon Dai & Naiyanetr, 1994, Heterochelamon Türkay & Dai, 1997, Indochinamon Yeo & Ng, 2007, Lacunipotamon Dai, Song, He, Cao, Xu & Zhong, 1975, Longpotamon Shih, Huang & Ng, 2016, Mediapotamon Türkay & Dai, 1997, Neilupotamon Dai & Türkay, 1997, Potamiscus Alcock, 1909, Qianguimon Huang, 2018, Sinolapotamon Tai & Sung, 1975, Somanniathelphusa Bott, 1968, Tiwaripotamon Bott, 1970, and Yarepotamon Dai & Türkay, 1997), Guangxi Zhuang Autonomous Region is second, in China, only to Yunnan Province in the number of freshwater crab genera (Shih and Ng 2011; Huang 2018; Huang et al. 2020d). From the number of new species described from this region in recent years (Zhu et al. 2010; Naruse et al. 2013; Do et al. 2016; Ng 2017; Huang 2018; Zou et al. 2018; Wang et al. 2019, 2020a, b), there is little doubt that many more remain to be discovered.

Stygomorphic potamid crabs are rare, and only a few species were previously known. *Chinapotamon clarkei* Ng, 2017 and *Diyutamon cereum* Huang, Shih & Ng, 2017b, from China; *Cerberusa tipula* Holthuis, 1979 and *Cerberusa caeca* Holthuis, 1979, from Borneo; *Erebusa calobates* Yeo & Ng, 1999, from Laos; and *Teretamon spelaeum* Absar, Mitra & Kharkongor, 2017, from India, all – exhibit varying degrees of stygomorphism. Of these, only *D. cereum* and *C. caeca* exhibit strong eye reduction and depigmentation–both appear to be blind and can be considered the most highly stygomorphic of known subterranean potamids.

In 2018, a local collector from Chongzuo City, Guangxi, China, alerted us to an unusual crab trapped from a karst spring. This crab, a female, was apparently a stygobite, lacking body pigmentation and having vestigial, unpigmented eyes. Many further attempts were made to collect more specimens of this unusual species, but they remained elusive. The collector only succeeded once in trapping a second specimen from the karst spring, but only half of the carcass remained when he checked the trap; the other half was apparently consumed by a specimen of the epigean crab *Lacunipotamon cymatile* Huang, Shih & Ahyong, 2020 (Huang et al. 2020c), which was also lured into the trap. It was not until over a year later that the collector discovered the primary habitat of the crab in a nearby cave system in which he successfully trapped more specimens. After we acquired and examined the specimens, it was obvious that these cave crabs were new to science: the combination of highly reduced and unpigmented eyes, long antennules, lack of body pigmentation, unique carapace features, and very wide male anterior thoracic sternum, immediately separate it from all other potamid genera. Molecular data derived from the mitochondrial 16S rDNA gene further support the taxon as new, and we herein describe it as a new genus and new species.

Materials and methods

Specimens were collected by hand, preserved in 75% ethanol, and deposited in the collections of the Sun Yat-sen Museum of Biology, Sun Yat-sen University, Guangzhou, China (SYSBM) and the Australian Museum, Sydney, Australia (AM). The terminology used primarily follows that of Dai (1999) and Davie et al. (2015). Carapace length (CL) was measured along the dorsal midline and carapace width (CW) was the greatest width measured across the branchial margins. The male gonopods 1 and 2 are abbreviated as G1 and G2, respectively. Measurements (mm) are of the carapace width and length, respectively.

A 16S sequence was obtained from the paratype (AM P.105524) following Shih et al. (2009), using the primers 16H10 and 16L29 (Schubart 2009), and aligned with the MUSCLE function of MEGA (vers. 10.0.5; Kumar et al. 2018) after verification with the complimentary strand. The sequence was deposited in NCBI GenBank under the accession number MW289910. A preliminary analysis showed that this genus belongs to the "China-East Asia Islands" Group within the "Eastern-Asia Subclade" of the subfamily Potamiscinae (Shih et al. 2009). Therefore, to confirm the phylogenetic position of the new genus and species, 24 additional 16S sequences from related genera from East Asia, Indochina and Southeast Asia in Shih et al. (2009), Huang et al. (2014, 2017a, b, 2018, 2020d), and Wang et al. (2019) were included for comparison. The variable regions in the loop regions of 16S that could not be aligned adequately for phylogenetic analysis were excluded (Shih et al. 2009).

The best-fitting model for sequence evolution of 16S was determined by PartitionFinder (vers. 2.1.1; Lanfear et al. 2017), selected by the Bayesian information criterion (BIC). The best model obtained, GTR+I+G, was subsequently applied for Bayesian inference (BI) and maximum likelihood (ML) analyses. The BI analysis was performed with MrBayes (vers. 3.2.2; Ronquist et al. 2012) using four chains run for 10 million generations, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist et al. 2005) and the first 1050 trees were discarded as burn-in accordingly. The ML analysis was conducted in RAxML (vers. 7.2.6; Stamatakis 2006). The GTR + G (i.e., GTRGAMMA) model was used for all subsets with 100 runs and found the best ML tree by comparing likelihood scores. The robustness of the ML tree was evaluated by 1000 bootstrap pseudoreplicates under the model GTRGAMMA. The uncorrected p-distances for genetic divergence between haplotypes were calculated by MEGA.

Taxonomy

Family Potamidae Ortmann, 1896 Subfamily Potamiscinae Bott, 1970

Phasmon gen. nov.

http://zoobank.org/0BD28B59-9BE1-4679-AD9C-268006C41E77 Figs 1–4

Type species. *Phasmon typhlops*, by present designation.

Diagnosis. Small sized (carapace width less than 30 mm). Carapace 1.6× wider than long; fronto-orbital width about twice width of posterior margin; dorsal surface weakly convex (Figs 1, 2A); frontal margin weakly sinuous, continuous with supraorbital margin, forming almost straight anterior margin of carapace in dorsal view (Fig. 1); postorbital and epigastric cristae almost indiscernible (Figs 1, 2A); orbit shallow, eyes vestigial, almost immovable, length about half orbital width; cornea reduced, unpigmented, facets absent; external orbital angle very wide, confluent with anterolateral margin (Figs 1, 2A). Epibranchial tooth inconspicuous. Median lobe of epistome broadly triangular (Fig. 2A). Maxilliped 3 ischium length less than twice width; exopod reaching beyond distal edge of ischium, flagellum well-developed (Fig. 3A). Cheliped fingers without gape when closed (Fig. 3D, E). Male anterior thoracic sternum very wide, around 2.3 times as wide as long (Fig. 2B). Male pleon triangular (Fig. 2C). G1 tapering anteriorly, tip narrow but blunt (Figs 3C, 4A, B). G2 distal segment tip pointed (Figs 3B, 4C, D). Female vulvae on sternite 6, reaching sutures of sternites 5/6 anteriorly, very widely spaced from one another (Fig. 2F).

Etymology. The genus name is an arbitrary combination of the Latin word "phasma", meaning ghost, which refers to the type species' pale appearance and dark habitat, and the genus name *Potamon*, which is the type genus of the family. Gender neuter.

Phasmon typhlops sp. nov.

http://zoobank.org/FEE83A41-2669-4B3F-B3A0-5E1F15F1DEB1 Figs 1–4

Type material. *Holotype:* SYSBM 001982, male (14.4×9.0 mm), Leiping Town, Daxin County, Chongzuo City, Guangxi Province, China, 22.65°N, 107.10°E, subterranean karst stream in cave, baited trap, coll. local collector, December 2019. *Paratype:* AM P.105524, female (22.1×13.7 mm), Leiping Town, Daxin County, Chongzuo City, Guangxi Province, China, karst spring, baited trap, coll. local collector, September 2018.

Description. Carapace broad, about 1.6 times as wide as long; fronto-orbital width about twice width of posterior margin; regions indistinct, dorsal surface slightly convex; surface finely pitted (Fig. 1A). Frontal margin weakly sinuous, continuous



Figure 1. *Phasmon typhlops* gen. nov. et sp. nov., male holotype (14.4 × 9.0 mm), SYSBM 001982 (**A**) female paratype (22.1 × 13.7 mm), AM P.105524 (**B**). Dorsal habitus.

with supraorbital margin, forming almost straight transverse margin in dorsal view (Figs 1A, B). Epigastric cristae and postorbital cristae almost indiscernible (Figs 1A, 2A). Branchial regions slightly swollen (Figs 1A, 2A). Cervical groove shallow (Fig. 1A). Mesogastric region slightly convex (Fig. 1A). External orbital angle obsolete, outer margin convex, almost indistinguishable from anterolateral margin (Figs 1A, 2A). Epibranchial tooth granular, inconspicuous (Fig. 1A). Anterolateral margin lined with 15–20 small, single or partially fused granules. Posterolateral margin posteriorly convergent (Fig. 1A); posterolateral surface generally smooth (Fig. 1A). Orbits shallow; supraorbital margins weakly cristate, infraorbital margins lined with granules (Fig. 2A). Eyes almost immobile, greatly reduced, tapering, length about half orbital width; peduncle short, stout; cornea vestigial, surface without facets, unpigmented (Figs 2A, 3F). Suborbital, pterygostomial and sub-hepatic regions generally smooth, pitted (Fig. 2A).



Figure 2. *Phasmon typhlops* gen. nov. et sp. nov., male holotype $(14.4 \times 9.0 \text{ mm})$, SYSBM 001982 (**A–D**) female paratype $(22.1 \times 13.7 \text{ mm})$, AM P.105524 (**E**, **F**). Cephalothorax, anterior view (**A**) anterior thoracic sternum (**B**) anterior thoracic sternum and pleon, ventral view (**C**) sterno-pleonal cavity with G1 *in situ*, ventral view (**D**) pleon, ventral view (**E**) vulvae, ventral view (**F**).

Antennules large, folded within broad fossae; antennae very short (Fig. 2A). Median lobe of epistome posterior margin broadly triangular, lateral margins sinuous (Fig. 2A).

Maxilliped 3 merus subtrapezoidal, with median depression, width about 1.2× length; ischium subtrapezoidal with shallow median sulcus, distomesial margin rounded, width about 0.6× length. Exopod reaching proximal one-third of merus; flagellum longer than half ischium length (Fig. 3A).

Chelipeds (pereiopod 1) subequal (Figs 1, 3D, E). Merus trigonal in cross section; margins slightly crenulated, surface generally smooth (Figs 1A, 2A). Carpus with sharp spine at inner-distal angle (Fig. 1 A). Major cheliped palm length about 1.5× height; dactylus 0.9× palm length (male) (Fig. 3D, E), as long as palm (female). Palm surface



Figure 3. *Phasmon typhlops* gen. nov. et sp. nov., male holotype $(14.4 \times 9.0 \text{ mm})$, SYSBM 001982. Left maxilliped 3 (**A**) left G2, pleonal view (**B**) left G1, pleonal view (**C**) major cheliped (**D**) minor cheliped (**E**) colour in life (**F**). Scale bars: 1.0 mm.

pitted (Fig. 3D, E). Dactylus as long as pollex (Fig. 3D, E). Occlusal margin of fingers with 18–20 irregular blunt teeth, without gape when closed (Fig. 3D, E).

Ambulatory legs (pereiopods 2–5) slender with very sparse short setae (Fig. 1). Pereiopod 3 merus 0.9× CL (male) (Fig. 1A), 0.8× CL (female) (Fig. 1B). Pereiopod 5 propodus length 2.8× height (male) (Fig. 1A), 3.4 height (female) (Fig. 1B), shorter than dactylus; dactylus length 6.1× height (male) (Fig. 1A), 6.2× height (female) (Fig. 1B).

Male thoracic sternum generally smooth, pitted; sternites 1–4 width about 2.3× length; sternites 1, 2 forming indistinguishably fused, broad triangle; fused sternites 1, 2 demarcated from sternite 3 by shallow transverse sulcus; sternites 3, 4 fused without indication of demarcation except for shallow lateral notch (Fig. 2B). Male sterno-pleonal cavity reaching anteriorly slightly beyond level of cheliped coxa articular condyle (Fig. 2B); deep median longitudinal groove between sternites 7, 8 (Fig. 2D). Male ple-



Figure 4. *Phasmon typhlops* gen. nov. et sp. nov., gonopods: left G1, abdominal view (**A**) left G1, sternal view (**B**) left G2, mesial view (**C**) left G2, pleonal view (**D**). Scale bar: 0.5 mm.

onal locking tubercle positioned at mid-length of sternite 5 (Fig. 2D). Female vulvae reaching sutures of sternites 5/6 anteriorly but not posteriorly to sutures of sternites 6/7, positioned widely apart from each other (Fig. 2F).

Male pleon broadly triangular; somites 3–6 progressively narrower; somite 6 width approximately 2.7× length; telson width 1.6× length; lateral margins slightly convex, apex rounded (Fig. 2C). Female pleon subovate (Fig. 2E).

G1 tapering, slightly sinuous, tip exceeding pleonal locking tubercle but not reaching suture between thoracic sternites 4/5 *in situ* (Fig. 2D); proximal segment length about 2.3× length of distal segment (Figs 3C, 4A, B). Distal segment slender, tapering anteriorly, slightly inclined towards midline; tip pointed upwards in dissected view (Figs 3C, 4A, B). G2 slender, almost straight, proximal portion with distal two-thirds subcylindrical, length about 2.4× length of distal portion (Figs 3B, 4C, D); distal portion flattened, apex acute, proximally with small triangular lobe.

Etymology. The species name is derived from the Greek words "typhlos" and "ops", meaning "blind" and "eyes", respectively. It refers to the greatly reduced and non-functional eyes of this species.

Colour in life. Pale yellowish-white all over (Fig. 3F).

Habitat. *Phasmon typhlops* gen. nov. et sp. nov. occurs in subterranean karst streams, but little is currently known about its precise habitat. According to the collector, subterranean streams in the dark zone of caves appear to be the primary habitat of *P. typhlops* sp. nov., where it has been found in shallow and still water as well as flowing streamways. However, some specimens have also been captured at night from a karstic spring that is immediately connected to the more extensive subterranean karst system. We only examined the two type specimens, of which the holotype was collected from the former habitat and the paratype from the latter. An epigean species, *Lacunipotamon cymatile*, inhabits the areas immediately adjacent to the spring and has been observed to prey on *Phasmon typhlops* gen. nov. et sp. nov. (Huang et al. 2020c).

Distribution. Chongzuo City, Guangxi Province, China.

Remarks. Phasmon typhlops gen. nov. et sp. nov. can be considered a true stygobite owing to its stygomorphic features, in particular the strong reduction of the eyes, body depigmentation and slightly elongated appendages, which are consistent with its subterranean lifestyle (Holthuis 1986; Ng and Goh 1987). Apart from P. typhlops gen. nov. et sp. nov., *Divutamon cereum* and *Cerberusa caeca* are the only other apparently blind stygomorphic potamid crabs known. We have not directly examined the eyes of C. caeca, but those of D. cereum and P. typhlops are unpigmented and the cornea is vestigial and without facets. Although we cannot exclude the possibility that the eyes of D. cereum and P. typhlops are capable of light detection, the absence of pigmentation or ommatidial facets indicates that the eyes are incapable of image formation. The enlarged antennules as present in Phasmon gen. nov. are otherwise seen in only a few cavernicolous freshwater crabs such as the gecarcinucids Sundathelphusa waray Husana, Naruse & Kase, 2009, and S. lobo Husana, Naruse & Kase, 2009 (Husana et al. 2009: figs 2B, 5B), and are likely a sensory compensation for the loss of vision (Culver et al. 1995). Other than these two species, there are other stygomorphic gecarcinucids from Asia, but these can be separated from the new species by obvious family-level characters.

Sexual dimorphism is evident in our two specimens of *P. typhlops*: the smaller male holotype has proportionally longer but stouter legs in comparison to the larger female. The anterior carapace of the larger female is also proportionately wider than the posterior than in the male. Although the differences in leg proportions follow the pattern of sexual dimorphism observed in other potamids (e.g., Huang et al. 2020b), whether this carapace difference is due to size, sex or general variation remains to be determined.

Taxonomically, the most striking features of *Phasmon* gen. nov. are its very wide carapace (CW/CL=1.6; Fig. 1) and wide male anterior thoracic sternum (width 2.3× length; Fig. 2B). These characters combined immediately separate *Phasmon* gen. nov. from all other potamid genera. *Diyutamon cereum* occurs in Guizhou, which is relatively close to the type locality of *P. typhlops* gen. nov. et sp. nov. *Phasmon typhlops* gen. n. et sp. n. can be separated from *D. cereum* by its proportionally wider carapace (CW/ CL=1.6 vs. 1.3–1.4 in *D. cereum*; Huang et al. 2017b: fig. 2A); granulate anterolateral carapace margins (Fig. 1) (vs. spinose in *D. cereum*; Huang et al. 2017b: fig. 2A); proportionally wider male anterior thoracic sternum (width $2.3 \times$ length vs. width $1.7 \times$

length in *D. cereum*; Huang et al. 2017b: fig. 6C); proportionally wider male pleon (compare Fig. 2C with Huang et al. 2017b: fig. 2C); male thoracic sternite 8 being fully concealed when the pleon is closed (Fig. 2C) (vs. partially exposed in *D. cereum*; Huang et al. 2017b: fig. 3E, F); and its relatively shorter and stouter walking legs (Fig. 1) (see Huang et al. 2017b: fig. 2A).

Phasmon typhlops gen. nov. et sp. nov. is similar to *Cerberusa caeca* in general physiognomy and size. However, the new species can immediately be distinguished by its proportionally wider carapace (CW/CL=1.6 vs. 1.3–1.4 in *C. caeca*; Holthuis 1979: pl. 8); almost indiscernible postorbital cristae (Fig. 1) (vs. low, indicated by a transverse row of granules in *C. caeca*; Holthuis 1979: fig. 3A); proportionally wider male pleon (compare Fig. 2C with Holthuis 1979: fig. 3C); and its slightly sinuous G1 (Figs 3C, 4A, B) (vs. strongly bent outwards in *C. caeca*; Holthuis 1979: fig. 3D).

The G1 characteristics of Phasmon gen. nov. are rather unremarkable and particularly similar to those of Chinapotamon and Diyutamon. Chinapotamon is also found in Guangxi and includes two cavernicolous species, C. dashiwei Ng, 2017 and C. clarkei Ng, 2017, of which the latter displays evidence of stygomorphism in reduced body pigmentation and well-developed, albeit proportionally smaller eyes than epigean congeners (Ng 2017). Phasmon gen. nov. is readily distinguished from Chinapotamon in: the proportionally wider carapace (CW/CL=1.6 vs. 1.3-1.4 in Chinapotamon; Ng 2017: figs 2, 6; Zou et al. 2018: fig. 2); the frontal margin being continuous with the supraorbital margin, forming an almost straight transverse margin in dorsal view (Fig. 1) (vs. supraorbital margin distinctly concave in dorsal view in *Chinapotamon*; Ng 2017: figs 2, 6; Zou et al. 2018: fig. 2); the vestigial, unpigmented eyes (Fig. 2A) (vs. well-developed, pigmented eyes in Chinapotamon; Ng 2017: fig. 6); the almost indiscernible epigastric cristae and postorbital cristae (Fig. 1) (vs. clearly discernible in Chinapotamon; Ng 2017: figs 2, 6; Zou et al. 2018: fig. 2); the proportionally wider male anterior thoracic sternum (width/length 2.3 vs. 1.6–1.7 in *Chinapotamon*; Ng 2017: figs 3A, 7A; Zou et al. 2018: fig. 3A); and the proportionally wider male pleon (compare Fig. 2C with Ng 2017: figs 3B, 7B; Zou et al. 2018: fig. 3B).

DNA analyses and discussion

A 512-basepair segment of the 16S rDNA gene, excluding the variable regions, was amplified and aligned from 25 potamid genera. The phylogenetic tree of the 16S sequences reconstructed using BI analysis is shown with support values from ML analysis (Fig. 5). The phylogenetic results place *Phasmon* gen. nov., albeit with weak support, in a basal position of the "China-East Asia Islands" Group. Although the sister group to *Phasmon* could not be robustly determined, *Phasmon* gen. nov. is clearly phylogenetic cally distant from the stygobitic *Diyutamon*, indicating that the two lineages independently colonized subterranean habitats. Treating *Phasmon* gen. nov. as a basal clade and applying the substitution rate of 0.88% for 16S rDNA for terrestrial *Sesarma* and other



Figure 5. Bayesian inference (BI) tree of 16S rDNA for the "China-East Asian Islands" Group of the subfamily Potamiscinae. Support values at nodes represent posterior probabilities and bootstrap proportions > 50% for BI and maximum likelihood (ML), respectively.

freshwater crabs (Schubart et al. 1998; Shih et al. 2006, 2009; Huang et al. 2017b), the divergence time between *Phasmon* gen. nov. and other genera in the "China-East Asia Islands" Group is estimated at 10.8 ± 1.0 mya (= million years ago) (with uncorrected p-distance of $9.49\% \pm 0.91\%$).

The divergence time of 10.8 mya estimated for *Phasmon* gen. nov. is much older than the 5.7 mya estimated for another Chinese cave crab genus, *Diyutamon* (Huang et al. 2017b). The karst caves of this region are estimated to have formed in the Pleistocene (2.58–0.0117 mya) based on animal and plant fossils (Mead et al. 2014; Yan et al. 2014; Li et al. 2020). If ancestral *P. typhlops* gen. nov. et sp. nov. entered subterranean karst caves at the beginning of the Late Miocene (11.63–5.333 mya), then previous age estimates of the regional karsts would be substantially too young. However, it seems also more likely that although the lineage to which *Phasmon* gen. nov. belongs diverged in the Late Miocene, the ancestors of *P. typhlops* gen. nov. et sp. nov. probably entered the subterranean environment after it was formed later in the Pleistocene. The present-day distribution of *Phasmon* gen. nov. is probably relictual, and given its isolated phylogenetic position in the "China-East Asia Islands" Group, *P. typhlops* possibly represents the last of an otherwise extinct lineage.

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RESEARCH ARTICLE



Caridina sinanensis, a new species of stygobiotic atyid shrimp (Decapoda, Caridea, Atyidae) from a karst cave in the Guizhou Province, southwestern China

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Abstract

From a biodiversity survey of a subterranean habitat near Sinan County, Guizhou Province, southwestern China, a new atyid shrimp of the genus *Caridina* H. Milne Edwards, 1837, *C. sinanensis* **sp. nov.** was discovered. The new species can be separated from other congeners based on a combination of characters including depigmentation in body and reduction of eyes, small pigment spot at the centre of the cornea, the shape of rostrum and the endopod of the 1st male pleopod, and the relatively longer appendix interna on the appendix masculina of the 2nd pleopod. Mitochondrial COI and 16S rRNA gene sequences also support the establishment of the new species. Information on the habitat, and the levels of threat are discussed to guide the conservation of *C. sinanensis* **sp. nov.**

Keywords

Caridina, COI and 16S rRNA, freshwater biodiversity, karst landform, southwestern China, spelaeology, taxonomy

^{*} These authors contributed equally to this paper.

Introduction

China's karst landforms, accounting for about a third of the territory, are one of the largest and most spectacular karst sceneries in world (Shui et al. 2015). In 2007, the China Southern Karst Region was established as a Natural World Heritage site by the UNESCO World Heritage Committee during the 31st World Heritage Conference held in Christchurch, New Zealand (Hou and Sun 2011). The extensive cave systems underlying the karst region harbor a vast variety of freshwater organisms, including atyid shrimp. Subterranean species are astonishing and bizarre outcomes of evolution over eons, either through regression or vicariance under natural selection; they have evolved to fully adapt to aquatic subterranean habitats, and they tend to exhibit conspicuous morphological adaptations and localized endemism (Culver et al. 2008). They are typically colorless, with reduced eyes, long antennae and ambulatory appendages (Mejía-Ortíz et al. 2006). More than 500,000 caves have been documented in China (Ran and Yang 2015), but only a small fraction of theses have been investigated. Knowledge on the distribution, abundance, life history, and ecology of atyid shrimp is inadequate, while the study of the cave-dwelling fauna remains in its initial stages. So far, 23 cave-dwelling atvid species are known in China from four genera of the family Atyidae: Caridina H. Milne Edwards, 1837, Mancicaris Liang, Guo & Tang, 1999, Neocaridina Kubo, 1938, and Typhlocaridina Liang & Yan, 1981. The majority of these species belong to the genus *Caridina*. Most cave atyids are highly specialized, phylogenetically unique, with restricted distributions and specialized habitat requirements. The epigean species, Neocaridina palmata, Macrobrachium nipponense and M. *superbum* are capable of surviving and reproducing in the caves.

During an inventory to evaluate the status of a variety of cave fauna along karst cave systems of Guizhou province, southwestern China in 2019–2020, specimens of atyid shrimp were collected from a cave river near Tangtou Town, Sinan County. After detailed examination of these specimens based on a combination of morphological and molecular features (COI and 16S rRNA), we are confident that our specimens have sufficient differences from known species to be recognized as a new species, *Caridina sinanensis* sp. nov. The present work provides detailed description, illustrations, molecular evidence, standardized diagnoses, colour photographs, and habitat information, as well as a distribution map. In addition, the conservation significance of this species is also briefly described. This raises the total number of subterranean atyid shrimp species known to date from China to 24 species.

Materials and methods

Cave description

The cave where the specimens were sampled is located in Pengjiaao, Tangtou Town, Sinan County, Guizhou Province, southwestern, China, at 27°44'10"N, 108°11'58"E



Figure 1. Map indicating rivers in Guizhou Province, China, with a triangle showing the sample site for *Caridina sinanensis* sp. nov.

(Fig. 1). The straight-line distance between the cave entrance and Provincial Road S203 is 6.4 m. The mouth of the cave is in a cliff along the north bank of the Shiqian River and at an elevation of 294.7 m. There is a dry artificial channel, 0.5 m in width and 0.8 m in height, in front of the cave entrance. Bare shale above the cave entrance is surrounded by scattered ferns, bryophytes, and vines. The arched opening of the cave is at the bottom of a crack in the substrate, 5.4 m in width and 10.4 m in height. The north oriented horizontal passage with a gravel and rocky floors extends to a hall about 80 m from the entrance. The passage lengths along the transect extending into the cave from the entrance were the light zone, weak light zone, twilight zone, and dark zone were 7.4, 6.7, 4.3, and 7.0 m, respectively. Two closely spaced conical stalactites are on the cave ceiling at 14 m from the cave's entrance, and three more at 21 m from the entrance. A widening river appears in the middle of the horizontal passage 8 m into the cave. The drop between the surface of river and the entrance of cave is about 1 m. According to the local residents, heavy rains causes flooding with river water flowing out from cave. At ordinary times, the water was quite transparent, with sediment containing fine particulate matter, seeds, and fragments of leaves. Under the light of the headlights, dozens of shrimps were observed swimming or clinging to the bottom. The water depth is about 0.3-1.5 m, but the far wall was undercut and sloped precipitously to unknown depths. Only 20 m of the river is accessible, as the top of the cave intersects the water surface. The geomorphological features of the cave are shown in Fig. 2.



Figure 2. Schematic diagram of the cave **A** plan view of the cave **B** left profile view of the cave **C** closeup view of cave entrance **D** the vegetation around the cave **E** light zone of cave **F** dim light zone of cave.

Habitat description

Geological coordinates and basic hydrological and physicochemical parameters of the cave (width, depth, temperature, humidity, pH, CO, CO₂, O₂, H₂S, and dissolved oxygen) were measured with the following instruments: Bosch GLM-30 Laser rangefinder,

		GenBank accession numbers			
Species	Sampling locality	COI	16S rRNA		
01.1.	China, from type locality	KP168788	KP168718		
C. previata	China, from type locality	KP168789	KP168719		
C. cantonensis	Qingyuan, China	KP168802	KP168720		
	Qingyuan, China	KP168803	KP168721		
C. huananensis	Yingde, Qingyuan	MN701607	MT446452		
	Yingde, Qingyuan	MN701608	MT446453		
C. lanceifrons	Dongfang, Hainan	MN701605	MT446450		
	Dongfang, Hainan	MN701606	MT446451		
C. mariae	Nankun Mountain, Huizhou	MN701601	MT446456		
	Nankun Mountain, Huizhou	MN701602	MT446457		
C. nanaoensis	China	KP168792	KP168755		
C. serrata	Dong'ao Island, Zhuhai	MN701595	MT446454		
	Dong'ao Island, Zhuhai	MN701596	MT446455		
	Sinan Guizhou	MT433962	MT434873		
C. sinanensis	Sinan Guizhou	MT433963	MT434874		
	Sinan Guizhou	MT433964	MT434875		
	Zhuhai China	KP168795	KP168765		
C. trijasciata	Zhuhai China	KP168796	KP168766		
C 1	Dong'ao Island, Zhuhai	MN701603	MT446448		
C. zhujiangensis	Dong'ao Island, Zhuhai	MN701604	MT446449		
	Yangshan, Qingyuan	MN701611	_		
M + due + -	Yangshan, Qingyuan	MN701611	_		
iv. paimata	China	_	KP168779		
	Hong Kong, China	-	KP168780		

Table 1. Species used in the molecular analysis, with details on sampling locations, GenBank accession numbers (COI, 16S rRNA).

eTrex Venture GPS locator, JWSA2-2 temperature and hygrometer, LB-MS4X portable gas detector, BDO820 portable dissolved oxygen meter, and BPH-220 pH meter. The environmental physicochemical parameters of the cave are shown in Table 2, while the vegetation around the cave entrance and habitat features of cave interior are displayed in Fig. 2.

Samples collection

We visited the cave three times, in January and March 2019 and February 2020. Each time some dozens of specimens were observed, and altogether 40 specimens were collected. Samples were collected with a sturdy, long-handled, fine-meshed dip net (mesh size 0.6 mm) and with the aid of headlamps. The sampling procedure was recorded with photographs and video-recordings. Specimens were placed in oxygenated polythene bags, anaesthetized with ice, and transported to the hotel, to be photographed, and fixed in 95% ethanol. Ethanol was changed after 24 h with fresh 75% ethanol.

Morphological analysis

Specimens were examined using a dissecting microscope (Olympus SZX7). Illustrations and morphometric measurement of selected characters were recorded using a

Environmental	Unit	it Light zone			Twilight zone			Dark zone		
parameters		2019.1.25	2019.3.18	2020.2.15	2019.1.25	2019.3.18	2020.2.15	2019.1.25	2019.3.18	2020.2.15
Temperature(air)	°C	9.2	15.5	9.8	9.8	17.7	14.2	14.8	18.4	15.1
Temperature(water)	°C	/	/	/	20.1	20.2	20.2	20.2	20.3	20.2
Humidity(air)	%	76	78	80	90	92	93	94	96	97
pH(water)		/	/	/	6.6	6.5	6.7	6.6	6.6	6.7
oxygen(air)	%	28	27	29	23	25	24	21	22	22
Hydrogen sulfide(air)	mg/kg	0.45	0.48	0.44	0.37	0.40	0.39	0.21	0.25	0.22
Carbon monoxide(air)	mg/kg	17.0	17.2	17.2	16.9	16.1	14.9	11.5	12.2	11.7
Carbon dioxide(air)	mg/kg	270	273	280	270	274	283	355	348	367
Dissolved oxygen(water)	mg/L	/	/	/	8.3	8.0	8.7	8.3	8.0	8.7

Table 2. Environmental physicochemical parameters of the cave.

digital camera (DP22) mounted on a stereomicroscope (Olympus SZX7) and Olympus CellSens Entry v. 1.18 software.

The following abbreviations are used throughout the text: alt (altitude), cl (carapace length, measured from the postorbital margin to the posterior margin of the carapace), rl (rostral length, measured from the rostral tip to the postorbital margin), and tl (total length, measured from the rostral tip to the posterior margin of the telson). All measurements are in millimeters.

Specimens were deposited in the Department of Animal Science, School of Life Science and Engineering, Foshan University (FU), Guangdong, China.

Molecular data collection and analysis

The abdominal muscle of the specimens were used for DNA extraction with an Easy-Pure Genomic DNA Kit (TransGen Biotech, Beijing, China) and then stored in a -20 °C freezer. To construct the molecular phylogeny of selected *Caridina* species, two mitochondrial gene fragments, 710 bp of the cytochrome oxidase subunit I (COI) and approximately 560 bp of the large ribosomal subunit (16S) were amplified and sequenced on an Applied Biosystems 3730 Analyzer (Applied Biosystems, Foster City, CA, USA) using COI primers LCO1490 and HCO2198 (Folmer et al. 1994), and 16S rRNA primers 16S-F-Car and 16S-R-Car1 (von Rintelen et al. 2007). All new sequences have been deposited in GenBank and the remaining sequences were downloaded from GenBank (Klotz and von Rintelen 2014) (Table 1).

As there are no *Caridina longshan* and *C. alu* sequences on the GenBank, and no *C. longshan* and *C. alu* specimens were collected, only 22 sequences in this paper were analyzed. It is mainly based on nine species of *Caridina* shrimp from China, so as to conduct molecular analysis with *C. sinanensis* sp. nov. All sequences were aligned with MAFFT v. 7.037 software using the auto strategy and normal alignment mode (Katoh and Standley 2013). Appropriate models of sequence evolution were selected using ModelFinder (Kalyaanamoorthy et al. 2017), and consequently the GTR+F+I+G4 (COI) and the HKY+F+G4 (16S rRNA) were employed. Phylogenetic tree was constructed by Bayesian inference approach using MrBayes v. 3.2.6 (Ronquist et al. 2012), with two parallel runs, 2000000 generations, in which the initial 25% of sampled data were discarded as burn-in. Genetic distances were calculated using the Kimura 2-parameter model in MEGA v. 7.0 based on COI and 16S rRNA, respectively (Kumar et al. 2016).

Results

Taxonomy

Systematic accounts Family Atyidae De Haan, 1849 Genus *Caridina* H. Milne Edwards, 1837

Caridina sinanensis sp. nov.

http://zoobank.org/AC2E06AB-1DFF-49D6-8D76-26DB850E7597 Figs 3–5

Material examined. *Holotype*: Adult male (FU, 2019-01-25-01), tl 16.7 mm, cl 4.8 mm, rl 1.5 mm; a cave river at Pengjiaao, Tangtou Town, Sinan County, Guizhou Province, southwestern, China (27°44'10"N, 108°11'58"E, alt. 294.7 m), 25 Jan. 2019. *Paratypes*: 1 male (FU, 2019-01-25-02) cl 5.4 mm; 1 male (FU, 2019-01-25-03) cl 6.8 mm; 1 male (FU, 2019-01-25-04) cl 4.8 mm; 2 males (FU, 2019-01-25-05), cl 4.2–6.2 mm; 20 females (9 ovigerous) (FU, 2019-01-25-05), cl 4.9–6.6 mm, sampled together with the holotype.

Comparative material examined. *Caridina semiblepsia* Guo, Choy & Gui, 1996. Adult male (FU, 1994-05-17-01), tl 17.5 mm, cl 4.5 mm, rl 0.7 mm; a cave river at Tongpatong, Baojing County, Hunan Province, China, 17 May 1994. Paratypes: 4 males (FU, 1994-05-17-02) cl 4.8–5.6 mm; 5 females (2 ovigerous) (FU, 1994-05-17-03), cl 4.7–6.3 mm, sampled together with the holotype.

Caridina ablepsia Guo & Jiang, 1992. Adult male (FU, 1989-05-23-01), tl 26.8 mm, cl 6.5 mm, rl 1.8 mm; a cave river at Xiaolongtong, Yunshun County, Hunan Province, China, 23 May 1989. Paratypes: 5 males (FU, 1989-05-23-02) cl 5.4–6.7 mm; 6 females (FU, 1989-05-23-03), cl 5.7–6.9 mm, sampled together with the holotype.

Diagnosis. Rostrum short, slightly sloping downwards, usually reaching to the end of the 2^{nd} segment, occasionally reaching to the end of the 1^{st} segment or the end of the 3^{rd} segment of antennular peduncle, rostral formula 4-10+10-16/3-11. 1^{st} pereiopod carpus $0.77-0.83 \times$ as long as chela, $1.6-1.7 \times$ as long as high; chela $1.9-2.2 \times$ as long as broad; fingers $1.2-1.3 \times$ as long as palm. 2^{nd} pereiopod carpus $1.2-1.3 \times$ as long as chela, $4.7-6.1 \times$ as long as high; chela $2.2-2.9 \times$ as long as broad; fingers $1.6-2.3 \times$ as long as palm. 3^{rd} pereiopod propodus $3.8-4.1 \times$ as long as dactylus, with 9-11 thin spines on



Figure 3. *Caridina sinanensis* sp. nov. **A**, **B** carapace and cephalic appendages, lateral view **C** eye **D** antennule **E** antenna **F** mandible **G** maxillula **H** maxilla **I** first maxilliped **J** second maxilliped **K** third maxilliped **A**, **C** holotype (FU, 2019-01-25-01) **D–K** paratype (FU, 2019-01-25-02) **B** paratype (FU, 2019-01-25-03).

the posterior and lateral margins. 5th pereiopod propodus 3.7–4.1 × as long as dactylus, with 11–13 thin spines on the posterior and lateral margins, dactylus terminating in one claw, with 38–44 spinules on flexor margin. Endopod of male 1st pleopod extending to 0.45–0.50× exopod length, distal half usually curved posteriorly in the natural, occasionally not bent backwards, wider proximally, subrectangular, 2.4–2.7× as long as wide, appendix interna well developed, arising from distal 1/3 of endopod, reaching beyond end of endopod. Appendix masculina of male 2nd pleopod rod-shaped, reaching to 0.51 length of endopod, appendix interna reaching to 0.93 length of appendix masculina. Uropodal diaeresis with 10–12 movable spinules. Eggs size (without eyespots) 0.67–0.82 × 1.29–1.38 mm, eggs size (containing embryos with eyes) 0.98–1.02 × 1.16–1.47 mm.

Description. *Body* (Fig. 5A–D): depigmented, slender and subcylindrical, medium-sized, males up to 22.7 mm tl, females up to 26.0 mm tl.

Rostrum (Fig. 3A, B): 0.25–0.47 of cl, reaching to the end of the 2^{nd} segment of antennular peduncle (75.8%, N = 33) in large specimens, or to the end of the 1^{st} segment (15.2%), or to the end of the 3^{rd} segment of antennular peduncle (9.0%), straight, slightly sloping downwards; armed dorsally with 14–26 teeth, including 4–10 on carapace, ventrally with 3–11 teeth; lateral carina dividing rostrum into two unequal parts, continuing posteriorly to orbital margin.

Eyes (Fig. 3A–C): small, partly reduced, with short stalk, cornea pigmentation variable, usually with pigment at centre of cornea, or totally absent (only one specimen).

Carapace (Fig. 3A, B): smooth, glabrous; antennal spine acute, fused with inferior orbital angle; pterygostomial angle subrectangular, slightly protrude forward; pterygostomian spine absent.

Antennule (Fig. 3D): peduncle short, reaching slightly short of scaphocerite; stylocerite short, reaching 0.75–0.88 length of basal segment; anterolateral angle reaching 0.20 length of the 2^{nd} segment; basal segment as long as combined length of the 2^{nd} and 3^{rd} segments, 2^{nd} segment as long as $0.53-0.61\times$ of basal segment, $1.29-1.32\times$ of the 3^{rd} segment; all segments with sub-marginal plumose setae.

Antenna (Fig. 3E): peduncle about $0.53 \times$ as long as scaphocerite; scaphocerite $3.0-3.1 \times$ as long as wide, outer margin straight, asetose, ending in a strong subapical spine, inner and anterior margins with long plumose setae.

Mandible (Fig. 3F): without palp, with well-developed incisor and molar processes; left and right mandible of similar size but differing in shape; left incisor process with single sharp tooth and a marginal transparent slice followed by patch of long setae, molar process strongly produced, ridged; right mandible incisor process with two long outer teeth and single short inner tooth, margin leading to molar process with 12 curving setae, followed by patch of long setae, molar process stout and with triturative surface.

Maxillula (Fig. 3G): lower lacinia broadly rounded, with several rows of plumose setae; upper lacinia elongate, medial edge straight, with 36–42 strong spinules and simple setae; palp simple, longer than wide, slightly expanded distally, with four long simple setae.

Maxilla (Fig. 3H): Scaphognathite well developed, tapering posteriorly, distally with regular row of long plumose setae and short marginal plumose setae continuing down proximal triangular process, furnished with numerous long plumose setae; upper and middle endites with marginal simple, denticulate and submarginal simple setae, distally with plumose setae; lower endite with long simple marginal setae; palp slightly shorter than the cleft of upper endite, wider proximally than distally, setose.

First maxilliped (Fig. 3I): Palp broad with terminal plumose setae; caridean lobe broad, with marginal plumose setae; exopodal flagellum well developed, with distally marginal plumose setae; ultimate and penultimate segments of endopod indistinctly divided; medial and distal margins of ultimate segment with marginal and sub-marginal rows of simple, denticulate, and plumose setae; penultimate segments with marginal long plumose setae.

Second maxilliped (Fig. 3J): endopodite ultimate and penultimate antennomeres fused, slightly concave, reflected against basal antennomeres, inner margin of ultimate, penultimate and basal segments with long setae of various types; exopod flagellum long, slender, with marginal plumose setae distally. Podobranchium is comb-like.

Third maxilliped (Fig. 3K): endopod three-segmented, reaching slightly beyond scaphocerite; penultimate segment $0.87-0.92 \times$ of basal segment; distal segment as long as penultimate segment, ending in a large claw-like spine surrounded by simple setae, preceded by about 6–9 spines on distal third of posterior margin, proximally with a clump of long and short simple and serrate setae; exopod flagellum well developed, about a third the length of penultimate segment of endopod, distal margin with long plumose setae.

First pereiopod (Fig. 4A): short, reaches end of eyes; chela length $1.9-2.2\times$ breadth, $1.2-1.3\times$ length of carpus; movable finger length $2.7-2.9\times$ breadth, $1.2-1.3\times$ length of palm, setal brushes well developed; carpus excavated disto-dorsally, length $1.6-1.7\times$ breadth, $0.90-0.93\times$ length of merus.

Second pereiopod (Fig. 4B): reaches about end of 3^{rd} antennular peduncle segment, more slender and longer than first pereiopod; chela length $2.2-2.9\times$ breadth, $0.79-0.85\times$ length of carpus; movable finger length $3.8-4.4\times$ breadth, and $1.6-2.3\times$ length of palm, setal brushes well developed; carpus length $4.7-6.1\times$ breadth, slightly excavated distally, $1.0-1.1\times$ length of merus.

Third pereiopod (Fig. 4C, D): reaches beyond end of scaphocerite; dactylus length 4.0–4.2× breadth, ending in prominent claw-like spine surrounded by simple setae, behind which bears 7–9 spines; propodus length 3.8–4.1× of dactylus, bearing 9–11 spinules on posterior margin, 11.2–12.2× breadth; carpus length 0.60–0.78× of propodus; merus length 1.9–2.1× of carpus, with about three large spines on the posterior margin.

Fourth pereiopod (Fig. 4E): reaches end of 3^{rd} segment of antennular peduncle; dactylus length 4.0–4.2× breadth, ending in prominent claw-like spine surrounded by simple setae, behind which bears 7–8 spines; propodus length $3.9-4.3\times$ of dactylus, bearing 11–16 spinules on posterior margin, $13.5-14.2\times$ breadth; carpus length $0.53-0.62\times$ of propodus; merus length $1.5-1.7\times$ of carpus, with about three strong spines on the posterior margin.



Figure 4. *Caridina sinanensis* sp. nov. **A** first pereiopod **B** second pereiopod **C** third pereiopod **D** dactylus of third pereiopod **E** fourth pereiopod **F** fifth pereiopod **G** dactylus of fifth pereiopod **H–J** first pleopod **K** second pleopod **L** telson **M** diaeresis of uropodal exopod **N** spermatophore **A–G**, **I–N** paratype (FU, 2019-01-25-02) **H** paratype (FU, 2019-01-25-04).

Fifth pereiopod (Fig. 4F, G): reaches the end of the 3^{rd} segment of antennular peduncle; dactylus length 4.9–5.4× breadth, ending in prominent claw-like spine surrounded by simple setae, behind which bears a comb-like row of 38–44 spines; propodus length $3.7-4.1\times$ of dactylus, bearing 11-13 spinules on posterior margin, $16.6-17.6\times$ breadth; carpus length $0.50-0.61\times$ of propodus; merus length $1.4-1.5\times$ of carpus, with about three strong spines on the posterior margin.

First four pereiopods with epipod. Branchial formula typical for genus.

First pleopod (Fig. 4H–J): endopod of male subrectangular, distal half usually curved posteriorly in the natural, occasionally not bent backwards, wider proximally, length 0.45–0.50× exopod length, 2.4–2.7× proximal breadth, ending broadly rounded; inner margin slightly concave, bearing long spine-like setae, outer margin slightly convex or straightly, proximally 1/3 naked and distally 2/3 bearing nearly equal length spine-like setae; appendix interna well developed, arising from distal 1/3 of endopod, reaching to or beyond end of endopod, distally with cincinulli.

Second pleopod (Fig. 4K): appendix masculina rod-shaped, reaching about 0.51× length of exopod, with numerous long spiniform setae proximally and distally, appendix interna well developed, almost same size as appendix masculina, reaching about 0.93× length of appendix masculina, distally with cincinulli.

Telson (Fig. 4L): $0.34-0.47 \times$ of cl, shorter than the 6th abdominal segment, $0.90-0.96 \times$ length of sixth abdominal segment, tapering posterior, with a median projection, dorsal surface with six pairs of stout movable spinules including the pair at poster lateral angles; posterior margin with four pairs of intermedial strong spiniform setae, sublateral pair shorter than lateral and inner pairs. Exopodite of the urpood bears a series of 10-12 movable spinules along the diaeresis, last one shorter than the lateral process.

Female carrying a number of 20–32 eggs, sized eggs $0.67-0.82 \times 1.29-1.38$ mm (without eyespots), and $0.98-1.02 \times 1.16-1.47$ mm (with eyespots).

Colouration (Fig. 5A–D): body and appendages translucent white; eyes with black spot at centre of cornea; internal organs (gonads and hepatopancreas) whitish or yellowish; eggs in females yellowish or blackish.

Etymology. *Caridina sinanensis* is named after Sinan County, where the type locality is located.

Remarks. Six *Caridina* species lacking body pigmentation and having a small black spot on each eye are known from Chinese subterranean aquatic habitats: *C. acuta, C. alu, C. demenica, C. longshan, C. semiblesia*, and *C. sinanensis* These taxa can be readily separated into two groups by the rostrum shape and indentation. In the first group including *C. acuta, C. demenica*, and *C. semiblesia*, the rostrums are similarly lanceolate and short, with fewer teeth or unarmed. In the second group including *C. alu, C. sinanensis*, the rostrums are long, reaching at least to the end of the 2nd antennular segment, mostly beyond the end of scaphocerite, and armed with dorsal and ventral teeth. *Caridina. sinanensis* is morphologically close to *C. longshan* in sharing a similar spination pattern, the anterior region of endopod on the 1st male pleopod folded backwards, and the variably pigmented cornea. *Caridina. sinanensis*



Figure 5. The cave dwelling organisms, colour in life **A–D** *Caridina sinanensis* sp. nov. **E** a blind millipede **F** a camel cricket.

can be distinguished from C. longshan by the relatively longer appendix interna on the appendix masculina of the 2nd pleopod (reaching about 0.93 of appendix masculina vs 0.80 in C. longshan), the length of 6th abdominal segment distinctly longer than the telson (vs same length of telson in C. longshan), and telson with posteromedian projection and lack of spinules on the surface of posterior telsonic spines (caudal spines) (vs lacking posteromedian projection and possessing spinules in C. longshan). Caridina. sinanensis can be easily separated from C. alu by its short rostrum (reaching to the end of the 2nd antennular peduncle vs reaching to the end of scaphocerite in *C. alu*), the carpus of 1st and 2nd pereiopods are slender (length to breadth ratio 1.6-1.7 and 4.7-6.1 versus 1.3 and 2.6 in C. alu), the telson with posteromedian projection (vs lacking in C. alu), and male with completely different shape of the endopod of 1st pleopods and appendix masculina of the 2nd pleopods. Caridina. sinanensis sp. nov. also shows close similarity with *C. semiblesia* in the ratios of various segments of the 1st and 2nd pereiopods, and the shape of endopod of the 1st pleopod in males. In addition to a longer rostrum that slopes downwards, C. sinanensis also differs from C. semiblesia in having the end of the palp of the 1st maxilliped being broadly rounded and without a fingerlike tip (vs ending in a finger-like tip in C. semiblesia), the smaller eggs $(0.98-1.02 \times 10^{-1})$ $1.16-1.47 \text{ mm vs} 1.05-1.15 \times 1.37-1.71 \text{ mm}$), and the stout and long appendix interna of the appendix masculina on the 2nd pleopod (appendix interna almost same size as appendix masculina and reaching about 0.93 of appendix masculina vs distinctly slender and reaching about 0.80 in C. semiblesia).



Figure 6. Bayesian inference (BI) tree of species of *Caridina* and outgroups (*Neocaridina*) based on COI gene. Support values at the nodes represent posterior probability.

Molecular phylogenetic results. Including the GenBank sequences, we analysed 22 COI sequences and 22 16S rRNA sequences in total. The new sequencing results are corrected for 621-bp (COI) and 487-bp (16S) for subsequent analysis. Three specimens of *Caridina sinanensis* were used in the molecular phylogenetic analysis shown in Figures 6, 7. Specimens assigned to *C. sinanensis* formed a clade distinct from other species. And the tree topologies derived from COI and 16S rRNA analyses were basically congruent. *C. sinanensis* sp. nov. is well isolated from other nine *Caridina* with a sequence divergence of 15.3–26.7% (COI) and 7.2–11.2% (16S), respectively. According to Hebert et al. 2003, the genetic distances support the molecular-based description of *C. sinanensis* as a new species.

Ecological notes. *Caridina sinanensis*, sp. nov. lives in an aphotic subterranean waterbody where the source of energy may come from allochthonous materials carried or washed into the cave, as there are particulates of vegetable debris in the water. Based on our observation on the shrimp's feeding behavior and intestinal contents, this species feeds on detritus and microorganisms from the bottom sediments with its brush-tipped chelae and mouthparts, and the full intestine suggests that the foods are relatively abundant (Fig. 5C, D).



Figure 7. Bayesian inference (BI) tree of species of *Caridina* and outgroups (*Neocaridina*) based on 16S rRNA. Support values at the nodes represent posterior probability.

Leeches co-occurred with atyid shrimp in the subterranean waters, camel crickets were common on the cave rocks, especially in the dark zone, and a blind unpigmented species of millipede was found crawling along the rocks in the dark zone (Fig. 5E,F). Some trogloxene animals, such as bats and birds, were occasionally encountered in the cave entrance area.

In general, the populations of other cave-dwelling species were very small, while shrimp were moderately abundant. Competition for food and habitat seems insignificant, possibly because the groundwater was enriched with particulate organic matter and predators (such as leeches) were not abundant. Leeches are the natural enemies of the shrimp; they attach to the carapace, branchial chambers and appendages where they feed on the hemolymph of the shrimps. Parasitism certainly confers negative impact on populations of the new species, but accurate population data on the shrimp are lacking.

The sex ratio and reproduction season were preliminary inferred based on three sampling times. On 25 January 2019, 18 individuals were collected, including two adult males, 10 adult females (six ovigerous), and six subadult females. The sex ratio (male to female) is 1:8, and the percentage of ovigerous females is 60%. On 18 March

2019, 18 individuals were caught, including five adult males, 10 adult females (three ovigerous), and three subadult females. The sex ratio is 1:2.6, and the percentage of ovigerous females is 30%. In 18 February 2020, four individuals were caught including one adult male and three adult females (one ovigerous). The sex ratio is 1:3, and the percentage of ovigerous females is 33%. These results showed that the number of males was significantly less than females in the population from January to March. The causes responsible for the skewed sex ratio in favor of females may worth further study.

The ovigerous females comprised 60%, 33%, and 30% of mature females, respectively, in populations from January to March. One male carrying a spermatophore on the intermediate of the fifth walking legs was observed from specimens collected in January (Fig. 4N). This cave dwelling species has the highest number of reproductive individuals recorded during the winter and spring months, suggesting that the peak reproductive period occurs from January to March.

Females carried 20–32 eggs, size of undeveloped eggs (without eyespots) were $0.67-0.82 \times 1.29-1.38$ mm, size of developed eggs (containing embryos with eyes) $0.98-1.02 \times 1.16-1.47$ mm. The females of this species carry a small number of large eggs and produce eggs with a large amount of yolk and reduced number of larval stages. It is believed that abbreviated larval development may occurs in this species, larval direct development into benthic hatchlings that resemble miniature adults.

We are trying to understand embryonic development and hatching of this species. On 20 March 2019, five ovigerous females were transported to the laboratory for rearing, but unfortunately, after 7 days, the shrimps died.

Conservation. Cave ecosystems are an invaluable resource, providing an ideal refuge for cave-dwelling species. Cave shrimp communities are particularly vulnerable to human disturbance, particularly groundwater pollution due to the local agricultural activities (fertilization, herbicide, and pesticide) and overexploitation (domestic usage and agricultural irrigation). These appear to be responsible for the pollution and degradation of subterranean habitat, but the extent of the impact is a little known. If groundwater become contaminated, local aquatic organisms certainly are at risk. Maintaining healthy groundwater shrimp communities requires the reduction of anthropogenic impacts, such as minimizing the use of agricultural pesticides, herbicides, and fertilizers by local farmers. It is suggested that the local government should ration the use of groundwater resources.

The Announcement of the Ministry of Agriculture and Rural Areas of China (CITES Appendix aquatic wild species of China, no. 69, 2018), fails to list freshwater shrimps in the CITES threatened categories. Since *Caridina sinanensis* is a new species, no conservation status has been assigned. According to the criteria listed in the IUCN Red List categories (IUCN 2019), *C. sinanensis* should be considered as a Critically Endangered species due to its exceptional rarity, restricted distribution in a single cave system, and the imminent threats from pollution. In order to better protect cave ecosystems, and their associated rare and threatened evolutionary relict fauna, it is critical and of great urgency to collect more baseline data on population and distribution patterns, delineate the importance and threatened status of cave fauna, and to devise corresponding conservation and management measures. Regular monitoring may be necessary to ensure populations are sustained in the face of further anthropogenic disturbances. Furthermore, cave biodiversity protection laws should be enacted as soon as possible.

Discussion

Caridina sinanensis sp. nov. is not blind, as the eyes still contain considerable pigmentation, although we found one male specimen lacking pigments in its cornea (Fig. 4B). The body is almost transparent but still shows a yellowish colour. Such presumably derived structures of the eyes and body coloration might represent an adaptation to aphotic cave environments. Therefore, the species has successfully adapted to the subterranean environment and can be considered a stygobiotic atyid.

The freshwater atyid species associated with caves are relatively numerous in China. The number of subterranean atyids recorded now totals 24, but only 13 species can be regarded as real troglobionts. Of these, five have reached the advanced stage in which the faceted cornea of the eyes is absent, namely: *Caridina alba, C. ablepsia, C. caverna, Mancicaris sinensis,* and *Typhlocaridina lanceifrons.* Eight species with reduced eyes but presence of pigmented cornea are: *C. acuta, C. alu, C. demenica, C. longshan, C. semiblesia, T. liui, T. semityhplata,* and *C. sinanensis* sp. nov. In order to contribute to the management of the vulnerable subterranean ecosystems and their highly specialized endemic stygofauna, there is an urgent need to accelerate scientific research, including but not limited to collecting the much-needed information on taxonomy, life history, ecology, and distribution, and to design monitoring programs of subterranean species, especially for those that are under the most intense threats from anthropogenic factors.

As molecular analysis has become a crucial step towards resolving taxonomic problems, delimitations, and the real biodiversity of atyids, a combined morphological and molecular data is necessary for the description of a new taxa. Ideally, this process will involve to comprehensive information on color, physiology, and ecology. In Chinese karst landforms, as many caves are explored and the molecular identification applied, the species list will increase with future surveys.

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Deep-sea clawed lobster Nephropsis stewarti Wood-Mason, 1872 species complex in the Indo-West Pacific (Crustacea, Decapoda, Nephropidae), with description of a new species

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Abstract

Nephropsis stewarti Wood-Mason, 1872 is the most common species of the deep-sea clawed lobster genus *Nephropsis* Wood-Mason, 1872 in the Indo-West Pacific. Morphological comparisons and genetic analyses of extensive material referred to this lobster revealed the presence of three species. The three species differ mainly in body size, development of the intermediate carina on the carapace, position of the lateral pair of rostral teeth, whether the pleonal tergum is granulate, and the spination on the large chelipeds. *Nephropsis stewarti* is restricted to the western central Indian Ocean, and a neotype is selected to fix its identity. The name *Nephropsis grandis* Zarenkov, 2006 is revived with neotype selection for the large form found in the West Pacific and northwestern Australia. The smaller form from southern Taiwan and the Philippines is described as *Nephropsis pygmaea* **sp. nov.**

Keywords

Deep-sea, DNA barcoding, lobster, taxonomy

Introduction

Among the 16 species in the deep-sea clawed lobster genus *Nephropsis* Wood-Mason, 1872 (Nephropidae) (Chan 2010, 2019; Chang and Chan 2020), the type species N. stewarti Wood-Mason, 1872 is probably the most well-known in the Indo-West Pacific because of its frequent records showing a wide geographical distribution, large size, and presence at shallower depths compared with other congeners (see for example Miyake 1982; Holthuis 1984, 1991; Baba 1986; Chan and Yu 1988, 1993; Macpherson 1990, 1993; Wadley and Evans 1991; Chan 1997, 1998; Jones and Morgan 2002; Zarenkov 2006; Dineshbabu 2008; Radhakrishnan et al. 2019; Chang and Chan 2019). Nephropsis stewarti is a unique species within the genus as it has only one pair of rostral teeth, no distinct spines on the subdorsal carina, a pleon lacking dorsal carina, a telson without erected dorsal spine, and uropodal exopods bearing well-developed diaeresis. Slight differences can be observed among the materials of *N. stewarti* from various localities (see Macpherson 1993), and Zarenkov (2006) considered a large specimen from the Arafura Sea, north of Australia, as a different species, described as N. grandis Zarenkov, 2006. Zarenkov's (2006) specimen (carapace length, including rostrum, 58 mm) is smaller than N. stewarti in many other localities (e.g., Macpherson 1990, 1993; Chan 1997; Zarenkov 2006), and the main diagnostic characteristic of *N. grandis* is the spination of the large chelipeds (see Chang and Chan 2019), which is missing in the holotype of N. stewarti (Wood-Mason 1873, 1874); therefore, Chan (2010, 2019) tentatively treated *N. grandis* as a junior subjective synonym of *N. stewarti*.

Many reports have illustrated the coloration of specimens identified with *Nephropsis stewarti* (e.g., Miyake 1982; Baba 1986; Chan and Yu 1988, 1993; Wadley and Evans 1991; Jones and Morgan 2002; Chang and Chan 2019). The body of *N. stewarti* was believed to primarily have a whitish color. However, during a recent survey on the decapod crustacean fauna in India carried out by the second author, about 10 *N. stewarti* specimens were observed in a local fishing port, and all were reddish in color. As *N. stewarti* was originally described in the Andaman Sea near India, it was suggested that the currently recognized *N. stewarti* might contain more than one species. This work compared extensive material of *N. stewarti* from various Indo-West Pacific localities, aided by molecular barcoding genetic analysis (Bucklin et al. 2011), and revealed the presence of three species. *Nephropsis stewarti* is restricted to the western central Indian Ocean; a neotype is selected to fix its identity. The name *Nephropsis grandis* can be applied to much of the material from the western Pacific to northwestern Australia; its identity was also fixed by the erection of a neotype. The third, undescribed species, which is smaller in the size compared with the two closely allied species, is present in southern Taiwan and the Philippines; it requires a new name.

Materials and methods

Samples

The present study was based mainly on the extensive collection of the *N. stewarti* species complex deposited at National Taiwan Ocean University, Keelung, Taiwan

(NTOU), supplemented with material from the Muséum national d'Histoire naturelle, Paris, France (MNHN); the Department of Aquatic Biology and Fisheries, University of Kerala, India (DABFUK); Natural History Museum and Institute, Chiba, Japan (CBM); and Senckenberg Museum, Frankfurt am Main, Germany (SMF). These materials included topotypic specimens of *N. stewarti* and *N. grandis*. Carapace length (cl) was measured dorsally from the orbital margin to the posterior margin of the carapace. The abbreviation (CP) preceding the station number indicates the type of the collecting gear (French beam trawl). Morphological terminology generally follows Macpherson (1990) and Holthuis (1991). *Nephropsis stewarti* s.l. is well known in the Indo-West Pacific, having many taxonomic accounts or reports, often with only brief descriptions and without mentioning any of the diagnostic characteristics of the three species identified in this study. Therefore, the synonymy provided is restricted to important taxonomic works related to this species complex.

Molecular analysis

Although the barcoding gene, cytochrome c oxidase I (*COI*), has better resolution for species delimitation (Bucklin et al. 2011), the universal primers (Folmer et al. 1994) failed to amplify the COI gene in many western Pacific material of N. stewarti (in GenBank there are only two COI sequences of N. stewarti s.l.: MH428010, 176 bp; LC309102, 713 bp). Therefore, another barcoding gene, 16S rRNA (16S), was used for the present analysis. Twelve of the present specimens from various Indo-West Pacific localities had their 16S newly sequenced. Genomic DNA was extracted from the pleonal somite VI or the pleopod V muscle tissue using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN). A partial sequence of the 16S rRNA gene was amplified by the primers 16SF (Tsang et al. 2014) and 16SR (Tsang et al. 2009). The PCR amplifications were performed in 25-µL reaction mixtures containing 50-250 ng of the DNA extract, 2.5 µL of 10× polymerase buffer, 3 mM of MgCl₂, 200 nM of each primer, 200 µM of dNTPs (PROTECH, Taipei, Taiwan), and 1U of ProTaq DNA polymerase (5U/µL, PROTECH). The PCR cycling conditions were as follows: 5 min at 94 °C for initial denaturation; followed by 30 cycles of 30 sec at 95 °C, 40 sec at 47 °C, and 40 sec at 72 °C; and a final extension step for 5 min at 72 °C. The quality of the PCR products was determined by running 5 µL of the reaction on a 1% agarose gel and then sending the sample to a commercial company for further purification (Geneaid) and sequencing (ABI 3730 XL automated sequencer). To examine the accuracy of each sequence, the complementary consensus sequences were aligned by Clustal W, implemented in Bioedit (Hall 1999). The obtained 16S sequences (399-425 bp) were then assembled and aligned by MUSCLE implemented in MEGA v.7 (Kumar et al. 2016), along with the 16S sequences of *Nephropsis* of more than 399 bp, and identified down to the species level in GenBank (Table 1). This revealed that the GenBank sequence EU882882 from a Taiwanese specimen (NTOU M00505) reported by Tshudy et al. (2009) was identical to another GenBank sequence, U96086, of a specimen from Natal, South Africa (Tam and Kornfield 1998). Re-amplification of the 16S gene of the

Species	Locality	Voucher no.	GenBank no.
N. stewarti	Andaman Sea	NTOU M02249*	MW301998
	India	DABFUK/AR-ACH-10	MW301999
	Mozambique	MNHN IU-2018-5063	MW302000
	Natal, S. Africa	Unspecified	U96086
N. grandis	Indonesia	MNHN IU-2017-9001*	MW302001
	South China Sea	NTOU M02163	MW302002
	the Philippines	NTOU M02251	MW302003
	N. Taiwan	NTOU M00505#	MW302004
	S. Taiwan	NTOU M02174	MW302005
N. pygmaea sp. nov.	S. Taiwan	NTOU M01898*	MW302006
	S. Taiwan	ZRC2002.0471	AY583891
	the Philippines	NTOU M02263	MW302007
	the Philippines	NTOU M02254	MW302008
N. serrata	South China Sea	NTOU M02162	MW302009
	Taiwan	NTOU M00157	EU882881
N. aculeata	Massachusetts, USA	Unspecified	U96085
	Unspecified	KC2117	DQ079727
	Mexico	CNCR-21650	EU882884
	Mexico	CNCR-21660	EU882885
N. rosea	Mexico	CNCR-21631	EU882886

Table 1. *Nephropsis* Wood-Mason, 1872 material for 16 rRNA sequence analysis. # refers to the same specimen as the sequence EU882882 in GenBank, but with 7.3% divergence. * indicates holotype or neotype.

same Taiwanese specimen (NTOU M00505) confirmed that the sequence EU882882 is incorrect (with 7.3% difference). Uncorrected pairwise divergences (*p* distance) among the specimens of *Nephropsis* were performed using MEGA v.7.

Taxonomy

Family Nephropidae Dana, 1852 Genus *Nephropsis* Wood-Mason, 1872

Nephropsis stewarti Wood-Mason, 1872

Figures 1, 4A–F

- Nephropsis Stewarti Wood-Mason 1872: 151 (type locality: Andaman Sea); 1873: 60; 1874: 40, pl. 4-1–3, 5, 7; 1885: 71; Alcock 1894: 230; 1901: 159; Anderson 1897: 96; Alcock and Anderson 1899: 286.
- Nephropsis stewartii.-Alcock and Anderson 1894: 161; 1896: pl. 27-figs 1, 1a; Lloyd 1907: 3; Ramadan 1938: 124, text-fig. 1; Thomas 1979: 43.
- Not Nephropsis Stewarti.-De Man 1916: 112, pl. 3-fig. 17. [= Nephropsis serrata Macpherson, 1993].
- Nephropsis stewarti.–Calman 1925: 21; Barnard 1950: 531; Holthuis 1991: 45 (in part), fig. 80; Macpherson 1990: 312 (in part), figs 5e, 10, 11c, d, 16e; Zarenkov 2006: 93 (in part); Radhakrishnan et al. 2019: 112, fig. 3.22.

? Nephropsis Stewarti.-Balss 1925: 208.

- Not *Nephropsis stewarti.*–Kubo 1965: 629, unnumbered fig.; Miyake 1982: 77, pl. 26-1; Baba 1986: 153, fig. 103; Chan and Yu 1988: 8, pl. 1A; 1993: 83, unnumbered photo.; Holthuis, 1991: 45 (in part); Wadley and Evans, 1991: 39, unnumbered photo; Macpherson 1993: 63; Chan 1997: 415; Jones and Morgan 2002: 83, unnumbered photo; Davie 2002: 391; Zarenkov 2006: 93 (in part), fig. 19; Chang and Chan 2019: 50 (in part), fig. 7. [= *Nephropsis grandis* Zarenkov, 2006].
- Not *Nephropsis stewarti.*—Macpherson 1990: 312 (in part). [? = *Nephropsis grandis* Zarenkov, 2006 and/or *Nephropsis pygmaea* sp. nov.].
- Not *Nephropsis stewarti.*-Chang and Chan 2019: 50 (in part). [= *Nephropsis pygmaea* sp. nov.].
- Not *Nephropsis stewarti.*–Chang and Chan 2019: 50 (in part), figs 2C, D. [= *Nephropsis serrata* Macpherson, 1993].

Material examined. *Neotype*: ANDAMAN SEA • male cl 46.2 mm; RV "Dr. Fridtjof Nansen" stn 135, 12°21.96'N, 96°37.32'E, 514 m, 23 May 2015 (NTOU M02249).

Other material. ANDAMAN SEA • 1 male cl 42.3 mm; RV "Dr. Fridtjof Nansen" stn 68, 14°03.72'N, 94°19.08'E, 457 m, 10 May 2015 (NTOU M02250) • 1 female cl 42.6 mm; commercial trawler, 09°34'65"N, 92°43'21"E, 320 m, 13 Nov. 2017 (DAB-FUK/AR-ACH-7). ANDAMAN ISLANDS • 1 male cl 38.4 mm, 1 female cl 33.7 mm; A185, commercial trawler, 13 Nov. 2017 (DABFUK/AR-ACH-8). INDIA • 1 male cl 47.0 mm; Sakthikulangara fishing harbor, Kollam district, Kerala, commercial trawler, Nov. 2013 (DABFUK/AR-ACH-9) • 1 male cl 50.8 mm, 2 ovigerous females 42.4 and 48.7 mm; 4 Mar. 2019 (DABFUK/AR-ACH-10) • 2 ovigerous females cl 46.8 and 49.2 mm (DABFUK/AR-ACH-11). MOZAMBIQUE • 1 male cl 52.7 mm; MAINBAZA stn CP3138, 25°12.13'S, 35°21.07'E, 700–707 m, 10 Apr. 2009 (MNHN IU-2018-5063).

Diagnosis. Rostrum bearing one pair of dorsolateral teeth usually situated near mid-length of rostrum. Carapace with subdorsal carinae granulate, without distinct spine or tooth-like process; supraorbital and antennal spines present, lacking post-supraorbital spine; post-cervical groove U-shaped in dorsal view; intermediate and lateral carinae well marked. Large cheliped (pereiopod I) with inner surface of palm lacking distinct spines; carpus with strong distoventral, ventro-outer distal (rarely absent), and dorso-inner distal spines, inner surface with dorsal margin generally bearing 2–4 spines, outer surface without distinct spines; merus bearing subdistal dorsal, subdistal outer and distoventral spines. Pleon finely granulate, without median carina; pleura lacking spine on anterior margins. Telson without erected dorsal median spine near base. Uropodal exopods with complete diaeresis.

Description. Body covered with long or short pubescence, rather thick on anterior two pereiopods, dorsal carapace, and pleonal tergum.

Carapace finely granulated (Fig. 1A, B); rostrum 0.4–0.5× as long as carapace, with 1 pair of teeth usually situated near mid-length of rostrum; subdorsal carinae granulate, without distinct spine or tooth-like process; supraorbital and antennal spines well developed, post-supraorbital spine absent; cervical, postcervical, and hepatic grooves



Figure 1. *Nephropsis stewarti* Wood-Mason, 1872, Andaman Sea, RV 'Dr. Fridtjof Nansen' stn 135, neotype male cl 46.2 mm (NTOU M02249) **A** carapace and pleon, dorsal **B** same, lateral **C** left pereiopod I, chela to distal part of merus, dorsal **D** same, lateral **E** same, ventral. Pubescence and setae omitted • Position of spines on inner surface of palm \diamond position of spines on dorsal margin of outer surface of carpus • position of spines on dorsal margin of inner surface of carpus **II** dorso-inner distal spine of carpus \triangle ventro-outer distal spine of carpus II distoventral spine \bigstar subdistal dorsal spine of merus \bigstar position of subdistal outer spine of merus.

present, with post-cervical groove U-shaped in dorsal view; intermediate and lateral carinae both well marked; gastric tubercle located near supraorbital spine, their distance being approximately $0.4\times$ distance between gastric tubercle and post-cervical groove; distance between orbital margin and post-cervical groove $1.2-1.5\times$ longer than the distance between post-cervical groove and posterior margin of carapace.

Large cheliped (pereiopod I), generally granulate (Fig. 1C–E) but less granular in females; inner surface of palm granular but without distinct spines; chela $3.0-4.0 \times$ as long as wide, males generally having relatively longer chela (> $3.5 \times$), whereas females

having shorter chela (< 3.5×); carpus with strong distoventral spine, ventro-outer distal spine (rarely absent) and dorso-inner distal spine, inner surface with dorsal margin bearing 2–4 (rarely 1) spines, outer surface without distinct spines; merus bearing subdistal dorsal spine, subdistal outer spine and distoventral spine.

Pereiopod II with carpus $0.5-0.7 \times$ palm length. Pereiopod III with carpus $0.4-0.5 \times$ as long as palm; merus $1.4-2.1 \times$ as long as carpus. Pereiopods IV and V with dactyli $0.4-0.6 \times$ as long as propodi.

Entire pleon finely granulate (Fig. 1A, B) without median carina; pleura lacking spine on anterior margins, each terminating ventrally into blunt to sharp spine. Telson without erected dorsal median spine near base. Uropodal exopods with complete diaeresis.

Eggs spherical and 2.2–2.7 mm in diameter.

Distribution. Known with certainty in the western to northeastern Indian Ocean from the eastern coast of South Africa to the Andaman Sea. Found at depths of 250–1520 m and perhaps even 1720 m, but mostly less than 1000 m (see Alcock 1901; Macpherson 1990; Zarenkov 2006).

Color in life. Body varies from whitish to reddish (Fig. 4; Radhakrishnan et al. 2019: fig. 3.22). Eyes and antennal peduncle always whitish. Distal parts of pereiopods II–V, pleopods always reddish. Large cheliped and pleonal tergum, whitish to orange. Pleonal pleura and uropods purple to reddish. Antennal and antennular flagella orange to reddish. Pubescence grayish to reddish. Eggs orange.

Remarks. Although the present Indian specimens have a very reddish color, a comparison with Andaman Sea topotypic specimens and material from Mozambique Channel revealed wide color variations in *N. stewarti*, from whitish to reddish (Fig. 4). Specimens with different colors in the western and northern Indian Ocean are genetically very similar, with 1.0% or less sequence divergence in 16S (Table 2). However, large genetic divergences (16S sequence divergence 3.8–7.3%) exist between the material from the western and northern Indian Ocean, and that from the western Pacific (including the South China Sea and Arafura Sea) and northwestern Australia (Table 2). Such genetic differences are greater than those between *N. rosea* Bate, 1888 and *N. aculeata* Smith, 1881 (3.1–3.7%) and between *N. serrata* Macpherson, 1993 and *N. stewarti* s.l. (lowest 2.8%).

Nephropsis grandis, previously considered to be a synonym of *N. stewarti*, has a type locality in the Arafura Sea (Zarenkov 2006). Of the three characteristics proposed by Zarenkov (2006: table 1) to separate *N. grandis* from *N. stewarti*, the shape of the distal part of the rostrum has been shown to be variable. The subdistal outer spine on the merus of the large cheliped is present in all of the western and northern Indian Ocean specimens (Fig. 1C, D) but can be present or absent in the western Pacific and northwestern Australia material (Figs 2C, D, 3C, D). The degree of development of the lateral carina on the carapace is similar in all the Indo-West Pacific material (Figs 1B, 2B, 3B). However, the intermediate carina on the carapace is well developed in all of the western and northern Indian Ocean material (Figs 1A, B) but is indistinct in the western Pacific and northwestern Australia specimens (Figs 2A, B, 3A, B). Moreover, a pair of lateral rostral teeth is usually situated around the mid-length of the rostrum in the

: the Nephropsis stewarti Wood-Mason, 1872 species complex and Nephropsis). Number in parentheses refers to number of individuals. Numbers in shade	
cted divergences (<i>p</i> -distance) of the 16S gene $(399-521 \text{ bp})$ amongst the N	e in GenBank (excluding those not identified to species and < 399 bp). Nun	ic divergences. * indicates holotype or neotype.
Table 2. Uncorre	sequences availabl	refer to intraspeci.

	16S		N. ster	varti			1	V. grandis			N. P.	ygmaea s	p. nov.	N. se	rrata		N. aculeata	
		Andaman	India	Mozam-	Natal	Indonesia *	South	Philippines	ż	s.	s.	s.	Philippines	South	Taiwan	Massa-	unspecified	Mexico
		Sea*		bique			China		Taiwan	Taiwan	Taiwan*	Taiwan	(2)	China		chusetts		(2)
							Sea							Sea				
N. stewarti	Andaman Sea*																	
	India	0.005																
	Mozambique	0.002	0.007															
	Natal, S. Africa	0.005	0.010	0.002														
N. grandis	Indonesia*	0.064	0.064	0.064	0.068													
	South China Sea	0.064	0.064	0.064	0.068	0.009												
	Philippines	0.064	0.064	0.064	0.068	0.009	0.005											
	N. Taiwan	0.069	0.068	0.068	0.073	0.016	0.019	0.019										
	S. Taiwan	0.069	0.068	0.068	0.073	0.016	0.019	0.019	0.000									
N. pygmaea	S. Taiwan*	0.040	0.040	0.040	0.044	0.075	0.075	0.075	0.085	0.085								
sp. nov.	S. Taiwan	0.048	0.048	0.048	0.051	0.084	0.084	0.084	0.094	0.094	0.007							
	Philippines (2)	0.038	0.038	0.038	0.041	0.078	0.078	0.078	0.083-	0.083-	0.012	0.019	0.005					
									0.087	0.087								
N. serrata	South China Sea	0.033	0.028	0.036	0.039	0.078	0.078	0.078	0.088	0.088	0.036	0.043	0.038					
	Taiwan	0.033	0.028	0.030	0.033	0.078	0.078	0.078	0.088	0.088	0.028	0.028	0.030	0.013				
N. aculeata	Massachusetts	0.066	0.061	0.066	0.068	0.073	0.073	0.073	0.080	0.080	0.073	0.080	0.071	0.054	0.063			
	unspecified	0.066	0.061	0.066	0.068	0.068	0.068	0.068	0.075	0.075	0.073	0.080	0.071	0.059	0.063	0.015		
	Mexico (2)	0.064	0.059	0.064	0.068	0.066	0.066	0.066	0.073	0.073	0.071	0.079	0.068	0.057	0.063	0.015	0.000	0.000
N. rosea	Mexico	0.053	0.047	0.054	0.059	0.064	0.069	0.069	0.071	0.071	0.059	0.067	0.057	0.050	0.053	0.037	0.032	0.031

western central Indian Ocean material (with only one exception; a cl 42.3 mm ovigerous female of DABFUK/AR-ACH-10) but mostly in a position distinctly posterior to the middle of the rostrum in the specimens from the western Pacific and northwestern Australia (except in three specimens; one in *N. grandis*: NTOU M02177, and two in *N. pygmaea* sp. nov.: NTOU M02168, NTOU M02262).

Nephropsis stewarti was originally described from a single female specimen lacking large chelipeds, and collected from Ross Island of the Andaman Islands (Wood-Mason 1873, 1874). Soon after its discovery, many more specimens of this species were collected in India (see Alcock 1901); however, the holotype (supposed to have registration number 1404) is no longer extant, although having been held by the Zoological Survey of India, Calcutta (S Mitra, Zoological Survey of India, Calcutta, personal communication). As the *N. stewarti* species complex has now been found to contain at least three species, in order to fix the identity of *N. stewarti*, a recently collected Andaman Sea specimen (NTOU M02249) with color (Fig. 4E) and genetic information (Table 1) and that is very close to the type locality, is herein selected as the neotype of this species. The neotype fits well with the description of the holotype (Wood-Mason 1873, 1874), particularly in terms of the eye being rudimentary, bearing one pair of lateral rostral teeth, exopod of uropod with distinct diaeresis, and generally being similar to the figures provided for the holotype (Fig. 1A, B; Wood-Mason 1874: pl. 4-1–3).

Because material from northwestern Australia in the eastern Indian Ocean is now considered to belong to *N. grandis* instead of *N. stewarti*, re-examination of the specimens from northeastern Sumatra in the eastern Indian Ocean, reported by Balss (1925), will be necessary to determine if they represent *N. stewarti*, despite being collected near the type locality of the latter species. Re-examination of the "*N. stewarti*" material, reported by Chang and Chan (2019), revealed that all but one female (NTOU M02162) from the South China Sea belong to either *N. grandis* or the new species described below. This particular female specimen has the subdorsal carina on the carapace bearing small spines; therefore, it actually represents *N. serrata* (also see Tables 1, 2).

Although a red or white body color is thought to be specific for *Nephropsis* (see Chang and Chan 2019), this is not the case for *N. stewarti*. The Indian material appears to be much redder (Fig. 4A, B; Wood-Mason 1885; Alcock and Anderson 1899; Radhakrishnan et al. 2019: fig. 3.22) and has more distinct granules on the pleon, sometimes even arranged like a median carina. Thomas (1979), however, mentioned that his *N. stewarti* material from the Gulf of Mannar had a greenish-yellow color and deep red appendages. Color photographs are available for two of the Andaman Sea specimens examined (NTOU M02249, M02250). One (Fig. 4F) has a rather white body like the Mozambique specimen (MNHN IU-2018-5063, Fig. 4C, D), except for the pereiopods II to V, which are entirely pale purple. The neotype (Fig. 4E) has a body that is generally pale orange (which is intermediate between red and white). Color information on more specimens from different areas of the central western Indian Ocean will be necessary to understand whether material from the same locality also exhibits large variations in body color for this species.

Nephropsis grandis Zarenkov, 2006

Figures 2, 5A, B

Nephropsis grandis Zarenkov 2006: 86, figs 5–7 (type locality: Arafura Sea).

Nephropsis stewarti.–Kubo 1965: 629, unnumbered fig.; Miyake 1982: 77, pl. 26-1; Baba 1986: 153, fig. 103; Chan and Yu 1988: 8, pl. 1A; 1993: 83, unnumbered photo; Holthuis 1991: 45 (in part); Wadley and Evans 1991: 39, unnumbered photo; Macpherson 1993: 63; Chan 1997: 415; Jones and Morgan 2002: 83, unnumbered photo; Davie 2002: 391; Zarenkov 2006: 93 (in part), fig. 19; Chang and Chan 2019: 50 (in part), fig. 7. [not Wood-Mason 1872].

? Nephropsis stewarti.-Macpherson 1990: 312 (in part). [not Wood-Mason 1872].

Material examined. *Neotype*: INDONESIA • male cl 64.1 mm; Tanimbar Islands, Arafura Sea, Karubar stn CP59, 08°20'S, 132°11'E, 405–399 m, 31 Oct 1991 (MNHN IU-2017-9001).

Other material. JAPAN • 1 male cl 43.5 mm; Suruga Bay, off Numazu, commercial trawler, 34°44.37'N, 138°41.13'E, 350 m, 20 Apr. 2016 (CBM-ZC 14212) • 1 male cl 46.0 mm, 1 female cl 36.0 mm; Tosa Bay, off Mimase, 16 Jan.-14 Feb. 1963 (SMF 18328) • 2 males CL 32.0, 41.0 mm, 1 female cl 27.5 mm; 1961–1963 (SMF 24678). TAIWAN • 1 female cl 44.8 mm; Dasi fishing port, Yilan County, commercial trawlers, 10 Sept. 1984 (NTOU M02165) • 1 male cl 45.3 mm, 2 females cl 39.7 and 39.8 mm; Sept. 1992 (NTOU M02171) • 1 male cl 38.9 mm; Aug. 2003 (NTOU M00505) • 1 male cl 41.9 mm; 29 May 2008 (NTOU M02177) • 1 female cl 32.4 mm; 12 Apr. 2012 (NTOU M02178) • 1 male cl 19.8 mm; 14 Aug. 2013 (NTOU M02179) • 1 male (carapace damaged), 1 female cl 40.7 mm; Nanfang-ao fishing port, Yilan County, commercial trawlers, 2 May 1985 (NTOU M02166) • 1 male cl 28.2 mm, 1 female cl 40.5 mm; 20 Apr. 1988 (NTOU M02167) • 1 male cl 31.8 mm; 12 Nov. 2004 (NTOU M02176) • 2 males cl 29.0 and 32.7 mm; Donggang fishing port, Pingtung County, commercial trawlers, 27 Dec. 1997 (NTOU M02174) • 1 male cl 31.1 mm, 2 females cl 30.2 and 40.6 mm; locality not specified, 1993 (NTOU M02172). SOUTH CHINA SEA • 1 female cl 45.9 mm; Dongsha (Pratas), Jun. 1991 (NTOU M02170) • 1 male cl 15.9 mm; ZHONGSHA 2015 stn CP4137, 19°53.059'N, 114°21.678'E, 536-524 m, 23 Jul. 2015 (NTOU M02161) • 1 male cl 12.8 mm; stn CP4155, 16°13.60'N, 115°01.61'E, 526–510 m, 28 Jul. 2015 (NTOU M02163). PHILIPPINES • 1 female cl 32.0 mm; PANGLAO 2005 stn CP2384, 8°46.2'N, 123°16.1'E, 647–613 m, 29 May. 2005 (NTOU M02251). INDONESIA • 1 female cl 51.1 mm; Tanimbar Islands, Arafura Sea, KARUBAR stn CP39, 07°47'S, 132°26'E, 477-466 m, 28 Oct. 1991 (MNHN IU-2017-9002) • 1 female cl 25.4 mm; stn CP59, 08°20'S, 132°11'E, 405–399 m, 31 Oct. 1991 (MNHN IU-2018-5062). NORTH WEST AUSTRALIA • 1 male cl 53.7 mm; 18°19'S, 117°149'E, 25 Feb. 1986 (NTOU M02252).

Diagnosis. Rostrum armed with a single pair of lateral teeth usually situated posterior to mid-length of rostrum. Carapace with subdorsal carinae granulate, lacking distinct spine; supraorbital and antennal spines present; post-supraorbital spine ab-



Figure 2. *Nephropsis grandis* Zarenkov, 2006, Tanimbar Islands, Arafura Sea, KARUBAR stn CP59, neotype male cl 64.1 mm (MNHN IU-2017-9001) **A** carapace and pleon, dorsal **B** same, lateral **C** left pereiopod I, chela to distal part of merus, dorsal **D** same, lateral **E** same, ventral. Pubescence and setae omitted • Position of spines on inner surface of palm \diamondsuit position of spines on dorsal margin of outer surface • position of spines on dorsal margin of inner surface **E** dorso-inner distal spine of carpus \bigtriangleup ventro-outer distal spine of carpus \square distoventral spine \bigstar subdistal dorsal spine of merus.

sent; postcervical groove U-shaped in dorsal view; intermediate carina weak, indistinct. Large cheliped (pereiopod I) with inner surface of palm usually armed with row of distinct spines; carpus with strong distoventral, ventro-outer distal, and dorso-inner distal spines, inner surface usually with 2–4 spines along dorsal margin and several small spines on ventral margin, both dorsal and ventral margins of outer surface spinose; merus with subdistal dorsal and anteroventral spine, generally also bearing a spine or sharp tubercle on subdistal outer surface. Pleon generally smooth and lacking mid-dorsal carina; pleura each with unarmed anterior margin. Telson without erected mid-dorsal spine near base. Uropodal exopods with complete diaeresis. **Description.** Body covered with long or short pubescence, rather thick on dorsal carapace, pleonal tergites and anterior two pereiopods. Carapace finely granulated (Fig. 2A, B); rostrum 0.4–0.8× as long as carapace (proportionally longer in small individuals), bearing 1 pair of lateral teeth usually situated posterior to mid-length of rostrum; subdorsal carinae granulate, lacking distinct spine; strong supraorbital and antennal spines present; post-supraorbital spine absent; cervical, postcervical, and hepatic groove well-marked, with post-cervical groove U-shaped in dorsal view; intermediate carina weak, indistinct; lateral carina distinct; distance between gastric tubercle and supraorbital spines 0.3–0.4× distance between gastric tubercle and postcervical groove; distance between orbital margin and postcervical groove 1.3–1.5 (rarely 1.6)× distance between post-cervical groove and posterior margin of carapace.

Large cheliped (pereiopod I), generally with smooth surface (Fig. 2C–E); chela 2.7-4.7 (mostly 2.9-3.8)× as long as wide; inner surface of palm generally armed with row of distinct spines except for very small individuals; carpus with strong distoventral spine, ventro-outer distal spine, and dorso-inner distal spine, inner surface having 2–4 spines (sometimes only one in very small individuals) along dorsal margin and several small spines on ventral margin, both dorsal and ventral margins of outer surface spinose (with fewer spines in small young specimens); merus with subdistal dorsal spine and distoventral spine, usually also bearing subdistal outer spine or sharp tubercle. Pereiopod II with carpus 0.5-0.7 (rarely 1)× palm length. Pereiopod III with carpus $0.4-0.5\times$ as long as palm; merus $1.8-2.1\times$ longer than carpus. Pereiopods IV and V with dactyli $0.3-0.6\times$ as long as propodi.

Pleon generally smooth (Fig. 2A, B), lacking median carina, only tergites I and VI granulate; pleura each with unarmed anterior margin and each terminating ventrally into a blunt or sharp spine. Telson without erected dorsal median spine near base. Uropodal exopods with complete diaeresis.

Eggs spherical and approximately 3 mm in diameter (Chan and Yu 1988).

Distribution. Western Pacific and northwestern Australia, known with certainty from Japan, Taiwan, South China Sea, the Philippines, Indonesia (Kai and Tanimbar Islands), Arafura Sea, and northern Australia (Queensland to NW Shelf); at depths of 312–647 m (Macpherson 1993; present study) and perhaps 170–821 m (see Remarks).

Color in life. Body, including eyes, generally whitish, pubescence grayish to grayish brown (Fig. 5A, B; Miyake 1982: pl. 26–1; Baba 1986: fig. 103; Chan and Yu 1988: pl. 1A; 1993: unnumbered photo; Wadley and Evans 1991: unnumbered photo; Jones and Morgan 2002: unnumbered photo). Rostrum and antennal flagella orange to reddish, sometimes anterodorsal carapace also orange. Antennular flagella whitish to reddish. Maxilliped III and pereiopods II to V white to orange-pink and with distal parts reddish. Large cheliped whitish to somewhat orange. Pleopods and margins of pleonal pleura whitish or reddish. Uropods and distal part of telson pinkish red to reddish. Eggs whitish (Chan and Yu 1988).

Remarks. Although the western Pacific and northwestern Australia material has been shown to be not the true *N. stewarti*, molecular genetic analysis suggests that there are two distinct species (16S sequence divergence as high as 7.5–9.4%, even

higher than 3.1-6.3% among N. serrata, N. aculeata, and N. rosea; Table 2). Careful comparisons revealed that these two genetic forms differ, detailed as follows. (1) The body size is large in one form (up to cl 64.1 mm, MNHN IU-2017-9001) but much smaller in the other (up to cl 28.0 mm NTOU M02259). (2) Although the pleon is concealed by thick pubescence, the surfaces of tergites II to V is rather smooth in the large size form (Fig. 2A, B) but distinctly granular in the smaller form (Fig. 3A, B). (3) The large cheliped is also concealed by long pubescence but is more spiny in the large form. In the large form, the carpus is heavily spinose on the outer surface and has 2-4 spines along the dorsal margin of the inner surface (Fig. 2C-E). The inner surface of the palm is also spiny (Fig. 2C, E), except in small young specimens (cl < 20 mm), and the merus generally has a subdistal outer spine or sharp tubercle (Fig. 2C, D) except in one female (MNHN IU-2018-5062). For the large cheliped of the smaller form, the carpus has an outer surface without distinct spines and the inner surface bears only one (mostly) or two spines on the dorsal margin (Fig. 3C-E). The inner surface of the palm lacks distinct spines (Fig. 3C, E), and there is no subdistal outer spine or sharp tubercle on the merus (Fig. 3C, D).

Nephropsis grandis was described from a single male collected near the Tanimbar Islands in the Arafura Sea (09°07.5'S, 131°14.9'E, Zarenkov 2006). The holotype is a large specimen (carapace length, including rostrum, 58 mm) and its pleonal tergites I-VI are without granules on the surfaces. The large cheliped in the holotype appears to be rather spinous; however, it is difficult to comprehend the exact spination on the various parts from the original description and illustrations. For example, the holotype appears to lack a subdistal outer spine at the merus of the large cheliped (Zarenkov 2006: table 1: subdistal spine on outer surface of merus of cheliped). Nonetheless, the larger form discussed above generally fits the characteristics described for N. grandis, and at present, only the larger form is found in the Arafura Sea. Currently, the holotype of N. grandis (ZMMU Ma 5157) cannot be located in the Zoological Museum of Moscow University (V Spiridonov, personal communication). As there are now three species in the N. stewarti species complex and each has only subtle differences, it is desirable to fix the identity of N. grandis. The current largest specimen (MNHN IU-2017-9001) from the Tanimbar Islands, and with genetic data available, was selected as the neotype of N. grandis, thus affixing this name on the larger form in the West Pacific and northwestern Australia. The neotype was collected from a locality very close to that of the holotype (both in the Arafura Sea) and is generally similar to the original figures provided for the holotype (Fig. 2A-C; Zarenkov 2006: figs 5, 6).

Nephropsis grandis is genetically distinct from N. stewarti, with 6.4–7.3% 16S sequence divergence (Table 2). Other than the differences described under the "Remarks" of N. stewarti, these two species also differ in the pleonal tergites II–V, being smooth in N. grandis (Fig. 2A, B) but granular in N. stewarti (Fig. 1A, B). In Nephropsis grandis the outer surface of the large cheliped carpus is spinose (Fig. 2C–E) but this is only granular and without distinct spines in N. stewarti (Fig. 1C–E). In large specimens, the inner surface of palm of the large cheliped is

also spinose in *N. grandis* (Fig. 2C, E) but still lacks spines in *N. stewarti* (Fig. 1C, E). Macpherson (1993) suggested that the shape of the large chela might be different among the species in the *N. stewarti* species complex. However, this is not supported by the present work, even though the large chelae have sexual dimorphism only in *N. stewarti*.

Nephropsis grandis is widely distributed from Japan to Australia. Photographs of the Japanese specimen identified as "N. stewarti" from Suruga Bay (CBM-ZC 14212) and with a very short 16S sequence for eDNA metabarcoding (LC430805, 163 bp; Komai et al. 2019) is now confirmed to represent N. grandis. The short 16S sequence of this specimen is also identical to the sequence of the present Taiwanese specimens (NTOU M02174, NTOU M00505) assigned to N. grandis. The Japanese specimens (SMF 18328), referred to "N. stewarti" by Zarenkov (2006), are rather small (two males and one female, cl, including rostrum, 32-41mm) but still have a distinct spine on the outer surface of the carpus of the pereiopod I (Zarenkov 2006: fig. 19A); therefore, they likely represent N. grandis instead. The SMF 18328 lot, however, consists of two moderately large specimens (cl 36.0 mm and 46.0 mm). One more lot of "N. stewarti" from Japan is held in the Senckenberg Museum (SMF 24678), and there are three specimens within the lot. Although the number and sex of the specimens in the SMF 24678 lot match those reported for SMF 18328 in Zarenkov (2006), their sizes (cl 27.5–41.0 mm) do not match. Nevertheless, photographs of all five of these Japanese specimens in the Senckenberg Museum clearly show that they are all *N. grandis* because of the weak intermediate carina, large cheliped with distinct spines on the outer surface of the carpus, and the merus having subdistal outer spine. For the published photographs of Japanese "N. stewarti", the one of Baba (1986: fig. 103) clearly shows the large cheliped with the inner surface of the palm and the outer surface of the carpus bearing distinct spines. The other photograph of Miyake (1982: pl. 26-1) also shows the large cheliped with the outer surface of the carpus armed with distinct spines, although the spination on the inner surface of the palm is unclear because of the covering of thick pubescence. Thus, it appears that only N. grandis is distributed in Japan among the N. stewarti species complex. Among the two sequenced Taiwanese specimens of this species, specimen NTOU M00505 was used in a clawed lobster phylogenetic study (Tshudy et al. 2009) as "N. stewarti" with GenBank no. EU882882, which has a sequence identical to U96086 from a specimen of N. stewarti in Natal, South Africa (Tam and Kornfield 1988). However, re-amplification of the 16S gene of the NTOU M00505 specimen (GenBank no. M302004) revealed that its sequence does not match EU882882 and belongs to the clade of N. grandis instead.

The present work revealed that among the *N. stewarti* species complex, both *N. grandis* and *N. pygmaea* sp. nov. are distributed in southern Taiwan and the Philippines, and the true *N. stewarti* is restricted to the Indian Ocean. Re-examination of the Philippines material (with a depth range of 170–821 m) reported as "*N. stewarti*" in Macpherson (1990) is necessary to determine which of these two species they belong to, and whether *N. grandis* can be found in waters as shallow as 170 m and/or as deep as 821 m.

Nephropsis pygmaea sp. nov.

http://zoobank.org/286FA460-CA9A-465F-B793-22F9F603D4BA Figures 3, 5C, D

Nephropsis stewarti.–Holthuis 1991: 45 (in part); Chang and Chan 2019: 50 (in part). [not Wood-Mason 1872].

? Nephropsis stewarti.-Macpherson 1990: 312 (in part). [not Wood-Mason 1872]

Material examined. *Holotype*: TAIWAN • male cl 25.6 mm; Donggang, Pingtung County, commercial trawler, 22°11.880'N, 120°22.213'E, 630 m, 2 Oct. 2014 (NTOU M01898).

Paratypes: TAIWAN • 1 male cl 23.4 mm; Donggang fishing port, Pingtung County, commercial trawler, Jul. 1975 (NTOU M02164) • 1 male cl 21.1 mm, 6 females cl 19.5–26.2 mm; 3 May. 1991 (NTOU M02168) • 2 males cl 19.2 and 21.7 mm; 14 May. 1991 (NTOU M02169) • 1 male cl 22.4 mm; 4 Jun. 1995 (NTOU M02173) • 1 male cl 21.8 mm; 27 Dec. 1997 (NTOU M02175).

Other material. PHILIPPINES • 4 males cl 12.5–18.1 mm, 1 female cl 19.1 mm; PANGLAO 2005 stn CP2333, 09°38.2'N, 123°43.5'E, 596-565.5 m, 22 May 2005 (NTOU M02253) • 1 male cl 16.2 mm; stn CP2335, 09°34.3'N, 123°37.8'E, 733-743 m, 22 May 2005 (NTOU M02254) • 1 male cl 14.9 mm, 2 females cl 20.0 and 22.1 mm; stn CP2336, 09°32.4'N, 123°39.3'E, 757-729 m, 22 May 2005 (NTOU M02255) • 1 male cl 12.0 mm, 1 female cl 22.9 mm; stn CP2341, 09°24.5'N, 123°49.7'E, 712–888 m, 23 May 2005 (NTOU M02256) • 1 female cl 11.1 mm; stn CP2351, 09°30.7'N, 124°3.0'E, 810–830 m, 24 May 2005 (NTOU M02257) • 1 male cl 9.0 mm; stn CP2352, 09°27.3'N, 124°3.1'E, 1260-1761 m, 24 May 2005 (NTOU M02258) • 3 males cl 11.8-20.4 mm, 5 females cl 15.7-28.0 mm; stn CP2358, 08°52.1'N, 123°37.1'E, 569-597 m, 26 May 2005 (NTOU M02259) • 1 male cl 16.2 mm; stn CP2358, 08°52.1'N, 123°37.1'E, 569-597 m, 26 May 2005 (NTOU M02260) • 6 males cl 10.4-21.9 mm, 1 ovigerous female cl 22.9 mm, 11 females cl 10.3-25.3 mm; stn CP2389, 09°27.9'N, 123°38.4'E, 784-782 m, 30 May 2005 (NTOU M02261) • 1 male cl 19.6 mm, 2 females cl 19.5 and 19.6 mm; stn CP2390, 09°27.4'N, 123°43.1'E, 627-613 m, 30 May 2005 (NTOU M02262) • 2 males cl 17.6 and 23.8 mm; stn CP2397, 09°34.9'N, 123°41.7'E, 669-712 m, 31 May 2005 (NTOU M02263) • 1 male cl 24.0 mm; stn CP2398, 09°32.6'N, 123°40.5'E, 731-741 m, 31 May 2005 (NTOU M02264) • 2 males cl 16.4 and 19.4 mm; stn CP2405, 09°39.0'N, 123°46.1'E, 387-310 m, 1 Jun. 2005 (NTOU M02265) • 1 female cl 16.6 mm; northern coast of Panglao Island, Jul. 2004–May. 2005 (NTOU M02266).

Diagnosis. Rostrum bearing one pair of lateral teeth usually situated behind midlength of rostrum. Carapace with subdorsal carinae granulate and lacking distinct spine; supraorbital and antennal spines strong; post-supraorbital spine absent; postcervical groove U-shaped in dorsal view; intermediate carina indistinct and lateral carina moderately developed. Large cheliped (pereiopod I) with inner surface of palm granular, lacking distinct spine; carpus with strong distoventral, ventro-outer (rarely absent) and dorso-inner distal spines, outer surface without distinct spine, inner surface bear-



Figure 3. *Nephropsis pygmaea* sp. nov., Donggang, Pingtung County, Taiwan, holotype male cl 25.6 mm (NTOU M01898) **A** carapace and pleon, dorsal **B** same, lateral **C** left pereiopod I, chela to distal part of merus, dorsal **D** same, lateral **E** same, ventral, pubescence and setae omitted • position of spines on inner surface of palm \diamond position of spines on dorsal margin of outer surface • position of spines on dorsal margin of inner surface **II** dorso-inner distal spine of carpus \triangle ventro-outer distal spine of carpus \Box distoventral spine \bigstar subdistal dorsal spine of merus, ***** position of subdistal outer spine of merus.

ing one or rarely two spines on dorsal margin; merus armed with anteroventral and subdistal dorsal spines, lacking subdistal outer spine or sharp tubercle. Pleon finely granulate, without mid-dorsal carina, pleura each with unarmed anterior margin. Telson without erected dorsal spine near base. Uropodal exopods with complete diaeresis.

Description. Body covered with long or short pubescence, those on anterior two pereiopods, dorsal carapace, and pleonal tergum quite dense. Carapace finely granu-



Figure 4. A–F *Nephropsis stewarti* Wood-Mason, 1872 A, B Sakthikulangara fishing harbor, India, ovigerous female cl 42.4 mm (DABFUK/AR-ACH-10) C, D Mozambique, MAINBAZA stn CP3138, male cl 52.7 mm (MNHN IU-2018-5063) E Andaman Sea, RV 'Dr. Fridtjof Nansen' stn 135, neotype male cl 46.2 mm (NTOU M02249) F Andaman Sea, RV 'Dr. Fridtjof Nansen' stn 68, male cl 42.3 mm (NTOU M02250) A, C, E, F dorsal habitus B, D lateral habitus E, F photographed by PN Psomadakis.

lated (Fig. 3A, B); rostrum $0.5-0.9 \times$ carapace length (proportionally longer in small individuals), bearing 1 pair of lateral teeth usually situated behind mid-length of rostrum, median groove extending anteriorly beyond lateral rostral teeth; subdorsal carinae granulate and lacking distinct spine; strong supraorbital and antennal spines present; post-supraorbital spine absent; cervical, postcervical, and hepatic groove well marked, with postcervical groove U-shaped in dorsal view; intermediate carina indistinct and lateral carina moderately developed; gastric tubercle near supraorbital spines, $0.3-0.4 \times$ distance between gastric tubercle and postcervical groove; distance between orbital margin and postcervical groove $1.5-1.8 \times$ distance between postcervical groove and posterior margin of carapace.

Large cheliped (pereiopod I) generally granulate (Fig. 3C–E); fingers 0.9–1.5 (mostly 1.0-1.3)× as long as palm; chela 2.6–4.1 (usually 2.8-3.2)× as long as wide and similar in both sexes, inner surface of palm granular but lacking distinct spine; carpus with strong distoventral spine, ventro-outer spine (rarely absent) and dorso-inner distal spine, outer surface without distinct spine, inner surface bearing 1 (mostly) or 2 spines on dorsal margin; merus armed with distoventral spine and subdistal dorsal spine, lacking subdistal outer spine or sharp tubercle. Pereiopod II chelate, smooth, carpus 0.5-0.7× palm length. Pereiopod III generally similar to pereiopod II but less stout; carpus 0.4-0.6× as long as palm; merus 1.6-2.2 (mostly 1.7-2.0)× as long as carpus. Pereiopods IV and V smooth, not chelate; dactyli 0.5-0.8 (mostly 0.5-0.7)× as long as propodi.

Entire pleon finely granulate (Fig. 3A, B), without mid-dorsal carina but bearing indistinct and medially interrupted transverse groove on tergites II–V and sometimes also on tergite I; pleura each with unarmed anterior margin, that of pleuron II strongly convex while those of pleura III–V only slightly convex, all terminating ventrally into sharp spine. Telson without erected dorsal spine near base.

Uropod generally smooth, exopods with distinct complete diaeresis.

Eggs spherical, 1.8–2.0 mm in diameter.

Color in life. Body generally whitish to pinkish white (Fig. 5C, D), with pleon sometimes pinkish orange. Eyes whitish. Anterodorsal carapace pinkish orange. Rostrum and antennal flagella pinkish orange to orange red. Antennular flagella and maxilliped III orange red. Large cheliped whitish to pinkish orange, distal parts of fingers always pinkish orange. Pereiopods II–V whitish with distal segments orange red or entirely orange red. Pleopods whitish to orange red. Tail fan whitish, sometimes with median parts rose red. Pubescence grayish brown.

Etymology. The Latin *pygmaea* (little) refers to the much smaller size of this species compared with other species in the *N. stewarti* species complex.

Distribution. Western Pacific and known with certainty from southern Taiwan and the Philippines, at depths of 310–888 m, and perhaps as shallow as 170 m (see "Remarks").

Remarks. This smaller form restricted to the northwestern Pacific has a maximum carapace length of 28.0 mm (NTOU M02259), with females bearing eggs attaining only 22.9 mm in the carapace length (NTOU M02261). The largest specimens of *N. stewarti* and *N. grandis* is 54 mm (Dineshbabu 2008) and 64.1 mm (present mate-



Figure 5. A, B *N. grandis* Zarenkov, 2006, Dasi fishing port, Taiwan, male cl 45.3 mm (NTOU M02171) **C, D** *N. pygmaea* sp. nov., Donggang fishing port, Taiwan, paratype female cl 24.4 mm (NTOU M02168) **A, C** dorsal habitus **B, D** lateral habitus.

rial) in the carapace length, respectively. The smallest ovigerous females recorded for *N. stewarti* and *N. grandis* are of carapace lengths approximately 24 mm (total length 80 mm, Dineshbabu 2008) and 38 mm (body length 105 mm, Chan and Yu 1988), respectively. Other than the difference in body size, *N. pygmaea* sp. nov. is unique in the *N. stewarti* species complex in that it lacks the subdistal outer spine or sharp tubercle on the merus of the large cheliped (Fig. 3C, D), which are present in *N. stewarti* and *N. grandis* (Figs 1C, D, 2C, D).

In spite of the restricted distribution to the northwestern Pacific, N. pygmaea sp. nov. is genetically closer to N. stewarti than N. grandis. The lowest 16S sequence divergence between N. pygmaea sp. nov. and N. stewarti is 3.8%, whereas the sequence divergence is almost double (7.5%) between N. pygmaea sp. nov. and N. grandis. Morphologically, N. pygmaea sp. nov. is also generally more similar to N. stewarti in the surface of the pleonal tergites distinctly granular (Figs 1A, B, 3A, B), and the large cheliped is relatively less spiny (with inner surface of palm and outer surface of carpus lacking distinct spine; Figs 1C, E, 3C, E). As such a male specimen from the Philippines (NTOU M02260) has the granules arranged somewhat like a median carina on the pleon, as in some Indian *N. stewarti* specimens. Nevertheless, *N. pygmaea* sp. nov. can also be separated from *N. stewarti* by the intermediate carina on the carapace indistinct (Fig. 3A, B; vs. well-marked, Fig. 1A, B), rostral teeth usually located posterior to the mid-length of the rostrum (Fig. 3A, B; vs. usually at mid-length of the rostrum, Fig. 1A, B), and the inner surface of the carpus of the large cheliped usually armed with one or occasionally two spines along the dorsal margin (Fig. 3C; vs. usually two to four spines, rarely one spine, Fig. 1C). Of the 62 specimens examined for N. pygmaea sp. nov., only 10 (16.1%) have two spines instead of one on the dorsal margin of the inner surface of the carpus of the large cheliped.

The present materials from southern Taiwan and the Philippines are generally very similar. Only one specimen (NTOU M02168) has three teeth instead of one on the right side of the rostrum. As both *N. grandis* and *N. pygmaea* sp. nov. occur in the Philippines, it is necessary to re-examine the Philippines "*N. stewarti*" material reported by Macpherson (1990) to determine their exact identities. Although most of the Philippines specimens described by Macpherson (1990) are rather small, a few of them (eg. carapace length, including rostrum, 70 mm, equivalent to a carapace length of approximately 47 mm) are larger than the present largest specimen (cl 28 mm) of *N. pygmaea* sp. nov. Moreover, a Philippines specimen identified by Macpherson (1990) was obtained from a depth of 170–200 m, exceptionally shallow for species of *Nephropsis*. Reexamination of this specimen may eventually reveal that the present species or *N. grandis* extends to such shallow depth.

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RESEARCH ARTICLE



A contribution to the knowledge of cavernicolous ground beetles from Sichuan Province, southwestern China (Coleoptera, Carabidae, Trechini, Platynini)

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Abstract

Two new genera and four new species of cave-adapted ground beetles are described from Sichuan Province, southwestern China. Uenoaphaenops gen. nov. is established to place the trechine species Qianotrechus fani Uéno, 2003 occurring in the limestone cave Hua'er Dong, southeastern Sichuan (Luzhou: Gulin). Chu pheggomisetoides gen. nov. & sp. nov., from the limestone cave Hanwang Dong, northeastern Sichuan (Guangyuan: Chaotian), is somewhat like the European cavernicolous trechine genus Pheggomisetes Knirsch, 1923, from Bulgaria and Serbia, in particular in the configurations of head and pronotum. Boreaphaenops lipuani sp. nov., also from Hanwang Dong, is the second representative of the genus and the first record in Sichuan. Agonotrechus sinotroglophilus Deuve, 1999, a troglophile, is reported from Sichuan for the first time. The other two new species belong to the platynine genus Jujiroa Uéno, 1952: J. uenoi sp. nov. from the cave Banche Dong on the northern side of the Dadu River (Leshan: Shawan) and J. wangzheni sp. nov. from the cave Hua'er Dong, which is sympatric with Uenoaphaenops fani (Uéno, 2003) comb. nov. A distribution map for the localities of all abovementioned caves and a key to Jujiroa species known in Sichuan are provided.

Keywords

Carabids, new combination, new genera, new species, subterranean, taxonomy

Introduction

Sichuan Province without question holds the richest specific diversity of Carabidae *sensu lato* in China. Over one fourth (1189) of the total species (3946) known in China occur in Sichuan (Anichtchenko et al. 2007–2020). On the contrary, the subterranean fauna of Carabidae is comparatively poor in this province, with the only exception, the troglobitic platynine genus *Jujiroa* Uéno, 1952 which is very rich in Sichuan. Five of the eight *Jujiroa* species known in mainland China were reported from Sichuan (Vigna Taglianti 1995; Uéno and Kishimoto 2001; Uéno 2007; Deuve and Pütz 2013; Tian and He 2020).

However, the cave fauna of the ground beetles in Sichuan is interesting. For example, *Troglopatrobus zhouchaoi* Deuve et al., 2020, known only from the cave Lianhua Dong in Pengzhou in the northern suburb of Chengdu, the provincial capital city, is morphologically highly modified and the only Patrobini species occurring in subterranean habitats in the world (Deuve et al. 2020). Species of the genus *Sichuanotrechus* Deuve, 2005, together with *Duvalioblemus* (*Shublemus*) *liyuani* Deuve et al., 2020 occur only in the Longmen Mountains of northern Sichuan (Deuve 2005; Uéno 2006, 2008; Huang and Tian 2015). *Qianotrechus fani* Uéno, 2003 is only found inside the cave Hua'er Dong in Gulin County, in the southeastern corner of the province (Uéno 2003; Deuve et al. 2020). *Agonotrechus sinotroglophilus* Deuve, 1999, a troglophilous species formerly recorded from Chongqing (Deuve 1999; Deuve and Tian 2016) was newly found in a cave in northeastern Sichuan. Another troglophilous species, *Trechiotes perroti* Jeannel, 1954, occurs in a large area in southwestern China including Sichuan (Deuve et al. 1999; Deuve and Tian 2011, 2016).

Thanks to the Sichuan Cave Exploration Team (SCET, Chengdu), in which the cave biology group is led by Li He (the second author of the present paper), our knowledge of cave ground beetles in Sichuan Province is quickly increasing. The majority of members of SCET are young and active cavers (Fig. 1). In recent years, they have conducted many cave surveys which resulted in important scientific discoveries in terms of cave invertebrates, especially ground beetles. For instance, they found one of the richest cave fauna of ground beetles recorded from China in the limestone cave Hanwang Dong, northeastern Sichuan (Guangyuan: Chaotian). Four cave-adapted carabid species in total have been collected in this beautiful limestone cave: a pterostichine species belonging to the *Pterostichus* subgenus *Huaius* (Tian & He, 2020), two new troglobitic trechine species and the troglophile *Agonotrechus sinotroglophilus* Deuve, 1999. They have re-discovered almost all species of the genus *Sichuantrechus*. From two caves in Leshan and Luzhou respectively, they found another two new *Jujiroa* species apart from *J. deliciola* Uéno & Kishimoto, 2001 and the recently described *J. zhouchaoi* Tian & He, 2020 (Tian and He 2020).

Qianotrechus fani Uéno, 2003 was the first troglobitic trechine beetle reported from Sichuan. This species was named in honour of the person who provided crucial support to Dr Shun-Ichi Uéno during his collecting travels in China from 1998 to 2010 based on a so-called international scientific collaborative project, which resulted in fruitful



Figure 1. Group photo of some members of the Sichuan Cave Exploration Team (SCET, Chengdu).

discoveries on subterranean ground beetles. *Qianotrechus fani* is a very peculiar member within the genus *Qianotrechus* Uéno, 2000 not only because of its locality which is far from those of other congeners, but also its morphological character states which are very different from the other species of the genus. Uéno (2003) tentatively treated it as a *Qianotrechus* species because only a female was available at that time. Thanks to Dr Yunchun Li (an expert of Pseudoscorpiones from China West Normal University, Nanchong), the first author received three individuals of *Q. fani* collected in the cave Hua'er Dong, the type locality. Later, Yuan Li and Zhen Wang (both are local amateur entomologists) who also surveyed in Hua'er Dong, successfully collected three specimens of the species. Further laboratory study revealed that there is no sexual dimorphism in this species, i.e., the protarsi are not modified in male, and abdominal ventrite VII is quadrisetose in both sexes. Furthermore, the male genital organ of *Q. fani* is very short and stout, contrary to the other *Qianotrechus* species in which it is always thin and long. These crucial features, plus other peculiar morphological characters, strongly support that *Q. fani* belongs to an unknown genus rather than *Qianotrechus*.

The aim of this paper is to establish a new genus for Uéno's *Qianotrechus fani*, describe another new genus and two new species of the tribe Trechini from the cave Hanwang Dong, give the first record of *Agonotrechus sinotroglophilus* for Sichuan Province, and describe two new species of the Platynini genus *Jujiroa*.

This paper is dedicated to the late Dr Shun-Ichi Uéno, a well-known cave biologist in National Science Museum (Natural History), Tokyo, who unfortunately passed away on October 3, 2020.

Material and methods

The material for this study were discovered from three caves in Sichuan Province, namely, Hanwang Dong, Hua'er Dong and Banche Dong (Fig. 2). Beetles were collected using an aspirator or trap baited with silkworm (*Bombyx mori* L., 1758) chrysalis meal, killed with ethyl acetate and kept in vials with 75% ethanol; a few specimens were kept in 95% ethanol, for DNA sequencing.

The specimens were examined with a Nikon SMZ1000 stereo-microscope in a solution of glycerin. All illustrations were completed using Adobe Illustrator CS 6.0 based on digital photos taken by means of a Keyence VHX-5000 digital microscope. The distribution map was drawn using MapInfo Professional 12.0 software.

Length of body is measured from the right mandible (when opened) to the apex of the elytra. Width of body is the maximum width of combined elytra. Abbreviations of measurements used in the text are as follows:

EL	length of elytra, from base of scutellum to elytral apex
EW	maximum width of combined elytra
HLI	length of head excluding mandibles, from front of labrum to base of head
HLm	length of head including mandibles, from apex of right mandible to base of head
HW	maximum width of head
PfW	width of pronotum at front
PbW	width of pronotum at base
PL	length of pronotum, through mid-line
PW	maximum width of pronotum

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

- CLH Collection of Li He, Chengdu, Sichuan, China
- CYL Collection of Yuan Li, Deyang, Sichuan, China
- CZW Collection of Zhen Wang, Chengdu, Sichuan, China
- SCAU South China Agricultural University, Guangzhou, China

Taxonomy

Tribe Trechini Bonelli, 1810

Uenoaphaenops gen. nov.

http://zoobank.org/20CF5C96-DFC5-419F-97D8-924D18433C3F Chinese name: 上野穴步甲属 Figs 2, 3, 4A, C, 5A, B

Type species. *Qianotrechus fani* Uéno, 2003 from the cave Hua'er Dong, Gulin, south-eastern Sichuan).



Figure 2. Map of Sichuan Province showing the locations of the related caves.

Generic characteristics. Medium-sized, aphaenopsian and depigmented; body moderately elongate, wholly pubescent. Head strongly elongate, much longer than wide, nearly parallel-sided; neck weakly-marked, ring-shaped; two pairs of supraorbital setiferous pores present; frontal furrows incomplete, parallel-sided in most part though briefly divergent posteriorly; frons and vertex convex; right mandibular tooth bidentate; mentum and submentum completely fused; mentum bisetose, and covered with short setae on basal area of mental tooth, and along the site of labial suture; base largely concave, uni-foveate, tooth short and simple at tip; submentum with a row of 12 setae; labial palpomere 2 much longer than 3 (1.3 times), bisetose on inner margin, without additional setae; maxillary palpomere 3 much longer than 4 (1.4 times) (Fig. 4A); antennae long and thin, extending to about 1/4 of elytra from apex; one pair of suborbital pores present. Prothorax distinctly expanded, propleura visible from above; pronotum elongate, as long as head excluding mandibles, wider than head, widest at about 1/3 from front, lateral margins sinuate before hind angles which is nearly rectangular, base slightly narrower than front; two pairs of latero-marginal setae present, disc moderately convex. Elytra ovate, shoulders obtuse, almost rounded, distinctly serrate at prehumeral part, while ciliate on other parts; disc extraordinarily convex though depressed near base; striae reduced though traceable; presence of two

dorsal pores along the 3rd striae and the preapical; prehumeral set of the marginal umbilicate pores not aggregated, the 5th pore much closer to 4th than to 6th. Protarsi not modified in male; tibiae without longitudinal sulci. Ventrites IV–VI each with pair of paramedian setae; ventrite VII quadrisetose in both sexes.

Male genitalia (Fig. 5A, B). Aedeagus very short and small, but thick, weakly sclerotized. The median lobe slightly arcuate at median portion, but strongly sinuate before apex which is obtuse, with a large round opening; base quite large, without a sagittal aileron; inner sac provided with a thick and long copulatory piece, which is about 2/5 as long as aedeagus; in dorsal view, apical lobe gradually contracted towards apex which is broadly rounded; parameres well-developed, but much shorter than the median lobe, truncate at apical margin, each armed with four long setae at apex.

Remarks. This peculiar species was put into the genus *Qianotrechus* Uéno, 2000 due to the lack of a male at that time. However, Uéno (2003) pointed out the following characteristics of this species which are not present in the congeners of *Qianotrechus*: body wholly pubescent; humeral margins of elytra strongly serrated; and the 5th pore of marginal umbilicate series forwardly and inwardly shifted, widely distant from the 6th pore. Hence, he mentioned that the above peculiarities may suggest a generic separation of this species from the Guizhou genus. Our examination of male individuals provided further evidence to support his opinion. First, protarsi are not modified in the male of *Qianotrechus fani*, while the 1st and 2nd protarsomeres are spurred inwards at the apices in all other *Qianotrechus*. Second, ventrite VII is quadrisetose in both sexes in *Qianotrechus fani*, vs. bisetose in males of other *Qianotrechus*. Third, the aedeagus of *Qianotrechus fani* is very small and stout, not the same type as in other *Qianotrechus* species, which are always large and elongate (Uéno 2000, 2003).

Etymology. "*Ueno*" + "*-aphaenops*". Dedicated to the late Dr Shun-Ichi Uéno who made a great contribution to the knowledge of Chinese subterranean ground beetles. Gender masculine.

Generic range. China (Sichuan) (Fig. 2). A monospecific genus only recorded from the cave Hua'er Dong, Gulin County, southeastern Sichuan.

Uenoaphaenops fani (Uéno, 2003) comb. nov.

Chinese name: 范氏上野穴步甲 Figs 2, 3, 4A, C, 5A, B, 6

Material. 3 females, the cave Hua'er Dong, Xiangdingshan, Xiangding, Shiping, Gulin, Luzhou, Sichuan (四川省泸州市古蔺县石屏镇向顶村象顶山华儿洞), 28.028931°N, 106.00716°E, 640 m, 2020-VI-22, leg. Yuan Li & Zhen Wang, in CLH, CYL and CZW, respectively; 2 males and 1 female, same cave, 2019-XI-3, leg. Yunchun Li, in SCAU.

Remarks. Uéno (2003) mentioned that the submentum of *Qianotrechus fani* is 8-setose. Actually, there are 12 setae in total in our exemplars, excluding the shorter pubescence along the site of labial suture.



Figure 3. Habitus of Uenoaphaenops fani (Uéno, 2003), comb. nov., female.



Figure 4. Cave trechine beetles **A** ventral head of *Uenoaphaenops fani* (Uéno, 2003), comb. nov., female **B** ventral head of *Chu pheggomisetoides* gen. nov. & sp. nov., female **C–E** elytral chaetotaxy of *Uenoaphaenops fani* (Uéno, 2003), comb. nov., female, *Chu pheggomisetoides* gen. nov. & sp. nov., female paratype, and *Boreaphaenops liyuani* sp. nov., female holotype. Scale bars: 1.0 mm.



Figure 5. Male genitalia of cave trechine species, lateral and dorsal views **A**, **B** *Uenoaphaenops fani* (Uéno, 2003), comb. nov. **C**, **D** *Chu pheggomisetoides* gen. nov. & sp. nov.

Distribution. China (Sichuan). Known only from the cave Hua'er Dong (Figs 2, 6). Uéno (2003) gave a detailed description of the cave Hua'er Dong in which there are two main entrances in opposite directions (Fig. 6A–D). The exemplars of *Ue*-

noaphaenops fani (Uéno, 2003) comb. nov. were collected on the wall or under stone in the moist areas about 20–30 m from the left entrance in dark zone. It is sympatric with *Jujiroa wangzheni* sp. nov. (Fig. 6E).

Chu gen. nov.

http://zoobank.org/323F87B4-17A4-48F4-B816-8423C9011A68 Chinese name: 初盲步甲属 Figs 2, 4B, D, 5C, D, 7, 8

Type species. *Chu pheggomisetoides* sp. nov., from the limestone cave Hanwang Dong in Guangyuan, northeastern Sichuan.

Generic characteristics. Medium-sized, somewhat similar to the Balkan genus Pheggomisetes Knirsch, 1923 in appearance especially head and pronotum (Fig. 7); anophthalmic and depigmented; body moderately elongate, with rather thin and slender appendages. Head strongly expanded laterally and convex though shorter than long, two pairs of supraorbital setiferous pores present; frontal furrows long and wellmarked; labrum widely emarginated at front, mandibles widened and developed, apices strongly hooked, right mandibular tooth tridentate; labial suture visible at side, completely disappeared medially (Fig. 4B); mentum bisetose, base largely concave, submentum 8-setose; antennae thin and very long, extending over apices of elytra. Propleura invisible from above; pronotum subcordate, transverse, hind angles very sharp and distinctly protruded backwardly which is similar in *Pheggomisetes*, only presence of the anterior pair of latero-marginal setae. Elytra elongated ovate, twice as long as wide, much longer than fore body; prehumeral angles rounded off; lateral margins well-bordered throughout, finely ciliate on shoulders, whereas smooth on other parts; disc moderately convex, striae noticeable though distinctly reduced; two pairs of dorsal and the preapical setiferous pores present; the humeral group of the marginal umbilicate pores not aggregated, the 1st pore inwardly shifted to the site of 7th stria, only the 2nd pore closest to marginal gutter, 4th shifted posteriad; 5th and 6th pores closely spaced (Fig. 4D). The 1st and 2nd protarsomeres modified in male, distinctly widened and bluntly denticulate inward at apices, and with spongy setae ventrally. Ventrite VII with one pair of apical setae in male, whereas two pairs in female. Male genitalia with median lobe almost straight, suddenly widened at apical part; in lateral view, apex notched dorsally (Fig. 5C, D).

Remarks. Although *Chu* gen. nov. resembles the Balkan cavernicolous genus *Pheggomisetes* Knirsch, 1923 (Knirsch 1923; Vrbica et al. 2017) in the shape of head and pronotum, this must be a case of convergence because the faunas in the Balkan Peninsula and China are completely different. Indeed, there are many cases of large gaps in cave trechines between Europe and China, e.g., *Doderotrechus* from Western Italy



Figure 6. Cave Hua'er Dong, the type locality of *Uenoaphaenops fani* (Uéno, 2003), comb. nov. and *Jujiroa wangzheni* sp. nov. **A–C** environs of the cave, left (yellow arrow) and right (red arrow) entrances **D** entrance to the right **E** a running individual of *Jujiroa wangzheni* sp. nov. in cave. (**A–D** by courtesy of Yuan Li).

vs. *Trechus* of the *dacaitranus* group (*Trechus bastropi*) from Tibet (Faille et al. 2013). Apart from the similarities of the head and pronotum, both *Chu* and *Pheggomisetes* are very different in many aspects including outlines of head, prothorax and elytra, and in particular the chaetotaxic pattern on head and elytra.

As the fauna of subterranean ground beetles are still not well known, the phylogenetic position of *Chu* gen. nov. among Trechini is unknown at present, like many other genera. Its peculiar characters, such as ellipsoidal head with deep emarginated labrum, stout mandible, subcordate pronotum with sharp and backwardly protruded hind angles, and almost straight median lobe which is enlarged and notched at the apex isolate *Chu* gen. nov. from all other Chinese trechines. Beside comparative morphological study, evidence from molecular analysis would be valuable in determining its position.

Etymology. "*Chu*" means "the first time" in Chinese, suggesting that this species was the first subterranean beetle discovered by Li He (the second author). Gender masculine.

Generic range. China (Sichuan) (Fig. 2). Monospecific genus, known only from the cave Hanwang Dong in Chaotian, Guangyuan.

Chu pheggomisetoides sp. nov.

http://zoobank.org/5CB92BFD-836E-4342-8ADD-70DA2C6C4A81 Chinese name: 汉王初盲步甲 Figs 2, 4B, D, 5C, D, 7, 8

Material. *Holotype*: male, cave Hanwang Dong, Zhongbai, Zengjia, Chaotian, Guangyuan, Sichuan (四川省广元市朝天区曾家镇中柏村汉王洞), 32.577297°N, 106.106979°E, 1210 m, 2020-VI-07, leg. Li He & Yuan Li, in SCAU. *Paratypes*: 1 male, *idem*, in SCAU; 1 female, same cave as above, 2020-VI-06, leg. Li He, Yuan Li & Yimei Wen, in CLH; 1 female, same cave, 2018-IV-06, leg. Li He, in SCAU.

Diagnosis. Medium-sized troglobitic beetles, eyeless and lacking pigmentation, somewhat similar to a *Pheggomisetes* species of Bulgaria and Serbia in Balkan Peninsula due to its convex head and subcordate pronotum with very sharpened hind angles.

Description. Length: 6.0-6.5 mm; width: 1.5 mm. Habitus as in Fig. 7.

Body yellow or brown, but antennae, palps and tarsi paler; surface glabrous and smooth though genae sparsely setose and elytra covered with a few, minute, pubescence on lateral margins. Underside of head with a few sparse setae, of thorax and abdominal ventrites glabrous. Microsculpture engraved meshes more or less polygonal on head and pronotum, and irregularly and densely striate on elytra.

Head slightly elongate, ellipsoidal, longer than wide, HLm/HW = 1.67–1.72, HLl/HW = 1.18–1.28; genae expanded laterally, widest a little behind middle of head excluding mandibles, neck constriction broad, moderately defined, frons and vertex strongly convex, anterior and posterior supraorbital setiferous pores narrowly spaced, frontal furrows deep and fairly long, strongly divergent posteriorly, ending beside posterior supraorbital pores; clypeus quadrisetose; labrum transverse, deeply and widely emarginated at frontal margin, 6-setose; mandible stout and widened, curved at apical


Figure 7. Habitus of *Chu pheggomisetoides* gen. nov. & sp. nov., male holotype.



Figure 8. Cave Hanwang Dong, the type locality of *Chu pheggomisetoides* gen. nov. & sp. nov. and *Boreaphaenops liyuani* sp. nov., and some sympatric cave animals **A**, **B** habitat inside the cave, arrow in **A** indicates the place where a *Chu pheggomisetoides* gen. nov. & sp. nov. was found, arrow in **B** indicates the place where the single female of *Boreaphaenops liyuani* sp. nov. was found **C** an individual of *Chu pheggomisetoides* gen. nov. & sp. nov. running in cave **D** a ground beetle's larva was trapped in web of spider *Pseudonesticus* sp. **E** a *Nepalella* millipede **F** a bat **G** a cricket **H** *Gammarus qinling* Hou & Li, 2018 **I** a dipluran.

1/3, strongly hooked at apices, right mandibular tooth very developed; labial suture disappearing medially, making mentum and submentum partly fused; mentum bisetose, base largely concave, tooth short and bifid at apex, about half as long as lateral lobes; submentum 8-setose; ligula 6-setose at apex; palps moderately elongate and glabrous but the 2nd labial palpomere bisetose on inner margin, with an additional seta at outer margin at subapex; 2nd labial palpomere slightly longer than 3rd; 3rd maxillary palpomere as long as 4th; suborbital pores located on ventral side of head, intermedial between neck constriction and submentum (Fig. 4B). Antennae with 10^{th} and 11^{th} antennomeres extending over elytral apices, 1^{st} antennomere smooth and stout, covered several long setae, pubescent from the 2^{nd} antennomere; relative length of each antennomere compared with the 2^{nd} in the holotype as: the $1^{\text{st}}(1.13)$, $2^{\text{nd}}(1.00)$, $3^{\text{rd}}(1.67)$, $4^{\text{th}}(1.73)$, $5^{\text{th}}(1.93)$, $6^{\text{th}}(1.87)$, $7^{\text{th}}(1.80)$, $8^{\text{th}}(1.57)$, $9^{\text{th}}(1.54)$, $10^{\text{th}}(1.43)$ and $11^{\text{th}}(1.57)$.

Pronotum wider than long, PnL /PnW = 0.76-0.83; much shorter than head without mandibles, PnL/HLI = 0.55-0.75; wider than head, PnW/HW = 1.07-1.11; lateral margins and front finely bordered, widest at about 1/3 from front, gently narrowed anteriorly and posteriorly, but strongly curved before base, forming a large and acute hind angle with the arcuate base; anterior latero-marginal setae at about 1/7 from front; front slightly emarginate, distinctly wider than base, Pnb/Pnf = 0.83-0.86; disc moderately convex, mid-line clear, both front and posterior transversal impressions moderately marked. Scutellum large.

Elytra much longer than fore body including mandibles, much longer than wide, EL/EW = 1.90-2.02; much wider than pronotum, EW/PrW = 1.65-1.67; base unbordered, prehumeral part widely rounded, lateral margins finely but well-bordered throughout, widest at about middle, gently and gradually contracted towards base and apices; disc moderately convex though slightly depressed on each elytron near base; striae faint but noticeable; basal pore present at side but behind of scutellum, anterior and posterior dorsal pores along the 3rd stria located at about basal 1/5 and apical 2/5 of elytra respectively, preapical pore at about apical 1/8 of elytra, much closer to suture than to apical margin; locations of the marginal umbilicate pores as in Fig. 4D.

Legs densely pubescent; the 1^{st} tarsomere much, and slightly shorter than $2^{nd}-4^{th}$ combined in fore and middle legs, respectively, whereas as long as in hind ones; tibiae without longitudinal sulci.

Ventrites pubescent; IV–V each with two pairs, VI with three pairs of paramedian setae, and several additional setae which are much shorter; VII bisetose in male, while quadrisetose in female.

Male genitalia (Fig. 5C, D). Median lobe and parameres long and thin, suddenly curved at basal one fourth, then nearly straight towards apex. Basal opening small, without a sagittal aileron; inner sac provided with a long copulatory piece, which is about 1/3 as long as aedeagus; in dorsal view, apical lobe suddenly narrowed before the enlarged apex which is broadly rounded; each paramere armed with four long setae at apex.

Etymology. Refers to the similarity of this new species with a *Pheggomisetes* species from Balkans.

Distribution. China (Sichuan). Known only from the cave Hanwang Dong in Guangyuan (Fig. 2), sympatric with *Boreaphaenops liyuani* sp. nov., *Agonotrechus sinotroglophilus* Deuve, 1999 and *Pterostichus (Huaius) hanwang* Tian & He, 2020.

The exemplars of *Chu pheggomisetoides* gen. nov. & sp. nov. were collected under a stone at the water edge in the innermost main passage in the cave (Fig. 8A–C). In addition to the four ground beetle species mentioned above, other animals found in Hanwang Dong are a *Pseudonesticus* spider, a *Nepalella* millipede, a *Gammarus* amphipod, bats, crickets and diplurans (Fig. 8D–I).

Boreaphaenops liyuani sp. nov.

http://zoobank.org/7FA4C38E-65A2-42F1-BB95-12784F9AFAB9 Chinese name: 李圆北盲步甲 Figs 2, 4E, 9, 10

Material. *Holotype*: female, the cave Hanwang Dong, Zhongbai, Zengjia, Chaotian, Guangyuan, Sichuan (四川省广元市朝天区曾家镇中柏村汉王洞), 32.577297°N, 106.106979°E, 1210 m, 2020-VI-6, leg. Li He, Yuan Li & Yimei Wen, in SCAU.

Diagnosis. An aphaenopsian, small-sized beetle, eyeless and depigmented, body distinctly elongate, with thin and long appendages, densely pubescent on head and elytra, presence of only a dorsal setiferous pore along the 3rd stria on each elytron.

Description. Length: 5.2 mm, width: 1.3 mm. Habitus as in Fig. 9.

Body brown, but antennae, palps and tarsi paler; head and elytra covered with dense pubescence, pronotum glabrous. Underside of head with a few sparse setae, of thorax and abdominal ventrites glabrous. Microsculpture engraved meshes more or less isodiametric on head, strongly transverse on pronotum and densely striate on elytra.

Head similar to B. angustus Uéno, 2002 from the cave Lenre Dong in Shenlongjia, western Hubei Province (Uéno 2002), but with only two pairs of supraorbital setiferous pores instead of three; elongate, much longer than wide, HLm/HW = 2.56, HLl/ HW = 1.58; nearly parallel-sided due to genae not convex instead of slightly expanded; widest at about middle of head excluding mandibles, neck constriction well-marked; frons moderately, and vertex strongly convex respectively; anterior and posterior supraorbital pores located at middle and basal 2/9 of head excluding mandibles, frontal furrows strongly divergent, ended near posterior supraorbital pores; clypeus 6-setose; labrum transverse, straight at frontal margin, 6-setose; mandible thin and elongate, gently hooked apically, right mandibular tooth bidentate though distinctly reduced; labial suture completely disappeared; mentum tooth very small, shorter than half of the lateral lobes, bifid at tip, with two setae on each side of base; ligula adnated with paraglossae, 8-setose at apex; basal foveae large and separated; submentum 10-setose; palps thin, slender and glabrous, but bisetose on inner margin of the 2nd labial palpomere which is very long and 1.70 times as long as 3rd; 3rd maxillary palpomere 1.15 times as long as 4th; suborbital pores intermediate between neck and submentum; antennae pubescent from the 2nd antennomere, 1st antennomere stouter covered with several long setae, slightly longer than 2nd; 3rd to 6th longer, subequal to one another; relative length of each antennomere compared with the 2^{nd} in the holotype as: the 1^{st} (1.05), 2nd (1.00), 3rd (1.82), 4th (1.82), 5th (1.82), 6th (1.82), 7th (1.82), 8th (1.64), 9th (1.64), 10th (1.46) and 11th (1.27).

Prothorax slightly tumid at sides, propleura medially visible from above, slightly wider than pronotum; pronotum similar in *B. angustus* but more elongate, fore angles distinct, hind ones nearly rectangular; much longer than wide, PnL/PnW = 1.32; slightly shorter than head without mandibles, PnL/HLl = 0.95; wider than head, PnW/HW = 1.19; widest at about 3/4 from base; lateral margins almost vanished at 1/4 portion from base, finely bordered in other parts; base and front nearly straight,



Figure 9. Habitus of *Boreaphaenops liyuani* sp. nov., female holotype.

unbordered, the former narrower than the latter, Pnb/Pnf = 0.69; anterior latero-marginal setae at about 1/6 from front, posterior ones before hind angles; disc moderately convex, mid-line clear, both front and posterior transversal impressions faintly marked. Scutellum small and elongated.

Elytra longer than fore body including mandibles, much longer than wide, EL/ EW = 1.94; nearly twice as wide as prothorax, EW/PrW = 1.96; base unbordered; similar in *B. angustus* but devoid of humeral angles, lateral margins finely but wellbordered throughout, smooth; widest at about middle, gently contracted towards base but strongly to apices; disc moderately convex though depressed near base just behind basal pores; striae faint but well-indicated from the 1st to 4th; basal pore present at sides of scutellum, only a median dorsal pore present along the 3rd stria at a little behind middle; preapical pore present at about apical 1/7 of elytra, much closer to suture than to apical margin; marginal umbilicate pores well-marked, 2nd (Fig. 4E).

Legs densely pubescent; 1st tarsomere much and slightly shorter than 2nd-4th combined in fore and middle legs, whereas as long in hind ones; tibiae without lon-gitudinal sulci. Abdominal ventrite IV–VI each with two pairs of paramedial setae, ventrite VII quadrisetose.

Male. Unknown.

Etymology. In honour of Mr. Yuan Li (Deyang, Sichuan), a co-collector of the type material.

Remarks. The cave Hanwang Dong is about 400 km in a straight line from Lengre Dong, the locality of B. angustus Uéno, 2002, though both localities belong to same range of the Daba-Micang Mountains. Although B. liyuani sp. nov. has several differences from *B. angustus* which are probably of generic importance, e.g. completely fused mentum and submentum (labial suture visible in the latter species), thin and straight mandibles with tooth distinctly reduced (well-developed in B. angustus), smooth elytral lateral margins (ciliate in B. angustus), and very long 2nd labial palpomere which is 1.7 time as long as the 3rd (such a feature never observed in other Chinese cave trechines), we prefer to describe it as a member of Boreaphaenops at present as only a single female exemplar is available. In addition, it also differs from B. angustus in having smaller body size, presence of only a pair of posterior supraorbital pores on the head instead of two, pronotum well-angulate on hind and fore angles instead of rounded, lack of prehumeral angles of elytra which have only a single dorsal setiferous pore along the 3rd stria instead of three in *B. angustus*, and with equidistant prehumeral pores of the marginal umbilicate series, vs. 4th pore far from 3rd in *B. angustus*.

Distribution. China (Sichuan). Known only from the cave Hanwang Dong in Guangyuan (Fig. 2).

Boreaphaenops liyuani sp. nov. is the first representative of the genus from Sichuan Province, living together with *Chu pheggomisetoides* gen. nov. & sp. nov., *Agonotrechus* sinotroglophilus Deuve, 1999 and *Pterostichus* (*Huaius*) hanwang Tian & He, 2020. It



Figure 10. An individual of Boreaphaenops liyuani sp. nov. running in cave.

is very rare in the cave: three surveys carried out by SCET led to the discovery of only a single female, which was collected under a stone at the water edge in the innermost main passage in the cave (Fig. 10).

Agonotrechus sinotroglophilus Deuve, 1999

Figs 2, 11

Deuve 1999: 152; Deuve and Tian 2016: 352

Material. 1 male, cave Hanwang Dong, Zhongbai, Zengjia, Chaotian, Guangyuan, Sichuan (四川省广元市朝天区曾家镇中柏村汉王洞), 32.577297°N, 106.106979°E, 1210 m, 2018-IV-06, leg. Li He, in SCAU; 1 female, same cave, 2020-VI-06, leg. Li He, Yuan Li & Yimei Wen, in CLH.

Diagnosis. A troglophilous species though depigmented, macrophthalmic, body stout, with short appendages, developed frontal furrows on head, humeral set of the marginal umbilicate pores aggregated. Habitus as in Fig. 11.

Remarks. This species was formerly reported from two limestone caves in Chongqing Municipality (Deuve 1999; Deuve and Tian 2016). This is the first record in Sichuan Province.

Distribution. China (Chongqing and Sichuan) (Fig. 2).

The exemplars of *Agonotrechus sinotroglophilus* Deuve, 1999 were collected under stone in the moist area about 100–200 m from the entrance in Hanwang Dong.



Figure 11. Habitus of Agonotrechus sinotroglophilus Deuve, 1999, male.

Tribe Platynini Bonelli, 1810 Genus *Jujiroa* Uéno, 1952

Jujiroa uenoi sp. nov.

http://zoobank.org/B466CD2F-3ABC-47E0-8EF9-3B4B7344A305 Chinese name: 上野穴胫步甲 Figs 2, 12, 13A, 14, 15

Material. *Holotype:* male, cave Banche Dong, Jianshanzi, north side of the Dadu River, Shawan, Leshan, Sichuan (四川省乐山市沙湾区大渡河北岸尖山子搬车洞), 29.21043°N, 103.58349°E, 670 m, 2020-V-30, leg. Li He, Yuan Li & Hao Long, in SCAU. *Paratype:* 1 female, *idem*, in SCAU.

Diagnosis. Medium-sized *Jujiroa* species, body depigmented, microphthalmic, head thin and slightly expanded at sides, antennae not extending to apices of elytra, fore angles of pronotum distinctly protruded, elytra mucronate at apices, striae finely punctate, presence of two dorsal pores along the 2nd stria, tarsi smooth.

Description. Length: 15.0–15.5 mm; width: 4.5 mm. Habitus as in Fig. 12.

Body concolorous yellow or brown, but a little darker on head, surface smooth and glabrous (though rough on sides and lateral margins of pronotum), moderately shiny. Microsculptural meshes finely and transversely striate on head, pronotum and elytra.

Head thin and elongate, much longer than wide, HLm/HW = 2.06-2.11, HLl/ HW = 1.53-1.48; widest just behind the level of eyes; genae convex, and slightly expanded at side; frontal furrows short and shallow, ending before the level of anterior supraorbital pores; two pairs of supraorbital pores present, anterior at about middle of head from labrum to neck, posterior about basal 2/7 of head; eyes very small and flat; clypeus bisetose, labrum bisinuate at front margin, 6-setose; mandibles elongated, teeth reduced; labial suture clear; mentum with two setae on each side just in front of the basal pits which are very small; median tooth short, about half as long as lateral lobes, bluntly bifid at tip; submentum with two setae on each side, inner ones longer; ligula short, widened and truncated at apical margin, bisetose; palpomeres long and slender, the 2nd labial palpomere bisetose on inner margin, 1.2 times as long as 3rd, the 3rd maxillary palpomere as long as 4th; antennae filiform, thin and very long, extended to apical 1/10 (male) or 1/9 (female) of elytra, the 1st to 3rd antennomeres glabrous, each of the 1st and 2nd with a seta near apex, pubescent from the 4th; the 2nd shortest, while 4th longest; relative length of each antennomere compared with the 2nd in the holotype as: the 1st (2.50), 2nd (1.00), 3rd (2.50), 4th (2.93), 5th (2.58), 6th (2.07), 7th (2.29), 8th (2.00), 9th (1.88), 10th (1.64) and 11th (1.71).

Pronotum subcordate, transverse, PL/PW = 0.91-0.92, but a little longer than wide measured through fore angles; much wider but slightly shorter than head, PW/HW = 1.09-1.12, PL/HLl = 0.86-0.96; widest at about 2/5 from front, lateral margins including front and hind angles widely and strongly reflexed throughout, gently and gradually narrowed towards hind angles which are nearly rectangular, fore angle extraordinarily and forwardly protruded, forming a obtuse lobe; basal foveae short but



Figure 12. Habitus of Jujiroa uenoi sp. nov., male holotype.



Figure 13. Elytral chaetotaxy of *Jujiroa* species **A** *J. uenoi* sp. nov., male holotype **B** *J. wangzheni* sp. nov., female holotype.

well-marked; only basal latero-marginal setae present, inserted just on the hind angles; entire lateral margins and front without borders, base finely bordered, slightly narrower than front including front angles, PbW/PfW = 0.87-0.88; both base and front nearly straight. Scutellum small.



Figure 14. Male genitalia of *Jujiroa uenoi* sp. nov. **A** median lobe, lateral view **B** apical part of median lobe, dorsal view **C** parameres **D** genital ring, ventral view.

Elytra elongate, amygdaloid, much longer than wide, EL/EW = 1.80-1.89; distinctly longer than forebody including mandibles, much wider than pronotum; base well-bordered (but unbordered against the 1st interval), shoulders nearly rounded; widest at about middle of elytra, apex distinctly protruded, mucronate; disc convex, marginal depressions well-defined and reflexed throughout, the 9th interval suddenly deepened and distinctly curved at about basal 1/4; striae entire, impressed by small but deep punctures, intervals almost flat; scutellar striole short; basal pores present; the 3rd interval with two setiferous pores close to 2nd stria at about apical 1/5 and 3/7 of elytra respectively; three pores present on 7th stria posteriorly; an apical pore present at apical anastomosis of the 1st and 4th striae; presence of 23–24 marginal umbilicate pores throughout, continuous (Fig. 13A).

Legs slender and elongate, procoxae asetose, mesocoxae unisetose, metacoxae trisetose (the inner setae present); each trochanter with a single seta; metafemur unisetose posteriorly; tibiae and tarsi smooth, without longitudinal sulci or striae externally; the 4th tarsomere bilobed in fore and middle legs, deeply emarginated in hind ones; protarsi not modified in male, but 1st -3^{rd} each with two spongy setae on ventral surface. Ventral surface smooth and glabrous. Each abdominal ventrite IV–VI bisetose, ventrite VII bisetose in male, quadrisetose in female.

Male genitalia (Fig. 14A–D). Median lobe of aedeagus very slender and elongate, gently arcuate in middle portion, then gradually narrowed towards apex which is bluntly pointed; base moderately opened, presence of a small sagittal aileron; parameres developed. In lateral view, apical lobe thin, slightly longer than wide.

Remarks. Similar to *Jujiroa zhouchaoi* Tian & He, 2020 and *J. satoi* Uéno, 2005, but having much longer antennae and distinctly mucronated apices on the elytra. In addition, it is easily distinguished from *J. zhouchaoi* by a broader head, flat intervals and large punctate striae of the elytra and from *J. satoi* by a slenderer body with a thin head, and the presence of dorsal pores on the elytra. Furthermore, *Jujiroa uenoi* sp. nov. has a peculiar character state: presence of an inner seta on each metacoxa, which is absent in other species of *Jujiroa*.

Etymology. Dedicated to the late Dr Shun-Ichi Uéno, Science Museum (Natural History), Tokyo.

Distribution. China (Sichuan). Known only from the cave Banche Dong in Leshan (Fig. 2).

Cave Banche Dong is about 1.6 km away in a straight line from Xiaodouyan Tiankeng (硝斗岩天坑), which is a well-known touristic site in Leshan. The opening of the entrance is so narrow that it allows only one person to crawl in at a time (Fig. 15A). The interior of the cave is small and moist (Fig. 15B). The two individuals of *Jujiroa uenoi* sp. nov. were found running on the ground inside cave (Fig. 15C–D). Other animals observed in the cave are *Leopoldamys* rats, crickets, moths and *Serriphaedusa* snails (Fig. 15E–H).

Jujiroa wangzheni sp. nov.

http://zoobank.org/12F04E26-A163-40D0-A8ED-DE16EEA5F6B4 Chinese name: 王震穴胫步甲 Figs 2, 6, 13B, 16

Material. Holotype: female, cave Hua'er Dong, Xiangdingshan, Xiangding, Shiping, Gulin, Luzhou, Sichuan (四川省泸州市古蔺县石屏镇向顶村象顶山华儿洞), 28.028931°N, 106.00716°E, 640 m, 2020-XI-24, leg. Yuan Li & Zhen Wang, in SCAU. Paratype: 1 female, idem, in SCAU.

Diagnosis. A small-sized *Jujiroa* species, depigmented, body and appendages elongate, microphthalmic, head thin and slightly expanded medially, fore angles of pronotum moderately protruded, elytral striae largely punctate, mucronate at apices, absence of dorsal pores, tarsi longitudinally sulcate.

Description. Length: 12.5 mm; width: 3.6 mm. Habitus as in Fig. 16.

Body concolorous yellow but a little darker on head, surface smooth and glabrous (though rough on sides and lateral margins of pronotum), moderately shiny. Microsculptural meshes transversely and densely striate on head, pronotum and elytra.



Figure 15. Cave Banche Dong, the type locality of *Jujiroa uenoi* sp. nov., and some sympatric cave animals **A** Li He in front of Banche Dong **B** environs inside cave, arrow indicating the place where a *J. uenoi* was found **C** Yuan Li is collecting **D** an individual of *J. uenoi* running in cave **E** *Leopoldamys edwardsi* (Thomas, 1882) **F** a cricket **G** a moth **H** *Serriphaedusa serrata* Deshayes, 1870.

Head thin and elongate, much longer than wide, HLm/HW = 2.00, HLl/HW = 1.48; widest exactly at the eyes location; frons nearly flat, vertex strongly convex medially, neck constriction wide; genae convex, and slightly expanded at side; frontal furrows short and shallow, ending before the level of anterior supraorbital pores; two pairs of supraorbital pores present, anterior near inner margin of eye; eyes very small and flat; clypeus bisetose, labrum bisinuate at front margin, 6-setose; mandibles elongate, teeth reduced; labial suture clear; mentum with two setae on each side, basal pits small; median tooth short, half as long as the lateral lobes, bifid at tip; submentum



Figure 16. Habitus of *Jujiroa wangzheni* sp. nov., male holotype.

with two setae on each side, inner ones much longer; ligula short, widened and truncated at apical margin, bisetose; palpomeres long and slender, the 2nd labial palpomere bisetose on inner margin, 1.2 times as long as 3rd, the 3rd maxillary palpomere slightly longer than 4th; antennae filiform, thin and very long, extended to apices of elytra, the 1st to 3rd antennomeres glabrous, the 1st and 2nd with a seta near apex, pubescent from the 4th; the 2nd shortest, while 5th longest; relative length of each antennomere compared with 2nd antennomere in the holotype as follows: the 1st (1.88), 2nd (1.00), 3rd (2.07), 4th (2.89), 5th (2.76), 6th (2.89), 7th (2.35), 8th (2.14), 9th (2.00), 10th (1.57) and 11th (1.64).

Pronotum subcordate, nearly as long as wide, PL/PW = 0.98; wider but slightly shorter than head, PW/HW = 1.38, PL/HLI = 0.88; widest at about 2/5 from front, lateral margins including front and hind angles widely reflexed throughout, gently and gradually straight towards hind angles, fore angles roundly and moderately protruded forwardly, hind angles rectangular; basal foveae large and shallow; only basal lateromarginal setae present, inserted on the hind angles; entire lateral margins and front without borders, base finely bordered, slightly wider than front, PbW/PfW = 1.10; both base and front nearly straight. Scutellum small, short.

Elytra elongate, amygdaloid, much longer than wide, EL/EW = 2.01; distinctly longer than forebody including mandibles, much wider than pronotum; base well-bordered (but interrupted against the 1st interval), shoulders nearly rounded; widest at about 4/7 of elytra from base, apex sharply protruded, distinctly mucronate; disc convex, striae entire, impressed by large and nearly rounded punctures; scutellar striole short; basal pores present; without setiferous pore on the 3rd interval, and absence of the preapical pore; two pores present on 7th stria posteriorly; an apical pore present on each elytron; marginal umbilicate pores present throughout, continuous (Fig. 13B).

Legs very thin and slender, procoxae asetose, mesocoxae unisetose, metacoxae bisetose (without inner setae); each trochanter with a single seta; metafemur bisetose posteriorly; tibiae and tarsi smooth, without longitudinal sulci externally; the 4th tarsomere bilobed in fore and middle legs, while deeply emarginated in hind ones.

Ventral surface smooth and glabrous. Each abdominal ventrite IV–VI bisetose, ventrite VII, quadrisetose.

Male. Unknown.

Remarks. More or less similar to *J. deliciola* Uéno & Kishimoto, 2001 (from two caves in Xingwen County, Yibin, southern Sichuan) (Uéno and Kishimoto 2001; Tian and He 2020) in having a thin and elongate body, and distinctly mucronate elytral apices, but *J. wangzheni* sp. nov. is easily recognized by a less shiny and less glabrous body, widened elytra base, strongly convex intervals and largely punctate striae of elytra which are devoid of dorsal pores on 3rd elytral interval and without preapical pores.

Etymology. The new species is dedicated to Mr. Zhen Wang (Chengdu, Sichuan), a co-collector of the type exemplars.

Distribution. China (Sichuan). Known from the cave Hua'er Dong in Gulin County, southeastern Sichuan (Fig. 2).

The specimens of *J. wangzheni* sp. nov. were collected by baited traps in a chamber at about 30–50 m inside of the left entrance of Hua'er Dong. The species is sympatric with *Uenoaphaenops fani* (Uéno, 2003) (Fig. 6E).

Key to species of the genus Jujiroa Uéno, 1952 from Sichuan Province

Large-sized, over 19.0 mm long; interval 3 with five discal pores
J. lingguanensis Deuve & Pütz
Medium, or small-sized, less than 16.0 mm long; interval 3 with at most
three discal pores
Apices of elytra not mucronate
Apices of elytra distinctly and sharply protruded, mucronate4
Head slightly expanded laterally, elytra without dorsal setiferous pores
Head thin, nearly parallel-sided; elytra with two dorsal setiferous pores
Eyes slightly convex, pronotum longer than wide
<i>J. deliciola</i> Uéno & Kishimoto
Eyes atrophied, flat, pronotum transverse5
Antennae long, reaching the apices of elytra, elytra without dorsal pores along
the 3 rd stria <i>J. wangzheni</i> sp. nov.
Antennae short, not extending to apices of elytra, elytra with dorsal pores
along the 3 rd stria
Metacoxae trisetose (inner setae present), head thin, pronotum with fore an-
gles strongly protruded forwardly, lobed, base narrower than front
Metacoxae bisetose (inner setae absent), head broad, pronotum with fore an-
gles moderately protruded forwardly, not lobed, base as wide as front
<i>J. iolandae</i> Vigna Taglianti

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RESEARCH ARTICLE



New Metalimnobia crane flies (Diptera, Limoniidae) from China with an update of species distributions

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Abstract

Two new species of the genus *Metalimnobia* Matsumura, 1911 from southwest China, *M.* (*M.*) *bicolor* **sp. nov.** and *M.* (*M.*) *caudifusca* **sp. nov.**, are described and illustrated. The new species can be distinguished from congeners by their wing patterns and male genitalia. New distributional data are given for the following species: *M.* (*M.*) *bifasciata* (Schrank, 1781), *M.* (*M.*) *quadrinotata* (Meigen, 1818) and *M.* (*M.*) *tenua* Savchenko, 1976. An updated key for all Chinese *Metalimnobia* crane flies is presented.

Keywords

Classification, distribution, identification key, Limoniinae, Limoniini, new species, taxonomy

Introduction

Metalimnobia Matsumura, 1911 is a small genus in the family Limoniidae with 48 known species/subspecies. The genus is characterized by the following characters: body medium-sized to large, wing length more than 10 mm; antenna with 12 or 13 flagel-lomeres, palpus with five segments; wing distinctly patterned with dark brown spots or markings and often with smoky areas; R_{1+2} and R_2 short and transverse, almost ending at the same level, basal section of CuA₁ before the fork of M; male gonocoxite with a large ventro-mesal lobe, inner gonostylus often divided into three lobes; ovipositor

with a short and small cercus and large hypovalva (Dienske 1987; Podenas and Gelhaus 2007; Salmela and Starý 2009; Mao and Yang 2010; Podenas and Byun 2016).

Members of the genus *Metalimnobia* are grouped into three subgenera: *Metalimnobia* (s. str.) (34 species/subspecies), *Tricholimonia* Alexander, 1965 (11 species) and *Lasiolimonia* Alexander, 1976 (three species). The nominotypical subgenus is known from the Palaearctic (15 species/subspecies), Nearctic (12 species) and Oriental (11 species/subspecies) regions. The other two subgenera are known only from the Afrotropical region (Oosterbroek 2020).

Nine *Metalimnobia* crane flies all belonging to the nominotypical subgenus have been recorded from China (Oosterbroek 2020), of which five were published by Mao and Yang (2010). In this paper, two new species are added to the Chinese fauna. Examination of specimens from several localities in China also revealed new distribution records for *M.* (*M.*) *bifasciata* (Schrank, 1781), *M.* (*M.*) *quadrinotata* (Meigen, 1818) and *M.* (*M.*) *tenua* Savchenko, 1976. A dichotomous key modified from Mao and Yang (2010) for all Chinese *Metalimnobia* crane flies is given.

Materials and methods

Specimens for this study were collected from several localities in China by different entomologists in the period 2009–2019. Adult crane flies were collected by insect net, Malaise trap and light trap. Type specimens of known *Metalimnobia* species deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (**USNM**), the Natural History Museum, London, UK (**NHM**) and the Entomological Museum of China Agricultural University, Beijing, China (**CAU**) were also examined. Type specimens of the new species were deposited in CAU. Other specimens were deposited in the Entomological Museum of Qingdao Agricultural University, Shandong, China.

Genitalic preparations of males were made by macerating the apical portion of the abdomen in cold 10% NaOH for 12–15 hours. Observations and illustrations were made using a ZEISS Stemi 2000-C stereomicroscope. Photographs were taken with a Canon EOS 77D digital camera through a macro lens. The morphological terminology mainly follows McAlpine (1981), and the venation is described after Alexander and Byers (1981). The following abbreviations in figures are used: **tg 9** = ninth tergite, **tg 10** = tenth tergite, **st 9** = ninth sternite, **goncx** = gonocoxite, **o gonst** = outer gonostylus, **i gonst** = inner gonostylus, **aed** = aedeagus, **pm** = paramere, **cerc** = cercus, **hyp vlv** = hypogynial valve.

Taxonomy

Key to Chinese Metalimnobia crane flies

1	Wing yellowish	2
_	Wing grayish or brownish (Figs 2, 5d)	3

2	Femora yellow with tips brown
_	Femora dark brown with subapical yellow rings
3	Wing with Sc ₁ ending at about 2/3 of Rs, inner gonostylus divided into four
	lobes
_	Wing with Sc ₁ ending close to or beyond fork of Rs (Figs 2, 5d), inner gono-
	stylus divided into three lobes (Figs 3, 6)4
4	Wing without spot at base (Fig. 5d)
_	Wing with one or more spots at base (Fig. 2)5
5	Mid and hind femora each with apical ring
_	Mid and hind femora each with two rings (Figs 1a, 5a)6
6	Subapical rings of mid and hind femora as dark and wide as apical rings
_	Subapical rings of mid and hind femora paler than apical rings and spreading
	over half of femora (Figs 1a, 5a)7
7	Wing spots at origin and fork of Rs with upper parts brown and lower parts
	brownish black (Fig. 2)
_	Wing spots at origin and fork of Rs uniformly dark brown (Fig. 5d)8
8	Pleuron of thorax mostly black, paramere with tuft of hairs at tip (Fig. 4h)
_	Pleuron of thorax mostly brownish yellow, paramere without hair at tip9
9	Outer gonostylus slender, nearly as wide as innermost lobe of inner gonosty-
	lus M. (M.) impubis Mao & Yang, 2010
_	Outer gonostylus broad, about twice as wide as innermost lobe of inner gono-
	stylus
10	Paramere with tip flattened
_	Paramere with tip narrow and acute (Fig. 4b, e)

Metalimnobia (Metalimnobia) bicolor sp. nov.

http://zoobank.org/59181930-7D58-414F-952B-3B4A56E74E0E Figures 1–3

Type material. *Holotype* male, China: Sichuan, Batang, Deda (30°17'43"N, 99°23'50"E, 3727 m), 2019.VII.17, Liang Wang. *Paratypes:* 1 male 1 female, same data as holotype.

Diagnosis. Pleuron brownish black with brownish yellow stripe extending from pronotum to base of wing. Femora each with two rings, apical ring brown, subapical ring slightly paler and spreading over half of femora. Wing brownish with one or more brown spots at base, two large spots at origin and fork of Rs with upper parts brown and lower parts brownish black. Sc₁ ending beyond fork of Rs, Sc₂ shorter than Sc₁;



Figure 1. *Metalimnobia (Metalimnobia) bicolor* sp. nov. **a** male habitus, lateral view **b** head, lateral view **c** thorax, dorsal view **d** ovipositor, lateral view. Scale bars: 2.0 mm (**a**); 0.5 mm (**b**, **c**); 0.2 mm (**d**).

basal section of CuA₁ before fork of M. Inner gonostylus divided into three lobes. Paramere with distal part long and nearly straight, tip flattened and bare.

Description. Male. Body length 10.5–10.8 mm, wing length 11.8–12.0 mm.

Head (Fig. 1b) brown with vertex and frons dark brown. Setae on head dark brown. Antenna length 2.5–2.8 mm, brownish black with scape dark brown. Scape cylindrical; pedicel nearly globose; flagellomeres oval with long setae, each flagellomere slightly narrower than previous one, terminal flagellomere elongated. Rostrum dark brown with dark brown setae. Palpus brownish black with brownish black setae.

Thorax (Fig. 1c). Pronotum brownish black with lateral margins brown. Prescutum brown with four broad, brownish black longitudinal stripes, two lateral stripes about 1/2 length of median stripes. Scutum brown with middle area brownish yellow; each lobe with two large brownish black spots, anterior spot connected with lateral stripe of prescutum. Scutellum brownish black with middle area brownish yellow. Mediotergite brownish black, posterior area with two nearly confluent pale brownish yellow spots. Pleuron (Fig. 1a) brownish black with a brownish yellow stripe extending



Figure 2. Variations of wing pattern of Metalimnobia (Metalimnobia) bicolor sp. nov. Scale bars: 1.0 mm.

from pronotum to base of wing. Setae on thorax brownish black. Coxae brown; trochanters pale yellow; femora each brownish yellow with two rings, apical ring brown, subapical ring slightly paler and spreading over half of femora; tibiae brownish yellow with tip dark brown; tarsi brownish black, basal 1/3-1/2 of first tarsal segments brownish yellow. Setae on legs brownish black. Wing (Fig. 2) brownish with brown to brownish black pattern: long oval, brown spot at wing base (Fig. 2a), often divided into two or three small spots (Fig. 2b, c); two large spots at origin and fork of Rs, each spot with upper part pale brown and lower part brownish black; stigma brown with each side darker; brown seams along cord, m-m, basal section of M₃ and base of CuA; obscure, irregular brownish clouds in most cells, darker near R₃. Veins brownish yellow, darker in clouded areas. Venation: Sc long, Sc₁ ending beyond fork of Rs; Sc₂ a greater distance before tip of Sc₁, Sc₁ 1.5–2 times as long as Sc₂; basal section of CuA₁



Figure 3. *Metalimnobia* (*Metalimnobia*) *bicolor* sp. nov. **a** male hypopygium, dorsal view **b** male hypopygium, ventral view. Scale bars: 0.2 mm.

1/2-2/3 of its own length before fork of M. Halter length 1.7–1.8 mm, white with knob dark brown.

Abdomen (Fig. 1a). Tergites 1–4 brownish black with lateral regions brown, tergites 5–8 black. Sternites 1–3 brown, sternites 4–8 black.

Hypopygium (Fig. 3). Posterior margin of ninth tergite emarginate, each lobe with several setae. Gonocoxite slender with a large, elongate and caudally curved ventromesal lobe. Outer gonostylus broad, arched at 2/3 length, outer third narrowing into a flattened spine. Inner gonostylus divided into three lobes: innermost lobe long, slender and curved; dorsal fleshy lobe fingerlike with long setae; ventral fleshy lobe oval with long setae. Paramere wide basally with distal part long and nearly straight, tip flattened and bare. Aedeagus long, slightly enlarged before tip.

Female. *Body* length 12.0 mm, wing length 11.0 mm. Similar to male, but tergites 6–9 brownish black with lateral regions brown, sternites 5–9 brown. Tenth tergite (Fig. 1d) brown. Cercus dark brown, base wide, tip pointed. Hypogynial valve dark brown with middle area of posterior half paler, tip reaching middle of cercus.

Distribution. China (Sichuan).

Etymology. The specific name refers to the wing spots at the origin and fork of Rs which have brown upper parts and brownish black lower parts.

Remarks. *Metalimnobia* (*M*.) *bicolor* sp. nov. can readily be distinguished from all other *Metalimnobia* crane flies known from China by the wing spots at the origin and fork of Rs. This species has a somewhat similar wing to the widespread *M*. (*M*.) *tenua* but can be easily distinguished from the latter by the antenna with dark brown scape and brownish black pedicel, the mostly brownish black pleuron, the brown coxae, and the paramere being wide basally with a long and nearly straight distal part and a flattened tip (Figs 3, 4a, 4d). In *M*. (*M*.) *tenua*, the scape and pedicel of the antenna are yellowish brown, the pleuron is mostly yellowish brown, the coxae are pale yellow, and the paramere is subtriangular with an acute tip (Fig. 4b, e).



Figure 4. Details of male genitalia of *Metalimnobia* **a**-**c** parametes and aedeagus, dorsal view **a** *M*. (*M*.) *bicolor* sp. nov. **b** *M*. (*M*.) *tenua* **c** *M*. (*M*.) *caudifusca* sp. nov. **d**-**h** tip of paramete **d** *M*. (*M*.) *bicolor* sp. nov. **e** *M*. (*M*.) *tenua* **f** *M*. (*M*.) *caudifusca* sp. nov. **g** *M*. (*M*.) *quadrimaculata* **h** *M*. (*M*.) *rectangularis* **g**, **h** after Mao and Yang 2010.

Metalimnobia (Metalimnobia) bifasciata (Schrank, 1781)

Tipula bifasciata Schrank, 1781: 429. Type locality: Austria, Linz *Limonia xanthoptera* Meigen, 1804: 56. Type locality: not given

Linonia xaninopiera ivieigen, 1804. 90. Type locality. not given

Metalimnobia vittata Matsumura, 1911: 63. Type locality: Russia, Sakhalin, Naiptchi Limnobia avis avis Alexander, 1918: 444. Type locality: Japan, Honshu, Shinano, Takuhara

Limnobia avis flavoabdominalis Alexander, 1918: 445. Type locality: Japan, Honshu

Specimens examined. 1 male, China: Sichuan, Yanyuan, Lugu Lake (27°43'00"N, 100°54'18"E, 2673 m), 2019.VII.19, Liang Wang (light trap).

Diagnosis. Pleuron yellow. Femora yellow with apical brown rings. Wing yellowish without spot at base. Sc_1 ending close to fork of Rs, Sc_2 longer than Sc_1 ; basal section of CuA_1 close to or beyond fork of M. Outer gonostylus broad, arched at 2/3 length, outer angle extended and darkened. Inner gonostylus undivided. Paramere with distal part long and nearly straight, tip blunt with several hairs on inside.

Distribution (new record in bold). China (Beijing, Guizhou, Hebei, Heilongjiang, Hubei, Jilin, Liaoning, Ningxia, Shaanxi, Shanxi, **Sichuan**); Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Rep., Denmark, Estonia, Finland, France, Germany, Georgia, Great Britain, Hungary, Ireland, Italy, Japan, Kazakhstan, ?Latvia, Lithuania, Luxembourg, Mongolia, Netherlands, North Korea, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, South Korea, Sweden, Switzerland, Tajikistan, Turkey, Ukraine (Oosterbroek 2020).

Remarks. *Metalimnobia* (*M.*) *bifasciata* is widely spread in the Palearctic and Oriental regions. In China, this species is known in many provinces and is now recorded in Sichuan for the first time. For descriptions and illustrations of this species, see Alexander (1918), Boardman (2007), Mao and Yang (2010), Matsumura (1911), Nakamura (2006), Podenas et al. (2006), Podenas and Gelhaus (2007) and Podenas and Byun (2016).

Metalimnobia (Metalimnobia) caudifusca sp. nov.

http://zoobank.org/FD4F8AD7-031D-48A5-8A7D-7D983C27F7EB Figures 5, 6

Type material. *Holotype* male, China: Xizang, Bayi, Tibet agriculture and Animal Husbandry University (29°39'46"N, 94°20'43"E, 3000 m), 2014.VIII.22–IX.18, Baohai Wang (Malaise trap). *Paratypes:* 1 male, same data as holotype. 1 male, China: Xizang, Bayi, Lulang (29°43'10"N, 94°42'06"E, 3800 m), 2009.VIII.2, Maoling Sheng.

Diagnosis. Pleuron yellow. Fore femur yellow with apical brown ring; mid and hind femora each with two rings, apical ring brown, subapical ring pale brown and spreading over half of femur. Wing grayish without spot at base. Sc_1 ending beyond fork of Rs, Sc_2 shorter than Sc_1 ; basal section of CuA_1 before fork of M. Inner gonostylus divided into three lobes; dorsal fleshy lobe arched at 2/3 length, tip pointed. Paramere with distal part long, slender and slightly curved outwards, tip angulate with tuft of hairs.

Description. Male. Body length 9.5-10.5 mm, wing length 11.5-13.0 mm.

Head (Fig. 5b) brownish yellow with vertex brown and frons yellow. Setae on head brown. Antenna length 2.2–2.5 mm, yellow with flagellomeres brown. Scape cylindrical; pedicel nearly globose; flagellomeres oval with long setae, each flagellomere slightly narrower than previous one, terminal flagellomere elongated. Rostrum brown with dark brown setae.

Thorax (Fig. 5c). Pronotum brown with lateral regions brownish yellow. Prescutum yellow with four broad, brown longitudinal stripes, lateral stripes about 1/2 length of median stripes. Scutum yellow, each lobe with two large pale brown spots, anterior spot connected with lateral stripe of prescutum. Scutellum yellow with lateral regions pale brown. Mediotergite yellow, base with two pale brown spots. Pleuron (Fig. 5a) yellow. Setae on thorax dark brown. Coxae yellow. Trochanters pale yellow. Fore femur yellow, tip with brown ring; mid and hind femora each yellow with two rings, apical ring brown, subapical ring pale brown and spreading over half of femur. Tibiae brown. Tarsi brown, bases of first tarsal segments slightly paler. Setae on legs dark brown.



Figure 5. *Metalimnobia (Metalimnobia) caudifusca* sp. nov. **a** male habitus, lateral view **b** head, lateral view **c** thorax, dorsal view **d** wing. Scale bars: 2.0 mm (**a**); 0.5 mm (**b**, **c**); 1.0 mm (**d**).

Wing (Fig. 5d) grayish with pale brownish yellow to pale brown pattern: large, pale brown spots at origin and fork of Rs; stigma pale brown with middle area paler; very pale brown seams along cord, m-m, basal section of M_3 and base of CuA; obscure, irregular pale brownish yellow clouds in most cells. Veins pale brownish yellow, darker in clouded areas. Venation: Sc long, Sc₁ ending beyond fork of Rs; Sc₂ a greater distance before tip of Sc₁, Sc₁ 1.2–3 times as long as Sc₂; basal section of CuA₁ 1/4–1/2 of its own length before fork of M. Halter length 1.7–1.9 mm, pale yellow.

Abdomen (Fig. 5a). Tergites 1–5 yellow to brownish yellow, tergites 6–8 brown. Sternites 1–6 yellow, sternites 6–8 brown.



Figure 6. *Metalimnobia* (*Metalimnobia*) *caudifusca* sp. nov. **a** male hypopygium, dorsal view **b** male hypopygium, ventral view. Scale bars: 0.2 mm.

Hypopygium (Fig. 6). Posterior margin of ninth tergite emarginate, each lobe with several setae. Gonocoxite slender with short, rounded and apically blunt ventro-mesal lobe. Outer gonostylus broad, arched at 2/3 length, outer third narrowing into a flattened spine. Inner gonostylus divided into three lobes: innermost lobe long, slender and curved; dorsal fleshy lobe long and stout with long setae, arched at 2/3 length, tip pointed; ventral fleshy lobe oval with long setae. Paramere wide basally with distal part long, slender and slightly curved outwards, tip angulate with tuft of hairs. Aedeagus long and slender.

Female. Unknown.

Distribution. China (Xizang/Tibet).

Etymology. The specific name refers to the caudal segments of the abdomen being uniformly brown.

Remarks. Some *Metalimnobia* crane flies that occur in China also have a tuft of hairs at the tip of the parameres (e.g., *bifasciata*, *quadrimaculata*, *rectangularis* and *xanthopteroides*). However, the wing of M. (M.) *caudifusca* sp. nov. is grayish, while the wings of both M. (M.) *bifasciata* and M. (M.) *xanthopteroides xanthopteroides* are yellowish. *Metalimnobia* (M.) *caudifusca* sp. nov. can also be easily distinguished from M. (M.) *quadrimaculata* and M. (M.) *rectangularis* by the wing pattern (*caudifusca* has no spot at the base of the wing, but *quadrimaculata* and *rectangularis* have a spot at the base of their wings) and the shape of the paramere (Fig. 4c, f–h). Notably, the type specimens of M. (M.) *yunnanica* deposited in USNM and NHM were examined and showed that its wing had pale spots at the base.

Metalimnobia (Metalimnobia) quadrinotata (Meigen, 1818)

Limnobia quadrinotata Meigen, 1818: 144. Type locality: not given (?near Stolberg [Germany])

Limnobia variegata Macquart, 1826: 153. Type locality: northern France

Specimens examined. 1 male, China: Jilin, Antu, Mount Changbai, Lvyuantan (42°03'43"N, 128°04'05"E, 1775 m), 2015.VIII.6, Zehui Kang. 1 male 1 female, China: Hebei, Xinglong, Mount Wuling, main peak (40°35'30"N, 117°29'29"E, 1780 m), 2017.VIII.9, Liang Wang. 2 males, China: Neimenggu, Genhe, Hanma National Nature Reserve, Central Management Station (51°37'29"N, 122°26'34"E, 1200 m), 2014.VIII.1, Li Shi.

Diagnosis. Pleuron brownish yellow. Femora each brownish yellow with two rings, apical ring black, subapical ring paler and spreading over half of femora. Wing brownish with one or more spots at base. Sc₁ ending beyond fork of Rs, Sc₂ longer than Sc₁; basal section of CuA₁ before fork of M. Inner gonostylus divided into three lobes. Paramere with distal part long and curved ventrally, tip flattened and bare.

Distribution (new records in bold). China (Gansu, Hebei, Heilongjiang, Jilin, Neimenggu, Xinjiang); Armenia, Austria, Belarus, Belgium, Czech Rep., Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Hungary, Ireland, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Macedonia, Mongolia, Montenegro, Netherlands, North Korea, Norway, Poland, Romania, Russia, Slovakia, Slovenia, South Korea, Spain, Sweden, Switzerland, Ukraine (Oosterbroek 2020).

Remarks. *Metalimnobia* (*M.*) *quadrinotata* is widespread in the Palearctic region. In China, this species was previously known in three provinces and is now recorded in Hebei, Jilin and Neimenggu for the first time. For descriptions and illustrations of this species, see Savchenko (1985), Podenas et al. (2006), Podenas and Gelhaus (2007), Mao and Yang (2010) and Podenas and Byun (2016).

Metalimnobia (Metalimnobia) tenua Savchenko, 1976

Metalimnobia quadrinotata tenua Savchenko & Krivolutskaya, 1976: 151. Type locality: Russia, several localities in Eastern Siberia and the Far East

Specimens examined. 1 male, China: Sichuan, Pingwu, Wanglang National Nature Reserve, Shuizhagou (32°54'16"N, 104°09'34"E, 2447 m), 2016.VII.20, Yizhe Li.

Diagnosis. Pleuron brownish yellow, variegated by darker areas. Femora each brownish yellow with two rings, apical ring black, subapical ring slightly paler and spreading over half of femora. Wing brownish with two relatively large spots at base. Sc_1 ending beyond fork of Rs, Sc_2 shorter than Sc_1 ; basal section of CuA_1 before fork of M. Inner gonostylus divided into three lobes. Paramere subtriangular, tip slightly acute and bare (Fig. 4b, e).

Distribution (new record in bold). China (Ningxia, **Sichuan**); Austria, Czech Rep., Italy, Finland, Japan, Kazakhstan, Mongolia, Norway, Russia, Slovakia, Sweden (Oosterbroek 2020).

Remarks. *Metalimnobia* (*M*.) *tenua* is widespread in the Palearctic region. In China, this species was previously only known in Ningxia and is now recorded in Sichuan for the first time. For descriptions and illustrations of this species, see Savchenko and Krivolutskaya (1976), Savchenko (1983), Podenas and Gelhaus (2007) and Mao and Yang (2010).

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RESEARCH ARTICLE



A new species of *Chromis* damselfish from the tropical western Atlantic (Teleostei, Pomacentridae)

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Abstract

Initially described in 1882, *Chromis enchrysurus*, the Yellowtail Reeffish, was redescribed in 1982 to account for an observed color morph that possesses a white tail instead of a yellow one, but morphological and geographic boundaries between the two color morphs were not well understood. Taking advantage of newly collected material from submersible studies of deep reefs and photographs from rebreather dives, this study sought to determine whether the white-tailed *Chromis* is actually a color morph of *Chromis enchrysurus* or a distinct species. Phylogenetic analyses of mitochondrial genes cytochrome b and cytochrome c oxidase subunit I separated *Chromis enchrysurus* and the white-tailed *Chromis* into two reciprocally monophyletic clades. A principal component analysis based on 27 morphological characters separated the two groups into clusters that correspond with caudal-fin coloration, which was either known or presumed based on the specimen's collection site according to biogeographic data on species boundaries in the Greater Caribbean. Genetic, morphological, and biogeographic data all indicate that the white-tailed *Chromis* is a distinct species, herein described as *Chromis vanbebberae* **sp. nov.** The discovery of a new species within a conspicuous group such as damselfishes in a well-studied region of the world highlights the importance of deep-reef exploration in documenting undiscovered biodiversity.

Keywords

Caribbean, coral reef, mesophotic, phylogenetics, rariphotic, systematics

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Introduction

Chromis enchrysurus Jordan & Gilbert, 1882 is a species of Pomacentridae found on reefs in the tropical and subtropical western Atlantic Ocean from 5–146 m depth (Emery and Smith-Vaniz 1982). The species was first described by Gilbert and Jordan (1882) based on three specimens from Pensacola on the northeast Gulf of Mexico coast of Florida, USA. Jordan later provided the etymology in 'The Fishes of North and Middle America' (Jordan and Evermann 1898) as $evgpv\sigma o \varsigma$ (enchrysos), meaning deep golden, and $opv\alpha$ (oura), meaning tail, indicating that the species was named for its bright yellow caudal fin. Studies dating back to at least Smith-Vaniz and Emery (1980) refer to this species as *Chromis enchrysura*, based on the fact that the genus *Chromis* is feminine (Emery 1975). However, Jordan and Gilbert (1882) did not specify whether *enchrysurus* was intended as an adjective or noun in apposition. Following article 31.2 of the International Code of Zoological Nomenclature, if it is unclear how the name was intended, the name should then be treated as a noun in apposition with the original spelling unchanged, and gender need not match that of the genus. Thus, the original name *enchrysurus* is retained.

Emery and Smith-Vaniz (1982) redescribed *C. enchrysurus* and analyzed the morphological variation between populations of the species across its range. They noted that *C. enchrysurus* occasionally possesses white instead of yellow on the caudal, pelvic, anal, and posterior portion of dorsal fins, and that the species comprises either two or three populations (Bermuda, Brazil and Caribbean plus USA) that are morphologically distinct. However, most specimens used in the study were not observed alive, so any correlation between caudal-fin color and morphology or location could not be determined. Furthermore, since no genetic data were available at that time, the white-tailed *Chromis* was assumed to be a color morph of *C. enchrysurus* that shared a geographic range and lacked significant differences in morphology (Emery and Smith-Vaniz 1982).

Some of the confusion around the distribution and general biology of the species stems from the white-tailed form being restricted to deep reefs at or below the lower boundary of conventional SCUBA diving (~ 40 m). However, research on deep-reef fishes has significantly expanded in the last decade due to advances in technical diving and the use of manned submersibles and remote operated underwater vehicles (ROVs) (Gilmore 2016; Baldwin et al. 2018a; Rocha et al. 2018). In the Caribbean, this has been driven largely by the Smithsonian's Deep Reef Observation Project (DROP). DROP uses manned submersibles to document in-life coloration, collect fresh specimens, and observe live specimens in their natural habitat. Such initiatives have led to the discovery that Caribbean mesophotic (~ 40–130 m) and rariphotic (~ 130–300 m) fish communities are taxonomically distinct from their shallow reef counterparts (Baldwin et al. 2018a; Rocha et al. 2018) and contain a wealth of undescribed biodiversity (Baldwin and Robertson 2013, 2014, 2015; Baldwin and Johnson 2014; Baldwin et al. 2016a, b, 2018b; Tornabene et al. 2016a, b, c; Tornabene and Baldwin 2017, 2019). Similar efforts at sites across the central and western Pacific have resulted
in the discovery of new deep-reef fishes in a variety of taxonomic groups, including the genus *Chromis* (e.g., Arango et al. 2019; Pinheiro et al. 2019; Tea et al. 2019). Many recently described deep-reef species had never been observed before; however, in some cases, individuals formally recognized as juveniles or color morphs of known species have been identified as new species through observations of fresh and live coloration of both juveniles and adults coupled with DNA analysis (e.g., Baldwin et al. 2016a). At the outset of this study, we considered that this may be the case for the two putative color morphs of *C. enchrysurus*.

To date, DROP researchers have made collections at five deep-reef sites spanning the eastern and western Caribbean and have documented various *Chromis* species at each site, including the white-tailed morph of *C. enchrysurus*. In addition, one of us (LAR) has recorded *Chromis* spp. from closed-circuit rebreather dives off oceanic islands and the coast of Brazil, south to São Paulo State. Through these observations and collections together with photographic records accumulated by Robertson and Van Tassell (2019), comparisons between the two color morphs of *C. enchrysurus* made it possible to evaluate whether they represent distinct species. We combine molecular data from the mitochondrial genes cytochrome b and cytochrome c oxidase I, color photographs, distribution data, and morphological data from specimens collected across the entire range of the species complex to demonstrate that the white-tailed color morph represents a distinct species of *Chromis*, which we describe here, that is largely allopatric with *C. enchrysurus*. The discovery of this species contributes to our growing understanding of underexplored deep-reef ecosystems.

Materials and methods

Geographic range estimation

To determine the geographic range for both color morphs we used data from Robertson and Van Tassell (2019), which includes georeferenced records based on a number of public data aggregators (i.e., OBIS [www.obis.org], GBIF [www.gbif.org], Fish-Net2 [www.fishnet2.net], iDigBio [www.idigbio.org]), museum specimen databases, and independent collection efforts from the authors and dozens of contributing photographers. Initial estimates of the location of each color morph were made based on the photo-verified records and eyewitness reports from contributors to Robertson and Van Tassell (2019). This was supplemented with data from literature surveys of ROV studies (Colin 1974, 1976; Luiz et al. 2008; Pinheiro et al. 2016; Rosa et al. 2016; Simon et al. 2016; Stefanoudis et al. 2019), and our own specimens collected/ observed from DROP surveys and rebreather dives, creating more comprehensive range estimates. Gaps in data were inferred based on estimates derived from typical biogeographic breaks and provinces in the Greater Caribbean region as described by Robertson and Cramer (2014).

Specimens

Four fresh yellow-tailed specimens of *Chromis enchrysurus* were collected from Marathon Key, Florida by Frank Young (Dynasty Marine, Inc; https://dynastymarine.net). Eleven fresh white-tailed specimens were collected from Curaçao and Sint Eustatius during submersible expeditions carried out by DROP. An additional eleven samples collected by DROP from Curaçao were represented only from tissue samples (vouchers were not retained), but white fins were noted from these specimens at the time of collection. DROP specimens were collected by the 'Curasub' crewed submersible, which was equipped with a quinaldine ejection system that was used to anesthetize the fish. A suction tube terminating in a holding tank was used to collect and retain the fish once sedated. Collections took place periodically from 2010 to 2019.

For parts of the species range where no fresh specimens were available, specimens were examined from the University of Kansas (**KU**), the Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (**FSBC**), the University of Florida (**UF**), Louisiana State University Museum of Zoology (**LZUMZ**), and the Field Museum of Natural History (**FMNH**); collection acronyms follow Sabaj (2020). Live coloration of preserved specimens was presumed based on estimated ranges of color morphs observed in georeferenced photographs. Eleven of the preserved specimens had associated tissue samples, allowing retroactive confirmation of color morph through genetic comparison with confirmed vouchers. Detailed information on specimens examined in this study is provided in Suppl. material 1: Table S1.

Morphology

Morphological data were collected from 15 specimens of white-tailed morphs and 32 specimens of yellow-tailed morphs following methods of Pyle et al. (2008). We did not measure caudal fin concavity due to the condition of specimens. Nearly all characters used here were also analyzed by Emery and Smith-Vaniz (1982). A total of 28 characters were measured (plus standard length), and seven characters were counted. Measurements were taken using digital calipers to the nearest 0.1 mm, and counts were made with the aid of a Zeiss Discovery v20 SteREO microscope and cyanine blue dye (Saruwatari et al. 1997) when necessary. Vertebral counts are total vertebral elements (precaudal + caudal vertebrae) not including the urostyle, and were taken from six specimens, three of each color morph, using micro computed tomography scans taken on a Bruker Skyscan 1173 micro-CT scanner at the Karel F. Liem imaging facility at Friday Harbor Laboratories, University of Washington. Due to the condition of some specimens, certain measurements and counts could not be obtained.

Morphological data were analyzed using a Principal Component Analysis (PCA) conducted in RStudio (RStudio Team 2015) after converting values to residuals via linear regression to correct for variation attributable to specimen size. All 28 morphometric variables except standard length were included in the PCA. Average values of a measurement for color morphs were used for specimens that were missing a specific

Molecular methods

DNA was extracted from tissue preserved in 95% ethanol using the Qiagen DNAeasy Blood and Tissue Kit (Qiagen, Valencia, California). For USNM specimens, DNA was extracted using an automated phenol:chloroform protocol on the Autogenprep965 (Autogen, Holliston, MA) using the mouse tail tissue protocol (Baldwin et al. 2009).

The mitochondrial gene cytochrome b (cytb) was targeted using primers Fishcytb-F and Trucytb-R (Sevilla et al. 2007). The mitochondrial gene cytochrome c oxidase I (COI) was targeted using FISHCO1LBC and FISHCO1HBC (Baldwin et al. 2009) or FishF-1 and FishR-1 (Ward et al. 2004). Both genes were amplified via PCR using GoTaq Hotstart Master Mix (Promega, Madison, Wisconsin) using thermal profile as described in Sevilla et al. (2007) and Weigt et al. (2012). Cytb amplification was successful for 24 specimens and COI amplification was successful for 23 specimens. Sanger sequencing was performed at MCLAB and Texas A&M University – Corpus Christi Genomics Core Facility.

Sequences were trimmed, aligned, and concatenated in Geneious version 10.2.6 (Kearse et al. 2012). Sequences for other members of the genus *Chromis* and outgroups were gathered from GenBank or sequenced from USNM samples (see Suppl. material 2: Table S2). The concatenated alignment consisted of 87 sequences representing 53 pomacentrid species and four genera. The cytb alignment consisted of 71 sequences representing 49 pomacentrid species and four genera. The COI alignment consisted of 41 sequences representing 19 species and four genera. All three alignments contain representatives of *Chromis* from the Atlantic, Pacific, and Indian oceans, in addition to eight species from three other genera as outgroups (*Chrysiptera, Dascyllus, Pomacentrus*).

Substitution models and codon-partitioning schemes for each gene were selected using PartitionFinder2 (Lanfear et al. 2016) on XSEDE (Towns et al. 2014) through CIPRES (Miller et al. 2010). Phylogeny was estimated using MrBayes version 3.2 (Ronquist et al. 2012) on XSEDE (Towns et al. 2014) through CIPRES (Miller et al. 2010). Bayesian phylogenetic analyses were run for individual gene alignments and concatenated alignment for burn-in periods of 10%. Resulting consensus trees with posterior probability were visualized using FigTree v1.4.4 (accessible at http://tree.bio. ed.ac.uk/software/figtree/). Genetic distance matrices for both within- and betweengroup distances for both gene alignments were calculated in MEGA-X (Kumar et al. 2018). Distance values were calculated as the average number of base differences per site over all sequence pairs between groups (uncorrected p-distance). Positions with less than 95% site coverage were eliminated from the analysis. The cytb analysis consisted of 63 nucleotide sequences representing 41 species, and 324 positions were used for the final calculations. The COI analysis consisted of 31 nucleotide sequences representing ten species, and 603 positions were used for the final calculations. The alignments are available on Dryad (https://doi.org/10.5061/dryad.h9w0vt4gr).

Results

Geographic range

Analysis of the geographic ranges of color morphs indicate little overlap between yellow- and white-tailed morphs (Fig. 1). The yellow-tailed individuals occupy the Gulf of Mexico to the eastern tip of the Yucatan Peninsula, western Cuba, Florida, and the U.S. southern Atlantic coast. This includes the species' type locality, Pensacola, Florida (Jordan and Gilbert 1882). The white-tailed form occurs from the Bahamas, Bermuda and the Caribbean, south along the coast of South America to São Paulo, Brazil, and the Brazilian oceanic islands (Atol das Rocas, Fernando de Noronha, St. Paul's Rocks, and Trindade), and was previously recorded in most of these locations as *C. enchrysurus* (Pinheiro et al. 2018). The exact boundary off



Figure 1. Observations and hypothesized ranges of *Chromis enchrysurus* and *Chromis vanbebberae*. Open circles and triangles represent locations of specimens examined in this study. Solid circles or triangles represent records from visual observations, database searches, or the literature. Red triangle is Curaçao, the type locality of *C. vanbebberae*.

Cuba between the two color morphs is uncertain due to limited data. There is an area of overlap in the Florida Keys near 24.785167, -80.6595 in which both color morphs occur but are segregated by depth: the yellow-tailed morph occurring in shallower water ($\sim 25-40$ m), and the white-tailed morph occurring in deeper water ($\sim 60-90$ m; Frank Young, Dynasty Marine, pers. comm). The extent to which this overlap extends up the US coast is unknown; to date, white-tailed individuals have only been observed in the Florida Keys.

Morphometrics

Sixty-eight percent of overall morphometric variation is explained by the first five principal components, of which 29.6% is explained by PC1 (Suppl. material 3: Table S3). Plotting the specimens using scores from PC1 against PC2 separates the two color morphs into well-defined groups (Fig. 2), with areas of overlap consisting primarily of white-tailed individuals smaller than 20 mm SL, suggesting that color morphs may become more distinguished with ontogeny. The strongest loadings in PC1 are, in order of descending absolute value, caudal fin length, longest dorsal soft ray, body depth, and



Figure 2. Morphological variation in *Chromis enchrysurus* (yellow) and *Chromis vanbebberae* (blue) specimens, showing PC1 and PC2. Each point represents one individual specimen. Points are scaled according standard length of specimen.



Figure 3. Bayesian phylogenetic analysis of concatenated dataset of pomacentrid species. Circles at nodes indicate posterior probability. Branches with less than 0.50 posterior probability are collapsed. Branch length units are expected number of substitutions per site. Blue and yellow coloring on branches refer to *C. vanbebberae* and *C. enchrysurus*, respectively.

first pelvic soft ray (Suppl. material 4: Table S4). The strongest loadings in PC2 are, in order of descending absolute value, pre-dorsal length, body depth, pre-anal length, and 6^{th} dorsal spine length (Suppl. material 4: Table S4). Yellow-tailed specimens exhibit overall negative scores for component one with a wide range of component two scores, whereas white-tailed specimens exhibit overall positive scores for component one and more positive scores for component two. Many of the individual measurements that contribute substantially to PC1 showed large overlap between the species when looked at individually; however, *C. vanbebberae* sp. nov. does have a significantly longer soft dorsal base (t-test, p = 0.0015), longer last dorsal spines (p = 0.012), longer dorsal rays (p = 2.94e-7), longer anal rays (p = 1.35e-8), a longer caudal-fin (5.597e-8), and longer first pelvic soft rays (p = 0.040).

Molecular analyses

The individual gene trees and the concatenated tree all recovered the yellow-tailed *Chromis* and white-tailed *Chromis* as reciprocally monophyletic sister taxa. The posterior probability values supporting this relationship are 1.0 in the concatenated tree (Fig. 3) and in both gene trees (Figs 4, 5). Together, the white- and yellow-tailed clade is sister to *C. alta* Greenfield & Woods, 1980, an eastern Pacific species, in all trees (posterior probability = 0.91-0.97).



Figure 4. Bayesian phylogenetic analysis of cytb dataset of pomacentrid species. Circles at nodes indicate posterior probability. Branches with less than 0.50 posterior probability are collapsed. Branch length units are expected number of substitutions per site. Blue and yellow coloring on branches refer to *C. vanbebberae* and *C. enchrysurus*, respectively.



Figure 5. Bayesian phylogenetic analysis of COI dataset of pomacentrid species. Circles at nodes indicate posterior probability. Branches with less than 0.50 posterior probability are collapsed. Branch length units are expected number of substitutions per site. Blue and yellow coloring on branches refer to *C. vanbebberae* and *C. enchrysurus*, respectively.

Analysis of genetic variation between and within groups shows that for both genes assessed, there is substantially more genetic variation between the two color morphs than there is within each. Average pairwise genetic distance in cytb sequences (Table 1) between color morphs was 0.0566, versus 0.0076 within the yellow-tailed group and 0.0218 in white-tailed group. Average genetic distance between the two groups in COI sequences (Table 2) was estimated to be 0.0362, versus 0.0071 within the yellow-tailed

		1	2	3	4	5	9	7	8	6	10	11	12	13	14	15	16	17	18	19	20
_	C. enchrysurus	0.008	0.057	0.122	0.136	0.119	0.124	0.140	0.146	0.121	0.143	0.169	0.161	0.128	0.158	0.129	0.132	0.141	0.129	0.117	0.177
2	C. vanbebberae	0.057	0.022	0.128	0.143	0.124	0.136	0.156	0.144	0.135	0.154	0.172	0.182	0.147	0.171	0.157	0.141	0.146	0.138	0.125	0.166
3	C. verater	0.122	0.128		0.099	0.074	0.086	0.136	0.142	0.111	0.145	0.139	0.136	0.086	0.154	0.120	0.114	0.139	0.099	0.086	0.146
4	C. xanthura	0.136	0.143	0.099		0.114	0.127	0.139	0.176	0.086	0.157	0.164	0.145	0.117	0.167	0.157	0.151	0.157	0.123	0.037	0.152
Ś	C. xanthochina	0.119	0.124	0.074	0.114		0.025	0.142	0.130	0.114	0.154	0.157	0.164	0.096	0.164	0.133	0.123	0.145	0.105	0.105	0.168
9	C. weberi	0.124	0.136	0.086	0.127	0.025		0.145	0.136	0.123	0.154	0.170	0.167	0.090	0.167	0.139	0.133	0.148	0.123	0.117	0.175
\sim	C. vanderbilti	0.140	0.156	0.136	0.139	0.142	0.145		0.164	0.130	0.056	0.160	0.136	0.127	0.105	0.127	0.123	0.142	0.133	0.127	0.165
~	C. ternatensis	0.146	0.144	0.142	0.176	0.130	0.136	0.164		0.154	0.136	0.176	0.157	0.157	0.179	0.157	0.160	0.093	0.145	0.157	0.171
6	C. opercularis	0.121	0.135	0.111	0.086	0.114	0.123	0.130	0.154		0.148	0.179	0.173	0.120	0.145	0.127	0.123	0.148	0.108	0.080	0.165
10	C. nigura	0.143	0.154	0.145	0.157	0.154	0.154	0.056	0.136	0.148		0.151	0.133	0.127	0.111	0.142	0.145	0.127	0.139	0.145	0.162
Ξ	C. iomelas	0.169	0.172	0.139	0.164	0.157	0.170	0.160	0.176	0.179	0.151		0.099	0.167	0.154	0.194	0.188	0.179	0.157	0.160	0.102
12	C. dimidiata	0.161	0.182	0.136	0.145	0.164	0.167	0.136	0.157	0.173	0.133	0.099		0.154	0.154	0.173	0.167	0.160	0.160	0.139	0.105
13	C. chrysura	0.128	0.147	0.086	0.117	0.096	0.090	0.127	0.157	0.120	0.127	0.167	0.154		0.148	0.133	0.127	0.148	0.083	0.105	0.146
14	C. acares	0.158	0.171	0.154	0.167	0.164	0.167	0.105	0.179	0.145	0.111	0.154	0.154	0.148		0.182	0.160	0.170	0.142	0.160	0.162
15	C. chromis	0.129	0.157	0.120	0.157	0.133	0.139	0.127	0.157	0.127	0.142	0.194	0.173	0.133	0.182		0.049	0.139	0.145	0.133	0.194
16	C. limbata	0.132	0.141	0.114	0.151	0.123	0.133	0.123	0.160	0.123	0.145	0.188	0.167	0.127	0.160	0.049		0.139	0.123	0.133	0.181
17	C. viridis	0.141	0.146	0.139	0.157	0.145	0.148	0.142	0.093	0.148	0.127	0.179	0.160	0.148	0.170	0.139	0.139		0.136	0.148	0.165
18	C. ovalis	0.129	0.138	0.099	0.123	0.105	0.123	0.133	0.145	0.108	0.139	0.157	0.160	0.083	0.142	0.145	0.123	0.136		0.117	0.156
19	C. anadema	0.117	0.125	0.086	0.037	0.105	0.117	0.127	0.157	0.080	0.145	0.160	0.139	0.105	0.160	0.133	0.133	0.148	0.117		0.133
20	C. caudalis	0.177	0.166	0.146	0.152	0.168	0.175	0.165	0.171	0.165	0.162	0.102	0.105	0.146	0.162	0.194	0.181	0.165	0.156	0.133	
21	C. atrilobata	0.149	0.154	0.120	0.151	0.136	0.139	0.151	0.160	0.154	0.151	0.151	0.148	0.117	0.154	0.170	0.145	0.127	0.127	0.130	0.140
22	C. randalli	0.114	0.124	0.111	0.139	0.114	0.130	0.145	0.160	0.130	0.151	0.170	0.164	0.096	0.148	0.167	0.154	0.142	0.077	0.111	0.143
23	C. punctipimis	0.142	0.141	0.146	0.178	0.127	0.140	0.178	0.175	0.124	0.181	0.219	0.213	0.162	0.184	0.156	0.152	0.175	0.140	0.162	0.197
24	C. flavapicis	0.116	0.138	0.090	0.111	0.080	0.093	0.142	0.133	0.123	0.130	0.157	0.139	0.096	0.170	0.130	0.120	0.154	0.108	0.099	0.156
25	C. bami	0.131	0.150	0.083	0.114	0.093	0.093	0.123	0.160	0.117	0.130	0.164	0.151	0.003	0.145	0.130	0.123	0.151	0.080	0.102	0.143
26	C. fatuhivae	0.178	0.167	0.145	0.154	0.170	0.170	0.164	0.173	0.164	0.164	0.108	0.108	0.145	0.160	0.191	0.179	0.170	0.154	0.136	0.006
27	C. fieldi	0.161	0.182	0.136	0.145	0.164	0.167	0.139	0.160	0.170	0.136	0.096	0.003	0.157	0.151	0.176	0.170	0.160	0.160	0.139	0.102
28	C. lepidolepis	0.195	0.225	0.167	0.182	0.198	0.198	0.173	0.194	0.188	0.182	0.182	0.179	0.188	0.160	0.188	0.204	0.170	0.204	0.176	0.194
29	C. delta	0.140	0.156	0.136	0.139	0.142	0.145	0.000	0.164	0.130	0.056	0.160	0.136	0.127	0.105	0.127	0.123	0.142	0.133	0.127	0.165
30	C. xanthopterygia	0.141	0.134	0.108	0.136	0.068	0.071	0.164	0.136	0.139	0.160	0.164	0.173	0.114	0.167	0.154	0.154	0.154	0.123	0.130	0.184
31	C. retrofasciata	0.157	0.160	0.136	0.154	0.179	0.182	0.139	0.176	0.179	0.148	0.093	0.105	0.157	0.160	0.170	0.167	0.148	0.164	0.145	0.117
32	C. nitida	0.132	0.145	0.114	0.123	0.093	0.108	0.160	0.170	0.142	0.160	0.157	0.148	0.099	0.179	0.167	0.164	0.160	0.108	0.117	0.162
33	C. multilineata	0.157	0.160	0.108	0.127	0.130	0.139	0.157	0.167	0.145	0.167	0.160	0.160	0.139	0.127	0.170	0.151	0.148	0.136	0.117	0.156
34	C. margaritifer	0.156	0.175	0.142	0.145	0.160	0.170	0.130	0.154	0.167	0.127	0.105	0.009	0.154	0.154	0.173	0.160	0.154	0.154	0.139	0.108
35	C. flavomaculata	0.139	0.149	0.105	0.139	0.108	0.117	0.139	0.157	0.133	0.154	0.167	0.157	0.090	0.139	0.139	0.123	0.145	0.062	0.127	0.146
36	C. cyanea	0.141	0.161	0.111	0.123	0.117	0.130	0.133	0.157	0.139	0.145	0.148	0.136	0.130	0.157	0.136	0.139	0.136	0.133	0.114	0.140
37	C. atripes	0.159	0.167	0.136	0.148	0.167	0.164	0.148	0.170	0.157	0.148	0.096	0.105	0.148	0.157	0.170	0.154	0.148	0.160	0.136	0.095
38	C. atripectoralis	0.151	0.159	0.136	0.154	0.136	0.142	0.167	0.043	0.151	0.145	0.173	0.154	0.139	0.167	0.151	0.154	0.111	0.136	0.142	0.168
39	C. amboinensis	0.163	0.147	0.136	0.176	0.164	0.176	0.164	0.173	0.160	0.160	0.086	0.114	0.154	0.154	0.176	0.170	0.170	0.154	0.151	0.073
40	C. agilis	0.146	0.150	0.136	0.151	0.157	0.164	0.136	0.173	0.167	0.151	0.090	0.096	0.154	0.176	0.157	0.154	0.160	0.157	0.145	0.108
41	C. alta	0.079	0.074	0.117	0.127	0.120	0.127	0.151	0.145	0.123	0.151	0.167	0.160	0.133	0.139	0.151	0.136	0.136	0.127	0.114	0.162

Table I. Average genetic distance in mitochondrial gene cytb between species of Chromis. The number of base differences per site from averaging over alls equence pairs between groups are shown. Average within-species p-distance are shown on the diagonal.

41	0.079	0.074	0.117	0.127	0.120	0.127	0.151	0.145	0.123	0.151	0.167	0.160	0.133	0.139	0.151	0.136	0.136	0.127	0.114	0.162	0.160	0.114	0.121	0.123	0.130	0.157	0.160	0.182	0.151	0.136	0.154	0.139	0.145	0.154	0.136	0.145	0.157	0.136	0.148	0.160
40	0.146	0.150	0.136	0.151	0.157	0.164	0.136	0.173	0.167	0.151	0.090	0.096	0.154	0.176	0.157	0.154	0.160	0.157	0.145	0.108	0.154	0.167	0.216	0.151	0.151	0.114	0.093	0.201	0.136	0.167	0.102	0.145	0.167	0.096	0.173	0.127	0.090	0.176	0.099	
39	0.163	0.147	0.136	0.176	0.164	0.176	0.164	0.173	0.160	0.160	0.086	0.114	0.154	0.154	0.176	0.170	0.170	0.154	0.151	0.073	0.145	0.145	0.181	0.160	0.151	0.080	0.111	0.198	0.164	0.173	0.111	0.167	0.148	0.120	0.151	0.148	0.105	0.157		0.099
38	0.151	0.159	0.136	0.154	0.136	0.142	0.167	0.043	0.151	0.145	0.173	0.154	0.139	0.167	0.151	0.154	0.111	0.136	0.142	0.168	0.154	0.145	0.168	0.127	0.136	0.170	0.157	0.191	0.167	0.142	0.167	0.160	0.157	0.154	0.142	0.151	0.157		0.157	0.176
37	0.159	0.167	0.136	0.148	0.167	0.164	0.148	0.170	0.157	0.148	0.096	0.105	0.148	0.157	0.170	0.154	0.148	0.160	0.136	0.095	0.160	0.173	0.203	0.160	0.145	0.105	0.102	0.182	0.148	0.173	0.093	0.167	0.151	0.105	0.157	0.142		0.157	0.105	0.000
36	0.141	0.161	0.111	0.123	0.117	0.130	0.133	0.157	0.139	0.145	0.148	0.136	0.130	0.157	0.136	0.139	0.136	0.133	0.114	0.140	0.142	0.127	0.171	0.117	0.127	0.142	0.136	0.179	0.133	0.139	0.151	0.139	0.127	0.136	0.136		0.142	0.151	0.148	0 177
35	0.139	0.149	0.105	0.139	0.108	0.117	0.139	0.157	0.133	0.154	0.167	0.157	0.090	0.139	0.139	0.123	0.145	0.062	0.127	0.146	0.130	0.086	0.143	0.108	0.086	0.145	0.157	0.188	0.139	0.136	0.160	0.120	0.133	0.148		0.136	0.157	0.142	0.151	0.173
34	0.156	0.175	0.142	0.145	0.160	0.170	0.130	0.154	0.167	0.127	0.105	0.00	0.154	0.154	0.173	0.160	0.154	0.154	0.139	0.108	0.148	0.157	0.206	0.142	0.151	0.111	0.012	0.185	0.130	0.182	0.111	0.151	0.160		0.148	0.136	0.105	0.154	0.120	960.0
33	0.157	0.160	0.108	0.127	0.130	0.139	0.157	0.167	0.145	0.167	0.160	0.160	0.139	0.127	0.170	0.151	0.148	0.136	0.117	0.156	0.114	0.130	0.184	0.142	0.136	0.154	0.160	0.188	0.157	0.151	0.160	0.145	-	0.160	0.133	0.127	0.151	0.157	0.148	167
32	.132 (.145 (.114 (.123 (.093 (.108 (.160 (.170 (.142 (0.160 (.157 (.148 () 660.0) 179 (.167 (.164 (0.160 (.108 (.117 (.162 (.154 (.114 (.171 (.117 () 660.0	.164 (.148 (.198 (.160 (.120 (.167 (<u> </u>	.145	.151 (.120 (.139 (.167 (0.160 (.167 (1 45
31	.157 0	.160 0	.136 0	.154 0	.179 0	.182 C	.139 0	.176 0	.179 0	.148 C	.093 0	.105 0	.157 0	.160 0	.170 0	.167 0	.148 C	.164 0	.145 0	.117 0	.154 0	.167 0	.219 0	.164 0	.154 0	.120 0	.108 0	.179 0	.139 (.173 0	0 !	.167	.160 0	.111 0	.160 0	.151 0	.093 0	.167 0	.111 0	, 001
30	141 0	134 0	108 0	136 0	068 0	071 0	164 0	136 0	139 0	160 0	164 0	173 0	114 0	167 0	154 0	154 0	154 0	123 0	130 0	184 0	151 0	145 0	162 0	117 0	111 0	185 0	173 0	213 0	164 0	0	173	120 0	151 0	182 0	136 0	139 0	173 0	142 0	173 0	0 271
50	140 0.	156 0.	136 0.	139 0.	142 0.	145 0.	000 0.	164 0.	130 0.	056 0.	160 0.	136 0.	127 0.	105 0.	127 0.	123 0.	142 0.	133 0.	127 0.	165 0.	151 0.	145 0.	178 0.	142 0.	123 0.	164 0.	139 0.	173 0.	0	164	139 0.	160 0.	157 0.	130 0.	139 0.	133 0.	148 0.	167 0.	164 0.	0 221
8	95 0.	25 0.	67 0.	82 0.	98 0.	98 0.	73 0.	94 0.	88 0.	82 0.	82 0.	79 0.	88 0.	60 0.	88 0.	204 0.	70 0.	204 0.	76 0.	94 0.	79 0.	88 0.	229 0.	91 0.	85 0.	88 0.	79 0.	0.	73	213 0.	[79 0.	98 0.	88 0.	85 0.	88 0.	79 0.	82 0.	91 0.	98 0.	0 100
7 2	61 0.1	82 0.2	36 0.1	45 0.1	64 0.1	67 0.1	39 0.1	60 0.1	70 0.1	36 0.1	96 0.1	03 0.1	57 0.1	51 0.1	76 0.1	70 0.2	60 0.1	60 0.2	39 0.1	02 0.1	48 0.1	64 0.1	13 0.2	42 0.1	54 0.1	11 0.1	0.1	79	39 0.1	73 0.2	08 0.1	48 0.1	60 0.1	12 0.1	57 0.1	36 0.1	02 0.1	57 0.1	11 0.1	03 07
2	78 0.1	57 0.1	45 0.1	54 0.1	70 0.1	70 0.1	54 0.1	73 0.1	54 0.1	54 0.1	0.0 80	0.0 80	ί5 0.1	50 0.1	0.1	79 0.1	70 0.1	54 0.1	36 0.1	0.1	<u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>	45 0.1	0.2	51 0.1	<u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>	0.1	=	38 0.1	54 0.1	35 0.1	20 0.1	54 0.1	54 0.1	11 0.0	£ 5 0.1	<u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>	0.1	70 0.1	30 0.1	00 91
26	1 0.17	0 0.10	3 0.14	4 0.15	3 0.17	3 0.17	3 0.10	0 0.17	7 0.10	0 0.10	4 0.10	1 0.10	3 0.14	5 0.10	0 0.19	3 0.17	1 0.17	0 0.15	2 0.13	3 0.00	0 0.14	6 0.14	9 0.19	3 0.15	0.1^{2}	2	4 0.1]	5 0.18	3 0.10	1 0.18	4 0.12 2	9 0.10	6 0.15	1 0.1	6 0.14	7 0.14	5 0.10	6 0.17	1 0.08	1 0 1
25	5 0.13	3 0.15	0.08	1 0.11	0.0 0	3 0.09	2 0.12	3 0.16	3 0.11	0.13	7 0.16	9 0.15	5 0.00	0.14	0.13	0.12	4 0.15	3 0.08	9 0.10	5 0.14	7 0.12	0.09	2 0.15	0.0	~	1 0.14	2 0.15	1 0.18	2 0.12	7 0.11	4 0.15	0.00	2 0.13	2 0.15	3 0.08	7 0.12	0.14	7 0.13	0.15	10 15
24	0.110	0.138	0.09(0.111	0.080	0.093	0.142	0.133	0.123	0.13(0.157	0.139	0.090	0.170	0.13(0.12(0.154	0.108	0.09	0.150	0.157	0.117	0.152		0.093	0.151	0.142	0.19]	0.142	0.117	0.16	0.117	0.142	0.142	0.108	0.117	0.16(0.127	0.16(0.151
23	0.142	0.141	0.146	0.178	0.127	0.140	0.178	0.175	0.124	0.181	0.219	0.213	0.162	0.184	0.156	0.152	0.175	0.140	0.162	0.197	0.184	0.140		0.152	0.159	0.194	0.213	0.229	0.178	0.162	0.219	0.171	0.184	0.206	0.143	0.171	0.203	0.168	0.181	9160
22	0.114	0.124	0.111	0.139	0.114	0.130	0.145	0.160	0.130	0.151	0.170	0.164	0.096	0.148	0.167	0.154	0.142	0.077	0.111	0.143	0.117		0.140	0.117	0.096	0.145	0.164	0.188	0.145	0.145	0.167	0.114	0.130	0.157	0.086	0.127	0.173	0.145	0.145	167
21	0.149	0.154	0.120	0.151	0.136	0.139	0.151	0.160	0.154	0.151	0.151	0.148	0.117	0.154	0.170	0.145	0.127	0.127	0.130	0.140		0.117	0.184	0.157	0.120	0.142	0.148	0.179	0.151	0.151	0.154	0.154	0.114	0.148	0.130	0.142	0.160	0.154	0.145	0.154
	C. enchrysurus	C. vanbebberae	C. verater	C. xanthura	C. xanthochira	C. weberi	C. vanderbilti	C. ternatensis	C. opercularis	C. nigura	C. iomelas	C. dimidiata	C. chrysura	C. acares	C. chromis	C. limbata	C. viridis	C. ovalis	C. anadema	C. caudalis	C. atrilobata	C. randalli	C. punctipinnis	C. flavapicis	C. bami	C. fatuhivae	C. fieldi	C. lepidolepis	C. delta	C. xanthopterygia	C. retrofasciata	C. nitida	C. multilineata	C. margaritifer	C. flavomaculata	C. cyanea	C. atripes	C. atripectoralis	C. amboinensis	C avilie
	-	5	3	4	Ś	9	\sim	8	6	10	1	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40

)	4))					
	C. enchrysurus	C. insolata	C. vanbebberae	C. scotti	C. lubbocki	C. xanthura	C. randalli	C. sanctaehelenae	C. multilineata	C. alta
C. enchrysurus	0.007	0.070	0.036	0.065	0.110	0.118	0.143	0.104	0.145	0.057
C. insolata	0.070		0.066	0.048	0.088	0.111	0.134	0.095	0.129	0.065
C. vanbebberae	0.036	0.066	0.004	0.058	0.105	0.106	0.138	0.100	0.142	0.051
C. scotti	0.065	0.048	0.058		0.080	0.109	0.134	0.091	0.121	0.053
C. lubbocki	0.110	0.088	0.105	0.080		0.124	0.136	0.085	0.143	0.090
C. xanthura	0.118	0.111	0.106	0.109	0.124		0.156	0.108	0.143	0.109
C. randalli	0.143	0.134	0.138	0.134	0.136	0.156		0.138	0.144	0.139
C. sanctaehelenae	0.104	0.095	0.100	0.091	0.085	0.108	0.138		0.156	0.093
C. multilineata	0.145	0.129	0.142	0.121	0.143	0.143	0.144	0.156		0.131
C. alta	0.057	0.065	0.051	0.053	0.090	0.109	0.139	0.093	0.13	

Table 2. Average genetic distance in mitochondrial gene COI between species of Chromis. The number of base differences per site from averaging over all sequence pairs between groups are shown. Average within-species divergences are shown on diagonal. group and 0.0042 within the white-tailed group. Taken together, patterns of genetic distance and phylogenetic relationships recovered by the Bayesian phylogenetic analyses support the hypothesis that the two color morphs represent genetically distinct sister species. These genetic differences are corroborated by the morphological differences (Fig. 1) and distinct geographic ranges overlapping in the Florida Keys.

Chromis vanbebberae sp. nov.

http://zoobank.org/21C7BAA1-2F99-4039-9389-A6069EBC774D Whitetail Reeffish Figures 6–9

Type material. *Holotype.* USNM 446947, 73.9 mm SL, CURASUB19-01, tissue no. CUR19001, 117 m, Substation Curaçao Downline, Bapor Kibra, Curaçao, 12.0832, -68.8991, C.C. Baldwin, L. Tornabene, B. Van Bebber, W.B. Ludt, 6 May 2019.

Paratypes. CURAÇAO: All collected at the type locality off Curaçao: USNM 414901, 33.4 mm SL, CURASUB12-15, tissue no. CUR12142, 123-160 m, A. Schrier, B. Brandt, C.C. Baldwin, A. Driskell, P. Mace, 10 Aug 2012; USNM 414902, 36.1 mm SL, CURASUB12-15, tissue no. CUR12141, 123-160 m, A. Schrier, B. Brandt, C.C. Baldwin, A. Driskell, P. Mace, 10 Aug 2012; USNM 413966, 24.7 mm SL, CURASUB13-03, tissue no. CUR13056, 53-189 m, C.C. Baldwin, A. Schrier, D.R. Robertson, C.I. Castilla, B. Brandt, 7 Feb 2013; USNM 413947, 23.4 mm SL, CURASUB13-02, tissue no. CUR13013, C.C. Baldwin, A. Schrier, D.R. Robertson, C.I. Castilla, B. Brandt, 6 Feb 2013; USNM 430030, 14.9 mm SL, tissue no. CUR13335, Substation Curaçao Crew, 9 July 2013; USNM 406206, 24.1 SL, CURASUB11-03, tissue no. CUR11206, 119-161 m, A. Schrier, M. van der Huls, C.C. Baldwin, D.R. Robertson, J. Oliver, 24 May 2011; CAS 247234, 90.7 mm SL, CURASUB19-02, tissue no. CUR19010, C.C. Baldwin, L. Tornabene, T. Christiaan, S. Yerrace, 7 May 2019; UW 200069, 98.4 mm SL, tissue no. CUR19003, 106 m, C.C. Baldwin, L. Tornabene, B. Van Bebber, W.B. Ludt, 6 May 2019; UW 200070, 97.1 mm SL, CURASUB19-02, tissue no. CUR19009, C.C. Baldwin, L. Tornabene, T. Christiaan, S. Yerrace, 7 May 2019; SINT EUSTATIUS: USNM 442658, 13.9 mm SL, CURASUB17-17, tissue no. EUS17005, South and southeast of R/V Chapman mooring, SW of island, Kay Bay, St. Eustatius, 17.4599, -62.9817, C.C. Baldwin, L. Tornabene, B. Brandt, J. Casey, 15 April 2017. See Suppl. material 1: Table S1 for non-type material examined.

Type locality. Curaçao, Netherland Antilles.

Diagnosis. Dorsal rays XIII, 12–13; anal rays II, 12–13; pored lateral-line scales 15–18 (usually 17; one paratype with ten and no apparent scale loss or damage); gill rakers 7–8+16–18. Proportional measurements expressed as percent standard length, unless otherwise noted as percent head length (HL): head length 30.2–41.0 (mean 35.4); predorsal length 31.1–42.0 (mean 34.9); orbit diameter 11.5–17.4 (mean 14.6), 39.0 (35.4–48.5) % HL; upper jaw length 9.1 (6.0–14.4), 30.0 (22.3–34.8)



Figure 6. Micro-CT scans A *Chromis vanbebberae*, Curaçao, paratype, USNM 414901, 33.4 mm SL B *C. enchrysurus*, South of Marathon, Florida, UW 200011, 41.5 mm SL.

% HL; snout length 7.8 (6.9–10.3), 26.0 (17.5–32.2) % HL; interorbital width 10.7 (8.6–12.8), 35.4 (21.1–37.4) % HL; body depth 41.6–57.8 (mean 51.8); caudal length 29.7–44.9 (mean 37.20); last dorsal spine 10.2–16.4 (mean 13); longest dorsal ray 21.1–26.5 (mean 23.3); longest anal ray 18.9–28 (mean 24.3); 1st pelvic soft ray 28.8–43.2 (mean 36.4). See Table 3. Live coloration with thin iridescent blue oblique stripe extending from snout, through eye, ending below origin of spinous dorsal fin, dorsal half of head dark blue to dusky gray, dark coloration continuing in oblique line across dorsal half of body to end of spinous dorsal fin; ventral half of body, soft dorsal fin, paired fins, and caudal fin white; no yellow pigmentation on body or fins.

Description. *Body* deep, 55.2 (41.6–57.8), laterally compressed, width 19.4 (16.6–21.6), oval in shape; eyes large, 11.8 (11.5–17.4), interorbital width 10.7 (8.6–12.1). Mouth small, upper jaw length 9.1 (6.0-14.4), terminal, and oblique. Head

	Chromis v	anbebberae		Chromis	enchrysura	
	Holotype USNM 446947	Average	Range	Holotype KU 27029	Average	Range
standard length	73.9	48.2	13.9-98.4	68	60.7	80.8-17.7
body depth	55.2	51.1	41.6-57.7	50.9	50.2	53.9-44
body width	19.4	19.1	16.5-21.6	17.8	17.5	19.2-13.8
head length	30.2	35.4	30.2-41	31.3	31.6	36-29.8
snout length	7.9	8.2	5.2-10.3	8.4	8.2	9.3-5.8
orbit diameter	11.8	14.6	11.5-17.4	11.3	11.7	14.7-10
interorbit width	10.7	10.6	8.6-12.1	10.6	10.6	14-9.2
caudal peduncle depth	16.1	15.1	13.3-16.4	14.7	14	15.6-9.8
upper jaw length	9.1	10	6.0-14.4	9.4	9.7	10.9-8
predorsal length	33.2	34	28.6-42	35.6	33.7	38.3-28.2
spinous dorsal base	48.6	44.1	35.5-50.2	45.9	46.7	50.8-36.6
soft dorsal base	18.9	16.5	13.4-18.9	16.9	14.6	18-10.4
1st dorsal spine	8.7	9.1	7.2-11.9	10.3	8.3	10.3-6.7
2 nd dorsal spine	12.9	14.3	11.4-17.5	14.9	12.6	16.2-10.6
3 rd dorsal spine	15.7	17.9	15.3-21.6	19.6	15.5	19.6-12.3
4 th dorsal spine	19.4	20.2	16.6-24.5	22.4	17.4	22.4-13.5
5 th dorsal spine	20.6	20.5	16.2-25.9	22.2	17.4	22.4-13.5
6 th dorsal spine	19.8	18.6	15.5-23.7	21.6	17	21.6-13.3
last dorsal spine	16.4	13.8	10.3-17.4	14.1	12.3	16.1-9.3
longest dorsal ray	23.8	23.2	21.1-28.5	21.3	19.1	23-16.1
preanal length	64.1	67	63.2-69.7	64.7	66.5	69.9-63.1
1st anal spine	9.3	8.7	5.8-11.6	9.9	8.1	9.9-5.5
2 nd anal spine	19.9	19.2	15.1-22.4	20.9	18.8	21.8-16
longest anal ray	23.4	24.1	18.9-28	22.1	19.9	26.3-16.3
caudal length	41	36.8	29.7-44.9	31.5	31.4	35.8-27.3
longest pectoral ray	34.2	33.8	31.1-38.1	30.2	31.2	33.7-28.6
prepelvic length	35.2	38.4	35.2-43.6	37.4	37.3	41.7-33.8
pelvic spine length	22.2	20.3	18.7-22.4	22.7	20	31.2-17.2
1st pelvic soft ray	40.9	35.4	28.8-43.2	36.3	23.4	36.8-30.8
dorsal rays	12	12.73	12-13	12	12.22	11-15
anal rays	12	12.57	12-13	12	12.06	11-13
pored lateral line scales	17	16.54	15-17	17	17.16	16-18
upper gill rakers	7	7.27	7–8	8	7.47	7–8
lower gill rakers	17	16.93	16-18	16	16.84	16-18

Table 3. Morphometrics and meristics of *Chromis vanbebberae* and *Chromis enchrysurus* specimens examined. Morphometric values are as percentage of SL.

large, 30.2 (30.2–41.0) and rounded with a convex forehead and short snout 7.8 (5.2– 10.3), snout length shorter than orbit diameter (snout ~ 1.8 times in orbit). Preopercle mostly smooth with slight serration at ventral angle; opercle possesses one large spine on dorsal posterior side. Suborbital bones mostly joined to cheek, save for second and third, which flex away from cheek with preorbital. Vertebrae 25 plus urostyle (Fig. 6). Gill rakers very long and slender, closely spaced, equal to or greater than the length of gill filaments, with very fine serrations, 7+17 (7–8+16–18). Teeth in both jaws short and conical, arranged in three rows anteriorly, outer row very slightly enlarged, becoming two rows posteriorly.

Dorsal fin XIII, 12 (12–13); longest dorsal ray 23.8 (21.1–28.5); last (13th) dorsal spine 16.4 (10.3–16.4); spinous dorsal base 48.6 (35.5–50.2); soft dorsal base 18.9 (13.4–18.9); pre-dorsal length 33.2 (31.1–42.0). Anal fin II, 12 (12–13); longest anal-fin ray 23.4 (18.9–28.0); pre-anal length 64.1 (63.2–69.0). Pectoral fin 18 (17–20)

and lacking free rays; longest pectoral ray 34.2 (31.1–38.1). Pelvic fin I, 5; with a very long first pelvic ray 40.9 (28.8–43.2); pre-pelvic length 35.2 (35.2–43.6). Caudal fin forked with length 41.0 (29.7–44.9).

Scales large, coarsely ctenoid, covering body and most of head, often densely clustered at base of dorsal and anal fins. Pored lateral-line scales 17 (15–18), total scales in lateral series 28 (26–28); one paratype (USNM 430030, 14.9 mm SL) with only 10 pored lateral-line scales, lateral line terminating below the 10th dorsal spine in all individuals, without apparent damage or scale loss. Scales above lateral line 4 (3–4). Scales below lateral line 10 (10–11). Circumpeduncular scales 14 (13–4). No obvious pored or pitted scales on caudal peduncle.

Live coloration (Fig. 7): Adults (Fig. 7A–C, F) charcoal gray, sometimes tinged with iridescent blue from head to end of spinous dorsal base, with an abrupt, oblique division between dark dorsal portion and light lower body starting at pectoral-fin base and extending to end of spinous dorsal fin; ventral portion of body, soft dorsal fin, paired fins, and caudal fin bright white with no yellow pigmentation. Head with short, oblique iridescent blue stripe originating on upper lip extending through upper edge of eye extending onto side of nape above pectoral fin. In larger individuals, blue stripe reduced, present only on snout. Juvenile (Fig. 7D, E) pigmentation same as adult except dark area distinctly tinged with more blue iridescence and terminating halfway along spinous dorsal fin (versus at end of spinous dorsal fin in adults), blue stripe on head much more prominent, and a second shorter blue stripe often present ventral to eye.

Coloration in freshly dead specimens (Fig. 8): Coloration similar to that of live specimens with little or no blue iridescence except in juveniles, where blue stripe through eye is visible. Paired fins, anal fin, and caudal fin pale to dusky, not vibrant white.

Coloration in preservation (Fig. 9): Base coloration of body pale yellow to golden brown, areas blue or grey in life dark brown; spinous dorsal fin uniformly dark brown,



Figure 7. Live coloration of *Chromis vanbebberae* **A** Curaçao, holotype, USNM 446947, 73.9 mm SL **B, C** Curaçao **D, E** St. Paul's Rocks, Brazil, juveniles **F** St. Paul's Rocks, Brazil. Photographs by Barry B. Brown (**A**), Yi-Kai Tea (**B**), D. Ross Robertson (**C, D**), Luiz A. Rocha (**E, F**).



Figure 8. Freshly collected *Chromis vanbebberae* **A** paratype, CAS 247234, 90.7 mm SL, Curaçao **B** paratype, USNM 414902, 36.1 mm SL, Curaçao. Photographs by Carole C. Baldwin.

soft dorsal fin, anal fin, and pelvic fin dusky light grey, pectoral fin pale, caudal fin light brown at base becoming pale posteriorly.

Distribution (Fig. 1). *Chromis vanbebberae* occurs off Bermuda, the Florida Keys, the Bahamas, scattered sites in the northwest, central, eastern and southern Caribbean, and south to at least São Paulo, Brazil, including the offshore islands of Rocas Atoll, St. Paul Rocks, Trindade, and Fernando de Noronha.



Figure 9. Preserved *Chromis vanbebberae* **A** paratype, CAS 247234, 90.7 mm SL, Curaçao **B** paratype, UW 200070, 97.1 mm SL, Curaçao. Photographs by Luke Tornabene.

Habitat. Chromis vanbebberae occurs on a variety of deep-reef habitats at depths between 49 and at least 178 m, including on rocky reef slopes, coral outcroppings, around sponges, boulders, and caves. In areas of colder water in southeastern Brazil (Espírito Santo, Rio de Janeiro and Sao Paulo states) they are seen in depths as shallow as 10 m. In Curaçao, individuals are often found near sporadic patches of rocks located on otherwise open sandy bottoms devoid of other structure, which they frequently co-occupy with the seabasses *Serranus phoebe* or *S. notospilus*. They are also frequently found around artificial substrates such as shipwrecks (e.g., the wreck Queen of Nassau in southeast Florida), tires, and derelict ropes and fishing gear. This species and *C. insolata* Cuvier & Valenciennes, 1830, are the two most common pomacentrids on lower-mesophotic and rariphotic reefs in the Caribbean. In Brazil, *C. insolata* is

replaced by its southern mesophotic counterpart, *C. jubauna* Moura, 1995, and the latter often schools with *C. vanbebberae* on coastal reefs; however, *C. vanbebberae* is the only mesophotic *Chromis* recorded in Brazilian oceanic islands.

Where *C. vanbebberae* and *C. enchrysurus* overlap in southeastern Florida, the two species segregate by depth, with *C. enchrysurus* occurring from (~ 25–40 m), and *C. vanbebberae* occurring in deeper water (~ 60–90 m). Emery and Smith-Vaniz (1982) reported a depth range of 5–146 m for *C. enchrysurus*, noting that most observations were from 40–70 m. The 146 m record was from Puerto Rico, and thus represents *C. vanbebberae*, not *C. enchrysurus*. Based on the confirmed records of *C. enchrysurus* from this study, the known depth range of that species is 5–97 m.

Etymology. The species epithet *vanbebberae*, Latinized from Van Bebber, honors Barbara Van Bebber, one of the most accomplished submersible pilots in the Caribbean. Van Bebber was one of several skilled pilots of the 'Curasub' that assisted DROP with observations and collections of many new species, including this species. The common name "Whitetail Reeffish" (castañeta coliblanca in Spanish) refers to the caudal-fin coloration that distinguishes the species from *Chromis enchrysurus*, the Yellowtail Reeffish.

Remarks. *Chromis vanbebberae* is easily distinguished from *C. enchrysurus* (Fig. 10) in having white versus yellow on the caudal fin, pelvic fins, anal fin, and posterior rays of the dorsal fin; however, this rapidly fades in death and preservation, making the two nearly indistinguishable. The two species are otherwise morphologically very similar, and species identity of preserved fishes can be most reliably determined based on locality of collection and genetics.

Chromis vanbebberae frequently co-occurs with C. insolata and C. scotti Emery, 1968, in the Caribbean, and with C. jubauna in Brazil. It can be distinguished from C. scotti in having an abrupt, diagonal dividing line between the dark dorsal portion of body and white ventral portion of the body (a diffuse horizontal dividing line in C. scotti), and in lacking the prominent iridescent light blue coloration that is present on most of the dorsal portion of the body of *C. scotti* (Fig. 11). In addition, the tail is dusky in C. scotti versus bright white in C. vanbebberae. The diagonal light/dark divide on the body of C. vanbebberae also distinguishes it from adult C. insolata, which has a horizontal division similar to C. scotti (Fig. 11). Chromis insolata and C. jubauna both differ from C. vanbebberae in number of anal rays: C. insolata typically possesses eleven anal rays and C. jubauna 9-11, in comparison to the typical 12 (rarely 11 or 13) of C. vanbebberae. In addition, C. insolata typically possesses 18–19 pored lateral line scales, whereas no C. vanbebberae specimens examined exceed 17. Adult C. jubauna have uniformly grey to black bodies with bright yellow caudal and soft dorsal fins, versus the dark/light bodies and white fins of C. vanbebberae. The juveniles of C. vanbebberae, C. insolata, C. scotti, C. enchrysurus, and C. jubauna also have dramatically different live coloration (Figs 7, 11). The juveniles of C. vanbebberae are similar to adults in coloration, except with slightly more blue iridescence, whereas juvenile C. scotti are almost entirely blue, juvenile C. insolata have prominent, wide yellow, purple, and white horizontal stripes, and juvenile C. jubauna are yellow dorsally and bright purplish-blue ventrally.



Figure 10. Live coloration of *Chromis enchrysurus* **A** dry Tortugas, Florida **B** off North Carolina **C** gulf of Mexico, Florida **D–F** Florida Keys, juveniles. Photographs by Alison and Carlos Estape (**A**, **D–F**), Frank Krasovec (**B**), and Bob and Carol Cox (**C**). No photographed fish were preserved.



Figure 11. Live coloration of *Chromis scotti and C. insolata* A *C. scotti*, adult, Roatan, Honduras
B *C. scotti*, juvenile, Tobago C *C. insolata*, adult, Florida Keys D *C. insolata*, juvenile, Florida Keys
E *C. jubauna*, adult, Laje de Santos Island, Brazil F *C. jubauna*, juvenile, Laje de Santos Island, Brazil.
Photographs by Mickey Charteris (A), Alison and Carlos Estape (B–D), and Osmar Luiz Jr (E, F).

Discussion

Genetic analyses support the hypothesis that yellow-tailed and white-tailed specimens represent distinct species. Bayesian phylogenetic analysis of both genes and of the concatenated sequences returned topologies splitting the two species into reciprocally monophyletic clades with high posterior probability values. Additionally, genetic distance analyses demonstrate that for both genes, sequence variation between species is greater than that within species. While the within-group genetic distance of *C. vanbebberae* cytb sequences is higher than the within-group distance of *C. enchrysurus*, both values are distinctly lower than the between-group variation for the vast majority of species in our analysis (Table 1). In the COI analysis, within-group distance of *C. vanbebberae* is similar to that of *C. enchrysurus*. Both within-group distances of *C. enchrysurus* and *C. vanbebberae* were at least one order of magnitude lower than any between-group value in the analysis (Table 2).

The genes used in this study are commonly used in phylogenetic and species-delimitation studies in fishes. Mitochondrial genes are especially useful in species identification and phylogenetic reconstruction due to their high number of copies compared to nuclear DNA, lack of recombination, and comparatively fast evolution (Teletchea 2009); however, having independent data from nuclear genes would be beneficial. Broader-scale relationships within the genus and family presented in this study align with those identified in previous phylogenetic analyses of Pomacentridae using both nuclear and mitochondrial DNA, including the recovery of a paraphyletic *Chromis*, with the genus *Dascyllus* nested within it (Jang-Liaw et al. 2002; Quenouille et al. 2004; Cooper et al. 2009).

Although the PCA does separate the two species on the basis of PC1, the morphometric differences are subtle and fail to perfectly separate the two species, especially when individuals are small (SL < 25 mm). While some characters have statistically significant differences between the two species (i.e., the length of soft dorsal base, length of last dorsal spine [p = 0.012], caudal fin length, etc.; see Morphometrics results above), these characters are not discrete, overlap substantially between species, and are not prominent when individuals are small. Collectively, this makes them largely impractical for diagnosing the two species. Coloration remains the most useful morphological character for distinguishing the species. The presence of sister species that are nearly morphologically identical and distinguished primarily by live coloration is increasingly observed in coral-reef fishes (Victor 2015). Unfortunately, this makes it challenging or impossible to retroactively assign species identity for preserved specimens when no data exist for live coloration or genetics. Although color is not always indicative of species-level differences between closely related reef-fish taxa (Dibattista et al. 2012; Schultz et al. 2007), live color is often the primary, or in some cases only, external character by which species can be distinguished (Luiz et al. 2009; Randall and Rocha 2009). Such differentiating characters are particularly troublesome for distinguishing species of deep-reef fishes, as for centuries, many were seldom observed live and, until recently, none had been sampled genetically.

Data suggest that C. vanbebberae and C. enchrysurus occupy distinct geographic ranges with little overlap, which indicates that collection locality can help inform species identity with reasonable certainty when genetic analysis cannot be performed. Species-range estimates of C. enchrysurus and C. vanbebberae based on collections, visual observations, and genetic data from georeferenced specimens agree well with the findings of Robertson and Cramer (2014) on biogeographic patterns and species distributions in the Greater Caribbean. Robertson and Cramer (2014) divided the region into three provinces, each with its own faunal assemblage: a northern province encompassing the Gulf of Mexico and southeastern United States; a central province encompassing the West Indies, Bermuda, and Central America; and a southern province encompassing northern South America. In the Greater Caribbean, the southernmost locality of specimens examined in this study was Curaçao, which falls into the central province, although many photographic records and specimens identified as C. enchrysurus exist from the Venezuelan coast and the east coast of South America as far south as Brazil. Thus, C. enchrysurus occupies the northern province and C. vanbebberae occupies the central and southern provinces of the Greater Caribbean plus Brazil.

A genetic break between sister species or populations occurring in the northern province of Robertson and Cramer (2014; i.e., Gulf of Mexico, eastern U.S.) and those occurring in the Caribbean or South America is a common phylogeographic pattern (Floeter et al. 2008). For example, sister species of Liopropoma basslets demonstrate a similar split: L. eukrines inhabits the Gulf of Mexico and the Atlantic coast of the southeastern U.S., and L. aberrans inhabits the Caribbean (Baldwin and Robertson 2014). Populations of Bathygobius soporator from the Gulf of Mexico and eastern U.S. have also been shown to be distinct from those in the Caribbean and Brazil (Tornabene et al. 2010; Tornabene and Pezold 2011; Rodríguez-Rey et al. 2017). Other examples of sister lineages occurring in the Caribbean versus the Gulf of Mexico/eastern U.S. can be found in the *Menticirrus americanus* species complex (Marceniuk et al. 2020), the Lutjanus campechanus and L. purpureus species pair (Pedraza-Marrón et al. 2019; da Silva et al. 2020), the Scartella cristata species complex (Araujo et al. 2020), the genus Bagre (Betancur-R 2009), and in Epinephelus adscensionis (Carlin et al. 2003). In many cases these speciation patterns are thought to be a product of environmental variation between provinces as opposed to hard barriers to gene flow between the regions (Rocha et al. 2005; Robertson and Cramer 2014). The northern province is a heterogenous, more temperate environment, whereas the central and southern provinces are both more uniform and stable. The central and southern provinces are also more similar to one another than to the northern province, despite the northern and southern provinces bearing similarities in eutrophication and upwelling. We did not have genetic samples from Brazil, and while photographs of C. vanbebberae appear similar to those from the Caribbean, it is possible that additional genetic breaks may occur near the Amazon outflow, or between mainland Brazil and off-shore islands (Joyeux et al. 2001; Floeter et al. 2008).

Many of the recently described species from the Greater Caribbean are cryptobenthic fishes that are often overlooked in biodiversity surveys. However, pomacentrids are some of the most conspicuous fishes on corals reefs. They occur on shallow and deep coral reefs in every geographic region, where they are often the most abundant fishes on a given reef (Quenouille et al. 2004). Thus, it may be surprising that two common species that can easily be distinguished when alive and occupy separate ranges have been thought to be the same species for decades. This almost certainly represents a gap in knowledge attributed to a lack of genetic data, coupled with the challenges of observing live fishes below the depth limit of safe conventional SCU-BA diving, and the fact that these species are morphologically conserved. Such gaps can result in an underestimation of the overall biodiversity in reef systems. Although reef-fish assemblages on deep and shallow reefs typically come from the same set of families, deep-reef assemblages are taxonomically distinct from shallow reefs at the species level and contain a wealth of previously unknown biodiversity that is still being uncovered (Baldwin et al. 2018; Rocha et al. 2018). Many undescribed species discovered on deep reefs are immediately recognizable as being new to science; however, there are other instances where a single deep-reef species that was described many years ago is revealed to be a complex of two or more species. For example, two new deep-reef basses previously thought to be *Liopropoma aberrans*, which was described in 1860, have since been described as new, splitting that species into three (Baldwin and Johnson 2014; Baldwin and Robertson 2014). Collection of fresh specimens, tissues, and photographs from deep reefs also led to the discovery that individuals previously thought to be juvenile color morphs of the grammatid basslet Lipogramma evides was in fact a distinct species, *L. levinsoni*, with the two species segregating by depth in areas of geographic overlap (Baldwin et al. 2016a). These examples, including the current study, highlight the importance of initiatives that document the fauna of deep reefs through collection of multiple types of data (i.e., photographs, specimens, tissue samples, habitat and depth data, etc.) to gain a more complete understanding of tropical marine biodiversity.

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Supplementary material I

Table S1. Material examined of Chromis vanbebberae and C. enchrysurus

Authors: Emily P. McFarland, Carole C. Baldwin, D. Ross Robertson, Luiz A. Rocha, Luke Tornabene

Data type: Specimen list

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Link: https://doi.org/10.3897/zookeys.1008.58805.suppl1

Supplementary material 2

Table S2. GenBank accession numbers for outgroup taxa

Authors: Emily P. McFarland, Carole C. Baldwin, D. Ross Robertson, Luiz A. Rocha, Luke Tornabene

Data type: Accession numbers

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Link: https://doi.org/10.3897/zookeys.1008.58805.suppl2

Supplementary material 3

Table S3. Contribution to overall variance by the first ten principle components

Authors: Emily P. McFarland, Carole C. Baldwin, D. Ross Robertson, Luiz A. Rocha, Luke Tornabene

Data type: Principal component contributions

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Supplementary material 4

Table S4. Loadings for the first five components from the Principal Component Analysis

Authors: Emily P. McFarland, Carole C. Baldwin, D. Ross Robertson, Luiz A. Rocha, Luke Tornabene

Data type: Principal component loadings

- Explanation note: Values in blue contribute positively and those in red contribute negatively to a given component. The greater the absolute value of the contribution, the more intense the color. Only the first five of 28 components have been included.
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Link: https://doi.org/10.3897/zookeys.1008.58805.suppl4

RESEARCH ARTICLE



A new species of the genus Leptobrachella Smith, 1925 (Anura, Megophryidae) from Guizhou, China

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Abstract

Asian leaf-litter toads of the genus *Leptobrachella* represent charismatic anuran diversification with 80 species, of which 25 are from China. Recent new discoveries suggest that the diversity of this genus is underestimated. Here, we describe a new species of *Leptobrachella*, *Leptobrachella bashaensis* **sp. nov.** from the Basha Nature Reserve, Congjiang County, Guizhou Province, China. The new species is distinguished from its congeners by the following suite of morphological traits: small body size (SVL 22.9–25.6 mm in six adult males and 27.1 mm in one adult female); head longer than wide; dorsal skin slightly shagreened with small tubercles; creamy-white chest and belly with irregular black spots; distinct ventrolateral glands forming a white line; finger webbing and fringes absent; toe webbing rudimentary and lateral fringes narrow; iris bicolored with bright orange in upper half and silver in lower half; dorsal surface of tadpole head dark brown with small, brown, irregular spot, air sac-shaped bulges on both sides of body. The new species differs from all known congeners by an uncorrected *p*-distance of >5.3% of the 16S rRNA gene fragment examined, and the phylogenetic analysis clusters the new species with *L. maoershanensis* and *L. laui*. At present, the new species is only known from a small range of montane evergreen secondary forests in Basha Nature Reserve approximately 900 m elevation. Its natural history and conservation status are discussed.

* Contributed equally as the first authors.

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Keywords

Integrated taxonomy, morphology, tadpole, vocalization

Introduction

At present, the megophryid genus *Leptobrachella* Smith, 1925 comprises 80 nominal species that are widely distributed from southwestern China to northeastern India, Southeast Asia and Myanmar, through mainland Indochina to peninsular Malaysia and the island of Borneo (Chen et al. 2018; Frost 2020). With the development of DNA barcoding technology and extensive fieldwork, more and more cryptic species in this genus have been reported. From 2016 to 2020, a total of 28 species were described (Eto et al. 2016, 2018; Rowley et al. 2016, 2017a, b; Yang et al. 2016, 2018; Yuan et al. 2017; Duong et al. 2018; Hou et al. 2018; Nguyen et al. 2018; Wang et al. 2018, 2019; Chen et al. 2019, 2020; Hoang et al. 2019; Li et al. 2020; Luo et al. 2020). The discovery of these species indicates that the species diversity of the genus is underestimated, and there still may be a large number of undiscovered cryptic species.

In China, the genus *Leptobrachella* is currently known to comprise 25 species, including *L. alpinus, L. bourreti, L. eos, L. laui, L. liui, L. mangshanensis, L. maoershanensis, L. nyx, L. oshanensis, L. pelodytoides, L. purpura, L. sungi, L. tenngchongensis, L. ventripunctatus, L. wuhuangmontis, L. yingjiangensis, L. yunkaiensis, L. shangsiensis, L. bijie, L. purpuraventra, L. chishuiensis, L. feii, L. flaviglandulosa, L. niveimontis,* and *L. suiyangensis,* occurs widely in Yunnan, Guangxi and Guizhou Province (Chen et al. 2018; Wang et al. 2018; Yang et al. 2018; Chen et al. 2019; Wang et al. 2019; Amphibian China 2020; Li et al. 2020; Luo et al. 2020). More than half of the species of this genus were described in last three years, with more potential new species suggested by previous studies (Chen et al. 2018).

From 2017 to 2019, we collected a series of specimens during the field surveys in Basha Nature Reserve, Guizhou Province, Southern China. These specimens were assigned to genus *Leptobrachella* based on a combination of the following characteristics: small body size, rounded fingertips, presence of an elevated inner palmar tubercle not continuous to the thumb, absence of vomerine teeth, and vertical bars on the anterior tip of the snout. However, distinct morphological and genetic differences were found between the specimens and all recognized species. Subsequent molecular analyses confirmed that these specimens represent an as yet unknown lineage within the *Leptobrachella*. Therefore, we describe these specimens as a new species here.

Materials and methods

Sampling

A total of seven specimens were collected during fieldwork in October 2017 and March to June 2019 within Basha Nature Reserve, Guizhou Province (Fig. 1). All specimens



Figure 1. Collection locality (Basha, red star) of *Leptobrachella bashaensis* sp. nov. specimens from Basha, Guizhou, China used in this study.

were euthanized using chlorobutanol solution, fixed in 10% formalin for 24 hours, then stored in 75% ethanol. Liver or muscle tissues were taken from the specimens before fixing, and preserved in 95% alcohol at -20 °C. These newly collected specimens were subsequently deposited in the Museum of Biology of the Guizhou Institute of Biology, Guizhou Academy of Sciences (GIB, GAS), Guiyang, China.

Morphology and morphometrics

All measurements were obtained to the nearest 0.1 mm (Watters et al. 2016) with digital calipers following the methods of Fei et al. (2009). The measurements of seven adult individuals were as follows:

- **SVL** snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
- HDL head length (distance from the tip of the snout to the articulation of jaw);
- HDW head width (greatest width between the left and right articulations of jaw);
- **SNT** snout length (from tip of snout to anterior corner of eye);
- **EYE** eye diameter (diameter of exposed portion of eyeball);
- **IOD** interorbital distance (minimum distance between the inner edges of the upper eyelids);
- **IND** internasal distance (minimum distance between the inner margins of the external nares);

- **UEW** upper eyelid width (measured as the greatest width of the upper eyelid);
- **NEL** nostril-eyelid length (distance from nostril to eyelid);
- **NSL** nostril-snout length (distance from nostril to snout);
- TMP tympanum diameter (horizontal diameter of tympanum);
- **TEY** tympanum-eye distance (distance from anterior edge of tympanum to posterior corner of eye);
- **TIB** tibia length (tibia length with hindlimb flexed);
- ML manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
- LAHL length of lower arm and hand (distance from tip of the third finger to elbow);
- HLL hindlimb length (distance from tip of fourth toe to vent);
- **FOT** foot length (from proximal edge of inner metatarsal tubercle to tip of fourth toe).

Morphometrics of a single tadpole followed Ohler et al. (2011), a total of nine morphometric characters of tadpole were measured:

- **BH** maximum body height;
- **BL** body length (distance from base of vent to the tip of snout);
- **BW** maximum body width;
- **ED** maximum eye diameter (diameter of exposed portion of eyeball);
- TH maximum tail height (maximum height between upper and lower edges of tail);
- **SS** snout to spiraculum (distance from the tip of the snout to the opening of the spiracle);
- TMW maximum tail muscle width;
- TL tail length (distance from base of vent to the tip of tail);
- TOL total length (distance from the tip of snout to the tip of tail).

DNA sequencing and analysis of sequences

Standard phenol-chloroform extraction with ethanol precipitation protocol was used to extract genomic DNA from tissues (Sambrook et al. 1989). We amplified a 565-567 bp fragment 16S rRNA using polymerase chain reactions (PCRs), using the primers, and cycling conditions given by Kocher et al. (1989). Amplified PCR products were purified with enzymatic purification using exonuclease 1 and shrimp alkaline phosphatase that was incubated at 37 °C for 15 min followed by 80 °C to inactivate the enzymes. Purified PCR products were used as templates for Big Dye (Applied Biosystems, Foster City, CA) cycle sequencing. We conducted all sequencing on an ABI 3100 automated sequencers (Applied Biosystems) at Suoqin, Beijing. Considering that the morphological characters of the newly collected specimens are closest to those of *L. maoershanensis*, 19 sequences representing 16 recognized species of the seven clades belonging to the genus *Leptobrachella* from southern China (A1–A7; Chen et al. 2018) were retrieved from GenBank and included in the following phylogenetic analyses (Table 1). *Megophrys major* Boulenger, 1908 was selected as the outgroup based on the

ID		Ilit	Variahan	CanPanhan
1	Jamaus	Vilabuly Sayannakhat Laos	NCSM 76039	MU055800
1	L. aereus	Savannakhet, Laos	NC3M 70030	VD018122
2	L. aereus	Savannaknet, Laos	VI70/6916	MU055966
3	L. aipinus I. hiiin	Piiis City Cuickey, China	SVS -007212	MF/1/522
4	L. Dijie I. bijie	Bijie City, Guizhou, China Bijie City, Guizhou, China	SVS 2007314	MK414532
6	L. Dijie I. houmati	Sana Lao Cai, Viatnam	1000 5660	KD827860
7	L. bourren	Bat Vat Lao Cai Vietnam	7MMU A 5636 02280	MH055872
/ 8	L. oburren I chishuigneis	Chishui City Guizhou Province, China	CIBC\$201905180/7	MT117053
0	L. chishuiensis	Chishui City, Quizhou Province, China	CIBC\$20190518047	MT117054
2 10	L. UNISMULENSIS	Boun Tay, Dhonggaly, Loos	NCSM 80551	MU055887
11	L. cos	Boun Tay, Phongsaly, Laos	MNHN-200/ 0277	INI8/8//8
12	L. COS I faii	Wenshan Prefecture Vunnan Province China	KI7032625	MT302635
13	L. jeii I feii	Honghe Yunnan Province, Chin	KIZ048973	MT302637
14	L. firthi	Ngoc Linh Nature Reserve, Kon Tum, Vietnam	AMS: R 176506	IO739207
15	L. flaviølandulosa	Xiaogiaogou Nature Reserve, Yunnan, China	KIZ016064	MT302624
16	I. flaviglandulosa	Xiaogiaogou Nature Reserve, Yunnan, China	KIZ032626	MT302633
17	L. isos	Gia Lai, Vietnam	AMS: R 176469	KT824767
18	L. khasiorum	Khasi Hills, Meghalava, India	SDBDU 2009.329	KY022303
19	L. laui	San zhoutian. Shenzhen. China	SYS a002540	MH055904
20	L. laui	Mt. Wutong, Shenzhen, China	SYS a003477	MH605576
21	L. liui	Mt. Wuvi, Fujian, China	SYS a002478	MH605573
22	L. liui	Mt. Huanggang, Jiangxi, China	SYS a001620	KM014549
23	L. manøshanensis	Mangshan, Hunan, China	MSZTC201702	MG132197
24	L. mangshanensis	Mangshan, Hunan, China	MSZTC201703	MG132198
25	L. maoershanensis	Maoershan, Guangxi, China	KIZ019386	KY986931
26	L. maoershanensis	Mao'er Shan, Guangxi, China	KIZ07614	MH055927
27	L. maoershanensis	Mao'er Shan, Guangxi, China	KIZ027236	MH055928
28	L. bashaensis sp. nov.	Basha Nature Reserve, Guizhou, China	GIB196403	MW136294
29	L. bashaensis sp. nov.	Basha Nature Reserve, Guizhou, China	GIB196404	MW136295
30	L. minimus	Doi Chiang Dao, Chiangmai, Thailand	THNHM07418	JN848402
31	L. minimus	Doi Suthep, Thailand	KUHE:19201	LC201981
32	L. nahangensis	Na Hang Nature Reserve, Tuyen Quang, Vietnam	ROM 7035	MH055853
33	L. nahangensis	Na Hang, Tuyen Quang, Vietnam	ZMMU-NAP-02259	MH055854
34	L. namdongensis	Thanh Hoa Provincen, Vietnam	VNUF A.2017.37	MK965389
35	L. niveimongtis	Daxueshan Nature Reserve, Yunnan, China	KIZ028276	MT302620
36	L. niveimongtis	Daxueshan Nature Reserve, Yunnan, China	KIZ028277	MT302621
37	L. nyx	Mount Tay Conn Linh, Ha Giang, Vietnam	AMNH A163810	DQ283381
38	L. nyx	Ha Giang, Ha Giang, Vietnam	ROM 36692	MH055816
39	L. oshanensis	Emei Shan, Sichuan, China	KIZ025776	MH055895
40	L. oshanensis	Emei Shan, Sichuan, China	YPX37492	MH055896
41	L. petrops	Cham Chu Nature Reserve, Tuyen Quang, Vietnam	VNMN:2016 A.06	KY459998
42	L. pluvialis	Fansipan, Lao Cai, Vietnam	ROM 30685	MH055843
43	L. puhoatensis	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	VNMN:2016 A.23	KY849587
44	L. puhoatensis	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	AMS:R184852	KY849588
45	L. purpuraventra	Bijie City, Guizhou, China	SYS a007081	MK414517
46	L. purpuraventra	Bijie City, Guizhou, China	SYS a007277	MK414518
47	L. shangsiensis	Guangxi Prov.,China	NHMG1401032	MK095460
48	L. shangsiensis	Guangxi Prov.,China	NHMG1401033	MK095461
49	L. suiyangensis	Suiyang County,Guizhou, China	GZNU20180606002	MK829648
50	L. suiyangensis	Suiyang County,Guizhou, China	GZNU20180606006	MK829649
51	L. sungi	Tam Dao, Vinh Phuc, Vietnam	ROM 20236	MH055858
52	L. tengchongensis	Gaoligong Shan, Yunnan, China	SYS a004598	KU589209
53	L. tengchongensis	Gaoligong Shan, Yunnan, China	SYS a003766	MH055897
54	L. ventripunctatus	Zhushihe, Yunnan, China	SYS a004536	MH055831
55	L. yunkaiensis	Dawuling Forest Station, Maoming City, Guangdong, China	SYS a004663	MH605584
56	L. yunkaiensis	Dawuling Forest Station, Maoming City, Guangdong, China	SYS a004664	MH605585
57	L. zhangyapingi	Chiang Mai, Thailand	K1Z07258	MH055864
58	Megophrys major	Kon Ium, Vietnam	AMS K173870	KY4/6333

Table 1. List of voucher specimens and GenBank accession numbers for all DNA sequences of the mitochondrial 16S rRNA gene fragments of *Leptobrachella* included in this study.

current phylogenetic hypothesis of *Leptobrachella* (Chen et al. 2018). All the sequences were aligned using MUSCLE v. 3.6 with the default settings (Edgar 2004).

Phylogenetic relationships were inferred using both maximum likelihood (ML) and Bayesian inference (BI). ML analysis was performed with RAx ML Black Box (Stamatakis et al. 2008) under the GTR + G model (1000 replicates). BI was performed using MrBayes v. 3.2.1 (Ronquist et al. 2012). The best-fit model was determined using the Akaike Information Criterion (AIC) computed with jModel Test 2 (Darriba et al.2012). Two independent runs were conducted for 30 million generations, sampling every 1000, with four independent chains and a burn-in of 25%. Convergence was assessed by checking stationary distribution and effective sample sizes (>200) using Tracer v. 1.6. (Rambaut et al.2014). We used MEGA v. 5.2 (Tamura et al. 2011) to calculate the interspecific mean uncorrected pairwise distances between the samples from Basha and its most closely related species based on the phylogenetic results. Besides, intraspecific distance was calculated among the samples from Basha.

Results

A total of 565-567 base pairs (bp) of the 16S gene were obtained in the final alignment. The two sequences belonging to the newly acquired specimens (GenBank accession numbers MW136294 and MW136295) shared a same haplotype. The alignment contained 242 variable sites and 186 parsimony informative sites. The observed interspecific uncorrected *p*-distances between the new population from Basha and all species from clade A based on the study of Chen et al. (2018) varied from 5.3% (*L. maoershanensis*) to 15.7% (*L. firthi*) (Table 2).

Both ML and BI (Fig. 2) analyses yielded near-identical results except for the poorly supported nodes (BS < 75% or BPP < 0.95). The relationships of the most clades (A1–A7) of *Leptobrachella* were not resolved, which is similar to previous studies (Yang et al. 2016; Yuan et al. 2017) due to limited information of 16S. The samples from Basha, Guizhou, formed a monophyletic group (Clade A4, Fig. 2) with *L. liui, L. laui, L. mangshanensis, L. maoershanensis*, and *L. yunkaiensis* with high support in both analyses, but the relationships among these species were not resolved. The samples from Basha formed a lineage that clustered with *L. maoershanensis*, but with low support (BPP < 95% and BS < 70%). In combination with morphological differences (see below), we conclude that the newly collected Basha specimens represent a distinct species, and we describe it herein.

Taxonomic account

Leptobrachella bashaensis sp. nov.

http://zoobank.org/6257CDCB-B124-4649-BE10-9A4874C67F20 Figs 3–7; Tables 1–3

Type material. *Holotype.* GIB196403, adult male, from Basha Nature Reserve, Congjiang, Guizhou Province, China (25.8534°N, 108.7356°E, elevation 900 m. a.s.l.;


Figure 2. Bayesian Inference of *Leptobrachella* inferred from a 567 bp fragment of the 16S rRNA gene. Node support is indicated on branches as Bayesian posterior probabilities (BPP) (displayed values > 0.95) and Maximum Likelihood support (displayed values > 70); "-" represents low support values. The scale bar represents 0.07 nucleotide substitutions per site.

Fig. 1), collected by Jingcai Lyu and Liangliang Dai on 4 June 2019. *Paratypes*. GIB196401–02, GIB196404, GIB196406–07 (five adult males) collected from the holotype locality by Jingcai Lyu on 4 June 2019.

Diagnosis. The new species is assigned to the genus *Leptobrachella* on the basis of the following characters: small size, rounded fingertips, presence of an elevated inner palmar tubercle not continuous to the thumb, presence of supra-axillary, femoral and ventrolateral glands, vomerine teeth absent, tubercles on eyelids, and pale vertical bar present on anterior tip of snout (Dubois 1980, 1983; Ohler et al. 2011; Rowley and Cao 2009; Rowley et al. 2013). Leptobrachella bashaensis sp. nov. is distinguished from its congeners by a combination of the following morphological characters: (1) small size (SVL 22.9–25.6 mm in six adult males and 27.1 mm in one adult female), (2) head longer than wide, (3) externally distinct tympanum, (4) dorsal skin slightly shagreened with small tubercles and irregular brown stripes, (5) distinct dark spots on the flank, (6) creamy-white chest and off-white belly with irregular black spots, (7) grey-pinkish to dark brownish-violet ventral skin of limbs with numerous whitish speckles, (8) distinct ventrolateral glands, forming a distinct white line, (9) finger webbing and fringes absent, (10) toe webbing rudimentary and lateral fringes narrow, (11) longitudinal ridges under toes and not interrupted at the articulations, (12) a distinctly bicolored iris, typically bright orange in upper half, fading to silver in lower half.

Description of the holotype. Adult male. SVL 24.0 mm; head slightly longer than wide (HDL/HDW = 1.10), rectangular in dorsal view (Fig. 3A, B); snout rounded in both ventral view and lateral view, protruding slightly beyond lower jaw (Fig. 3D); nostril oval, located closer to tip of snout than to eye; loreal region oblique;

)	2		7	4		•					7			2		
	Species	1	2	3	4	5	9	7	8	6	10	11	12	13	14	15	16
-	L. bashaensis sp. nov.	I															
7	L. maoershanensis	0.053	I														
3	L. mangshanensis	0.073	0.064	I													
4	L. liui	0.089	0.070	0.026	I												
Ś	L. laui	0.068	0.081	0.081	0.085	I											
9	L. yunkaiensis	0.082	0.086	0.078	0.082	0.082	I										
~	L. flaviglandulosa	0.061	0.074	0.066	0.061	0.077	0.062	I									
œ	L. niveimontis	0.112	0.117	0.131	0.108	0.116	0.113	0.087	I								
6	L. chishuiensis	0.103	0.121	0.130	0.112	0.120	0.121	0.087	0.049	I							
10	L. namdongensis	0.107	0.119	0.116	0.112	0.099	0.130	0.104	0.131	0.116	I						
11	L. suiyangensis	0.120	0.130	0.148	0.139	0.138	0.130	0.112	0.057	0.053	0.143	I					
12	L. bijie	0.116	0.134	0.135	0.117	0.121	0.134	0.095	0.053	0.015	0.125	0.057	I				
13	L. purpuraventra	0.103	0.104	0.130	0.113	0.129	0.121	0.095	0.054	0.034	0.117	0.049	0.045	I			
14	L. shangsiensis	0.107	0.107	0.126	0.122	0.129	0.125	0.095	0.140	0.148	0.131	0.166	0.157	0.139	I		
15	L. feii	0.086	0.090	0.130	0.124	0.120	0.135	0.091	0.131	0.131	0.130	0.135	0.140	0.135	0.091	I	
16	L. trngchongensis	0.098	0.103	0.108	0.095	0.086	0.125	0.099	0.103	0.111	0.070	0.119	0.119	0.102	0.116	0.121	I
17	L. oshanensis	0.105	0.104	0.113	0.096	0.127	0.126	0.091	0.062	0.077	0.132	0.086	0.082	0.057	0.131	0.133	0.108
18	L. eos	0.107	0.107	0.125	0.108	0.115	0.107	0.082	0.042	0.069	0.121	0.073	0.073	0.053	0.134	0.139	0.094
19	L. bourreti	0.060	0.067	0.080	0.062	0.075	0.073	0.047	0.025	0.032	0.081	0.040	0.043	0.022	0.077	0.069	0.063
20	L. alpinus	0.095	0.117	0.122	0.116	0.107	0.113	0.074	0.053	0.042	0.126	0.049	0.057	0.049	0.140	0.109	0.107
21	L. zhangypingi	0.115	0.132	0.121	0.125	0.124	0.127	0.086	0.148	0.138	0.130	0.150	0.147	0.141	0.133	0.125	0.111
22	L. sungi	0.111	0.116	0.134	0.121	0.133	0.121	0.112	0.140	0.138	0.104	0.139	0.143	0.130	0.116	0.112	0.091
23	L. nahangensis	0.098	0.103	0.121	0.099	0.115	0.116	0.082	0.117	0.108	0.096	0.126	0.121	0.108	0.073	0.086	0.104
24	L. pluvialis	0.087	0.078	0.105	0.095	0.115	0.098	0.074	0.130	0.130	0.131	0.130	0.148	0.122	0.073	0.082	0.126
25	L. ventripunctatus	0.112	0.108	0.127	0.104	0.124	0.128	0.091	0.144	0.148	0.118	0.176	0.158	0.149	0.082	0.082	0.113
26	L. пух	060.0	0.095	0.109	0.091	0.107	0.121	0.078	0.099	0.112	0.109	0.139	0.121	0.121	0.078	0.065	0.104
27	L. aereus	0.086	0.086	0.117	0.099	0.119	0.134	0.082	0.126	0.113	0.113	0.139	0.122	0.122	0.082	0.049	0.116
28	L. miniums	0.079	0.079	0.105	0.101	0.113	0.100	0.075	0.119	0.118	0.096	0.127	0.136	0.109	0.063	0.075	0.105
29	L. puhoatensis	0.111	0.121	0.126	0.121	0.116	0.148	0.130	0.153	0.129	0.062	0.157	0.147	0.129	0.141	0.148	0.087
30	L. petrops	0.129	0.124	0.117	0.109	0.125	0.140	0.118	0.150	0.143	0.086	0.143	0.153	0.134	0.154	0.139	0.082
31	L. khasiorum	0.156	0.139	0.148	0.126	0.143	0.138	0.144	0.167	0.166	0.135	0.186	0.185	0.176	0.182	0.191	0.125
32	L. isos	0.134	0.139	0.154	0.134	0.155	0.153	0.121	0.144	0.167	0.135	0.158	0.177	0.158	0.149	0.148	0.131
33	L. firthi	0.157	0.172	0.153	0.130	0.143	0.169	0.130	0.143	0.152	0.141	0.162	0.166	0.162	0.153	0.153	0.131

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Tab	le 2. Continued.																	
	Species	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
-	L. bashaensis sp. nov.																	
2	L. maoershanensis																	
3	L. mangshanensis																	
4	L. liui																	
Ś	L. laui																	
9	L. yunkaiensis																	
~	L. flaviglandulosa																	
8	L. niveimontis																	
6	L. chishuiensis																	
10	L. namdongensis																	
11	L. suiyangensis																	
12	L. bijie																	
13	L. purpuraventra																	
14	L. shangsiensis																	
15	L. feii																	
16	L. trngchongensis																	
17	L. oshanensis	I																
18	L. eos	0.061	I															
19	L. bourreti	0.043	0.025	I														
20	L. alpinus	0.074	0.057	0.030	I													
21	L. zhangypingi	0.139	0.128	0.090	0.120	I												
22	L. sungi	0.139	0.134	0.075	0.139	0.081	I											
23	L. nahangensis	0.113	0.108	0.043	0.104	0.120	0.099	I										
24	L. pluvialis	0.122	0.130	0.069	0.117	0.112	0.099	0.069	I									
25	L. ventripunctatus	0.141	0.144	0.077	0.144	0.112	0.103	0.061	0.066	I								
26	L. nyx	0.122	0.116	0.045	0.108	0.120	0.095	0.053	0.074	0.062	I							
27	L. aereus	0.129	0.134	0.065	0.108	0.124	0.098	0.061	0.074	0.068	0.046	I						
28	L. miniums	0.110	0.105	0.050	0.110	0.103	0.079	0.055	0.059	0.063	0.062	0.071	I					
29	L. puhoatensis	0.142	0.152	0.092	0.143	0.153	0.114	0.105	0.131	0.136	0.127	0.144	0.111	I				
30	L. petrops	0.149	0.134	0.094	0.139	0.152	0.126	0.118	0.134	0.150	0.123	0.140	0.115	0.082	I			
31	L. khasiorum	0.167	0.152	0.124	0.176	0.175	0.157	0.136	0.154	0.168	0.159	0.176	0.114	0.135	0.122	I		
32	L. isos	0.141	0.149	0.098	0.149	0.153	0.149	0.112	0.153	0.129	0.134	0.145	0.132	0.151	0.170	0.193	I	
33	L. firthi	0.149	0.162	0.097	0.152	0.153	0.140	0.107	0.144	0.117	0.117	0.137	0.133	0.146	0.156	0.156	0.136	I



Figure 3. Holotype of *Leptobrachella bashaensis* sp. nov. (GIB196403) in life **A** frontolateral view **B** lateral view **C** iris coloration **D** W-shaped marking **E** dorsal view of thighs **F** posterioventral view of thighs **G** ventral view.

canthus rostralis distinct; eye large (EYE/HDL = 0.35), diameter slightly smaller than snout length (EYE/SNT = 0.88), notably protuberant in both dorsal and lateral view; pupil vertical; tympanum distinct, rounded, diameter smaller than that of eye (TMP/



Figure 4. Holotype of *Leptobrachella bashaensis* sp. nov. (GIB196403) in life **A**, **B** ventral and dorsal view of the hand **C**, **D** dorsal and ventral views of the foot.

EYE = 0.60) (Fig. 3C); vomerine teeth absent; vocal sac openings slit-like, located posterolateral on floor of mouth; tongue long and moderately wide, with a shallow notch at posterior tip; supratympanic ridge distinct, running from eye towards axillary with raised tubercles. Forelimb long and slender, fingers long and slender, without webbing and lateral fringes; relative length of fingers II<I<IV<III; tips of fingers rounded and slightly swollen; nuptial pad absent; subarticular tubercles absent in fingers; inner metacarpal tubercle large and rounded, separated from laterally compressed and much smaller outer metacarpal tubercle (Fig. 4A, B). Hindlimb moderately long; tibia half of snout-vent length (TIB/SVL = 0.50); tibiotarsal articulation of adpressed limb reaching snout, well beyond anterior margin of eye, but not beyond snout tip; relative toe lengths I<II<V<IIII<IV; toe tips rounded and thickened; greatly reduced basal webbing present between all five toes; narrow lateral fringes present only on II and III toes (Fig. 4C, D); subarticular tubercles hardly discernible under toes II and III; dorsal skin slightly rough with small tubercles and irregular pustules; ventral skin smooth; oval supra-axillary gland present at forelimb base on ventral surface of axillary region (Fig. 3F); oval femoral glands distinct on posteroventral surface of thigh, closer to knee than to vent; ventrolateral glands forming a distinct line on flanks.

Coloration of holotype in life. Dorsal surface of head and trunk brown with small, dark brown, irregularly shaped markings; large reverse-triangle dark brown marking between eyes, connected to the W-shaped mark between axillae (Fig. 3); supratympanic ridge present; elbow to upper arm reddish in color; fine, distinct reddish tubercles scattered on upper eyelids, dorsal surfaces of head, body, and limbs. Ventral surface of throat and belly off-white, with faint spots; chest creamy white, with



Figure 5. Holotype of *Leptobrachella bashaensis* sp. nov. (GIB196403) in preservation **A** dorsal view **B** ventral view **C** volar view of hand **D** plantar view of foot.

irregular black spots (Fig. 3G, H); ventral surfaces of lip and limbs covered with irregular white speckles; ventral surface of thighs grey-brown with white spots. Supra-axillary gland, ventrolateral glands, and femoral glands white. Iris bicolored: bright orange in upper half, silver in lower half, with black reticulations throughout. Fine, faint transverse dark brown bars on dorsal surface of fingers and toes, lower arms, tarsus, thighs, and tibia (Fig. 4).

Coloration of holotype in preservation. In preservative (75% ethanol), dorsal surface dark grey-brown, and ventral and lateral white glands not evident (Fig. 5); ventral surface of throat, chest, belly, and interior portions of arms fade to creamy white, ventral surface of thighs dark brown with white spots; bars and blotches on dorsum and limbs are dark brown and less apparent. The color of the tympanum fades to brown.

Tadpoles. Brown, narrow; BH 4.4 mm; BL 14.1 mm; BW 6.2; ED 2.0 mm; TH 3.8; SS 4.9 mm; TMW 3.4 mm; TL 29.3 mm; TOL 43.8 mm. TAL/TOL = 0.67, with a I:3+3/1+1:II labial tooth row formula (Fig. 6). In life, dorsal surface of head dark brown with small, brown, irregularly shaped spot; air sac-shaped bulges on both sides of the body; upper lip and lower lip nearly round shape. Tadpoles were collected in the field from a stream surveyed on 16 October 2017 in Basha Nature Reserve by Lyu.



Figure 6. Tadpole of *Leptobrachella bashaensis* sp. nov. in preservative **A** dorsal view **B** ventral view **C** dorsal view **D** labial tooth.

Etymology. The specific epithet, "*bashaensis*"/Basha Zhang Tu Chan (岜沙掌突蟾 in Chinese), refers to the location where the specimens were collected, Basha Nature Reserve of Guizhou Province, China.

Morphological variation. Variation in size and body proportions of the type series are shown in Table 3. Representative photographs of paratypes in life are shown in Fig. 7. In life, the dorsal surface of the head and trunk varies from olive-brown to reddish; the ventral surface of the lower jaw among these individuals varies from cream-yellow, pink, to grey; the ventral surface of chest and belly is grey-pink to white. There is variation among individuals in the shape of tubercles, pustules, black ventrolateral blotches, and dark stripes, bars on the dorsum or dorsal surface of limbs. The W-shaped marking between axillae is distinct in all individuals.

Natural history. All specimens were collected at night in small streams in Basha Nature Reserve approximately 900 m elevation (Fig. 8). Calling males were found along the streams, perching on large rocks, in rocky crevices, or under dead wood. Insect-like calls could be heard in June. The breeding season of this species is likely to occur from June to July, as females collected during these months were gravid, and males were heard calling only from June to the beginning of July. During both surveys, the number of males observed was much greater than females (males:females = 12:1).

Comparisons. *Leptobrachella bashaensis* sp. nov. differs from all known *Leptobrachella* species distributed north of the Isthmus of Kra by a combination of male body size,

Voucher number	GIB196401	GIB196402	GIB196403	GIB196404	GIB196406	GIB196407	GIB196405
Sex	Male	Male	Male	Male	Male	Male	Female
SVL	22.9	23.0	24.0	24.6	25.6	24.2	27.1
HDL	8.4	8.3	9.4	8.7	9.4	9.1	9.6
HDW	8.0	7.5	8.7	8.1	8.2	8.0	8.2
SNT	3.3	3.4	3.5	3.5	4.0	3.8	3.8
IND	2.5	2.4	2.4	2.7	2.7	2.5	2.9
IOD	3.2	3.5	3.3	3.3	3.3	3.2	3.3
UEW	2.3	2.1	2.2	2.2	2.3	2.5	2.3
EYE	2.8	2.7	2.9	3.1	3.5	3.3	3.6
NEL	1.5	1.6	1.8	2.0	1.9	1.8	2.0
NSL	1.9	1.8	1.7	1.7	1.9	1.6	1.9
TEY	0.7	0.9	1.1	0.9	1.0	1.0	1.1
ML	6.2	5.6	6.1	6.0	6.0	6.3	6.3
TMP	2.1	1.6	2.2	1.9	1.9	1.7	1.9
LAHL	11.7	10.4	10.8	10.9	11.5	11.6	11.9
HLL	37.4	37.6	40.7	40.3	38.8	39.7	41.3
TIB	12.1	11.4	12.6	12.3	12.1	13.2	12.9
FOT	11.0	10.9	11.2	11.3	10.6	11.6	11.8

Table 3. Morphometric measurements (in mm) of *Leptobrachella bashaensis* sp. nov. Abbreviations are defined in the text.



Figure 7. Paratypes of Leptobrachella bashaensis sp. nov. in life (GIB196404).



Figure 8. Habitat at the type locality of *Leptobrachella bashaensis* sp. nov., Basha Nature Reserve, Congjiang, Guizhou province, China.

externally distinct tympanum, and black spots on the flank, plus ventral coloration, degree of webbing and fringing on the toes, iris coloration, and dorsal skin texture.

Care was taken to differentiate *Leptobrachella bashaensis* sp. nov. from the five most morphologically and molecularly similar species, *L. maoershanensis*, *L. laui*, *L. man-gshanensis*, *L. yunkaiensis*, and *L. liui* from China.

Leptobrachella bashaensis sp. nov. differs from its sister taxon *L. maoershanensis* by the following: smaller body size (SVL 22.9–25.6 mm in males, 27.1 mm in female vs 25.2–30.4 mm in males, 28.6 mm in female); bicolored iris with bright orange in upper half, fading to silver in lower half (vs typically bright orange-red in upper half, fading to silver in lower half); vertical and distinct narrow pupil (vs vertical and wide pupil); narrow lateral fringes present only on II and III toes (vs narrow lateral fringes present on all toes).

Leptobrachella bashaensis sp. nov. differs from *L. laui* in having slightly a smaller body size (SVL 22.9–25.6 mm in males, 27.1 mm in female vs 24.8–26.7 mm in males, 28.1 mm in female); bicolored iris with bright orange in upper half, fading to silver in lower half (vs uniformly coppery orange with fine black reticulations throughout); vertical and distinct narrow pupil (vs vertical and wide pupil); narrow lateral fringes present only on II and III toes (vs wide lateral fringes present on all toes); distinct black spots on the flank (vs indistinct black spots on the flank).

Leptobrachella bashaensis sp. nov. differs from *L. mangshanensis* by having slightly smaller body size (SVL 22.9–25.6 mm in males, 27.1 mm in female vs 22.2–27.8 mm in males, 30.2 mm in female); bicolored iris with bright orange in upper half, fading to silver in lower half (vs bright orange in upper half, greyish cream in lower half); narrow lateral fringes present only on II and III toes (vs weak lateral fringes on toes).

Leptobrachella bashaensis sp. nov. differs from *L. yunkaiensis* by having smaller body size (SVL 22.9–25.6 mm in males, 27.1 mm in female vs 25.9–29.3 mm in males, 34.0–35.3 mm in females); bicolored iris with bright orange in upper half, fading to silver in lower half (vs coppery orange in upper half and silver in lower half); narrow lateral fringes present only on II and III toes (vs wide lateral fringes present on all toes); distinct black spots on the flank (vs indistinct black spots on the flank).

Leptobrachella bashaensis sp. nov. differs from *L. liui* in having slightly smaller body size (SVL 22.9–25.6 mm in males, 27.1 mm in female vs 23.0–28.7 mm in males, 23.1–28.1 mm in females); vertical and distinctly narrow pupil (vs vertical and wide pupil); narrow lateral fringes present only on II and III toes (vs wide lateral fringes present on all toes).

Discussion

Chen et al. (2018) suggested that *Leptobrachella* may contain hidden species diversity based on multiple nuclear DNA markers assessed using larger sample sizes, combined with morphological, nuclear gene, and bioacoustic data. With the development of DNA barcoding technology and extensive field work, more and more cryptic species in this genus have been reported. The number of *Leptobrachella* species recorded in

China is now up to 25, including the new species. The discovery of this species reaffirms that the diversity within the genus is underestimated, with many species yet to be discovered.

Guizhou Province is located in the typical Karst landform area of southern China with an abundance of species diversity. So far, eight species of the genus *Leptobrachella* (*L. liui, L. oshanensis, L. pelodytoides, L. ventripunctatus, L. bijie, L. purpuraventra, L. chishuiensis*, and *L. suiyangensis*) were recorded in Guizhou province, while *L. bashaensis* sp. nov. has not been included in previous studies, which indicates that there may potentially be more new species to be found by further field research (Wu et al. 1987; Wang et al. 2019).

We observed a restricted distribution of the species in Basha Natural Reserve. We believe that *L. bashaensis* sp. nov. has a specific niche associated with small streams. The following threats were observed: reduced forest coverage, soil erosion, habitat destruction, and local people using the tadpole of *L. bashaensis* sp. nov. for food. Hence, greater protection is required for this endemic species inhabiting this high-diversity region.

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