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Taxonomic study of the genus *Unkanodes* (Hemiptera, Fulgoroidea, Delphacidae) from Pakistan, with description of a new species

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Abstract

Unkanodes (*Kwonianella*) *malamjabbensis* **sp. nov.** (Hemiptera, Delphacidae) is described and illustrated and *U. latespinosa* (Dlabola, 1957) is newly recorded from Malamjabbah, Swat, Pakistan. These two species represent the first records of the genus *Unkanodes* Fennah, 1956 from Pakistan. A key to the world's species of the genus *Unkanodes* is provided.

Keywords

Distribution, Fulgoromorpha, key, morphology, taxonomy

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Introduction

The planthopper family Delphacidae currently consists of 2217 species in 427 genera (Bourgoin 2020). Delphacids are small insects that can be easily distinguished by the presence of a large, movable spur (the "calcar") at the apex of the hind tibiae (Bartlett 2014). Most delphacids are grass-feeders, although some feed on other monocots such as sedges and rushes, and some feed on dicots (Bartlett 2019). A number of species feed on economically important crops, such as sugarcane, maize and rice (Wilson and O'Brien 1987; Wilson et al. 1994). The plant order Poales accounts for more than 70% of their hosts, while all other plant orders provide only 3%. In the Poales group, the family Poaceae has the highest (52%) percentage of hosts, followed by Cyperaceae (16.5%), while the remaining families account for only 3% of host records (Bourgoin 2020).

The delphacid fauna of Pakistan has been poorly studied, with only ten species previously recorded from this country (Bourgoin 2020). This figure, consisting of about 0.4% of the world's described species, likely largely underrepresents the actual diversity of delphicids in this country. The genus *Unkanodes* was established by Fennah (1956) with the type species *Unkanodes sapporona* (Matsumura, 1935) from Che-Kiang (Zhejiang Province of China). Currently, this genus comprises nine species (Bartlett 2019) occurring in Afghanistan, Alaska, Austria, China, Denmark, Estonia, Finland, Germany, Greece, Iran, Japan, Lithuania, Mongolia, Poland, Russia, South Korea, Sweden, Taiwan, Turkey, Ukraine, Yugoslavia, and the U.S.A. (Bourgoin 2020). The genus *Unkanodes* is economically important and its members are vectors of many diseases in rice and cereals and also the causative agents of hopper burn diseases. *Unkanodes albifascia* is responsible for transmission of NCMV (Northern cereal mosaic virus), RBSDV (Rice black-streaked dwarf virus) and stripe disease. *Unkanodes sapporona* is involved in the transmission of NCMV, RSV (Rice stripe tenuivirus), and RBSDV. *Unkanodes tanasijevici* is reported to be a vector of IMMV (Iranian Maize Mosaic Nucleorhabdovirus), MIMV (Maize Iranian Mosaic Virus), MRDV (Maize Rough Dwarf Fiji Virus), and RBSDV (Bartlett 2019).

In this study, *U.* (*Unkanodes*) *latespinosa* (Dlabola, 1957) is recorded for the first time from Pakistan and a new species *U.* (*Kwonianella*) *malamjabbensis* sp. nov., is described.

Materials and methods

Specimens were collected from Pakistan and deposited at the Entomological Museum of Northwest A&F University (**NWAFU**) Yangling, Shaanxi, China. Morphological terminology follows Asche (1985), Wilson (2005) and Bartlett et al. (2014). The method for genitalia preparation and clearing follows Wilson and McPherson (1980) and Wilson (2005). Morphological characters were observed using the stereomicroscope Olympus SZX10. Measurements of characters are given in millimeters (mm). Photographs of the adults were taken using a Zeiss AxioCam ICc 5. Adobe Photoshop was used for labeling and plate composition of the obtained images.

Taxonomy

Family Delphacidae Leach, 1815
Subfamily Delphacin.ae Leach, 1815
Tribe Delphacini Leach, 1815

Genus *Unkanodes* Fennah, 1956

Unkanodes Fennah, 1956.
Unkanodes sapporona (Matsumura, 1935), comb. by Fennah 1956: 474.

Type species. *Unkana sapporona* Matsumura, 1935: 131, by original designation.

Diagnosis. Relatively slender, head slightly narrower than pronotum. Vertex longer than broad, its width at base not exceeding width of an eye, shallowly rounded at apical margin; carinae of vertex and frons distinct. Frons parallel-sided, about 2.0–2.5 times as long as wide, lateral margins parallel, narrowing upwards in apical 1/3; median carina of frons bifurcates near fastigium. Lateral carinae of pronotum diverging, vanishing before reaching posterior margin. Calcar with 10–20 well-developed teeth; apical tooth separate from the remaining teeth. Posterior margin of pygofer with a cut on the sides. Segment X (anal tube) with a pair of teeth or teeth absent. Styli flattened, diverging or more or less parallel beyond middle, with complex apices, zigzag-shaped bent and wide or narrowed and slanting outwards. Armature of diaphragm (bridge of pygofer) bearing a pair of teeth directed upwards or a projection with 2 apices. Aedeagus more or less straight, or bent ventrad, elbow-shaped, slightly asymmetrical due to location of gonopore and arrangement of teeth on aedeagal shaft (after Fennah 1956 and Anufriev and Emeljanov 1988).

Checklist of species of the genus *Unkanodes* Fennah

Subgenus *Unkanodes* (*Chilodelphax* Vilbaste, 1968)

Unkanodes* (*Chilodelphax*) *silvaticus Vilbaste, 1968
Unkanodes silvaticus Vilbaste, 1968: 24.
Chilodelphax silvaticus (Vilbaste, 1968); comb. by Kwon 1982: 4.
Unkanodes (*Chilodelphax*) *silvaticus* Vilbaste, 1968; comb. by Anufriev and Emeljanov 1988: 409.

Subgenus *Unkanodes* (*Unkanodes* Fennah, 1956)

Unkanodes* (*Unkanodes*) *excisa (Melichar, 1898)
Liburnia excisa Melichar, 1898: 67.
Delphax excisa (Melichar, 1898); comb. by Puton 1899: 108.
Liburnia elymi Jensen-Haarup, 1917: 3; syn. by Jensen-Haarup 1920: 53.
Delphacodes excisa (Melichar, 1898); comb. by Metcalf 1943: 436.
Elymodelphax excisa (Melichar, 1898); comb. by Wagner 1963: 167.
*Unkanodes excis*a (Melichar, 1898); comb. by implication Dlabola 1965: 86.

Unkanodes* (*Unkanodes*) *latespinosa (Dlabola, 1957)
Calligypona latespinosa Dlabola, 1957.
Unkanodes latespinosa (Dlabola, 1957), comb. apparently by Dlabola 1964: 240 (see also Dlabola 1967: 53; Emeljanov 1977: 113).
Unkanodes* (*Unkanodes*) *paramarginata (Dlabola, 1961: 275)
Unkanodes* (*Unkanodes*) *sapporona (Matsumura, 1935)
Unkana sapporona Matsumura, 1935.
Unkanodes sapporona (Matsumura, 1935), comb. by Fennah 1956: 474.
Unkanodes* (*Unkanodes*) *tanasijevici (Dlabola, 1965)
Elymodelphax tanasijevici Dlabola, 1965.
Calligypona zeravshanica Dubovsky, 1967; syn. by Emeljanov 1982: 98.
Ribautodelphax notabilis Logvinenko, 1970

Subgenus *Unkanodes* (*Kwonianella* Anufriev, 1988)
Unkanodes* (*Kwonianella*) *albifascia (Matsumura, 1900: 268)
Liburnia albifascia Matsumura, 1900: 268.
Delphax albifascia (Matsumura, 1900); comb. by Oshanin, 1907: 330.
Delphacodes albifascia (Matsumura, 1900); comb. by Metcalf 1943: 400.
Unkanodes (*Chilodelphax*) *albifascia* (Matsumura, 1900); comb. by Vilbaste 1968: 26.
Chilodelphax albifascia (Matsumura, 1900); status by Kwon 1982: 4.
Unkanodes (*Kwonianella*) *albifascia* (Matsumura, 1900); comb. by Anufriev and Emeljanov 1988: 409.
Unkanodes* (*Kwonianella*) *insularis Anufriev, 1988
Unkanodes (*Kwonianella*) *insularis* Anufriev & Emeljanov, 1988: 409.
Unkanodes* (*Kwonianella*) *sympaticus Anufriev, 1988
Unkanodes (*Kwonianella*) *sympaticus* Anufriev & Emeljanov, 1988: 409.

Key to subgenera and species of *Unkanode*s of the world

This key is modified from Anufriev and Emeljanov (1988). Bartlett and contributors (2017) treated *Ribautodelphax notabilis* Logvinenko, 1970 as a synonym of *Unkanodes tanasijevici* (Dlabola, 1965) based on Nast (1987). In the present key, characters mentioned for *U. tanasijevici* are from the description of Ding (2006). *Unkanodes* (*Unkanodes*) *paramarginata* is not included in the key due to limited literature.

1 Armature of diaphragm with a pair of teeth slanting upwards or directed back; genital style with relatively wide apices; segment X (anal tube) with large widely-spaced processes; sub genus *Unkanodes* (*Unkanodes*).....**2**
– Armature of diaphragm without teeth or with a tooth bifurcate at apex; genital style with narrow apices; segment X (anal tube) with or without such processes..... **5**
2 Process of anal tube spaced more widely, weakly or strongly diverging; genital style with strong subapical lobe, apex wider **3**

– Processes of anal tube spaced less widely, more or less parallel; genital style with weak subapical lobe, apex narrower (Anufriev and Emeljanov 1988: fig. 310: 1–12) ..... ***U.* (*Unkanodes*) *excisus***

3 Process of segment X widely spaced weakly diverging; apex of genital style relatively wider (Anufriev and Emeljanov 1988: fig. 310: 13–17) ..... ***U.* (*Unkanodes*) *sapporona***

– Process of segment X widely-spaced strongly diverging; apex of genital style comparatively less wider..... **4**

4 Aedeagus elbow-shaped; process of segment X posteroventrally curved (Figs 6–11)..... ***U.* (*Unkanodes*) *latespinosa***

– Aedeagus straight with a strong tooth on dorsal aspect, ventrally with a weak lobe below the tooth; segment X not curved (Ding 2006: fig. 338A–L)..... ***U.* (*Unkanodes*) *tanasijevici***

5 Dorsal and posterior margin of pygofer forming an obtuse angle in lateral view; segment X (anal tube) with large widely-spaced teeth; apical half of aedeagus straight; subgenus *Chilodelphax* (Anufriev and Emeljanov 1988: fig. 311: 1–15) ..... ***U.* (*Chilodelphax*) *silvaticus***

– Dorsal and posterior margin of pygofer forming an acute angle in lateral view; segment X (anal tube) without or with narrowly spaced teeth; apical half of aedeagus directed dorsad (Anufriev and Emeljanov 1988); subgenus *Kwonianella*..... **6**

6 Process of pygofer bridge very short, directed downwards, sometimes bifurcate at apex; genital style comparatively short with wide subapical lobe..... **7**

– Process of pygofer bridge slightly long, bifurcated and directed backwards; genital style longer with narrow subapical lobe or short with wide subapical lobe..... **8**

7 Aedeagus near bent with a pair of long teeth perpendicular to shaft, the length of aedeagus matches with thickness of shaft (Anufriev and Emeljanov 1988: fig. 312: 17–19) ..... ***U.* (*Kwonianella*) sympatricus**

– Aedeagus near bent without long teeth, the length of which matches with thickness of the shaft (Anufriev and Emeljanov 1988: fig. 312: 1–11) ..... ***U.* (*Kwonianella*) *albifascia***

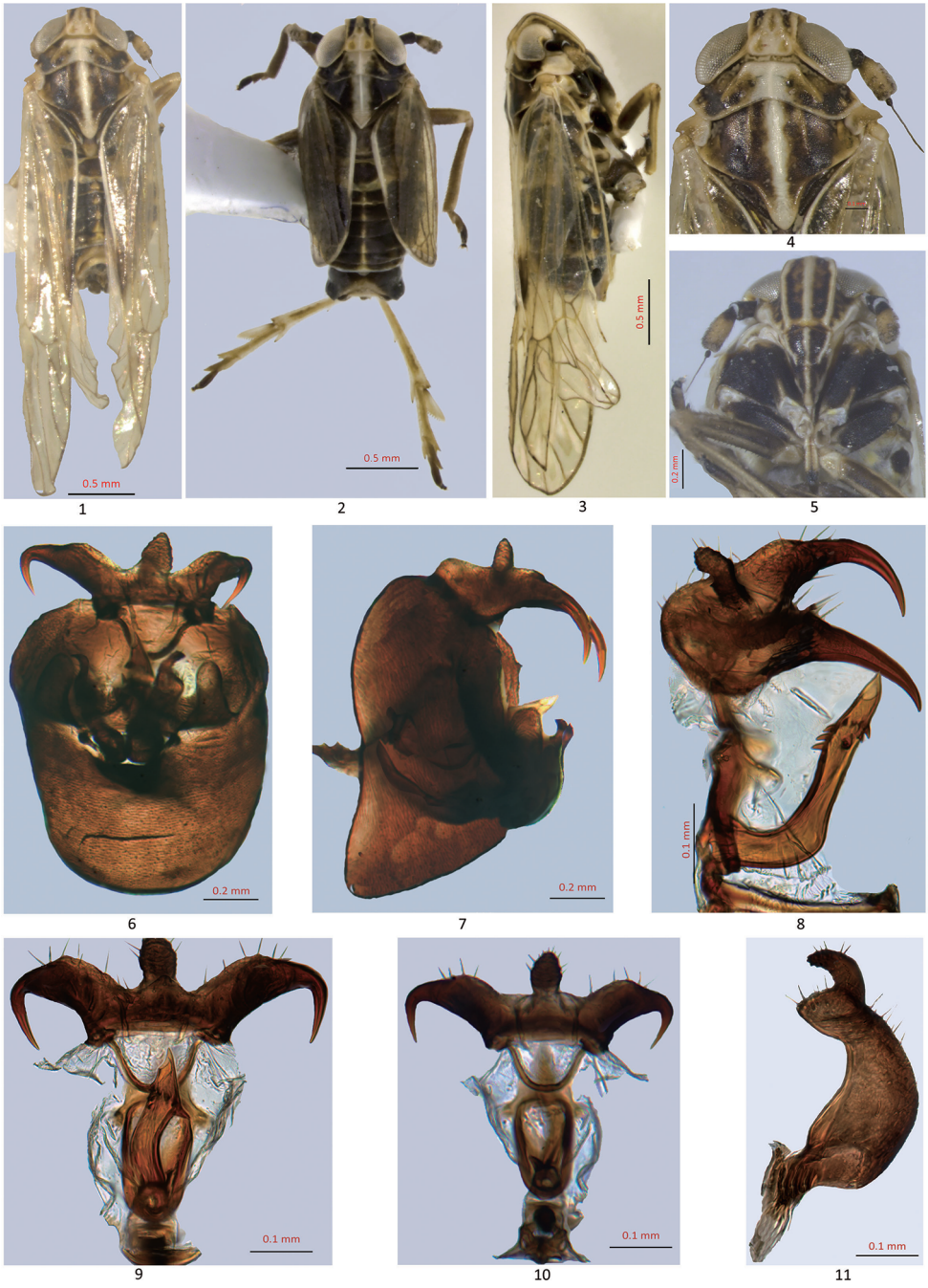
8 Aedeagus narrowing abruptly in apical 1/3, with a lobe-like process on ventral aspect in lateral view (Fig. 21) ..... ***U.* (*Kwonianella*) *malamjabbensis* sp. nov.**

– Aedeagus not narrowing abruptly in apical 1/3, without a lobe on ventral aspect in lateral view (Anufriev and Emeljanov 1988: fig. 312: 12–16) ..... ***U.* (*Kwonianella*) *insularis***

Unkanodes (*Unkanodes*) *latespinosa* (Dlabola, 1957)

Figs 1–11

Remarks. Dlabola (1957) described this species based on specimens from Afghanistan and provides a detailed description. It can be distinguished from other species of *Unkanodes* by the large, widely-spaced processes of segment X (anal tube) and elbow-shaped aedeagus.



Figures 1–11. *Unkanodes* (*Chilodelphax*) *latespinosa* (from Pakistan) **1** adult, dorsal view (macropterous) **2** adult, same species (brachypterous) **3** adult (macropterous), lateral view **4** vertex, pronotum and mesonotum, dorsal view **5** frons, ventral view **6, 7** male genitalia, caudal and lateral views **8–10** anal segment and aedeagus, lateral, dorsal and ventral views **11** genital style, lateral view.

Material examined. 3♂♂ (brachypterous), 7♂♂ (macropterous) Malamjabbah, Swat-Khyber Pakhtunkhwa, Pakistan, 35°13'21.76"N, 72°25'32.93"E, 2993.39 m, 5 vii 2018, sweeping grasses, coll. Kamran Sohail. The area has a very diverse habitat for fruits and vegetables, and this species was collected in grasses near vegetable fields. This species is newly recorded for the fauna of Pakistan.

Distribution. Previously recorded from Afghanistan, Iran, Mongolia, Turkey and Yugoslavia. In this study it is recorded from Swat, Khyber Pakhtunkhwa-Pakistan.

***Unkanodes* (*Kwonianella*) *malamjabbensis* sp. nov.**
http://zoobank.org/A9EFB5A5-AD3B-41B6-8599-94F3A4B3AC49

Description. Length of male (n=2) 1.4–1.6 mm.

Colour. General body colour dark brown to black. Vertex pale, compartments with three distinct yellow spots. Carina on frons pale, intercarinal region dark brown, gena concolourous with intercarinal region, compound eyes greyish. Antenna yellowish slightly darker at junction of scape and pedicel. Pronotum and mesonotum medially with a white stripe; darker at adjoining areas, extreme lateral margins and median carina white, lateral carina concolourous with adjoining regions. Forewings dark brown to black, apical and anal margins pale. Legs yellowish, spines with black apices. Abdominal tergites darker, segments IX and X lighter, pygofer brown.

Structure. Head narrower than pronotum, eyes extending beyond posterior margin of vertex (Figs 12, 14). Vertex ca. 2X longer than wide; stem of Y-shaped carina of vertex obsolete, lateral and posterior margins distinct, arms of submedian carina meeting at fastigium (Fig. 14). Frons parallel-sided; widest near basal 1/4 of eyes, narrower in apical 1/3, median carina bifurcate near fastigium (Fig. 15). Antennal scape about as long as wide, ca 1/2 x length of pedicel, pedicel bearing many sensory pits arranged in longitudinal rows dorsally from base to apex (Figs 13, 15). Frontoclypeal suture distinct, slightly arched; median carina on postclypeus visible, rostrum elongate, reaching hind coxae (Fig. 15). Pronotum much wider than long at midlength; lateral carinae strongly diverging, vanishing before reaching posterior margin and not in line with mesonotal lateral carinae, anterior margin straight at vertex, posterior margin slightly concave medially (Fig. 14). Mesonotum tricarinate, subequal to length of pronotum; median carina not extending to apex of scutellum, lateral carinae slightly diverging reaching hind margin, tegula inconspicuous (Fig. 14). Forewing covering only half of abdomen; veins granulate (Figs 12, 16). Metatibiae with two lateral spines on shaft, first near tibiofemoral articulation, second after middle. Metatibial spur tectiform, distally narrowed bearing row of 18 black-tipped teeth on outer margin, inner margin straight (Fig. 17). Spinal formula of hind leg 5/7/4.

Male genitalia. In caudal view, pygofer wider than long widest at mid length, dorsolaterally nearly straight (Figs 18, 20); diaphragm armature well-developed, V-shaped, pair of lobes located near the parameres directed upwards, pygofer bridge bearings two

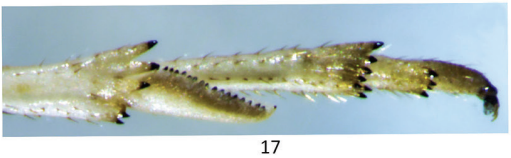
12

13

14

15

16



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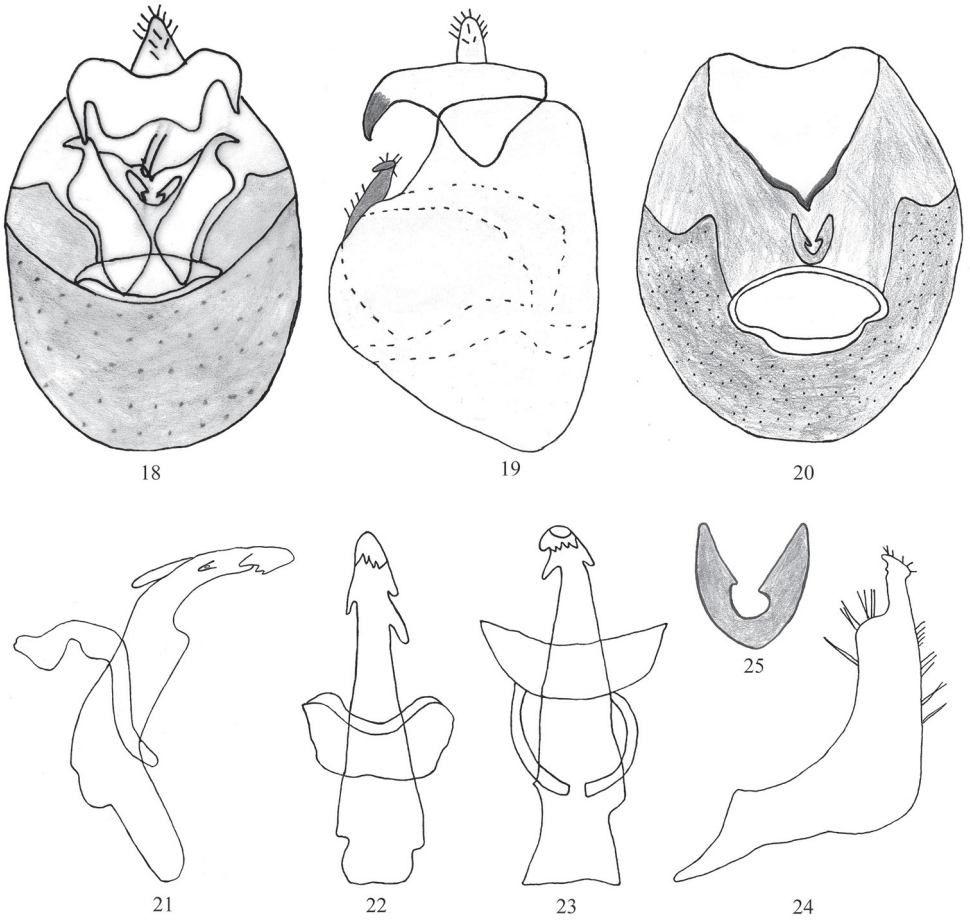
Figures 12–17. *Unkanodes* (*Kwonianella*) *malamjabbensis* sp. nov. **12, 13** adult, dorsal and lateral views **14** head and thorax, dorsal view **15** frons, ventral view **16** forewing **17** metatibial spur.

distinct tooth-like processes widely diverging, directed backwards forming blunt apex (Fig. 20). In lateral view, anterior margin nearly straight gradually arched apically, dorsal and posterior margins acutely rounded (Fig. 19). Segment X (anal tube) bearing



Figures 18–25. *Unkanodes* (*Kwonianella*) *malamjabbensis* sp. nov. **18, 19** male genitalia, caudal and lateral view **20** pygofer, caudal view **21** aedeagus, lateral view **22, 23** same, dorsal and ventral views **24** genital style, lateral view **25** medioventral process of armature of diaphragm.

large, widely spaced posteroventrally curved acute processes (Figs 18, 19). Parameres longer than wide, apically narrow, subapical lobe wider, posterior margin straight (Fig. 24). Aedeagus elongate and narrow, basal 1/3 straight, bent gradually forming an obtuse angle, apical 1/3 gradually curved ventrad (Fig. 21); in lateral view, with lobe or hump-like process on ventral aspect, with a large tooth just above the lobe on the dorsal aspect (Fig. 21). Suspensorium angling circled laterally, apically wider (Figs 22, 23).

Type materials. ***Holotype*:** ♂ Malamjabbah, Swat-Khyber Pakhtunkhwa, Pakistan, 35°13'21.76"N, 72°25'32.93"E, 2993.39 m, 5 vii 2018, sweeping grasses, coll. Kamran Sohail. Paratype: 1♂, same data as holotype.

Remarks. This new species was collected in a grass habitat near ponds. The Type locality is an understudied habitat for fulgoroids and the region reflects a true diversity of planthoppers for future prospects.

Female. Unknown.

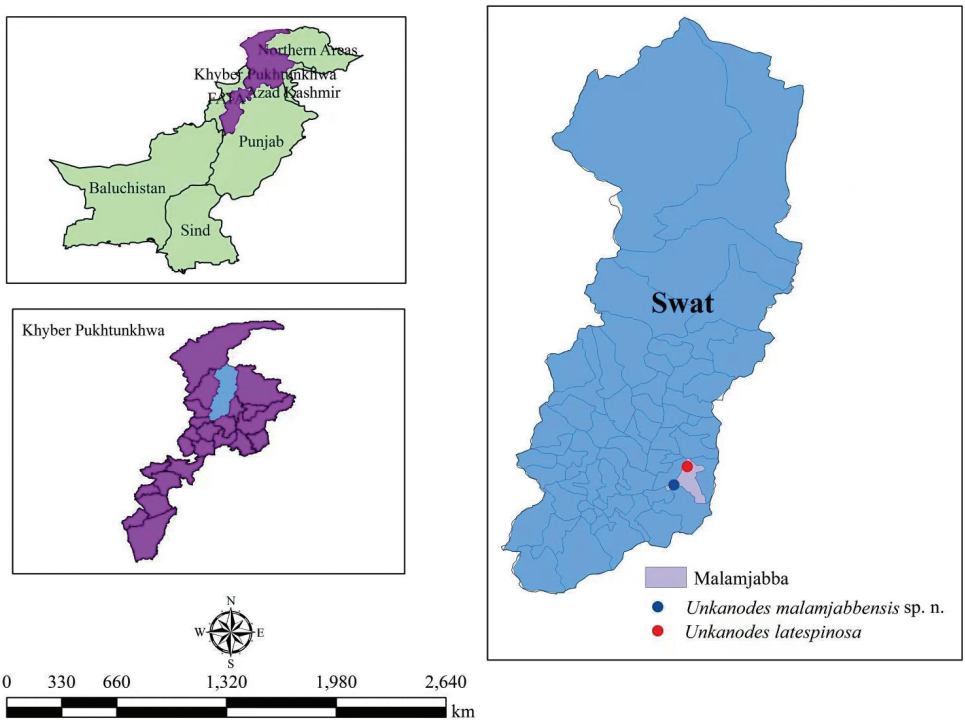


Figure 26. Distribution map of species of *Unkanodes* in Pakistan.

Etymology. The new species is named after the type locality 'Malamjabba'.

Diagnosis. The new species is externally similar to *U.* (*Kwonianella*) *albifascia* which also has a white stripe on the thorax and median margins of the forewings. However, it can be separated by the distinctly separated process of the pygofer bridge, widely diverging in *U.* (*Kwonianella*) *malamjabbensis* sp. nov. but very short and bifurcate at the apex in *U.* (*Kwonianella*) *albifascia* (Anufriev and Emeljanov 1988, Figs 2, 6; p. 412); and apical half of aedeagus gradually curved ventrad bearing a lobe-like process on the ventral aspect in *U.* (*Kwonianella*) *malamjabbensis* sp. nov. versus the apical half of the aedeagus slanting dorsad without a lobe in *U.* (*Kwonianella*) *albifascia* (Anufriev and Emeljanov 1988, Fig. 4; pp. 409, 412). *Unkanodes* (*Kwonianella*) *malamjabbensis* sp. nov. is also close to *U.* (*Kwonianella*) *insularis* Anufriev and *U.* (*Kwonianella*) *sympatricus* Anufriev in external appearance but can be distinguished by the distinct shapes of the aedeagus and parameres.

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East Asian *Cryphalus* Erichson (Curculionidae, Scolytinae): new species, new synonymy and redescriptions of species

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Abstract

Cryphalus Erichson, 1836 is a taxonomically challenging genus. It is particularly speciose in Asia. Many species are minor pests of fruit tree crops and forest products. We review collections from East Asia, using external morphology, internal morphology and genetic markers with a focus on sub-tropical species from fruit trees. Four new species are described; *Cryphalus gnetivorus* Johnson, **sp. nov.**, *C. itinerans* Johnson, **sp. nov.**, *C. morivorus* Johnson, **sp. nov.**, and *C. paramangiferae* Johnson, **sp. nov.** Ten species are redescribed to enable accurate identification: *C. artocarpus* (Schedl, 1939), *C. dilutus* Eichhoff, 1878, *C. dorsalis* (Motschulsky, 1866), *C. exiguus* Blandford, 1894, *C. kyotoensis* Nobuchi, 1966, *C. lipingensis* Tsai & Li, 1963 (= *C. kesiyae* Browne, 1975, **syn. nov.**), *C. mangiferae* Stebbing, 1914 (= *C. artestriatus* Browne, 1970, **syn. nov**.), *C. meridionalis* (Nobuchi, 1975), *C. scopiger* Berger, 1917, and *C. viburni* Stark, 1936. Additional records from new localities and new hosts are also presented.

Keywords
Bark beetle, broadleaf, fruit tree, *Hypocryphalus*, mango, mulberry, new species, new taxa, pest, pseudocryptic species

Introduction

Global forest health is threatened by a global redistribution of species introducing novel pathogens or vectors, coupled with stress from land management and climate change. Understanding the current biodiversity is a critical step in recording, reporting, and mitigating introductions of pests which affect the health of forest and agricultural systems. Bark and ambrosia beetles (Curculionidae: Scolytinae) are key drivers of change because of their roles in tree mortality, either via attacks overwhelming plant defences, or as vectors of disease (Six 2020). While a few bark beetle groups receive substantial attention of researchers, many are neglected. This has resulted in lack of revisionary systematics, difficulties in identification and taxonomic and nomenclatural complexity in most bark beetle groups.

Cryphalus Erichson, 1836 contains 252 known species of minute beetles ranging from 0.8 mm to 3.0 mm. Most of their diversity occurs in Asia, Australasia, and Oceania, with fewer species known from Africa and Europe, and a small number of species occurring in the Americas. Additionally, five putative species are introduced to the Americas (including *C. brasiliensis* Schedl, 1976 and *C. robustus* Eichhoff, 1872, but are only known from types and may represent extinct ephemeral populations).

Cryphalus typically live on recently dead or dying plant tissue under bark, but a few species are known to attack weakened (but still living) parts of trees and/or can vector plant pathogens (Jankowiak and Kolařík 2010). Known examples include *Ceratocystis* diseases of mango in the Middle East (Iqbal and Saeed 2012), and *Cryphalus* sp. attacking *Calophyllum* spp., vectoring *Verticillium calophylli* (Wiehe) W. Gams in the Seychelles (Wainhouse et al. 1998). *Cryphalus* have also been associated with the decline of other commodity trees such as figs (*Ficus carica* L.) (Faccoli et al. 2016), mulberry (*Morus* spp.) (Luo 2002) and loquat (*Eriobotrya japonica* (Thunb.) Lindl.) (Zheng et al. 2019), but without investigation to the role of pathogens.

Identification of *Cryphalus* is difficult, and they are often left without species identities (e.g., Zhao et al. 2004; Masuya et al. 2007; Hu et al. 2019), excluded in biodiversity surveys (e.g., Hulcr et al. 2007) and taxonomic work, or described with limited material and inadequate descriptions for diagnosis (e.g., Schedl 1942). Even economically important species, such as those on mango, have widespread identification errors which span decades (Johnson et al. 2017).

The beetles are challenging to identify due to their small size and soft bodies. Museum specimens are often discoloured and imploded, require high magnification microscopes to clearly see the diagnostic characters, or require dissection of the proventriculus or aedeagus, for which most species do not have adequate references to enable identification.

East Asia has a long history of taxonomic work on *Cryphalus*, from the meticulous work by Pang-hwa Tsai, Chao-lin Li, Huifen Yin, and Akira Nobuchi (e.g., Tsai and Li 1963; Nobuchi 1975; Yin et al. 1984). However, the subject of most of these studies were the species attacking coniferous forests, with very minimal treatment of species from broadleaf hosts. Taxonomic confusion with the now synonymised *Hypocryphalus* Hopkins, 1915 also contributed to the lack of treatment of species from broadleaf hosts- species previously classified in this genus, despite being morphologically very similar to certain species within the genus *Cryphalus*.

Developments in molecular tools give promise to enable accurate and rapid identification of difficult taxa, but rely on studies linking the identification of vouchered material, thorough descriptions, or sequence data with vouchered representatives (Cognato et al. 2020). The focus of this study is to provide data to enable accurate detection and study of *Cryphalus* species breeding in angiosperm trees in East Asia. Here, four species are described, ten species are redescribed, two names are synonymised, notes on the taxonomic history and new records are given for several East Asian species.

Materials and methods

Specimens were obtained from various collection methods, including hand collecting from plant material and traps by the authors.

Specimens were initially sorted and identified with a stereo microscope (Olympus SZX16). Photographs were taken with taken with a digital SLR (Canon rebel t3i) mounted on an Olympus UIS2 system (BX53 microscope) with 5× – 40× objectives, illuminated by diffused halogen lights. Photographs were stacked with Helicon Focus (Helicon Soft) using the pyramid stacking algorithm (method:C), and edited in Photoshop (version CC2015, adobe.com). The edits were limited to correcting colour and removal of background objects.

Material was studied in ethanol, partially dried for photography, and later side-mounted on card points using Gelva ethanol soluble PVA (no longer manufactured). Pieces of dissected specimens were stored in ethanol or used for attempted DNA extraction.

Specimen data are maintained with unique identifiers in the UFFE collection database, which may refer to specimens owned and/or physically housed elsewhere. A UFFE id refers to one or a group of specimens housed in the same place sharing collection and identification information. If known, other repository identifiers are provided for specimens from other collections. Format of the material examined includes with records separated by a bullet point, and localities arranged by their current name, from larger administrative regions to smaller. Historic locations, where misinterpretations of localities are possible, have the label data quoted verbatim in addition to the locality. The following systems were used: Revised Romanization of Korean; Hanyu Pinyin (ISO 7098, Chinese); BGN/PCGN Romanization of Russian.

Taxonomic work follows the most recent review and reclassification treating *Cryphalus* Erichson (Johnson et al. 2020), which contains the full references and quota-

tions of species treated and their synonyms. Holotypes were deposited in a state-supported taxonomic institution within each respective originating country, and paratypes are distributed to various international collections, particularly those in Asia where they would be useful to identify locally collected specimens. Types of existing specimens were examined directly or from photographs. Type material examined was clearly labelled as such and compared to the original description.

The following acronyms are used for specimens.

FSCA	USA, Florida, Gainesville, Florida State Collection of Arthropods.
IOZ	China, Beijing, Chinese Academy of Sciences, National Zoological Museum of China, Institute of Zoology.
MZB	Indonesia, LIPI Research Center of Biology, Division of Zoology, Museum Zoologicum Bogoriense, Widyasatwaloka, Cibinong.
NHMUK	United Kingdom, London, The Natural History Museum.
NIAES	Japan, Ibaraki, Tsukuba, National Institute of Agro-Environmental Sciences (ITLJ).
NMNS	Taiwan, Taichung, National Museum of Natural Science.
RIFID	South Korea, Namyangju, Research Institute of Forest Insect Diversity.
UFFE	USA, Florida, Gainesville, University of Florida Forest Entomology Collection.
USNM	USA, Washington D.C., National Museum of Natural History.
ZIN	Russia, St. Petersburg, Russian Academy of Sciences, Zoological Institute.

Cryphalus are very diverse and many undetermined specimens were observed. To avoid description of aberrant morphotypes or names which cannot be reliably identified in the future, new species were only described based on large series (at least 20 mature individuals) from multiple locations and for samples for which a DNA sequence had been obtained. Additional descriptions were provided for some species with economic significance.

Species are primarily described with a morphological species concept. To be considered a species, individuals have a distinct set of morphological characters based on a reasonable sample which the authors assume represents an evolutionary unit. Taxonomic changes are registered with ZooBank (Polaszek et al. 2005). For species of likely economic importance, a vernacular name is suggested in Chinese with an English translation. Chinese scientific literature uses vernacular names widely but sometimes inconsistently (Chen et al. 2017), so a name is suggested to promote consistency of use and prevent the establishment of non-informative or misleading names.

DNA sequence data were obtained when possible, to facilitate future molecular identification and corroborate species delimitation. The method used are as used by Johnson et al. (2017). Briefly, DNA was extracted from specimens using Qiagen DNeasy extraction kit (United States). DNA was amplified and sequenced with primers for 28 S (28S_A4285R: CCTGACTTCGTCCTGACCAGGC, 28S_S3690F: GAGAGTTMAASAGTACGTGAAAC; Sequeira et al. 2000) and COI (COI-LepF1: ATTCAACCAATCATAAAGATATTGG, COI-LepR1: TAAACTTCTGGATGTC-

CAAAAAATCA; Hebert et al. 2004). An approximate phylogeny was estimated using an alignment of 28S sequences listed in Table 1, inferred with MrBayes (version 3.2.7, Ronquist et al. 2012) using default settings.

Morphological terminology follows contemporary literature for bark and ambrosia beetles. Particularly, the funiculus includes the pedicel. Serrations are the asperities along the anterior margin of the pronotum. Converging aciculations refers to the texture appearing scratched, with irregular grooves and ridges, typically on the frons of some *Cryphalus* spp. converging on the epistoma. Lengths and proportions are given assuming a natural positioning of the beetle and given when viewed dorsally (e.g., pronotal length is from the apical margin to the base when viewed dorsally, not the diagonal distance). The pronotal profile refers to the shape when viewed dorsally, particularly where the pronotum is widest, which is usually at the base or in line with the summit. For the proventriculus, the closing teeth are the long teeth between the apical teeth and the masticatory brush.

The recorded host plants summarise the plant species from which the beetles were collected, primarily based on the material examined, with additional records from literature listed explicitly as uncertain. Similarly, the summarised distribution is based on examined material, listed as countries, with states or provinces listed specifically for the United States, Russia, and China. Relevant unconfirmed distribution records are explicitly cited as such.

An identification key is not provided at this time for the following reasons: all species in the region have not been thoroughly studied, especially the large diversity associated with coniferous trees, and the rate of species discovery is high and there are likely many more species yet to be reported, so keys may lead to misleading identifications. This study contributes towards a larger effort to make identification resources by providing thorough, complete descriptions and likely diagnostic characters with the intention to produce a key at a later date. Some types of species elsewhere are in poor condition and may correspond to aberrant individuals of the species included here but cannot be easily diagnosed. This approach is justified taxonomically, because it is important that names exist of the species in a timely manner. It is also justified economically, as several of the species here have been reported as pests, are related to pests, or are introduced elsewhere. Widely deposited type material, high resolution photographs and sequence data should ensure that conspecific specimens are identifiable in the future, enabling better descriptions of the distribution and biology of the species included, and enabling mitigation of potential pests.

Results

Phylogeny

The estimated phylogeny reveals some notable discoveries: Specimens from Thailand and China determined as *C. kesiyae* and *C. lipingensis* respectively are genetically identical, corroborating their morphological similarity (Figure 1). *Cryphalus exiguus* and

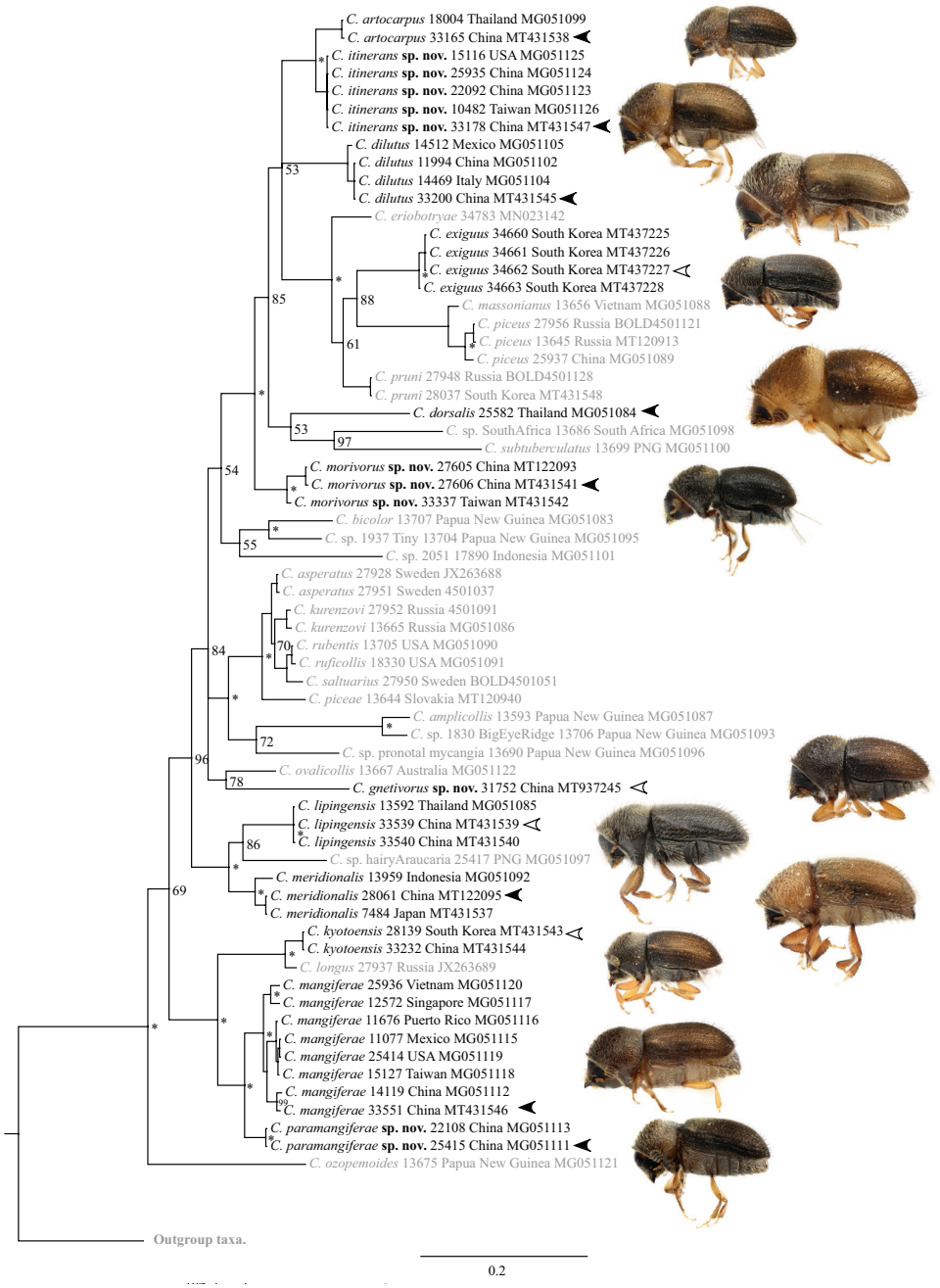

Figure 1. Preliminary phylogeny of *Cryphalus* spp. based on 28S, inferred using MrBayes using default parameters. Node labels indicate posterior probability (as %), 100% indicated with an asterisk. Outgroup taxa are listed in Table 1. Black text indicates treated species. Arrows correspond to the photographs, filled arrows indicate the exact specimen photographed, empty arrows indicate a specimen from the same collection being used.

another species from mulberry are distinct corroborating the morphological and distribution differences. *Cryphalus mangiferae* collected of *Choerospondias axillaris* (Roxb.) B.L. Burtt & A.W. Hill are genetically identical to specimens collected on *Mangifera indica* L. confirming *Choerospondias* as an alternative host.

Systematics

Curculionidae Latreille, 1802
Scolytinae Latreille, 1804
Cryphalini Lindemann, 1877

Cryphalus Erichson, 1836

Diagnosis. Eye deeply emarginated; antennal club flat with three sutures marked by setae; third tarsal segment emarginated; the hypomeron with bifurcating setae (rare exceptions); proventriculus with large flat apical plates, tight median suture, often with sutural teeth, and distinct apical teeth, moustache-like, in multiple transverse layers; aedeagus with paired tegminal apodemes (few exceptions) (Johnson et al. 2020). Spiculum gastrale a simple, curved rod.

Cryphalus artocarpus (Schedl, 1939)

Figures 2A, 3A, 4A–I

Ericryphalus artocarpus Schedl, 1939: 432 (Malaysia).
Cryphalus artocarpus Schedl, 1958: 498 (Malaysia).
Cryphalus brownei Wood, 1992: 432 (unnecessary replacement name).

Type material examined. Malaysia • 1 ♀ ***Holotype*** *Cryphalus artocarpus* Schedl, 1958; Sarawak, Siburan, Semenggoh; 23 Aug. 1957; F. G. Browne leg.; ex. *Artocarpus*; on branch of *Artocarpus*; 5833; UFFE: 26193; (NHMUK).

Other material examined. China • 4 ♀♀, 4 ♂♂; Hainan; 儋州市宝岛新村试验场七队桑园 [Danzhou, Baodaoxincun, No.7 farm, Mulberry field]; 19.51°N, 109.49°E; 14 Mar. 2019; Fuping Lu, and Shengchang Lai leg.; ex. *Morus*; "; samples degraded.; UFFE:33536; (UFFE) • 2 ♀♀, 1 ♂; Yunnan, Xishuangbanna, Sanchahe Nature Reserve; 22.1631°N, 100.8709°E; 30 May 2008; Anthony I. Cognato leg.; ex. *Ficus*; vial 133; Sanchahe Nature Reserve; collect from host tree phloem; 30/May/2008; Cognato coll; UFFE:11411; (UFFE) • 1 ♀; Yunnan, Xishuangbanna, Xishuangbanna Tropical Botanical Garden; 21.92°N, 101.27°E; 12 Jul. 2014; Craig Bateman leg.; EtOH trap; moved from vial 7778; UFFE:33166; (UFFE) • 1 ♂; same collection data; UFFE:33167; (UFFE) • 1 ♂; same collection data; DNA: 28S:MT431538; UFFE:33165; (UFFE) • 12 ♂♂, 12 ♀♀; Yunnan, Xishuangbanna, Xishuangbanna

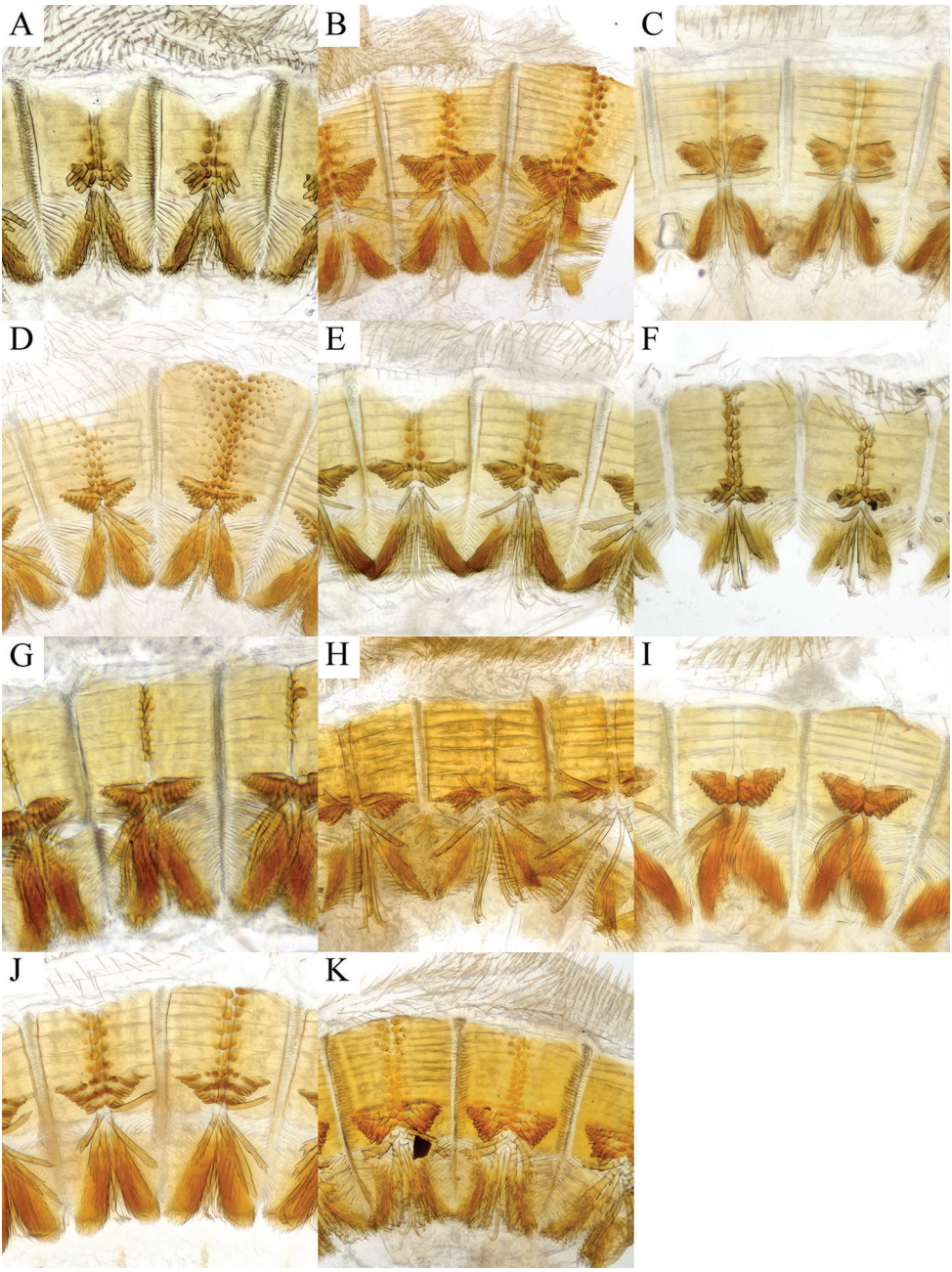

Figure 2. Proventriculus of **A** *Cryphalus artocarpus*, UFFE:33165 **B** *C. dilutus*, UFFE:34684 **C** *C. dorsalis*, UFFE:25581 **D** *C. gnetivorus*, UFFE:31753 **E** *C. itinerans*, UFFE:33178 **F** *C. kyotoensis*, UFFE:33232 **G** *C. lipingensis*, UFFE:28048 **H** *C. mangiferae*, UFFE:34966 **I** *C. meridionalis*, UFFE:28061 **J** *C. morivorus*, UFFE:27604 **K** *C. paramangiferae*, UFFE:34965.

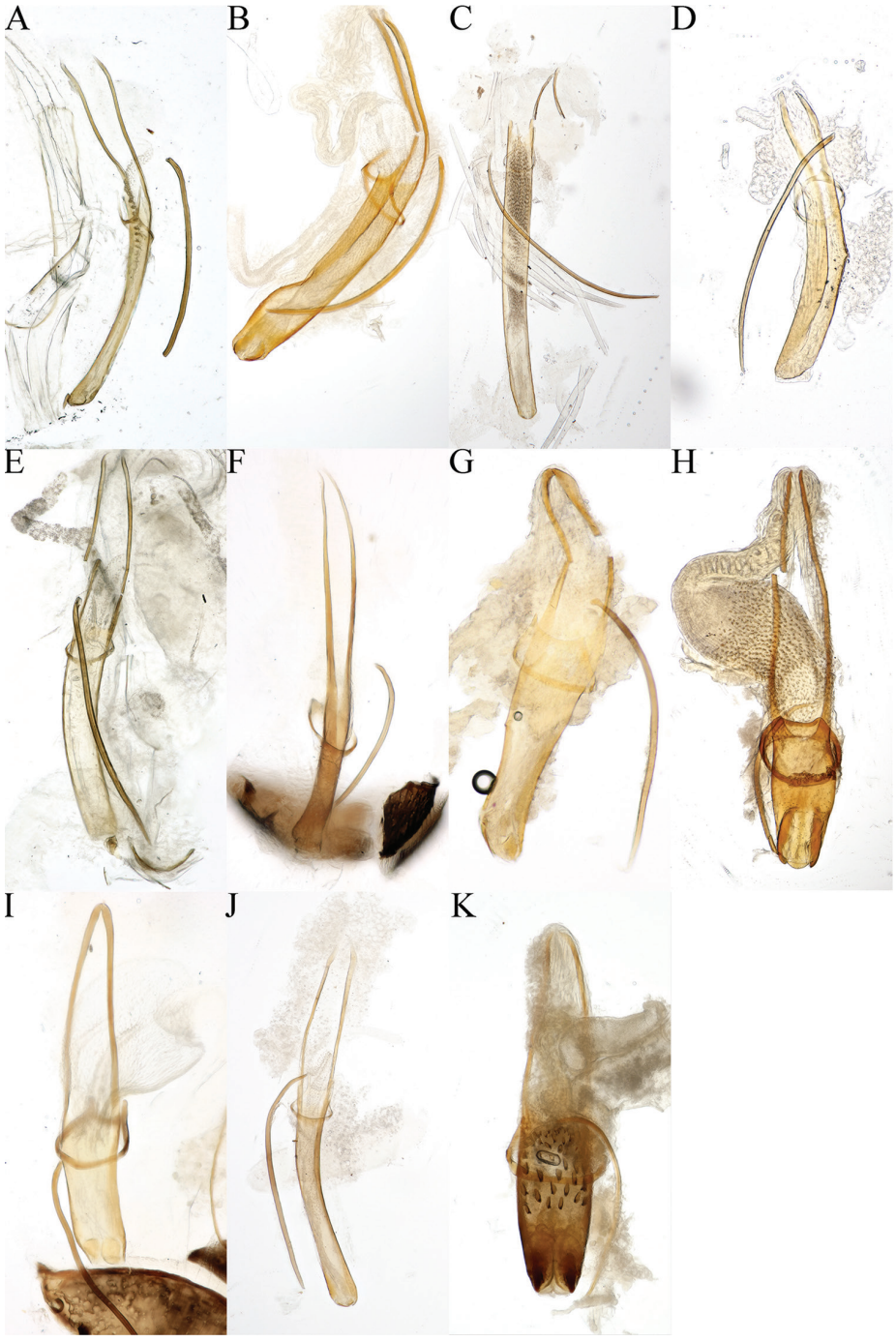

Figure 3. Aedeagus of **A** *Cryphalus artocarpus*, UFFE:33165 **B** *C. dilutus*, UFFE:34684 **C** *C. dorsalis*, UFFE:25581 **D** *C. gnetivorus*, UFFE:31753 **E** *C. itinerans*, UFFE:33178 **F** *C. kyotoensis*, UFFE:31745 **G** *C. lipingensis*, UFFE:28048 **H** *C. mangiferae*, UFFE:34966 **I** *C. meridionalis*, UFFE:28061 **J** *C. morivorus*, UFFE:27604 **K** *C. paramangiferae*, UFFE:34965.

Tropical Botanical Garden; 21.92°N, 101.27°E; 12 Jul. 2014; Craig Bateman leg.; EtOH bottle trap; UFFE:14088; (NHMUK, 1♀, 1♂; FSCA, 1♀, 1♂; MZB, 1♀, 1♂; NIAES, 1♀, 1♂;NMNS, 1♀, 1♂; IOZ, 1♀ IOZ(E)2057936, 1♂ IOZ(E)2057937; RIFID, 1♀, 1♂; UFFE, 2♀♀; USNM, 1♀, 1♂; ZIN, 1♀, 1♂).

Thailand • 1 ♂; Phatthalung, Srinagarindra District, Khao Banthat wildlife sanctuary; Jun. 2015; S. Steininger, and W. Sittichaya leg.; DNA: 28S:MG051099; UFFE:18004; (UFFE)

Diagnosis. This species can be diagnosed from similar *Cryphalus* in East Asia by the small size (1.10–1.30 mm), by the large pronotal disc one third of the length of the pronotum, by the stout elytra with a finely tuberculate surface texture, and by the male protibiae with similar setae to the females.

Female. Length 1.10–1.30 mm (holotype 1.25 mm). Proportions 2.1× as long as wide. Frons with minute aciculations, barely visible, converging to the epistoma. Antennal club with three weakly procurved sutures marked by coarse and long setae. Antennal funiculus with four segments, length shorter than the scape. Gular surface with evenly spaced hair-like setae. Protibiae and protarsi with only straight, hair-like setae. Pronotal colour dark brown on slope, sometimes a lighter brown on disc. Pronotal profile broadly rounded, slightly wider in line with summit. Pronotal margin armed with six to ten serrations, separated by approximately their width, the outer one or two pairs smaller. Pronotal declivity with more than 40 asperities (holotype has 51). Pronotal disc approximately one third the length of the pronotum, gently sloped, weakly tuberculate surface texture (obscured by scale-like setae). Pronotal vestiture on anterior and lateral slope hair-like. Pronotal vestiture on disc and postero-lateral regions mixture of scale-like and paddle-like, the scale-like setae 1× long as wide with a tridentate tip. Suture between pronotum and elytra weakly sinuate. Scutellum very small, barely visible. Elytra 1.5× as long as pronotum, brown to translucent yellow-brown, darker at base, broadly rounded with no clear elytral disc or a transition to the declivity, slightly angulate on lateral regions of the declivity on interstriae 5. Elytral surface densely punctures with small tubercles, especially on the basal half. Striae barely visible as rows of punctures and hair-like setae. Interstrial bristles erect, flattened with rounded tips, shorter towards the elytral suture. Interstrial ground vestiture tridentate, approximately one to two × as long as wide, translucent brown with a weak iridescence, sometimes light brown near the base of the elytra. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, more than distance between metacoxae. Proventriculus not examined.

Male. Similar to female except: Length 1.05–1.20 mm. Frons weakly aciculate with a glossy transverse carina above upper level of eyes. Gular surface shining around suture, surrounded by sparse setae. Pronotal profile widest in line with summit. Pronotal profile anterior to summit triangular. Pronotal margin with four to six marginal asperities, spaced approximately by twice their width. Pronotal declivity with almost straight slope, fewer and smaller asperities than the female. Pronotal disc with strongly tuberculate surface obscured by scale-like setae. Elytral surface densely punctured and strongly tuberculate, especially on basal half. Protibiae and protarsi with hair-like se-

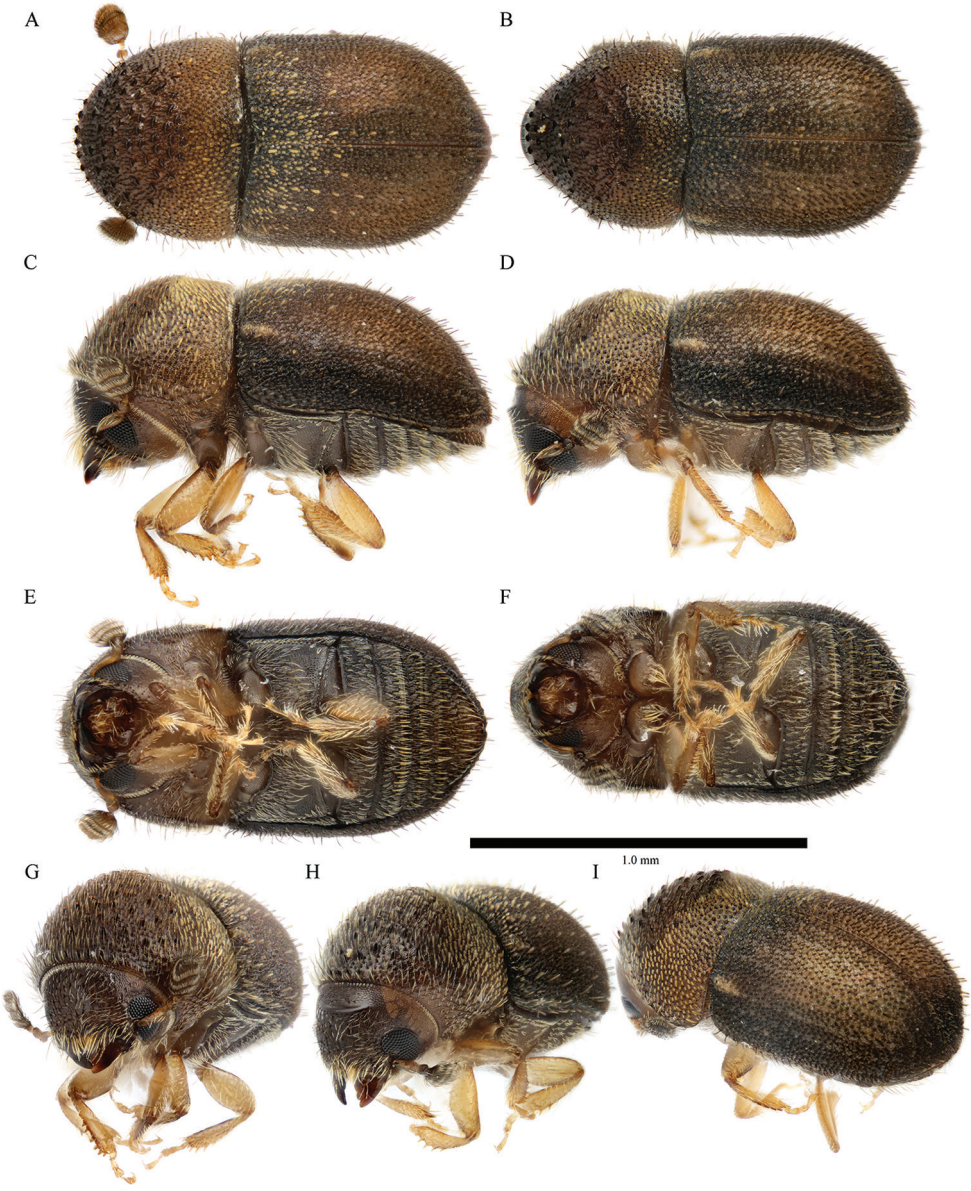

Figure 4. *Cryphalus artocarpus* **A, C, E, G** female, UFFE:33166 **B, D, F** male, UFFE:33167 **H, I** male, UFFE:33165.

tae which are curved, slightly larger than on female. Last abdominal ventrite clearly emarginated. Proventriculus sutural teeth rounded, in two overlapping longitudinal rows. Apical teeth extend laterally to less than two thirds of the plate. Closing teeth short, mix of palmate shorter teeth and longer, tapered, branched teeth extending beyond masticatory brush. Masticatory brush slightly less than half of the proventricular

length. Aedeagus long, weakly sclerotised. Penis apodemes approximately two thirds as long as penis body. Tegmen with two ventral apodemes, which are longer than distance between them. End plates indistinct, barely sclerotised.

Distribution. China (Yunnan, Hainan); Thailand; Malaysia.

Recorded plant hosts. Moraceae: *Artocarpus* sp., *Artocarpus elasticus* Reinw. ex Blume., *Ficus* sp., *Morus* sp.

Remarks. Abundant in ethanol bottle trap samples in Yunnan. This species was listed as "spThailandScaly" in Johnson et al. (2017).

Cryphalus dilutus Eichhoff, 1878a

Figures 2B, 3B, 5A–I

Cryphalus dilutus Eichhoff, 1878a: 384 (Myanmar); Eichhoff, 1878b: 490 (Myanmar).

Type material examined. Myanmar • 1 ♂ ***Holotype***; "Hindostan"; UFFE:14961; (NHMW).

Other material examined. China • 1 ♀; Guangdong, Shenzhen, Yantian; 22.5889°N, 114.2842°E; 08 Apr. 2017; Wei Lin leg.; dissected; moved from vial 17740; DNA: 28S:MT431545, COI:MT431649; UFFE:33200 • 1 ♀; Guangdong, Shenzhen, Yantian; 22.5889°N, 114.2842°E; 08 Apr. 2017; Wei Lin leg.; UFFE:33240; (UFFE) • 1 ♂; 深圳水库 [Shenzhen Reservoir]; 22.5945°N, 114.1797°E; 11 Jul. 2018; 阮用颖 [Yongying Ruan leg.]; light trap; UFFE:34064; (UFFE) • 1 ♀, 1 ♂; Guangdong, Zhuhai, Shixi Park; 22.2847°N, 113.5539°E; 01 Aug. 2018; Wei Lin, and You Li leg.; UFFE:34963 (IOZ, 1♀ IOZ(E)2057934, 1♂ IOZ(E)2057935) • 1 ♂; same collection data; dissected; UFFE:34684 (UFFE) • 1 ♂; specific origin unknown; 17 Dec. 2013; ex. *Ficus*; Interception at USA, Oberlin, from shipment of *Ficus* sp.; DNA: 28S:MG051102, COI:MG051150; UFFE:11994; (UFFE) • 1 ♀; Yunnan, Jinghong, Yunnan Institute of Tropical Crops; 22°N, 100.78°E; 13 Mar. 2019; Quan Zhou, and Shengchang Lai leg.; 20190313008 trap; UFFE:34041; (UFFE).

Mexico • 1 ♀; Tabasco, La Frontera; 18.6138°N, -92.5749°E; 19 Aug. 2014; Thomas H. Atkinson, and S. Burgos leg.; ex. *Mangifera indica*; shaded-out branches, 3–5 cm. diameter; UFFE:14892; (UFFE) • 1 ♂; same collection data; UFFE:14893; (UFFE).

Oman • 1 ♀; Mar. 2005; Randy Ploetz leg.; ex. *Mangifera indica*; labelled: "Oman: III 2005; Randy Ploetz; Ex. Mangifera indica"; UFFE:12479; (FSCA).

Diagnosis. This species can be diagnosed by the combination of the transverse carina on the male frons, by the pronotal margin which projects slightly, by the scale-like setae on the pronotal disc, by the barely apparent striae, and by the long spatula-shaped setae on the protibia and the spur on the mesofemur of males. The spine on the mesofemur is known exclusively in this species among all Scolytinae.

Female. Length 1.50–2.20 mm. Proportions 2.05× as long as wide. Frons simple, convex, with sparse but evenly distributed setae pointing towards its centre. Antennal club with three sutures on the basal half marked by coarse and long setae, the first

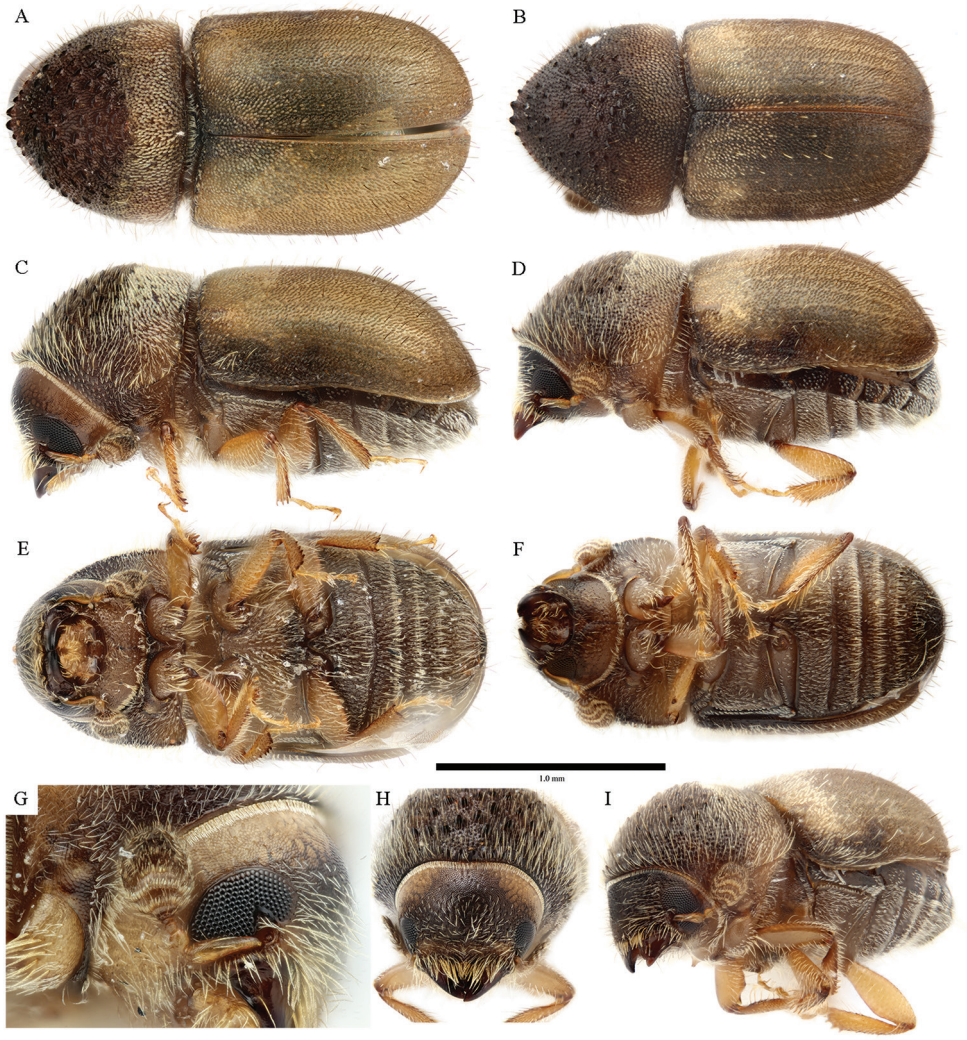

Figure 5. *Cryphalus dilutus* **A, C, E, G, H** female, UFFE:33200 **B, D, F, I** male, UFFE:34684.

weakly procurved, second and third more strongly procurved, and a weakly visible procurved line of coarse setae in the upper (distal) half. Antennal funiculus with four or five segments, the pedicel is slightly shorter than the other segments combined. Gular surface with evenly spaced hair-like setae. Pronotal colour brown, typically similar to head and elytra. Pronotal profile slightly triangular, widest in line with centre of pronotal disc. Pronotal margin rounded but protruding downward slightly, armed with four to eight serrations, the median pair distinctly larger, contiguous or separated by approximately half of their width, the outer pairs smaller separated by more than their width. Pronotal declivity with approximately 60 asperities. Pronotal disc approximately one third the length of the pronotum, gently sloped, with surface texture rugose/

closely punctured, partially obscured by scale-like setae. Pronotal vestiture hair-like and dark coloured on anterior and lateral slope, and a mixture of blonde scale-like and bristle-like, on disc and postero-lateral regions, with the scale-like setae 1–2× long as wide with a tridentate tip. Suture between pronotum and elytra weakly sinuate. Scutellum very small, barely visible when elytra closed. Elytra 1.6× as long as pronotum, brown to translucent yellow-brown, broadly rounded with no clear elytral disc or a transition to the declivity. Striae barely visible as rows of punctures and hair-like setae. Interstrial bristles erect, coarse bristles, shorter towards the elytral suture, evenly distributed across elytra. Interstrial ground vestiture tridentate, approximately 1–2× as long as wide, translucent brown with a weak iridescence, except the basal/anterior third of elytra which is blonde. Antero-lateral margin of elytra with some blonde hair-like setae, more dense than the interstrial bristles over the rest of the elytra. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, more than distance between metacoxae. Mesofemur with a slightly raised patch in the centre of the ventral face. Proventriculus sutural teeth of irregular size, confused, in two or more longitudinal rows. Apical teeth extend laterally over the entire segment. Closing teeth weakly branched, tapered, extending beyond masticatory brush. Masticatory brush with fine teeth, short, less than half the proventricular length.

Male. Similar to female except: Length 1.50–2.20 mm (type is 1.67 mm). Frons with straight transverse carina and sulcus above the level of eyes. Pronotal profile triangular, protruding apically. Pronotal declivity almost flat (not broadly rounded). Protibiae and protarsi with large spatula-shaped setae. Mesofemur with a distinct spine in the centre of the ventral surface. Last abdominal ventrite clearly emarginated. Proventriculus same as female. Aedeagus long. Penis apodemes shorter than penis body. Tegmen with paired apodemes about as long as distance between them. End plates sclerotised.

Distribution. China (Guangdong, Yunnan); Myanmar; also recorded from Malta; Italy; Tunisia; UAE; Oman; India; Pakistan; Bangladesh; Mexico (all Johnson et al. 2017).

Recorded plant hosts. Anacardiaceae: *Mangifera indica* L.; Moraceae: *Ficus carica* L., *F. retusa* L., *F. microcarpa* L.f., *Ficus bengalensis* L. (no new material from plant hosts, all cited in Johnson et al. 2017).

Suggested vernacular name. Chinese: 刺足梢小蠹 [= spur-footed twig bark beetle]; English: Spurred bark beetle.

Remarks. This species is associated with diseases of mango and die-off of edible fig. These new records confirm that it is widespread in Southern China; previously it was only known from an interception.

Cryphalus dorsalis (Motschulsky, 1866)

Figures 2C, 3C, 6A–H

Hypoborus dorsalis Motschulsky, 1866: 403 (India).
Hylesinus sericeus Motschulsky, 1866: 402 (Sri Lanka).
Hypoborus nebulosus Motschulsky, 1866: 403 (India).

Cryphalus indicus Eichhoff, 1878a: 384 (Myanamar).
Cryphalus indicus Eichhoff, 1878b: 489 (Myanamar).

Other material examined. China • 1 ♀; Hainan, Qiongzhong, Wanling; 19.2115°N, 109.9548°E; 26 Oct. 2016; You Li leg.; Light trap with ethanol; UFFE:34070; (UFFE) • 1 ♂; Yunnan, Xishuangbanna, Xishuangbanna Tropical Botanical Garden; 27 Jul. 2014; Craig Bateman leg.; EtOH live trap; specimens with crystalline structure, degraded; UFFE:21476; (UFFE).

Thailand • 1 ♂; Phatthalung, Srinagarindra District, Khao Banthat wildlife sanctuary; Jun. 2015; S. Steininger, and W. Sittichaya leg.; UFFE:25581; (UFFE) • 1 ♀; same collection data; DNA: 28S:MG051084; UFFE:25582; (UFFE).

Vietnam • 1 ♀; Hải Châu District, Da Nang; 10 Dec. 1966; H. P. Schurtleff leg.; S.L.Wood Collection; UFFE:12188; (USNM).

Diagnosis. The combination of the size (1.60–1.90 mm), body proportions (1.75 × as long as wide), the transverse ridge on the male frons, scale-like setae on the pronotal disc, the very short pronotal disc, the smooth elytra with barely visible rows of strial punctures, and the presence of sparse interstrial bristles on only odd-numbered interstriae, and long spatula-shaped setae on the male protibiae distinguish this species from others in East Asia.

Female. Length 1.60–1.90 mm. Proportions 1.75× as long as wide. Frons simple, convex, with sparse, evenly distributed erect setae. Antennal club with three procurved sutures marked by coarse and long setae, the most distal slightly more procurved. Antennal funiculus with four segments, the pedicel is slightly shorter than the other segments combined. Gular surface with evenly spaced hair-like setae. Pronotal colour brown, lighter on apical third. Pronotal profile slightly triangular, widest in line with centre of pronotal disc, 0.65× as long as wide. Pronotal margin rounded, armed with four to eight serrations, the median pair slightly larger, contiguous, or separated by approximately half of their width, the outer pairs smaller separated by more than their width. Pronotal declivity with approximately 50 asperities, otherwise smooth. Pronotal disc approximately one quarter the length of the pronotum, gently sloped, with surface texture weakly tuberculate. Pronotal vestiture hair-like and dark coloured on anterior portion pointing towards summit, and scale-like setae over pronotal disc. Suture between pronotum and elytra weakly sinuate. Scutellum very small, barely visible when elytra closed. Elytra 1.6× as long as pronotum, brown to translucent yellow-brown, elytral disc short, less than 1/3 length of elytra, and broadly rounded. Striae weakly visible as rows of punctures and hair like setae. Interstrial bristles erect, coarse bristles, shorter with a rounded tip on apical half, becoming longer and pointed on the declivity and lateral regions, only present on interstriae 1, 3, and 5 on declivity. Interstrial ground vestiture tridentate, approximately 2× as long as wide, translucent brown with a weak iridescence, except a few on the basal area near scutellum, which are blonde, antero-lateral margin with hair-like setae shorter than interstrial bristles. Procoxae with coarse, hair-like setae. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, more than dis-

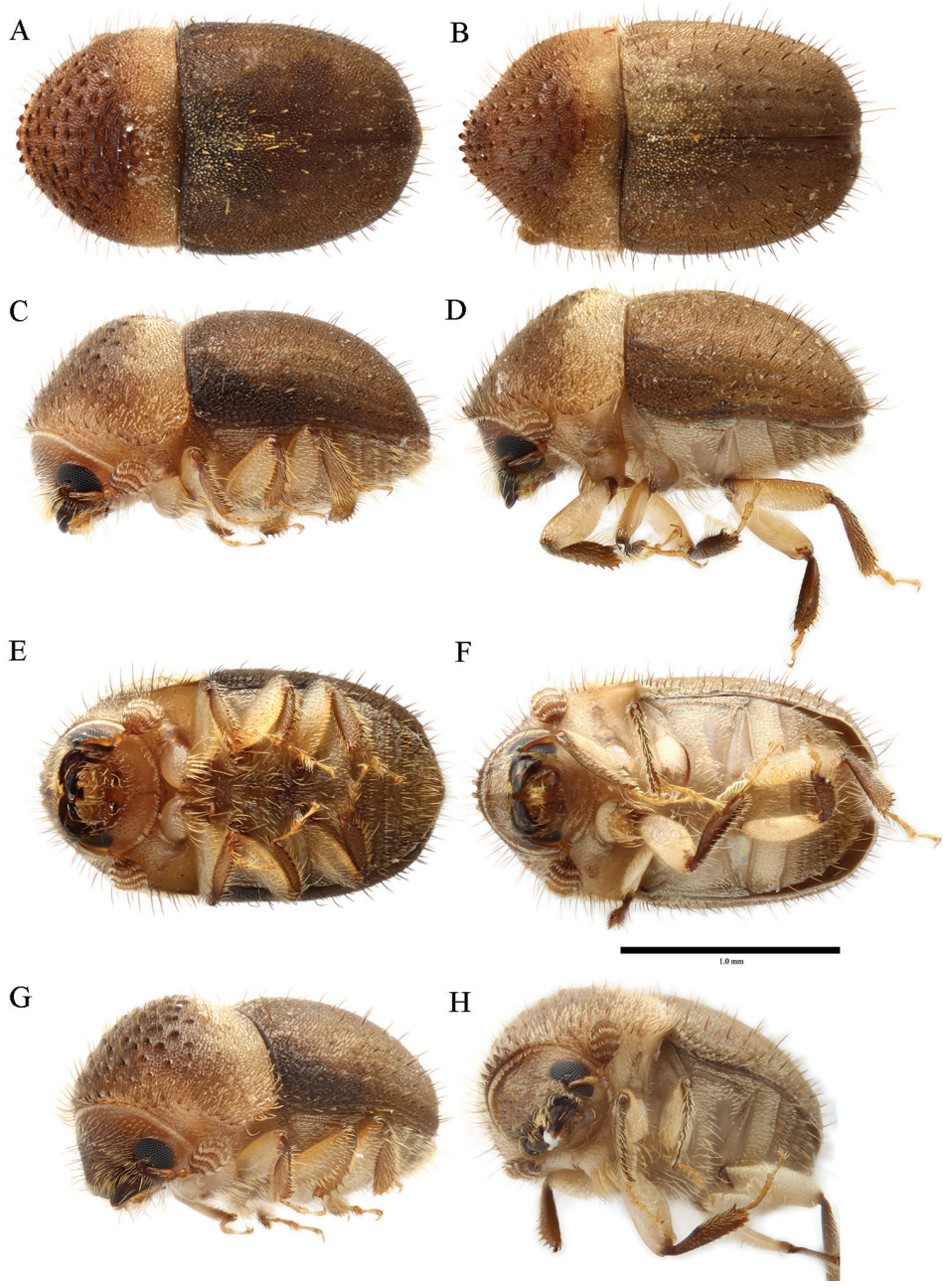

Figure 6. *Cryphalus dorsalis* **A, C, E, G** female, UFFE:34070 **B, D, F, H** male, UFFE:21476.

tance between metacoxae. Proventriculus sutural teeth in multiple irregular rows. Apical teeth extend two thirds the width of the segment. Closing teeth longer than masticatory brush, branched near tips. Masticatory brush with fine teeth, short, less than half the segment length.

Male. Similar to female except: Length 1.60–1.80 mm. Frons with straight transverse carina and sulcus above the level of eyes. Gular surface impressed and glabrous, surrounded by sparse hair-like setae. Pronotal profile triangular, almost constricted on antero-lateral edges. Pronotal declivity almost flat (not broadly rounded). Procoxae with large feather-like setae. Protibiae and protarsi with large spatula-shaped setae along inner margin. Last abdominal ventrite emarginated. Proventriculus same as female. Aedeagus not examined.

Distribution. China (Hainan, Yunnan); Thailand; Vietnam.

Remarks. Yin et al. (2002) listed *Cryphalus indicus* Stebbing, 1902, a primary homonym replaced by *Cryphalus strohmeyeri* Stebbing, 1914, as from Hainan. This record has subsequently been included in catalogues (Alonso-Zarazaga et al. 2017). Based on the descripted in Yin et al. (2002), the identity was probably intended as *Cryphalus indicus* Eichhoff, 1878a, a junior synonym of *C. dorsalis*. The description in Yin et al. (2002) clearly indicates the stout proportions (1.8× as long as wide) and the transverse ridge on the male frons, which correspond to *C. dorsalis* as we found in Hainan. Yin et al. (2002) listed the host as *Abies*, probably from other records or the original description, which does not grow in Hainan. Specimens of *C. strohmeyeri* were studied from elsewhere in China (USNM).

Cryphalus exiguus Blandford, 1894

Figure 7A–I

Cryphalus exiguus Blandford, 1894: 82 (Japan).

Type material examined. Japan • 1 ♂ ***Holotype***; Fukushima, not recorded; 29 Jul. 1891; C. Lewis leg.; labelled "Japan // C. Lewis // 1910–320 ///// Fukushima // 26.VII-29. VII.91 // *Cryphalus exiguus* Bland. // NHMUK 010805962"; UFFE:26205; (NHMUK).

Other material examined. Japan • 1 ♀; Yamagata, Tendo; 06 May 1982; I Ueno leg.; Photographed by SP; UFFE:28638; (NIAES).

Russia • 1 ♀; Sakhalin Oblast, South Kuril urban district, Kunashir Island, Tret'yakovo; 29 Jul. 2008; Michail Yu. Mandelshtam leg.; ex. *Morus australis*; small twigs, river valley; UFFE:31832; (FSCA).

South Korea • 1 ♂; Gangwon-do, Inje-gun, Inje-eub, Nambuk-ri; 38.0753°N, 128.1336°E; 23 Apr. 2013; Sangwook Park leg; UFFE:28122; (RIFID) • 1 ♀; same collection data; UFFE:28123; (RIFID) • 1; Gangwon-do, Inje-gun, Buk-myeon, Yongdae-ri; 38.2075°N, 128.3382°E; 08 May 2018; Sangwook Park leg.; specimen S3 by sequence only,; DNA: 28S:MT437225; UFFE:34660; (RIFID) • 1; Gangwon-do, Inje-gun, Buk-myeon, Hangye-ri; 38.1316°N, 128.3004°E; 08 May 2018; Sangwook Park leg.; specimen S5 sequence only; DNA: 28S:MT437226; UFFE:34661; (RIFID) • 1; Gangwon-do, Inje-gun, Buk-myeon, Hangye-ri; 38.1316°N, 128.3004°E; 08 May 2018; Sangwook Park leg.; specimen S6 by sequence only; DNA: 28S:MT437227; UFFE:34662; (RIFID) • 1; Gangwon-do, Pyeongchang-gun, Yongpyeong-myeon, Soksa-ri; 37.6673°N, 128.497°E; 20 May

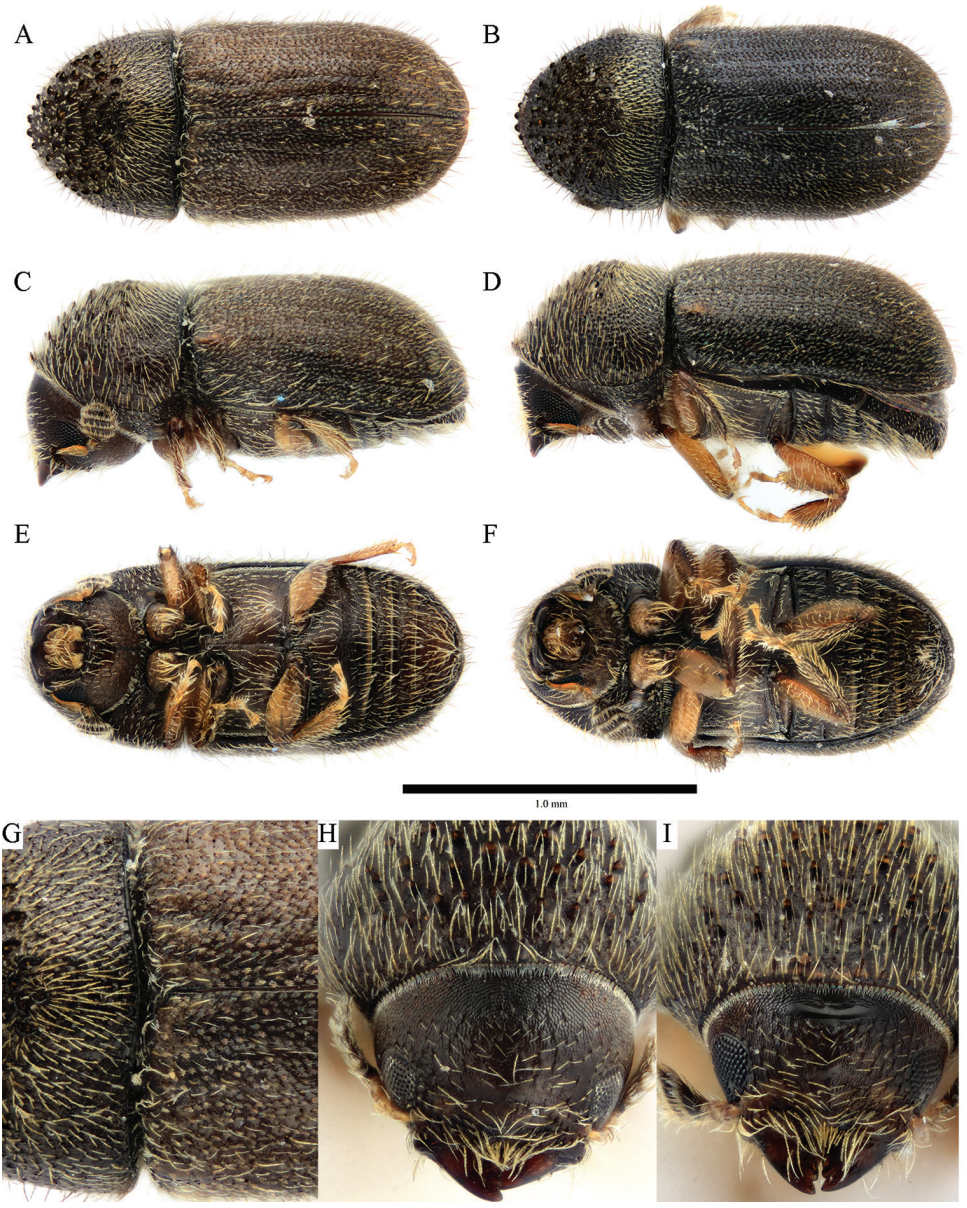

Figure 7. *Cryphalus exiguus* **A, C, E, G** female, UFFE:34070 **B, D, F, H** male, UFFE:21476.

2017; Sangwook Park leg.; specimen S11 sequence voucher; DNA: 28S:MT437228; UFFE:34663; (RIFID).

Diagnosis. This species is diagnosed from others in East Asia by the size (1.30–1.55 mm), by the proportions (2.20× as long as wide), by the male frons with a short transverse carina, by the antennal sutures (approximately horizontal), by the setae on the pronotal disc (entirely hair-like), and by the elytral striae devoid of ground vestiture.

This species is very similar to *Cryphalus mandschuricus* Eggers, 1929, and differs by the transverse carina on the male frons (*C. exiguus*: narrower, not much more than two thirds of the width between eyes; *C. mandschuricus*: wider, almost as long as width between eyes), by the sutures on the antennal club (*C. exiguus*: almost straight, recurved at edges; *C. mandschuricus*: procurved, especially third suture), by the length of setae on the pronotal disc (*C. exiguus*: much longer than distance between setae; *C. mandschuricus*: barely as long as distance between setae), and by the length of the elytral disc of females (*C. exiguus*: about two thirds of the length of elytra, declivity steep; *C. manschuricus*: only half of elytral disc, declivity slope gradual). The aedeagus of *C. exiguus* was not examined, but that in *C. mandschuricus* has the tegmen apodemes much longer than the distance between them.

Cryphalus morivorus, described below, is similar and can be distinguished by the characters therein.

Female. Length 1.40–1.55 mm (holotype 1.50 mm). Proportions 2.20× as long as wide. Frons simple, convex, flat and shining up to the level of the eyes. Antennal club with three straight sutures marked by a mixture of coarse short setae and longer setae with length approximately the same as distance between sutures. Antennal funiculus with four funicular segments. Pronotal colour dark brown, similar to elytra and head. Pronotal profile widest at base. Pronotal margin armed with four or five serrations, irregularly spaced and sometimes partially fused. Pronotal declivity broadly rounded, with approximately 50 asperities. Pronotal disc occupies approx. one third of the length of the pronotum, gently sloped, uniformly weakly asperate. Pronotal vestiture of only gold, hair-like setae. Suture between pronotum and elytra very weakly v-shaped. Scutellum small, triangular. Elytra 1.85× as long as pronotum, dark brown, broadly rounded with no clear transition to the declivity. Elytral texture mostly smooth, with punctures from the ground vestiture much smaller than the diameter of the strial punctures, and interspaces smooth. Striae visible as rows of punctures, with no scale-like setae between strial punctures. Interstrial bristles erect, hair-like, slightly flattened with pointed tips, those near the elytral suture are shorter and broader than those in lateral regions. Interstrial ground vestiture tridentate, 1–2× as long as wide, with a weak iridescence. Protibiae and protarsi with only simple hair-like setae. Mesocoxae narrowly separated, only slightly more than metacoxae. Proventriculus not examined.

Male. Similar to female except: Length 1.30–1.50 mm. Frons with a smooth transverse carina above the level of the eyes. Pronotal declivity with asperities slightly more sparse and slope is straight rather than broadly curved. Protibiae and protarsi with coarse, long setae on the proximal face, near the apex. Last abdominal ventrite clearly emarginated. Proventriculus not examined. Aedeagus not examined.

Distribution. Russia (Southern Kuriles: Kunashir and Shikotan Isles in Sakhalin Oblast); Japan; South Korea, North Korea (Ju, 1964, unconfirmed), China (North-East, Krivolutskaya, 1996, unconfirmed).

Recorded plant hosts. Moraceae: *Morus australis* Poir., *M. alba* L.

Suggested vernacular name. Chinese: 北桑梢小蠹 [Northern mulberry twig bark beetle]; English: Northern mulberry bark beetle; Korean: 뽕나무애나무좀; Russian: крифал шелковичный.

Remarks. Frequently misspelled as "*exignus*", with the first instance of this misspelling in Niisima (1909). The name *Cryphalus pilosus* Sasaki, 1899 has been treated as a junior synonym of *C. exiguus* (Wood and Bright 1992; Alonso-Zarazaga et al. 2017), based on the apparent synonymy by Niisima (1909). Upon inspection of the referenced material (Sasaki 1899), the synonymy is deemed to be a misinterpretation. The original publication, in Japanese, describes a pest of mulberry with the tentative identification as "Hylesinus (Xlechinus) pilosus?" [Sic], presumably referring to *Xylechinus pilosus* (Ratzeburg 1837) (Scolytinae, Hylurgini). There was no type material deposited or any indication that this was intended as a new species. Niisima's remark was intended to correct the identification, rather than suggesting that the two species names are synonymous.

The original description (Blandford 1894) indicates that the pronotum contains scale-like setae ("…with a thin covering of scales and hairs"). This is not visible on the holotype and any other specimens seen from northern and central Japan. Crucially, this is the opposite of a character state which diagnoses this species (having only hair-like setae). We consider this an error in the species description rather than either damage or accidental replacement of the holotype. The weakly asperate texture of the pronotal disc can give the illusion of scales in non-diffuse lighting. Another diagnostic character for *C. exiguus* is the clearly visible striae devoid of ground vestiture, which is included in the original species description.

We did not see any specimens representing this species in China (see remarks for *C. morivorus*), but it is likely to be present in the north-eastern regions. The key by Tsai and Li (1963) clearly indicated prominent striae, suggesting specimens of *C. exiguus* were used when compiling the key and comparing to *C. manschuricus*.

Cryphalus gnetivorus Johnson, sp. nov.

http://zoobank.org/320AFB38-5A27-458D-9A92-317274D8FC95
Figures 2D, 3D, 8A–I

Type material examined. China • 1 ♀ ***Holotype***; Guangdong, Shenzhen, Dapeng dam; 22.6316°N, 114.4624°E; 12 Apr. 2018; You Li leg.; ex. *Gnetum luofuense* ; reared from dead vines; IOZ(E)225671; UFFE:31751 (IOZ) • 1 ♂ ***Paratype***; same collection data; dissected; UFFE:31753; (UFFE) • 1 ♂ ***Paratype***; same collection data; dissected; DNA: 28S:MT937245, COI:MT937225; UFFE:31752 (UFFE) • 1 ♂ ***Paratype***; same collection data; UFFE:31750 (UFFE) • 8 ♀♀, 7 ♂♂ ***Paratypes***; same collection data except 01 Jun. 2018; UFFE:34924 (NHMUK, 1♀, 1♂; FSCA, 1♀; MZB, 1♀, 1♂; NIAES, 1♀, 1♂; NMNS, 1♀, 1♂; IOZ, 1♂ IOZ(E)225672; RIFID, 1♀; USNM, 1♀, 1♂; ZIN, 1♀, 1♂).

Other material examined. China • 1; Guangdong, Shenzhen, Yantian; 22.5889°N, 114.2842°E; 08 Apr. 2017; Wei Lin leg.; UFFE:33241.

Type locality. China, Guangdong, Shenzhen, Dapeng dam (22.6316°N, 114.4624°E).

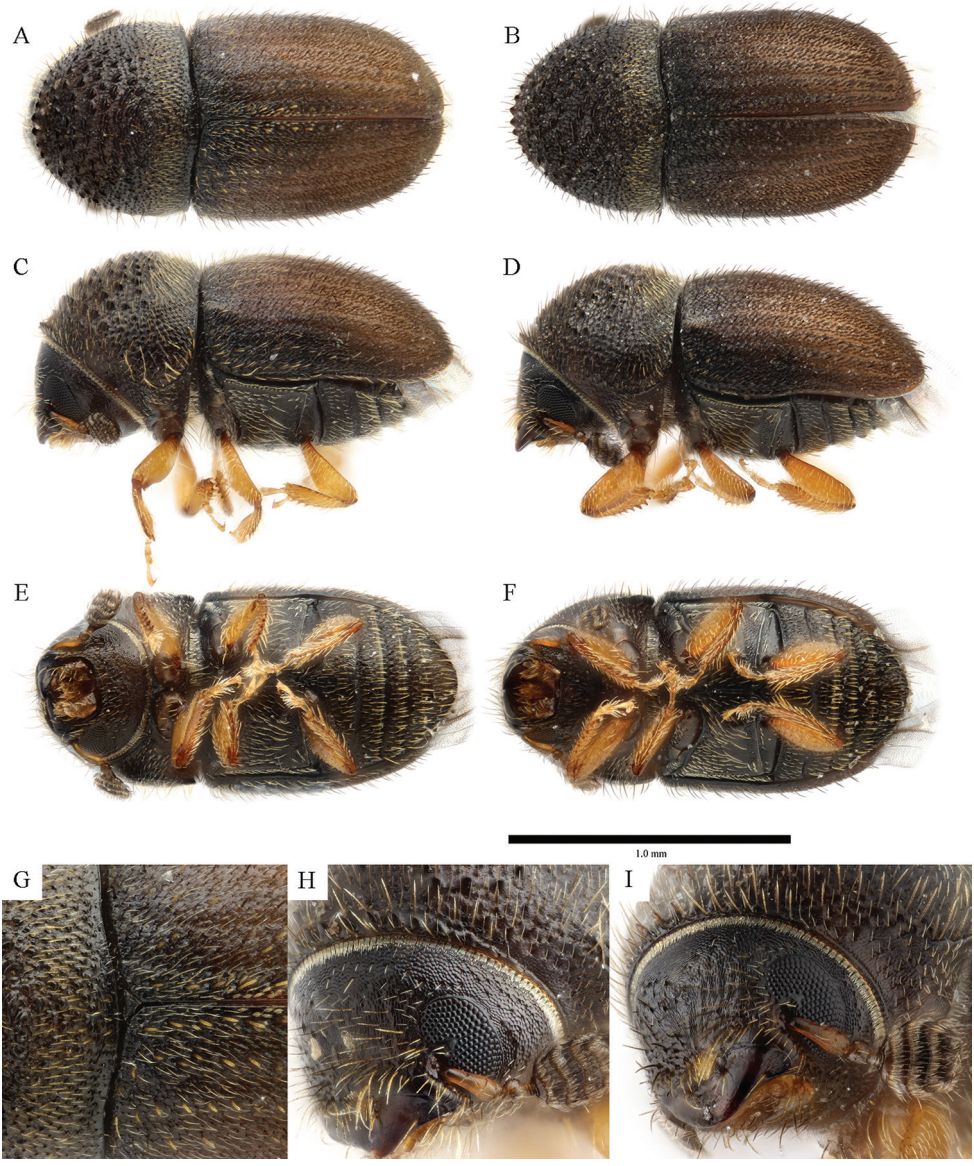

Figure 8. *Cryphalus gnetivorus* **A, C, E, G, H** holotype, female, UFFE:31751 **B, D, F, I** paratype, male, UFFE:31753.

Diagnosis. *Cryphalus gnetivorus* can be distinguished from most of the East Asian *Cryphalus* by the combination of the size (1.20–1.50 mm), the proportions (twice as long as wide), by the male frons with minute transverse aciculations in upper median area, by the short elytral disc and long, gradual declivity, by the interstrial bristles of approximately even length, widened and rounded at tips, pointing posteriorly, by the female interstrial ground vestiture which is hair like near base and tridentate scale-like

on declivity, and by the proventriculus with a wide area of sutural teeth, occupying half of the segment width.

Female. Length 1.25–1.50 mm (holotype 1.45 mm). Proportions 2.0× as long as wide. Frons with weak aciculations and punctures, and a weak median keel on lower half. Antennal funiculus with four or five segments, the last being short and wide. Antennal club with three straight or weakly procurved sutures. Pronotal colour dark brown. Pronotal profile broadly rounded, widest in line with summit. Pronotal margin armed with 6–8 wide, rather blunt serrations, larger at median. Pronotal declivity with 60–75 wide and blunt asperities (holotype has 71). Pronotal disc approx. three tenths of the pronotal length, asperate texture, most pronounced at median. Pronotal vestiture with golden hair-like setae, with some longer, coarse setae along baso-lateral margin. Suture between pronotum and elytra weakly sinuate. Scutellum small, triangular. Elytra very broadly rounded, disc occupying less than one third of elytral length, and a gradually sloping declivity. Elytra colour dark brown on disc, becoming chestnut-brown on declivity. Striae impressed on disc, gradually becoming less apparent and less impressed on declivity, visible only as row of shallow punctures on lower area of declivity. Interstrial bristles short, flattened near apex with a rounded tip, of a similar length on disc and declivity, curved pointing posteriorly, arranged somewhat irregularly on disc and in a row on declivity. Interstrial ground vestiture completely hair-like at base, barely indistinguishable from strial setae, becoming entirely tridentate scale-like on declivity. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, a little more than distance between metacoxae. Ventrites with mostly single hair like, and some bifurcated setae. Proventriculus not examined.

Male. Similar to female except: Length 1.20–1.45 mm. Frons with minute transverse aciculations in the median upper portion of the frons. Interstrial ground vestiture tridentate scale-like on disc and declivity, more elongate and intermixed with a few hair-like setae near base. Protibiae and protarsi with a few coarse curved setae. Last abdominal ventrite not emarginated. Proventriculus sutural teeth numerous, occupying about half of width of segment, sometimes in indistinct transverse rows of three or more, with an indistinct transition to the apical teeth. Apical teeth almost extending width of segment. Closing teeth extending beyond masticatory brush, branched and finely tapered at tips. Masticatory brush of a similar length to apical plate. Aedeagus short, without obvious end plate. Penis apodemes less than half of the length of penis body. Tegmen with short paired apodemes.

Etymology. The name is an adjective derived from a combination of *gnet* the stem scientific name of the host plant (*Gnetum*), a linking vowel *-i-* and an adjectival suffix *vorus*, meaning eater.

Distribution. China (Guangdong).

Recorded plant hosts. Gnetaceae: *Gnetum luofuense* C.Y. Cheng.

Remarks. *Gnetum* is an unusual leafy gymnosperm distributed in Asia through to New Guinea, which grows as vines or small shrubs. *Cryphalus gnetivorus* is unusual among the *Cryphalus* in the area of study in the shape, with a robust pronotum and

smaller elytra which is slightly tapered and mostly a gentle declivity, somewhat similar to *Eidophelus darwini* Eichhoff, 1878, or various Xyloctonini Eichhoff, 1878.

Using the key of Tsai and Li 1963, this species would key to "subgenus *Cryphalus*" and fail at couplet 2/7 if *Gnetum* is considered a conifer, and couplet 15/16 if *Gnetum* is considered a broadleaf. The colour and proportions are similar to *Cryphalus eriobotryae* Johnson, 2019, but can easily be distinguished by the antennal sutures (*C. gnetivorus*: evenly spaced and procurved; *C. eriobotryae*: unevenly distributed, the third much more procurved than first two), and using the diagnoses above.

***Cryphalus itinerans* Johnson, sp. nov.**

http://zoobank.org/A52892B8-430C-4113-9D1C-FCF118A06149

Figures 2E, 3E, 9A–I

Type material examined. China • 1 ♀ ***Holotype***; Hong Kong, Kadoorie Farm; 22.4294°N, 114.1146°E; Jun. 2017; James Skelton, P Carlson, You Li, and Jiri Hulcr leg.; IOZ(E)225673; ex vial 20306.; UFFE:33170; (IOZ) • 1 ♂ ***Paratype***; same collection data; ; dissected; DNA: 28S:MT431547, COI:MT431650; UFFE:33178; (UFFE) • 1 ♂ ***Paratype***; Hong Kong, Kadoorie Farm; 22.4294°N, 114.1146°E; Jun. 2017; James Skelton, P Carlson, You Li, and Jiri Hulcr leg.; UFFE:33174; (UFFE). .• 8 ♀♀, 8 ♂♂ ***Paratypes***; same collection data ; UFFE:33179; (NHMUK, 1♀, 1♂; FSCA, 1♀, 1♂; MZB, 1♀, 1♂; NIAES, 1♀, 1♂;NMNS, 1♀, 1♂; IOZ, 1♂ IOZ(E)225674; RIFID, 1♀, 1♂; USNM, 1♀, 1♂; ZIN, 1♀).

Other material examined. China • 7; Fujian, Quanzhou, Yongchun, Diyiyan; 25.3177°N, 118.2809°E; 22 Nov. 2015; You Li leg.; beetle walking on the bark of *Broussonetia papyrifera*; DNA: 28S:MG051123, COI:MG051178; UFFE:22092; (UFFE) • 12; Guangdong, Shenzhen, Yantian; 22.5889°N, 114.2842°E; 06 Apr. 2017; Wei Lin leg.; UFFE:31618; (UFFE) • 2; Guangdong, Zhuhai, Jialinshan; 22.185°N, 113.4784°E; 05 Sep. 2018; Wei Lin, and You Li leg.:34039; (UFFE) • 2; Guangxi, Shangsi, Shiwandashan; 21.9278°N, 107.9282°E; 27 Mar. 2018; You Li, and Shengchang Lai leg.; near pine plantation; DAE; UFFE:34230; (UFFE) • 1 ♂; Hainan, Danzhou, near Botanic Garden; 19.5174°N, 109.4994°E; 22 Oct. 2016; You Li leg.; mix spp; DAE; UFFE:34187; (UFFE) • 1 ♂; Hainan, Qiongzhong, Wanling; 26 Oct. 2016; You Li leg.; Light trap; DNA: 28S:MG051124, COI:MG051179; UFFE:25935; (UFFE).

Taiwan • 7; Nantou County, Sun Moon Lake; 22 Apr. 2013; Ching-Shan Lin leg.; DNA: 28S:MG051126, COI:MG051180; UFFE:10482; (UFFE) • 1; Taichung City, Wufeng District, Jingtonlin; 24.0457°N, 120.788°E; 10 Jan. 2019; Ching-Shan Lin leg.; ex. *Ficus erecta Thunb. var. beecheyana*; UFFE:32769; (UFFE).

United States • 1 ♂; Florida, Escambia County, Pensacola, Ellyson industrial park; 30.5198°N, -87.2109°E; 20 Jul. 2012; J. Welch leg.; UFFE:15115; (FSCA) • 1 ♂; same collection data; DNA: 28S:MG051125; UFFE:15116; (UFFE) • 1 ♀; same

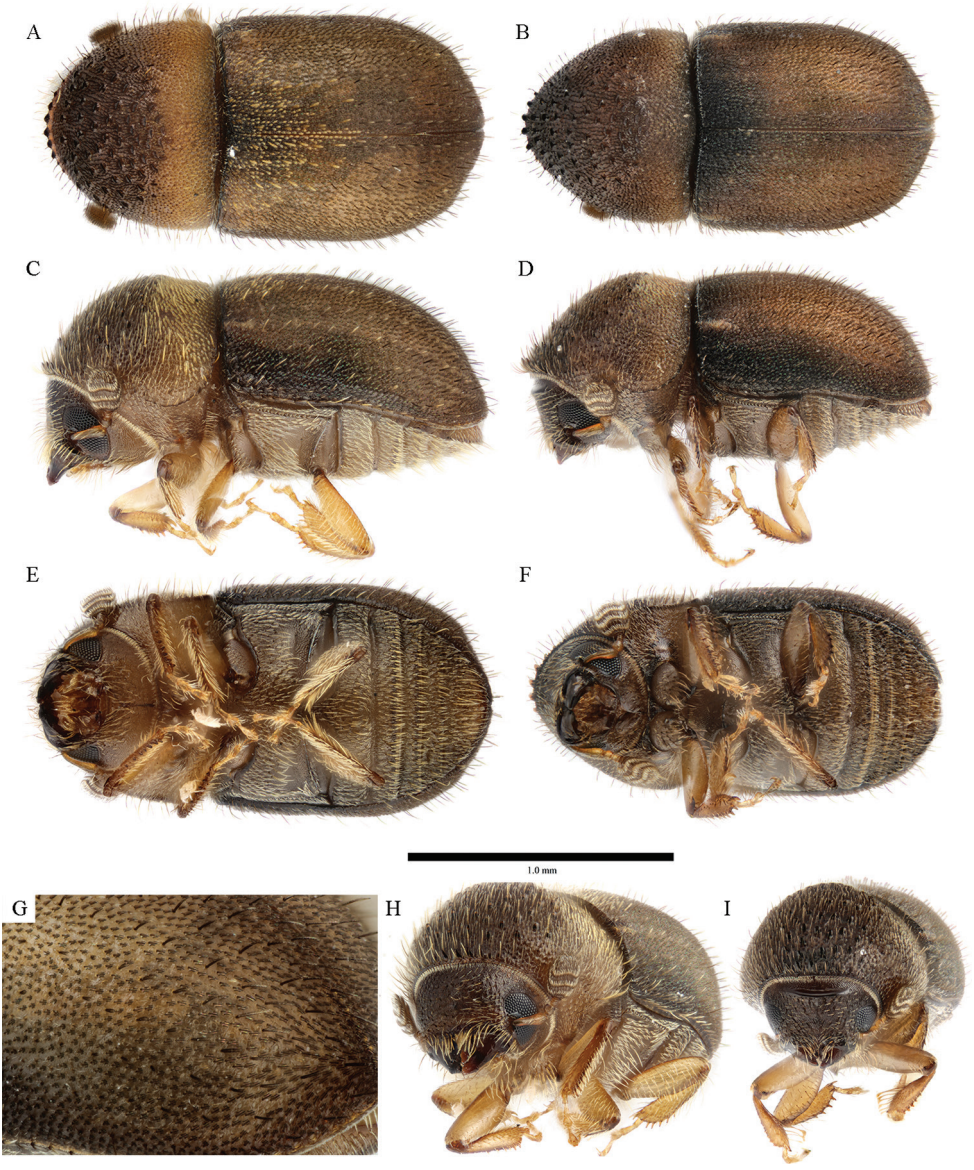

Figure 9. *Cryphalus itinerans* **A, C, E, G, H** holotype, female UFFE:33170 **B, D, F, I** paratype, male, UFFE:31746.

collection data; UFFE:15117; (FSCA) • 1 ♀; Florida, Escambia County, Pensacola, Navy Point park; 21 May 2013; J. Brooks leg.; UFFE:15114; (FSCA).

Type locality. China, Hong Kong, Kadoorie Centre (22.4294°N, 114.1146°E).

Diagnosis. *Cryphalus itinerans* can be distinguished from most of the East Asian *Cryphalus* by the combination of the size (1.3–1.8 mm), by the pronotal summit, which is about a third of the length, viewed dorsally, and by abundant scales over all of the pronotal disc.

This species is very similar to *Cryphalus artocarpus* but differs by the size (*C. itinerans*: 1.3–1.8 mm, versus *C. artocarpus*: 1.05–1.30 mm), by the setae on the male protibiae (*C. itinerans*: scythe-shaped, versus *C. artocarpus*: hair-like, barely larger than on female), and by the proventriculus apical teeth (*C. itinerans*: more than two thirds segment width, versus *C. artocarpus*: less than two thirds segment width).

This species is also very similar to *C. dilutus* and can be diagnosed by the setae on the male protibiae (*C. itinerans*: scythe-shaped, versus *C. dilutus*: spatula-shaped); by the mesofemora of the males (*C. itinerans*: unarmed, versus *C. dilutus*: armed with a large spine).

Female. Length 1.40–1.80 mm (holotype 1.70 mm). Proportions 2.0× as long as wide. Frons with minute aciculations, barely visible. Antennal club with three procurved sutures marked by coarse and long setae. Antennal funiculus with four or five funicular segments (holotype has four). Pronotal colour dark brown on slope, light brown on disc. Pronotal profile broadly rounded, slightly wider in line with summit. Pronotal margin armed with five to eight serrations (holotype has six), separated by approximately their width, and sometimes flanked by one or two pairs of smaller serrations. Pronotal declivity with more than 50 asperities (holotype has 65). Pronotal disc approximately one third the length of the pronotum, gently sloped, weakly tuberculate surface texture (obscured by scale-like setae). Pronotal vestiture hair-like on anterior and lateral sloped, and a mixture of scale-like and hair-like on disc and postero-lateral regions, with the scale-like setae 2–3× as long as wide with a tridentate tip. Suture between pronotum and elytra weakly sinuate. Scutellum very small, barely visible. Elytra 1.5× as long as pronotum, translucent yellow-brown, broadly rounded with no clear transition to the declivity. Striae barely visible as rows of punctures and hair-like setae. Interstrial bristles erect, weakly flattened with rounded tips, some uniform in length and some wider near tip. Interstrial ground vestiture tridentate, approximately 1–2× as long as wide, translucent brown with a weak iridescence, sometimes light brown near the base of the elytra. Apex of elytra barely obtuse. Gular surface with evenly spaced hair-like setae. Protibiae and protarsi with only straight, hair-like setae. Protibiae and protarsi with only simple hair-like setae. Mesocoxae moderately separated, more than distance between metacoxae. Proventriculus not examined.

Male. Similar to female except: Length 1.30–1.70 mm. Frons weakly aciculate with a glossy carina above the level of the eyes. Gular surface shining around suture, surrounded by sparse setae. Pronotal profile widest in line with summit. Anterior to the summit, the profile is triangular. Pronotal margin with four to six marginal asperities, spaced approximately by twice their width. Pronotal declivity with almost straight slope, with more than 45 asperities, asperities smaller than the females. Pronotal disc with strongly tuberculate surface obscured by scale-like setae. Protibiae and protarsi with long scythe-shaped setae. Last abdominal ventrite clearly emarginated. Proventriculus sutural teeth rounded, in two overlapping rows. Apical teeth extending the width of a segment. Closing teeth very long, extending beyond masticatory brush, tapered, few branches. Masticatory brush short, less than length or apical plate. Aedeagus long, weakly sclerotised. Penis apodemes approximately two thirds as long as penis body. Tegmen with two ventral apodemes, which are longer than distance between them.

Etymology. The name is derived from the Latin *itinerāns* meaning traveller, referring to the apparent ability to establish in new areas. It is invariable.

Distribution. China (Hainan, Fujian, Guangdong, Yunnan, Hong Kong); Taiwan; United States (Florida).

Recorded plant hosts. Moraceae*: Ficus carica* L., *F. erecta* Thunb. var. *beecheyana*, *Broussonetia papyrifera* (L.) Vent.

Suggested vernacular name. Chinese: 华南梢小蠹 [South China twig beetle].

Remarks. This species is weakly attracted to ethanol-quercivorol traps. It was observed making cave-like galleries in material 2–5 cm diameter.

This species was referred to as *Hypocryphalus* "sp.1422" in Johnson et al. (2017) where some of the distribution records and diagnostic characters were described. This species is abundant and widespread across Southern China and neighbouring regions. It is surprising that this species is not already described; it is perhaps because much of the work on *Cryphalus* in China has focused on species in the North or on coniferous hosts, as well as building upon work by researchers in the Russian Far East, not in tropical or sub-tropical regions. Several species from the Philippines such as *Cryphalus obesus* Hopkins, 1915 are similar but have a much shorter pronotal disc. *Cryphalus discretus* Eichhoff, 1878, from India and Myanmar, likely present in China, is also very similar, and differs by having a short elytral disc.

Using the key of Tsai and Li (1963), this species would be reach and match 23, *Cryphalus mandschuricus*, though the proportions and overall appearance differ greatly.

Cryphalus kyotoensis Nobuchi, 1966

Figures 2F, 3F, 10A–I

Cryphalus kyotoensis Nobuchi, 1966: 53 (Japan).

Type material examined. Japan • 1 ♀ ***Holotype***; Kyoto, Kyoto City, Mizoro-ga-ike; 30 Dec. 1957; A. Nobuchi leg.; ex. *Alnus firma*; UFFE:34947 (NIAES).

Other material examined. China • 1 ♀; Fujian, Longyan, Songmao Ridge, Liancheng; 25.57°N, 116.59°E; 03 Sep. 2019; Ling Zhang leg.; ex. *unknown*; 20190903001; AJJ also saw 1m and 1f from same collection, returned; UFFE:34068; (UFFE) • 1; Guangdong, Shenzhen, Yantian; 22.5889°N, 114.2842°E; 08 Apr. 2017; Wei Lin leg.; EtOH trap; UFFE:33203; (UFFE) • 1 ♀, 1 ♂; Jiangxi, Xunwu, Xiangshan; 24.9129°N, 115.8538°E; 10 Oct. 2018; You Li leg.; ex. *Rhus chinensis*; (IOZ, 1 ♀ IOZ(E)2057938, 1 ♂ IOZ(E)2057939); UFFE:31746; • 1 ♀; same collection data; DNA: 28S:MT431544, COI:MT431648; UFFE:33232 • 1 ♂ ; same collection data; UFFE:31745; (UFFE) • 9 ♀♀, 9 ♂♂ ; same collection data; (NHMUK, 1♀, 1♂; FSCA, 1♀, 1♂; MZB, 1♀, 1♂; NIAES, 1♀, 1♂; NMNS, 1♀, 1♂; RIFID, 1♀, 1♂; UFFE, 1♀, 1♂; USNM, 1♀, 1♂; ZIN, 1♀, 1♂); UFFE:31741.

South Korea • 1 ♀; Chungcheongbuk-do, Cheongju-si, Oksan-myeon, Guksa-ri; 02 Jul. 2008; Sangwook Park leg.; UFFE:28140; (UFFE) • 1 ♂; same collection data;

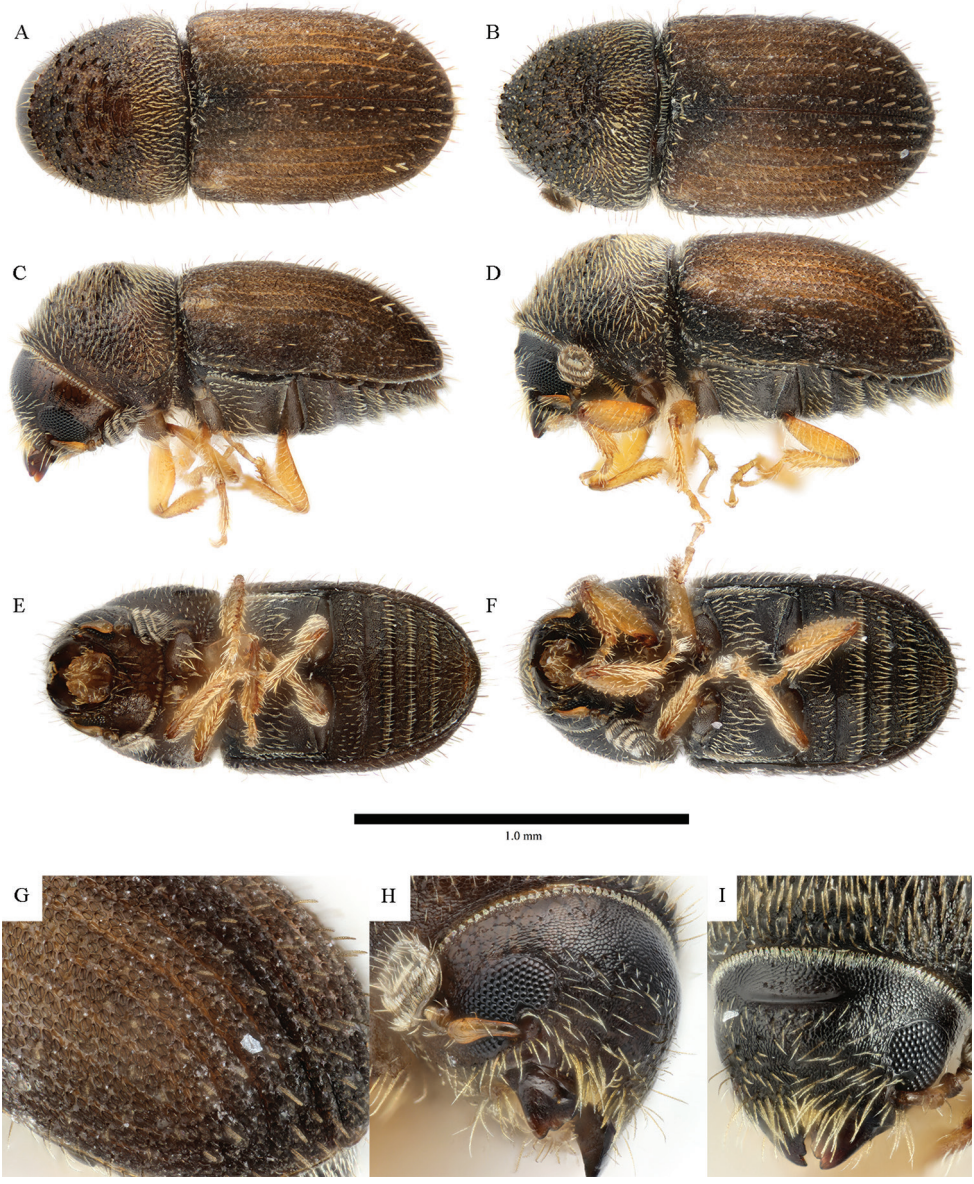

Figure 10. *Cryphalus kyotoensis* **A, C, E, G, H** female UFFE:33170 **B, D, F, I** male, UFFE:31745.

DNA: 28S:MT431543, COI:MT431647; UFFE:28139; (UFFE) • 5; same collection data; UFFE:28138; (UFFE) • 65 ♀♀, 77 ♂♂; Chungcheongnam-do, Gongju-si, Banpo-myeon, Maam-ri; 36.4252°N, 127.1979°E; 02 Aug. 2009; Sangwook Park leg.; UFFE:34708; (RIFID) • 1 ♀, 1 ♂; Gangwon-do, Chuncheon-si, Dong-myeon, Gamjeong-ri; 37.8952°N, 127.8218°E; 04 May 2017; Sangwook Park leg.; UFFE:34705; (RIFID) • 1 ♀, 2 ♂♂; Gangwon-do, Goseong-gun, Toseong-myeon, Wonam-ri;

38.2136°N, 128.4707°E; 15 Apr. 2016; Sangwook Park leg.; UFFE:34698; (RIFID) • 1 ♂; Gangwon-do, Inje-gun, Inje-eub, Nambuk-ri; 38.0753°N, 128.1336°E; 23 Apr. 2013; Sangwook Park leg.; UFFE:34701; (RIFID) • 2 ♀♀, 7 ♂♂; Gangwon-do, Inje-gun, Buk-myeon, Hangye-ri; 38.1357°N, 128.2763°E; 17 Apr. 2018; Sangwook Park leg.; UFFE:34702; (RIFID) • 2 ♀♀, 4 ♂♂; Gangwon-do, Inje-gun, Buk-myeon, Hangye-ri; 38.1357°N, 128.2763°E; 08 May 2018; Sangwook Park leg.; UFFE:34707; (RIFID) • 1 ♂; Gangwon-do, Samcheok-si, Mapyeong-dong; 37.4244°N, 129.1345°E; 12 Sep. 2013; Sangwook Park leg.; UFFE:34696; (RIFID) • 1 ♂; Gangwon-do, Samcheok-si, Mapyeong-dong; 37.4268°N, 129.1351°E; 16 Jun. 2016; Sangwook Park leg.; UFFE:34704; (RIFID) • 3 ♀♀; Gangwon-do, Yangyang-gun, Seo-myeon, Yeongdeok-ri; 37.9914°N, 128.5304°E; 15 Apr. 2016; Sangwook Park leg.; UFFE:34700; (RIFID) • 1 ♂; Gangwon-do, Yangyang-gun, Seo-myeon, Seorim-ri; 37.9634°N, 128.5178°E; 17 Apr. 2018; Sangwook Park leg.; UFFE:34697; (RIFID) • 1 ♂; Gangwon-do, Yangyang-gun, Seo-myeon, Osaek-ri; 38.0746°N, 128.4818°E; 08 May 2018; Sangwook Park leg.; UFFE:34699; (RIFID) • 6 ♂♂; Gyeonggi-do, Gapyeong-gun, Seolak-myeon, Seonchon-ri; 37.6769°N, 127.4716°E; 15 Apr. 2016; Sangwook Park leg.; UFFE:34706; (RIFID) • 1; Gyeongnam-do, Sangcheong-gun, Sicheon-myeon, Jungsan-ri; 35.1802°N, 127.4511°E; 01 Aug. 2008; Sangwook Park leg.; UFFE:28143; (UFFE) • 8 ♀♀, 6 ♂♂; Gyeongsangbuk-do, Sangju-si, Sabeol-myeon, Deokga-ri; 36.5091°N, 128.2241°E; 13 Apr. 2018; Sangwook Park leg.; UFFE:34695; (RIFID) • 1 ♀; Gyeongsangbuk-do, Sangju-si, Sabeol-myeon, Deokga-ri; 36.5091°N, 128.2241°E; 25 Apr. 2018; Sangwook Park leg.; UFFE:34703; (RIFID) • 1; Gyeongsangnam-do, Sangcheong-gun, Sicheon-myeon, Jungsan-ri; 35.1802°N, 127.4511°E; 01 Aug. 2008; Sangwook Park leg.; UFFE:28142; (UFFE).

Diagnosis. This species can be identified by the combination of the size (1.10–1.30 mm), the proportions (2.15× as long as wide) and the scale-like interstrial ground vestiture which is fused to the elytra.

Female. Length 1.20–1.30 mm (holotype 1.15 mm). Proportions 2.15× as long as wide. Frons simple, convex, with a small fovea in the centre. Antennal club with three recurved sutures marked by coarse setae. Antennae with three funicular segments. Pronotal colour dark brown. Pronotal profile widest at base, broadly rounded. Pronotal margin armed with eight serrations. Pronotal declivity with approx. 40–50 asperities, some of which are joined near the summit. Pronotal disc approximately one third of the pronotal length, sloping weakly from the summit. Pronotal vestiture coarse hair-like, light golden brown. Suture between pronotum and elytra weakly sinuate, marked with a carina at the base of the pronotum. Scutellum V-shaped, with hair-like setae. Elytra 1.6× as long as pronotum, translucent yellow-brown, broadly rounded with no clear transition to the declivity. Striae clearly visible as rows devoid of ground vestiture, and weakly visible punctures. Interstrial bristles erect, hair-like with blunt tips. Interstrial ground vestiture scale-like, recumbent, appearing fused to the elytra, sitting convex with a median keel; less than 1.5× as long as wide. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae separated, barely more than metacoxae. Proventriculus not examined.

Male. Similar to female except: Length 1.10–1.30 mm. Frons with a slightly deeper fovea, and a distinct transverse carina above the level of the eyes. Last abdominal ventrite not emarginated, similar to female. Proventriculus sutural teeth irregularly sized and shaped, though mostly rounded/conical, in two or more confused rows. Apical teeth extending only about half of segment width. Closing teeth long, barely branched near tip. Masticatory brush shorter than apical plate. Aedeagus long, bulbous at the base. Penis apodemes longer than penis body. Tegmen without paired apodemes.

Distribution. China (Fujian, Guangdong, Jiangxi); Japan; South Korea.

Ecology. Collected from branches 4–10 cm diameter.

Recorded plant hosts. Anacardiaceae: *Rhus chinensis* Mill.; Betulaceae: *Alnus firma* Siebold & Zucc.

Remarks. This species is phylogenetically similar to *C. longus* Eggers, 1926 from Primorskiy Kray and Japan (and likely present in northern China), which shares the unusual recumbent setae, but differs in the proportions.

The aedeagus is somewhat unusual among *Cryphalus* for having no paired apodemes on the tegmen, which is usually present and unique to *Cryphalus*.

Cryphalus lipingensis **Tsai & Li, 1959**

Figs. 2G, 3G, 11A–I

Cryphalus lipingensis Tsai & Li, 1959: 90 (China).
Cryphalus kesiyae Browne, 1975: 288 (Thailand), syn. nov.

Type material examined. China • 1 ♂ ***Lectotype*** [here designated]; 陕西黎坪 [Shaanxi, Liping town]; 15 Jun. 1958; 宋士美[Shimei Song leg].; 华山松 [*Pinus armandii*]; IOZ(E) 213228; UFFE:34756; (IOZ) • 33 ***Paralectotypes*** ; same collection information and labels; IOZ(E) 213218 to 213221; 213223 to 213250, 213436, and 213437; UFFE:34755; (IOZ).

Thailand • 1 ♂ ***Holotype*** *Cryphalus kesiyae* Browne, 1975; Chang Mai, Doi Pui; 15 Feb. 1971; Roger A. Beaver leg.; ex. *Pinus kesiya*; NHMUK 010805965; UFFE:26208; (NHMUK) • 1 ♀ ***Paratype***; Chang Mai, Doi Pui; 15 Feb. 1971; Roger A. Beaver leg.; ex. *Pinus kesiya*; UFFE:10407; (NHMUK).

Other material examined. China • 1 ♂; Guizhou, Weining, Maanshan; 26.822°N, 104.6282°E; 20 Oct. 2015; You Li leg.; ex. *Pinus armandii*; dissected; UFFE:28048; (UFFE) • 1 ♂; same collection data; UFFE:28041; (UFFE) • 1 ♀; same collection data; UFFE:28042; (UFFE) • 11 ♀♀, 12 ♂♂; Guizhou, Weining, Meihua village; 26.7251°N, 104.6027°E; 16 Oct. 2015; You Li leg.; ex. *Pinus armandii*; reared from multiple branches; (NHMUK, 1♀, 1♂; FSCA, 1♀, 1♂; MZB, 1♀, 1♂; NIAES, 1♀, 1♂; NMNS, 1♀, 1♂; IOZ, 1 ♀ IOZ(E) 2057940, 1 ♂ IOZ(E) 2057941; RIFID, 1♀, 1♂; UFFE, 2♀♀, 3♂♂; USNM, 1♀, 1♂; ZIN, 1♀, 1♂); UFFE:32956 ; same; same• 1 ♂; Yunnan, Kunming; 24.8801°N, 102.8329°E; 30 May 2013; Bateman leg.; UFFE:10939; (UFFE).

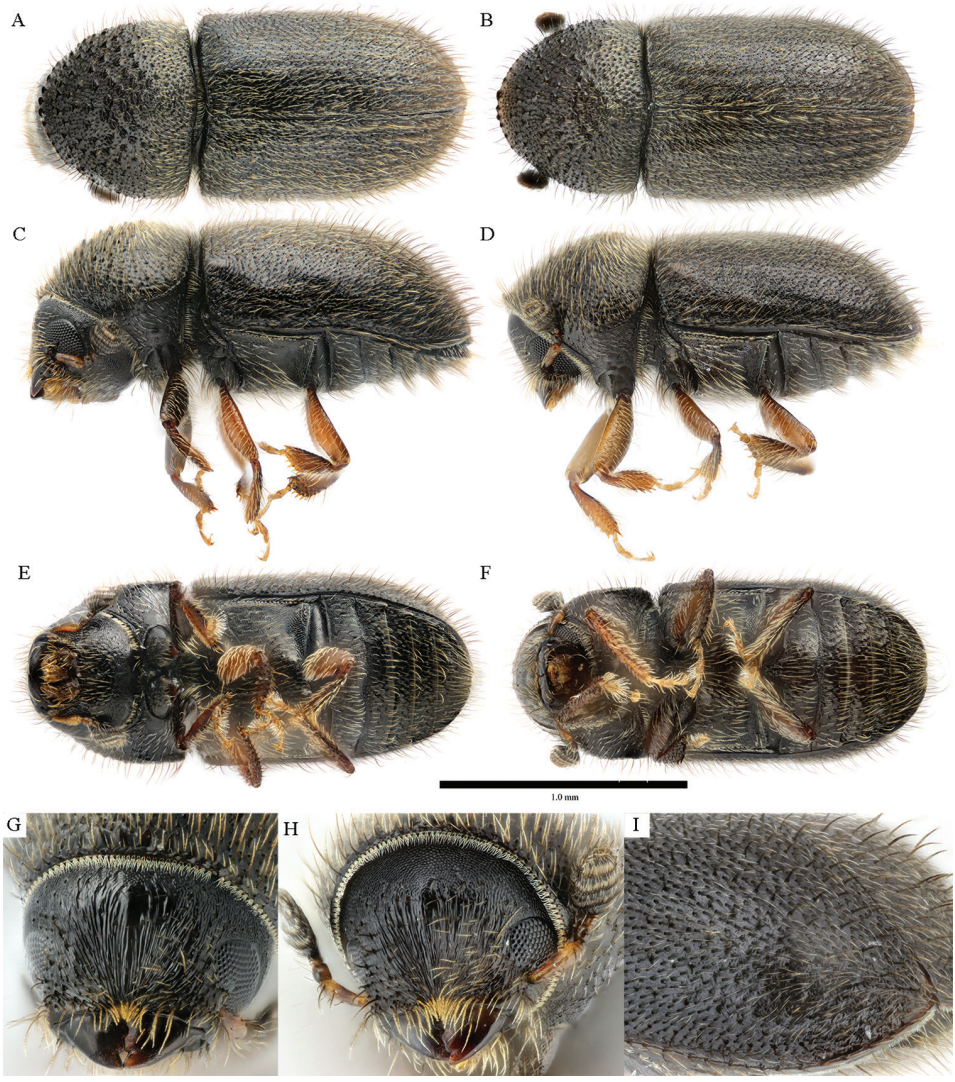

Figure 11. *Cryphalus lipingensis* **A, C, E, G** female, UFFE:28042 **B, D, F, H, I** male, UFFE:28041.

Thailand • 1; Chang Mai, Nanthaburi District, Omkoi Wildlife Sanctuary; 28 Jun. 2013; Bateman leg.; DNA: 28S:MG051085, COI:MG051133; UFFE:13592; (UFFE) • 1; Chiang Mai, Nanthaburi District, Omkoi Wildlife Sanctuary; 28 Jun. 2013; Bateman leg.; from vial 6101; voucher; UFFE:28529; (UFFE).

Diagnosis. This species can be easily distinguished from all other Chinese species by the combination of the strong aciculations on frons, by the male frons without transverse ridge or sulcus, by the long hair-like ground vestiture (only slightly widened at the base) and by the proventriculus with single row of sutural teeth.

Distribution. China (Guizhou, Sichuan, Shaanxi, Yunnan); Thailand.

Female. Length 1.70 mm (types 1.5–1.9 mm). Proportions 2.25× as long as wide. Frons distinctly aciculate, converging to the epistoma. Antennal club with three weakly recurved sutures marked by coarse and long setae, the distance between the suture 3 and the apex similar to the distance between sutures 2 and 3. Antennal funiculus with four segments, length shorter than the scape. Gular surface with evenly spaced hair-like setae. Pronotal colour dark brown, similar colour to elytra. Pronotal profile broadly rounded, slightly triangular, widest in line with summit, approx. 0.8× as long as wide. Pronotal margin armed with six serrations, often irregular and asymmetrical in size. Pronotal declivity with more than 70 asperities. Pronotal disc approximately one quarter the length of the pronotum, sloped, weakly tuberculate surface texture, larger tubercles near summit. Pronotal vestiture entirely hair-like setae. Suture between pronotum and elytra weakly sinuate. Scutellum very small, barely visible. Elytra 1.95× as long as pronotum, orange brown to brown, broadly rounded with no clear elytral disc or a transition to the declivity. Striae barely visible as rows of punctures and slightly impressed. Interstrial bristles erect, hair-like, with pointed tips, slightly longer and more dense on the declivity Interstrial ground vestiture hair like with pointed tips, denser on declivity. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, a little more than distance between metacoxae. Ventrites each with tooth on the postero-lateral corners. Proventriculus not examined.

Male. Similar to female except: Length 1.50–1.70 mm. Proportions 2.30× as long as wide. Frons convex, without transverse ridge or sulcus. Gular surface simple with few hair-like setae. Pronotal declivity more flat than female. Protibiae and protarsi with only hair-like setae, almost the same as female. Last abdominal ventrite weakly emarginated. Proventriculus sutural teeth small, in a single row on either side of the suture. Apical teeth extend the width of the entire segment, of typical proportions in the median. Closing teeth mostly shorter than length of masticatory brush, barely branched, and rounded tips. Masticatory brush about half of total length. Aedeagus short. Penis apodemes about as long as penis body, fused at tip. Tegmen with paired apodemes much shorter than distance between. End plate barely visible as two sclerotised plates.

Recorded plant hosts. Pinaceae: *Pinus kesiyae*, *P. armandi*, *P. yunnanensis*.

Suggested vernacular name. Chinese: 华山松梢小蠹 [Huashan pine twig bark beetle] (Tsai and Li 1959).

Remarks. We examined the holotype and paratypes of *Cryphalus kesiyae* Browne, 1975, and photographs of types of *C. lipingensis*, plus specimens from Thailand and China. There is little variation among the specimens including the type material, and more recent exemplars from near the type localities are genetically similar. Despite being from a similar region, Browne (1975) did not mention the similar species, but the descriptions were in Chinese and all of the large type series was held in China.

It appears that a holotype for *Cryphalus lipingensis* was never designated. The original description included two localities, "Liping", Shaangxi Province, from which it was named, and also Nanjiang, Sichuan Province. All of the specimens at IOZ were labelled as paratypes with a printed yellow label "PARATYPE", and all are from "Liping", presumed as the intended type locality. A similar issue exists for the other species

described by Tsai and Li in 1959, where no holotype is located or even mentioned. The specimens labelled as paratypes are therefore assumed to be syntypes, and to promote stability, a lectotype for *Cryphalus lipingensis* is hereby designated as specimen with the label "IOZ-(E) 213228".

***Cryphalus mangiferae* Stebbing, 1914**

Figures 2H, 3H, 12A–I

Cryphalus inops Eichhoff, 1872: 131 (Guadeloupe).
Hypothenemus griseus Blackburn, 1885: 194 (Hawaii).
Cryphalus mangiferae Stebbing, 1914: 542 (India).
Hypocryphalus mangiferae Eggers, 1928: 85 (Brazi).
Cryphalus subcylindricus Schedl, 1942: 16 (Indonesia).
Cryphalus mimicus Schedl, 1942: 17 (Indonesia).
Hypocryphalus opacus Schedl, 1942; 20 (Indonesia).
Taenioglyptes artestriatus Browne, 1970: 553 (Uganda), syn. nov.

Type material examined. India • 1 ♀ ***Lectotype*** *Cryphalus mangiferae* Stebbing, 1914; 1902; labelled "India// E.P.Stebing//902–309. //// mango twigs // *Cryphalus mangiferae* Stb. // C. Beeson det. //// Lectotype *Cryphalus mangiferae* // S.L.W. 1976 Stebb. ////NHMUK 010805929"; UFFE:26286; (NHMUK) • 1 ♂? ***Paralectotype***; 1902; UFFE:10426; (NHMUK).

Uganda • 1 ♀ ***Holotype*** *Taenioglyptes artestriatus* Browne, 1970; Central Region, Wakiso, Zika; 0.12°N, 32.52°E; 21 May 1961; K. W. Brown leg.; "***Holotype***//// Uganda//Zika//in light trap//K. W. Brown //29/5/61 //B.1715(g) ////1666 ////Taenioglyptes artestriatus// Browne ♀ //***Holotype***////Brit. Mus. 1973–70////NHMUK 010806013"; UFFE:26192; (NHMUK).

Other material examined. China • 1 ♀; Fujian, Quanzhou, Nan'an, Pushan village; 25.1189°N, 118.4276°E; May 2017; You Li leg.; ex. *Mangifera indica*; UFFE:33207; (UFFE) • 1 ♂; same collection data; UFFE:33208; (UFFE) • 9 ♀♀, 8 ♂♂; same collection data; UFFE:33206; (NHMUK, 1♀, 1♂; FSCA, 1♀; MZB, 1♀, 1♂; NIAES, 1♀, 1♂; NMNS, 1♀, 1♂; IOZ 1♀ IOZ(E)2057932, 1♂ IOZ(E)2057933; RIFID, 1♀, 1♂; USNM, 1♀, 1♂; ZIN, 1♀, 1♂) • 1 ♂; same collection data; dissected; UFFE:34966 (UFFE); • 1 ♀; Hunan, Changsha, Yuelushan; Aug. 2019; You Li leg.; ex. *Choerospondias axillaris*; DNA: 28S:MT431546; UFFE:33551 • 57; Hunan, Changsha, Yuelushan; 28.1897°N, 112.9329°E; Aug. 2019; You Li leg.; ex. *Choerospondias axillaris*; UFFE:33529; (UFFE) • 1; Yunnan, Mengla XTBG; C. Bateman leg.; DNA: 28S:MG051112, COI:MG051163; UFFE:14119; (UFFE) • 1; Yunnan, Menglun XTBG; 20 Jul. 2017; Craig Bateman leg.; DNA: COI:MG051162; UFFE:25419; (UFFE).

Guadeloupe • 1 ♂; Basse Terre, Point a Lezard; 20 May 2012; R. Turnbow leg.; UFFE:12501; (FSCA).

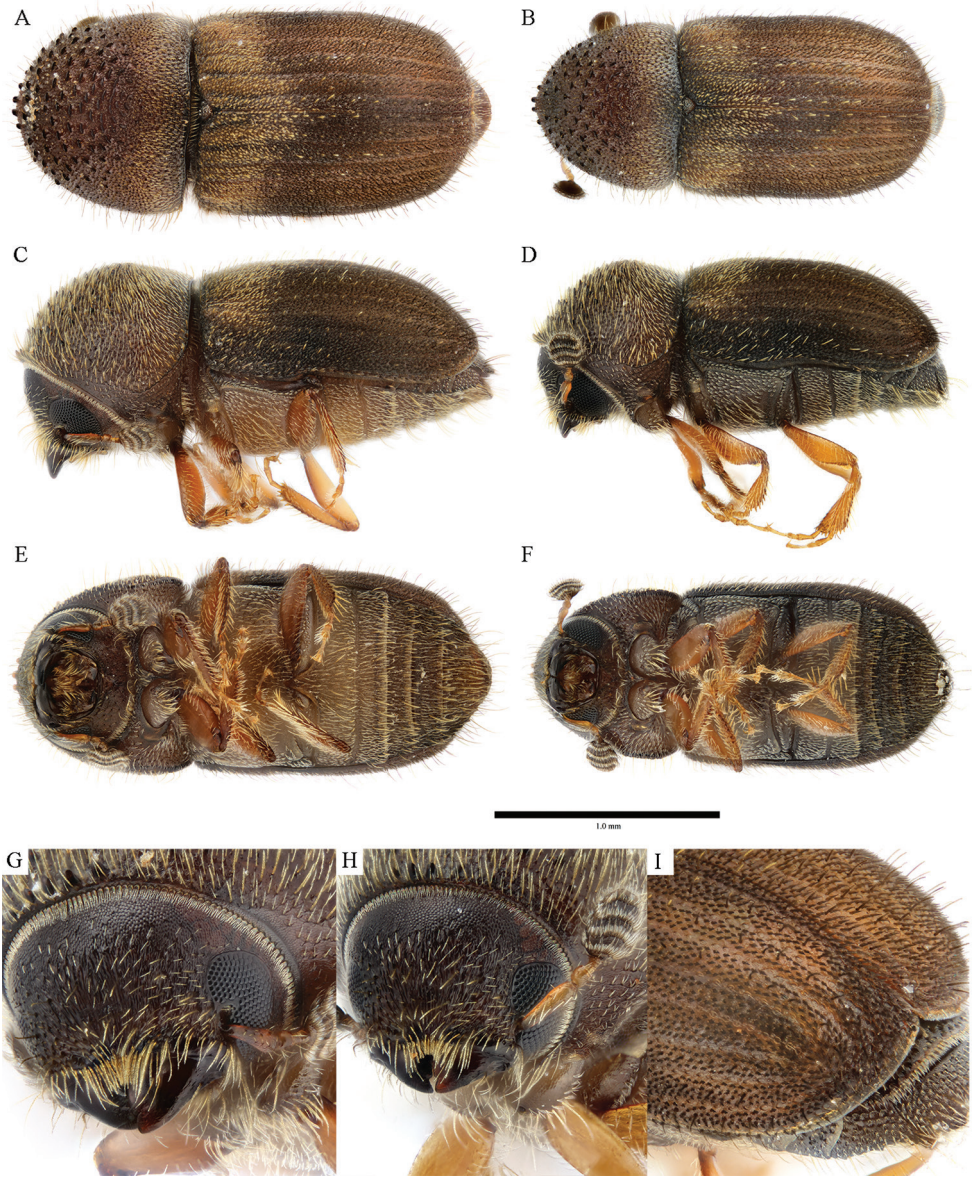

Figure 12. *Cryphalus mangiferae* **A, C, E, G** female, UFFE:33207 **B, D, F, H, I** male, UFFE:33208.

Taiwan • 1; Kaohsiung, Liouguei; Tom Harrington, and Caroline Wuerst leg.; Lindgren trap with ethanol; DNA: 28S:MG051118, COI:MG051172; UFFE:15127; (UFFE).

United States • 1; Florida, Homestead; 29 Jun. 2015; Thomas H. Atkinson leg.; ex. *Mangifera indica*; from vial 9444; DNA: 28S:MG051119, COI:MG051174; UFFE:25414; (UFFE) • 18; Florida, Miami Dade, Homestead, TREC; 23 Jul. 2018;

Andrew J. Johnson leg.; ex. *Mangifera indica*; UFFE:29770; (UFFE). • 1; Florida, Dade County, Homestead; 2012-Apr-05; L. Bradshaw leg.; ex. *Codiaeum variegatum*.

Vietnam • 1 ♀; TamDao; Michelle Jusino, and James Skelton leg.; quercivorol and ethanol trap; DNA: 28S:MG051120, COI:MG051175; UFFE:25936; (UFFE).

Diagnosis. This species is distinguished from other similar *Cryphalus* by the frons with a finely aciculate texture, the pronotal disc which is long, and has coarse hair-like setae, the elytral striae which are barely impressed but apparent by the rows without ground vestiture, and by the shape of ground vestiture which have tapered tips.

See notes for diagnosing the very similar *Cryphalus paramangiferae*, described below, under the diagnosis for that species.

Female. Length 1.6–2.2 mm. Proportions 2.2× as long as wide. Frons with weak converging aciculations. Antennal funiculus with four or five funicular segments. Antennal club with three evenly procurved sutures mostly marked by coarse setae. Pronotal profile widest in line with summit. Pronotal margin armed with four to six widely spaces serrations, the median pair larger. Pronotal declivity with more than 60 asperities. Pronotal disc approximately one third of the length, gently sloped. Pronotal vestiture entirely hair-like or dagger-like, very few setae bifurcating in baso-lateral area Suture between pronotum and elytra weakly sinuate. Scutellum shaped as a rounded triangle, almost semi-circular, with sparse, pale, hair-like setae. Elytra 1.6× as long as pronotum, usually a similar colour to pronotum, translucent yellow-brown, sometimes to dark brown, broadly rounded with no clear transition to the declivity. Striae weakly visible as rows of punctures and hair-like setae with almost no ground vestiture, barely impressed Interstrial bristles erect, curving posteriorly, of approximately even diameter. Interstrial ground vestiture near triangular, dagger-like, tapering to a fine point, longer on declivity, ground vestiture on basal third are usually a light brown/cold colour. Mesocoxae moderately separated, much more than metacoxae. Ventrites with mostly hair-like setae. Last abdominal ventrite with margin of rounded tubercles Proventriculus sutural teeth weakly sclerotised, rounded, irregular, one indistinct row each side of suture. Apical teeth in multiple rows, extending almost the width of a segment. Closing teeth long, barely branched. Masticatory brush short, less than length or apical plate.

Male. Similar to female except: Length 1.5–2.2 mm. Frons identical to females, with granulate texture on median of upper level. Pronotal profile slightly more triangular, widest nearer base. Pronotal vestiture hair like, with a few bifurcating setae on baso-lateral areas, slightly more than on female. Protibiae and protarsi with several larger, coarse curved setae on the proximal edge near the apex. Last abdominal ventrite weakly emarginated. Aedeagus long, penis body sclerotised, tapered to a point at apex, ejaculatory duct with small, evenly sized spinulae, end plates sclerotised. Penis apodemes 2.5× as long as penis body. Tegmen broad, with very small, almost obsolete paired apodemes.

Distribution. China (Fujian, Guangdong, Hunan, Yunnan); Taiwan; Thailand; Malaysia; Indonesia; India; Nepal; United States (Florida, Hawaii); Mexico; Puerto Rico; Cuba; Kenya, Uganda; American Samoa, Australia.

Suggested vernacular name. Chinese: 芒果梢小蠹 [Mango twig bark beetle]; English: Mango bark beetle.

Recorded plant hosts. Anacardiaceae: *Mangifera indica* L., *M. odorata* Griff. (Kalshoven 1958), *Choerospondias axillaris* (Roxb.) B.L.Burtt & A.W.Hill; Euphorbiaceae: *Codiaeum variegatum* (L.) A.Juss. [not confirmed reproductive host].

Remarks. *Choerospondias axillaris* is a specialty crop in southern China, and it represents a new reproductive host record. A record from *Codiaeum variegatum* from Florida suggests that the beetle might have a broader host range than previously thought, though *Codiaeum* was not confirmed as a reproductive host. Kalshoven (1958) also lists *Zizyphus oenopila* (L.) Mill. (Rhamnaceae), *Theobroma cacao* L. (Malvaceae), *Lansium parasiticum* (Osbeck) Sahni & Bennet (Meliaceae) and *Canarium commune* L. (Burseraceae), but the associated vouchers were not examined for accuracy. Beaver (1990) also recorded this species from an unknown angiosperm not *Mangifera*. No host outside of Anacardiaceae has been verified as a reproductive host.

The holotype of *Taenioglyptes artestriatus* Browne, 1970, while not from the regions of study, is recognised as a synonym. All diagnostic characters are visible. The antennae have four funicular segments, which previously distinguished the genera *Cryphalus* and *Hypocryphalus*, probably leading to this error. Several examples from China had similar antennae, and one specimen from Guadeloupe.

Cryphalus meridionalis (Nobuchi, 1975)

Figures 2I, 3I, 13A–I

Taenioglyptes meridionalis Nobuchi, 1975: 55 (Japan).

Type material examined. Japan • 1 ***Holotype***; Okinawa, Kunigami District, Yona Experimental Forest; 26.763°N, 128.216°E; 08 Jul. 1965; K. Takahashi leg.; UFFE:28614; (NIAES).

Other material examined. China • 1 ♂ ; Fujian, Quanzhou, Yongchun, Diyiyan; 25.3176°N, 118.279°E; 02 May 2017; You Li leg.; ex. *Schefflera heptaphylla*; DNA: 28S:MT122095; UFFE:28061; (UFFE) • 1 ♀ ; same collection data; UFFE:28062; (UFFE) • 1 ♂ ; same collection data; UFFE: 34962; (UFFE) • 1 ♀, 1 ♂; same collection data; (IOZ, 1 ♀ IOZ(E)2057942, 1 ♂ IOZ(E)2057943) • 1; Fujian, Zhangzhou, Yunxiao, Jiangjunshan Mt.; 23.952°N, 117.312°E; 25 Jul. 2019; Ling Zhang leg.; light trap with EtOH; 20190725–28001; UFFE:34069; (UFFE).

Japan • 1; Okinawa, Kunigami District, Yona Experimental Forest; 26.763°N, 128.216°E; Nov. 2010; Jiri Hulcr leg.; ex. Moraceae ?; teneral adult; DNA: 28S:MT431537; UFFE:07484; (UFFE)• 1 ♀; same collection data; UFFE:33236; (UFFE).

Diagnosis. *Cryphalus meridionalis* can be distinguished from other East Asian *Cryphalus* by the barely aciculate frons with a fine sharp median keel, a short transverse sulcus of the male frons, by the pronotal slope with wide, barely protruding asperities, by the short pronotal disc, by the ground vestiture of which the setae are widened near the base and hair-like at the tips, and by the proventriculus without any sutural teeth.

Female. Length 1.70–2.10 mm (holotype 1.80 mm). Proportions 2.1× as long as wide. Frons aciculate, converging to the epistoma, with a weak median keel. Antennal

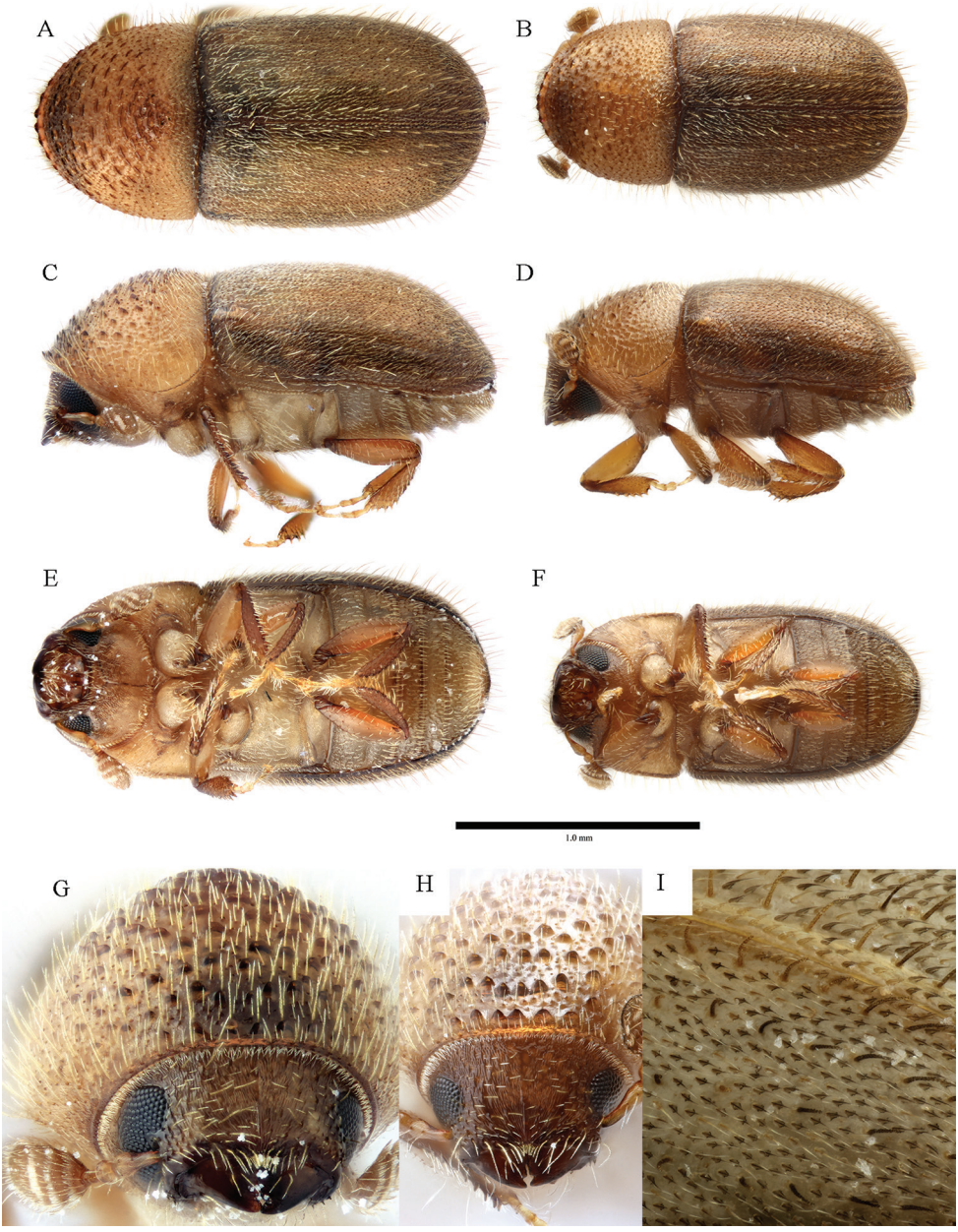

Figure 13. *Cryphalus meridionalis* **A, C, E, G** female, UFFE:28062 **B, D, F, H** male, UFFE:34962 **I** female, UFFE:33236.

club with three weakly procurved sutures marked by coarse and long setae, the distance between the suture 3 and the apex approximately twice the distance between sutures 2 and 3. Antennal funiculus with four segments, length shorter than the scape. Gular surface with evenly spaced hair-like setae. Pronotal colour orange-brown to brown,

slightly lighter than elytra. Pronotal profile broadly rounded, slightly triangular, widest in line with summit, approx. 0.75× as long as wide. Pronotal margin armed with six to eight serrations, the median pair or a similar size to the others. Pronotal declivity with more than 70 asperities (holotype has approx. 77), each wide and barely protruding. Pronotal disc approximately one quarter the length of the pronotum, sloped, weakly tuberculate surface texture. Pronotal vestiture entirely hair-like setae. Suture between pronotum and elytra weakly sinuate. Scutellum very small, barely visible. Elytra 1.8 × as long as pronotum, orange brown to brown, broadly rounded with no clear elytral disc or a transition to the declivity. Striae not apparent. Interstrial bristles erect, hair-like, with rounded tips, slightly shorter on disc. Interstrial ground vestiture wide at base, with tapered, hair-like tips. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, more than distance between metacoxae. Proventriculus not examined.

Male. Similar to female except: Length 1.65–2.00 mm Proportions 2.0× as long as wide Frons with converging aciculations and a fine median keel up to a short carina and shining sulcus above the level of the eyes. Gular surface simple with few hair-like setae. Pronotal profile rounded triangular, protruding slightly more than of the female. Pronotal declivity more flat than in female, with smaller asperities. Protibiae and protarsi with only hair-like setae, almost the same as female. Last abdominal ventrite emarginated. Proventriculus sutural teeth completely absent. Proventriculus apical teeth coarse, the median teeth are wider and rounded Proventriculus closing teeth mostly shorter than length of masticatory brush, barely branched, and rounded tips Proventriculus masticatory brush about half of total length Aedeagus short. Penis apodemes longer than penis body, fused at tip. Tegmen with paired apodemes shorter than distance between. End plate visible as two sclerotised plates.

Distribution. China (Fujian), Indonesia (Java), Japan (Ryukyu Islands)

Remarks. The original spelling of the name, as "*merdionalis*", was used throughout the original description. This was amended to "*meridionalis*" in subsequent publications, the correct spelling to refer to the species distribution in the southern region of Japan. No explicit correction was made following correct procedures (ICZN art. 33.2.1), but in all subsequent publications, with exception to a website listing types, the amended spelling is used clearly referencing the original publication, and this is considered sufficient prevailing usage under 33.2.3.1 to consider the change a justified emendation.

Recorded plant hosts. Araliaceae: *Schefflera* spp., "Moraceae"[?]

Cryphalus morivorus Johnson, sp. nov.

http://zoobank.org/2CA70B2D-F6BA-441C-B6D0-5CF60A0553EB
Figures 2J, 3J, 14A–I

Type material examined. China • 1 ♀ ***Holotype***; Hebei, Chengde, Shuangqiao; 41.021°N, 117.943°E; 08 Jul. 2017; You Li leg.; ex. *Morus*; IOZ(E)225669; UFFE:31722; (IOZ) • 1 ♂ ***Paratype***; same collection data; UFFE:31723; (UFFE) • 14 ♀♀, 13 ♂♂ ***Paratypes***; same collection data; UFFE:34757; (NHMUK, 1♀,

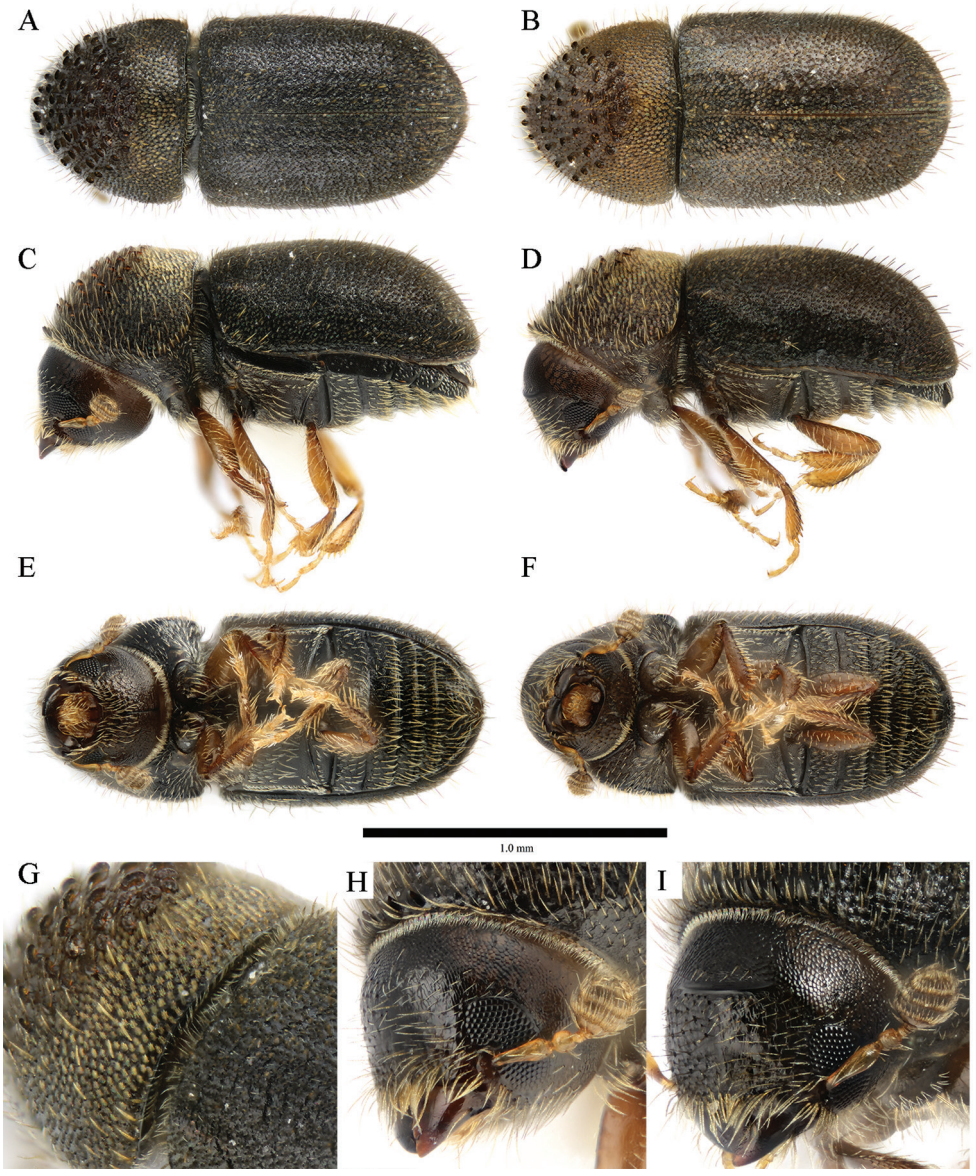

Figure 14. *Cryphalus morivorus* **A, C, E, G, H** holotype, female, UFFE:31722 **B, D, F, I** paratype, male, UFFE:31723.

1♂; FSCA, 1♀, 1♂; MZB, 1♀, 1♂; NIAES, 1♀, 1♂; NMNS, 1♀, 1♂; IOZ, 1♂ (IOZ(E)225670); RIFID, 1♀, 1♂; UFFE, 6♀♀, 3♂♂; USNM, 1♀, 1♂; ZIN, 1♀, 1♂) • 1 ♂ ***Paratype***; Hebei, Chengde, Shuangqiao; 41.021°N, 117.943°E; Aug. 2017; You Li leg.; ex. *Morus*; field notes: mulberry fresh cut twig with green bark; specimen dissected; DNA: 28S:MT431541, COI:MT431646; UFFE:27606; (UFFE).

Other material examined. China • 1 ♀; Hebei, "Peking" [Beijing], "Pataling" [Badaling]; 11 Jul. 1972; Fusheng Huang leg.; ex. *Morus alba*; UFFE:12190;

(USNM) • 1; Hebei, Chengde, Shuangqiao; 41.021°N, 117.943°E; Aug. 2017; You Li leg.; ex. *Morus*; dissected; DNA: 28S:MT122093; UFFE:27605; (UFFE) • 1 ♂; same collection data; UFFE:27604; (UFFE) • 1 ♀; 河北蓟县 [Hebei, Jixian]; 21 Jun. 1993; 于丽辰采集 [Lichen Yu leg.]; 桑树[*Morus*]; UFFE:33460; (IOZ) • 1 ♂; 河北蓟县 [Hebei, Jixian]; 21 Jun. 1993; 于丽辰采集 [Lichen Yu leg.]; 桑树 [*Morus*]; IOZ(E)701629; UFFE:33461; • 2; Shandong, Taian, Shandong Agricultural University campus; 36.168°N, 117.1542°E; 16 Oct. 2018; You Li leg.; ex. *Morus alba*; from twig, old cut; UFFE:31712; (UFFE) • 3; Shanghai, Shanghai Academy of Landscape Architecture Science and Planning; 31.1527°N, 121.4493°E; Dec. 2017; Lei Gao leg.; ex. *Morus*; UFFE:29907; (UFFE).

South Korea • 1 ♂; "경남 함양 마천 백무동" [Gyeongsangnam-do, Hamyang-gun, Macheon-myeon, Baekmudong valley]; 07 Nov. 1982; "추 호 렬" [Choo Ho Yul leg.]; "기주: 뽕나무" [ex. *Morus alba*]; UFFE:34726; (UFFE) • 1 ♂; same collection data; UFFE:34727; (UFFE) • 6 ♂♂; same collection data; UFFE:34782; (RIFID).

Taiwan • 1 ♂; Taichung, Taichung, Caohu; 24.079°N, 120.6903°E; 02 May 2018; Ching-Shan Lin leg.; ex. *Morus australis*; UFFE:33336; (UFFE) • 1 ♂; same collection data; DNA: 28S:MT431542; UFFE:33337; (UFFE).

Type locality. CHINA, Hebei, Chengde, Shuangqiao, 41.0210°N, 117.9430°E.

Diagnosis. This species can be distinguished from other *Cryphalus* in East Asia by the size (1.35–1.70 mm), by the proportions (2.25× as long as wide), by the male frons with a transverse carina, by the many scale-like setae on the pronotal disc extending to the lateral margins, and by the barely apparent elytral striae.

Cryphalus morivorus is very similar to *C. exiguus* and can be distinguished by the pronotal disc (*C. morivorus*: covered by scale-like setae, versus *C. exiguus* with entirely coarse hair-like setae), by the elytral striae (*C. morivorus*: barely apparent due to intermixed ground vestiture, versus *C. exiguus*: clearly visible as rows of punctures and fine hair like setae devoid of ground vestiture).

Female. Length 1.35–1.70 mm (holotype 1.40 mm). Proportions 2.25× as long as wide. Frons simple, convex, flat and shining up to the level of the eyes. Antennal club with three straight or barely procurved sutures marked by a mixture of coarse short setae and longer setae with length approximately the same as distance between sutures. Antennal funiculus with four funicular segments. Pronotal profile widest in line with the centre of the disc. Pronotal margin armed with six serrations, irregularly spaced and sometimes partially fused. Pronotal declivity broadly rounded, with approximately 50 asperities. Pronotal disc occupies approx. one third of the length of the pronotum, gently sloped, uniformly weakly asperate. Pronotal vestiture mostly of golden scale-like setae which are approximately as long as wide, with sparse longer dagger-like setae. Suture between pronotum and elytra almost straight. Scutellum very small, triangular. Elytra 1.8× as long as pronotum, dark brown, broadly rounded with no clear transition to the declivity. Elytral texture rugose, with punctures from the ground vestiture approximately half of the diameter of the strial punctures, and interspaces irregularly rugose. Striae barely apparent, visible as rows of slightly larger punctures and fine, white, hair-like setae. Interstrial bristles erect, weakly flattened with pointed tips, those near the elytral suture are shorter and broader than those in lateral regions. Interstrial

ground vestiture tridentate, 1–2× as long as wide, brown, with a weak iridescence, and distributed across the elytra including between strial punctures. Mesocoxae moderately separated, more than distance between metacoxae. Proventriculus not examined.

Male. Similar to female except: Length 1.30–1.60 mm. Frons with a smooth transverse carina above the level of the eyes. Pronotal declivity straight, asperities slightly more sparse. Elytra less rugose than female. Protibiae and protarsi with coarse, long setae on the proximal face, near the apex. Last abdominal ventrite clearly emarginated. Proventriculus sutural teeth of irregular size, confused, in two or more rows. Aedeagus very long, weakly sclerotised. Penis apodemes almost as long as penis body. Tegmen with paired penis apodemes approximately as long as they are spaced apart.

Recorded plant hosts. Moraceae: *Morus alba* L., *M. australis* Poir.

Distribution. China (Hebei, Shandong, Shanghai); Taiwan; South Korea.

Etymology. The name is an adjective derived from a combination of the stem of the Latin noun *mōrus* for mulberry, a linking vowel *-i-* and an adjectival suffix *vorus*, meaning eater.

Suggested vernacular name. Chinese: 南桑梢小蠹; English: Southern mulberry bark beetle; Korean: 남방뽕나무애나무좀.

Remarks. This species has widely been misidentified and misspelled as "*Cryphalus exignus*", with the first instance of the spelling Niisima (1909) referring to Japanese specimens likely of *C. exiguus*. Since the authority is clearly indicated in the earliest instance, we consider this a misspelling of *exiguus*, rather than a *nomen nudum*.

Under the name *C.* "*exignus*", which probably referred to this species, Yin and Li (1984) present photos of the proventriculus and aedeagus. The proventriculus differs slightly in the width of the patch of apical teeth. No voucher information for these specimens exist, and it is unclear if this variation is from within this new species or included specimens of *C. exiguus*.

Wood and Bright (1992) list "*Broussonetia kazinoki*, *Celtis sinensis*, *Diospyros kaki*, *Evodia rutaecarpa*, *Ficus* spp., *Morus alba*, *Morus bombysis* [= *australis*] and *Salix* sp." under *C. exiguus* which may refer to this species. It is also unclear whether these hosts are suitable reproductive hosts.

This species has been widely described as a pest of *Morus*. Named as *exignus*, this species was described to feed on developing buds of mulberry in early spring, sometimes reaching significant numbers to affect yields (Yin et al. 1984).

Using the key by Tsai and Li (1963), specimens of this species would key to *C. manschuricus*, but would differ in the scales on the pronotum, the size, proventriculus and aedeagus.

Cryphalus paramangiferae **Johnson, sp. nov.**

http://zoobank.org/C5D52474-B833-4564-AE40-FD56BEBA6155
Figures 2K, 3K, 15A–I

Type material examined. China • 1 ♀ ***Holotype***; Fujian, Quanzhou, Yongchun, Diyiyan; 25.3169°N, 118.2751°E; 26 Nov. 2015; You Li leg.; ex. *Mangifera indica*;

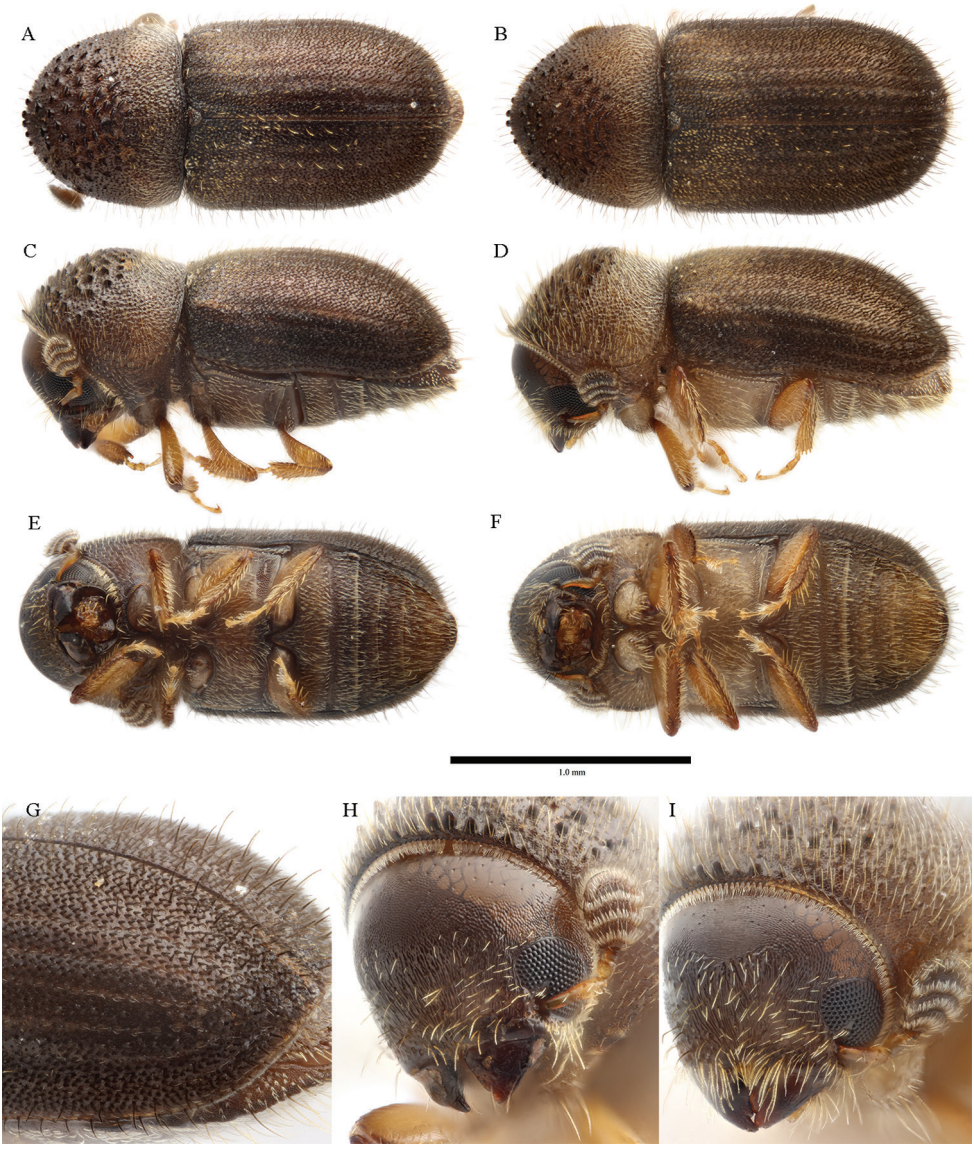

Figure 15. *Cryphalus paramangiferae* **A, C, E, G, H** holotype, female, UFFE:34967 **B, D, F, I** paratype, male, UFFE:34969.

IOZ(E)225765; ex v12347; UFFE:34967; (IOZ) • 1 ♂ ***Paratype***; same collection data; IOZ(E)225766; UFFE:34969; (IOZ) • 1 ♀, 1 ♂ ***Paratypes***; same collection data; pair in gallery with larvae; 1 larva used for DNA extraction without voucher; DNA: 28S:MG051113; UFFE:22108; (UFFE) • 5 ♀♀, 5 ♂♂ ***Paratypes***; same collection data; ex v12352; UFFE:34970; (NHMUK, 1♀, 1♂; FSCA, 1♀, 1♂; MZB, 1♀, 1♂; NIAES, 1♀, 1♂; NMNS, 1♀, 1♂) • 6 ♀♀, 6 ♂♂ ***Paratypes***; Fujian, Quanzhou, Nan'an, Pushan village; 25.1189°N, 118.4276°E; 01 May 2017; You Li leg.; ex.

Mangifera indica; UFFE:34974; (RIFID, 1♀, 1♂; UFFE, 3♀♀, 3♂♂; USNM, 1♀, 1♂; ZIN, 1♀, 1♂).

Other material examined. China • 1 ♂; Fujian, Quanzhou, Yongchun, Diyiyan; 25.3172°N, 118.2798°E; 17 Nov. 2015; You Li leg.; ex. *Mangifera indica*; UFFE:22062; (UFFE) • 1 ♂; Fujian, Quanzhou, Yongchun, Diyiyan; 25.3169°N, 118.2751°E; 26 Nov. 2015; You Li leg.; ex. *Mangifera indica*; ex v12348; dissected; UFFE:34909 (UFFE) • 1 ♂; same collection data; destructively extracted; DNA: 28S:MG051111; UFFE:25415; (UFFE) • 1 ♂; same collection data; UFFE:34968; (UFFE) • 3; Fujian, Quanzhou, Nan'an, Pushan village; 25.1189°N, 118.4276°E; 28 Apr. 2017; You Li leg.; ex. *Mangifera indica*; UFFE:26956 (UFFE) • 1 ♂ ; same collection data; dissected; mango 5; UFFE:34965 • 2 ♀♀; Guangdong, Shenzhen, Yantian; 22.5889°N, 114.2842°E; 08 Apr. 2017; Wei Lin leg.; EtOH trap; UFFE:33204; (UFFE).

Type locality. China, Fujian, Quanzhou, Yongchun, Diyiyan; (25.317°N, 118.275°E).

Diagnosis. This species is distinguished from other similar *Cryphalus* by the frons with an aciculate texture, the frons of the male which also has a shining patch in the median made from a fine file-like structure, the pronotal disc which is long, and has fine hair-like setae with some bifurcating setae on baso-lateral areas, the elytral striae which are barely impressed, and the striae 1, 2 and 3 which on the declivity, is barely apparent.

This species is externally very similar to *C. mangiferae*. They can be distinguished by the frons of the male (*C. paramangiferae*: with fine shining file like structure versus *C. mangiferae*: identical to female), by a subtle difference in the vestiture of the pronotum (*C. paramangiferae*: very fine and hair like versus *C. mangiferae*: coarse hair like), by a subtle difference in striae 1, 2, and 3 on the declivity (*C. paramangiferae*: slightly impressed and barely apparent with some ground vestiture between punctures versus *C. mangiferae*: apparent without ground vestiture between punctures), by the setae near the dorso-anterior corner of the metaventrite (*C. paramangiferae*: fewer than 10 multifurcate setae not distinct from hair-like setae versus *C. mangiferae*: 20 or more multifurcate setae, all distinctly smaller than other setae), and by the spinulae on the ejaculatory duct inside the aedeagus (*C. paramangiferae*: large spinulae versus *C. mangiferae*: many small spinulae).

Female. Length 1.4–1.9 mm (Holotype 1.8 mm). Proportions 2.2× as long as wide. Frons with converging aciculations and weakly emarginated at epistoma. Antennal funiculus usually with 4 segments. Antennal club with three evenly procurved sutures, slightly wider at apex. Pronotal profile widest in line with summit, approx. 0.85× as long as wide. Pronotal margin armed with four to six serrations, the median pair larger and near contiguous. Pronotal declivity with more than 60 asperities (holotype has 68). Pronotal disc approximately one third of the length, gently sloped. Pronotal vestiture entirely fine hair-like, some bifurcating setae bifurcating in baso-lateral area. Suture between pronotum and elytra weakly sinuate. Scutellum shaped as a rounded triangle, almost semi-circular, with sparse, pale, hair-like setae. Elytra 1.6× as long as pronotum, usually slightly darker colour than pronotum, medium-brown, broadly rounded with no clear transition to the declivity. Striae weakly

visible as rows of punctures and hair like setae, slightly impressed. Interstrial bristles erect, curving posteriorly, slightly wider at base. Interstrial ground vestiture near triangular, dagger-like, tapering to a fine point, longer on declivity, ground vestiture on basal third are usually a light brown/cold colour. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, much more than metacoxae. Ventrites with mostly hair-like setae. Last abdominal ventrite with margin of rounded tubercles. Proventriculus sutural teeth very weakly sclerotised, barely visible, rounded, in multiple irregular rows each side of suture. Apical teeth in multiple rows, extending almost the width of a segment. Closing teeth long, barely branched, extending beyond masticatory brush with fine, pointed branched tips. Masticatory brush slightly shorter than apical plate.

Male. Similar to female except: Length 1.4–1.9 mm. Frons with similar aciculations to females, but upper levels with glabrous patch with extremely fine transverse aciculations. Pronotal profile slightly more triangular, widest nearer base. Pronotal vestiture with fine hair-like setae and some bifurcating setae on baso-lateral areas. Protibiae and protarsi with coarse, curved setae on proximal edge of protibia and on the first few tarsi. Last abdominal ventrite very weakly emarginated. Aedeagus shorter, penis body sclerotised, only slightly tapered to a broadly rounded point at apex, ejaculatory duct with very large, rose-thorn-shaped spinulae, especially along portion inside penis body, end plates large and strongly sclerotised. Penis apodemes 1.5× as long as penis body. Tegmen broad, with very short apodemes.

Distribution. China (Fujian, Guangdong)

Etymology. The name is derived from a combination of the Ancient Greek *παρά*, meaning near, next to or besides, and *mangiferae*, the specific epithet of the species *Cryphalus mangiferae*, a species which is phylogenetically and morphologically very similar. It is invariable.

Suggested vernacular name. Chinese: 伪芒果梢小蠹 ; English: False mango bark beetle.

Recorded plant hosts. Anacardiaceae: *Mangifera indica* L.

Remarks. Johnson et al. (2017) noted a high genetic diversity of *Cryphalus mangiferae* in Asia, suggesting that there are likely cryptic and near-cryptic species present. Two of the specimens used in the molecular study (specimen 54 and 71) are of this newly described species, from the type locality.

The morphological differences are sexual characters, likely to be a reproductive barrier between the two species. In a location near the type locality (Nan'an), a sample reared from one log was of a mixture of *C. mangiferae* and *C. paramangiferae*, though no obvious differences in the biology were noted. All individuals collected in pairs corroborate these differences.

None of the examined types of synonyms of *C. mangiferae* have the fine hair-like setae on the pronotum or less distinct striae.

Based on the key for Chinese *Cryphalus* by Tsai and Li (1963), specimens of this species would fail on couplet 2/3, because the male frons does not have a prominent carina and the spinulae on the ejaculatory duct are distinctly spine-like rather than setiform.

***Cryphalus scopiger* Berger, 1917**
Fig 16 A–D

Cryphalus scopiger Berger, 1917: 228 (Russia).

Type material examined. Russia • 1 ♂ ***Lectotype***; "Ю.-Уссур. Край, окр. г. Владивост., ст. Седанка" [Southern Ussuriyskiy Kray, now Primorskiy Kray, near Vladivostok city, Sedanka station]; 1915; "В. Бергеръ" [V. M. Berger leg.]; ex. *Juglans mandshurica*; UFFE:34758 (ZIN).

Other material examined. China • 1 ♀; 辽宁清源N5 [Liaoning, Qingyuan N5]; 01 May 1987; 宋友文 [Youwen Song leg.]; 核桃楸 [*Juglans mandshurica*]; IOZ(E)700992; UFFE:33471; (IOZ) • 1 ♂; 辽宁清源N5 [Liaoning, Qingyuan N5]; 01 May 1987; 宋友文 [Youwen Song leg.]; 核桃楸 [*Juglans mandshurica*]; IOZ(E)700978; UFFE:33472; (IOZ).

Russia • 2 ♀♀; "228. Майх. Оп. Л." [Primorskiy Kray, Shkotovskiy District, Maikhe educational and experimental forest]; "усох ветки *Jugl. mand*." [dry limbs of *Juglans mandshurica*]; label in A.V. Mishin's handwriting; UFFE:34763; (ZIN) • 1 ♀ "дол. р. Майхэ, Шкотовск. р. ДВК [Far Eastern Kray, now Primorskiy Kray, Shkotovskiy District, Maikhe River valley]; "V. 931" [May 1931]; "Куренцов" [A.I. Kurentsov leg,]; UFFE:34760; (ZIN) • 1 ♂; "248". [Primorskiy Kray, Shkotovskiy District, Maikhe educational and experimental forest]; "21.VIII.31" [21 Aug 1931]; "A.M." [A.V.Mishin leg.]; ex. *Juglans mandshurica*; Specific locality data not provided, interpreted from specimens with otherwise similar collection data; UFFE:34764; (ZIN) • 1 ♀; same collection data except "28.VIII.31" [28 Aug 1931]; UFFE:34765; (ZIN) • 1 ♀; "р. Майхэ, лесн. Шкотовск. р. ДВК" [Far Eastern Kray, now Primorskiy Kray, Shkotovskiy District, Maikhe educational and experimental forest, Maikhe river]; "28./VIII 931" [28 Aug 1931]; "Шабліовский и Любарский" [V. V. Shabliovskiy, and L. V. Lyubarskiy leg.]; UFFE:34762; (ZIN)• 1 ♀; Primorskiy Kray, Shkotovskiy District, Forest district No. 11; 11 Sep. 1931; A. V. Mishin leg.; ex. *Juglans mandshurica*; no.169; UFFE:31840; (FSCA) • 1 ♀; same collection data; UFFE:31841; (FSCA) • 3 ♀♀, 4 ♂♂; "дол. р. Майхэ, Шкотовск. р. ДВК" [Far Eastern Kray, now Primorskiy Kray, Shkotovskiy District, Maikhe river valley]; "V.932" [May 1932]; "Любарский" [L.V. Lyubarskiy leg.]; "из коллекции В.Н. Старка" [from V.N. Stark collection]; UFFE:34761; (ZIN) • 1 ♀, 1 ♂; " дол. р. Малазы, Сучан. р., Усс. кр." [Ussuriyskiy Kray, now Primorskiy Kray, Suchan District, Malasa River valley]; "V.931" [May 1931]; "Куренцов" [A. I. Kurentsov leg.]; "32а// из коллекции В.Н. Старка" [from V.N. Stark collection]; UFFE:34759; (ZIN).

Diagnosis. This species can be distinguished from others in East Asia by the size (usually 1.70–2.0 mm), by the weakly aciculate frons without apparent sexual dimorphism, by the antennae with nearly straight sutures, by the pronotal disc occupying one third of the pronotal length, with entirely hair like setae, and by the interstrial ground vestiture of the female at the apex of the declivity, which is dense and elongate forming a brush.

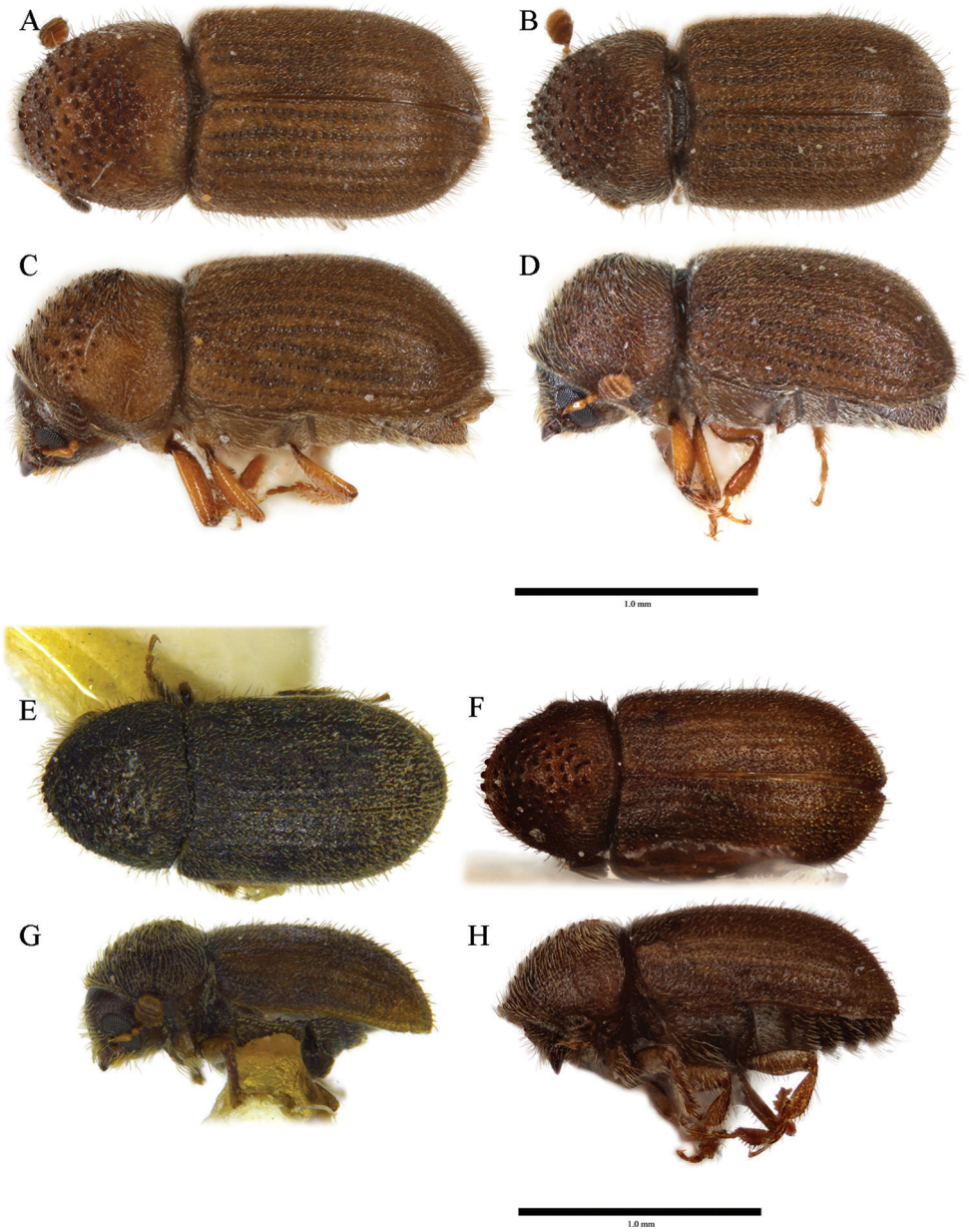

Figure 16. A–D *Cryphalus scopiger* **A, C** female, UFFE:33471 **B, D** male, UFFE:33472 **E–H** *Cryphalus viburni* **E, G** paralectotypes (2 different specimens, sex unknown), UFFE:34767 **F, H** male, UFFE:31842 [**H** is flipped horizontally].

Female. Length 1.70–2.05 mm. Proportions 2.23× as long as wide. Frons simple, convex, in the lower portion with aciculations converging at epistoma, and with indistinct longitudinal median keel, in the upper portion with sparsely set punctures, faintly

shining, covered by golden hairs, longer above epistoma. Antennal club with three nearly straight sutures marked by setae (fourth suture only indistinctly feebly marked) at outer surface and three strongly procurved sutures at inner surface. Antennal funiculus with four funicular segments, the pedicel is shorter than the other segments combined. Pronotal colour brown, similar to head and elytra (may be most beetles are incompletely coloured since the original description (Berger 1917) gives mature beetles colour as black). Pronotal profile 0.82 as long as wide, slightly triangular, widest in posterior third, distinctly narrowing towards the base, rather strongly narrowing anteriorly, posterior angles strongly rounded. Pronotal margin rounded, armed with six serrations at the anterior margin, central denticles larger than lateral. Pronotal declivity with approximately 50 asperities. Pronotal disc approximately one third of the pronotal length, sloping rather strongly from the summit. Pronotal vestiture long, golden and hair-like on disc, with dense, long hairs on the postero-lateral margin. Suture between pronotum and elytra weakly sinuate, base of the pronotum is marked with fine carina. Scutellum V-shaped. Elytra 1.47× as long as wide, 1.9× as long as pronotum, 1.05× wider than pronotum, parallel-sided for more than 3/4 of length, then broadly rounded toward apex. Striae impressed, interstriae convex, minutely punctured; punctures in striae round, with flat bottom, densely set, but not fusing with neighbouring punctures. Interstrial bristles erect, hair-like with pointed apices. Interstrial ground vestiture scale-like, formed by elongated pointed scales 2.0× as long as wide arranged in four rows at each interstria. Apex of elytra obtuse, slightly projecting, with dense brush of short hair-like brown setae. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae moderately separated, a little more than distance between metacoxae. Proventriculus (according to Berger 1917, sex of specimen dissected not indicated) typical of the genus. Proventriculus sutural teeth in a narrow band, rather numerous, blunt and rounded tubercles arranged in two irregular rows along each side of the suture. Apical teeth arranged in four rows, central teeth long, faintly curved, lateral teeth much smaller in size and disappear beyond the centre of each half of apical plate, not extending up to its lateral margins. Closing teeth usually in number of ten deeply palmate, longer than masticatory brushes. Masticatory brush as long as apical plate.

Male. Similar to female except: Length 1.70–1.95 mm (lectotype 1.75 mm). Elytra slightly stouter than in the female, about 1.39–1.43 as long as wide. Apex of elytra with hair-like setae similar to rest of declivity. Aedeagus (according to Berger 1917) long, penis body with developed end plates. Penis apodemes 1.5× as long as penis body. Tegmen with short paired tegminal apodemes.

Recorded plant hosts. Juglandaceae: *Juglans mandshurica* Maxim.; Oleaceae: *Fraxinus mandshurica* Rupr. (Krivolutskaya 1996)

Distribution. Russia (Primorskiy Kray); North Korea (Ju 1964); China (Liaoning).

Suggested vernacular name. Russian: Ореховый крифал; Chinese: 胡桃楸梢小蠹

Remarks. *Cryphalus scopiger* is a very common species that builds galleries in thin bark of stems and drying limbs of Manchurian walnut in river valleys. Wu et al. 1991 reported *Cryphalus viburni* as a pest of *Juglans mandshurica* in northern China, with detailed descriptions of the phenology and excavation of non-reproductive galleries.

We found specimens deposited in IOZ with the corresponding collection information (i.e., implicit vouchers) labelled as *C. viburni*, which were determined to be *C. scopiger*. This is corroborated with the host plant information. The descriptions provided were very similar in wording and content to those by Yin et al. (1984), and did not match the specimens vouchered, presumably not based on directly examined material. Song et al. (1998) also reports the same species on *Prunus*, though specimens were not verified.

The size range has been described as 1.50–1.90 (original description, Berger 1917) and 1.50–2.00 (Krivolutskaya 1996). The smallest examined was 1.7 mm, based on the material examined listed above and 200 additional specimens deposited at ZIN.

Cryphalus viburni **Stark, 1936**

Figure 16E–H

Cryphalus viburni Stark, 1936: 151 (Russia) Eggers, 1942: 30 (Russia).

Type material examined. Russia • 1 ***Lectotype***; "Шкотово" [Primorskiy Kray, Shkotovo]; "VI.1929" [Jun.1929], "Шабловск." [V.V. Shabliovskiy leg.]; "*Cryphalus viburni* Stark. Typ. V. Stark det. 1932 //Lectotypus *Cryphalus viburni* Stark Michalski J. 1965 [des.]"; UFFE:34766 (ZIN) • 7 ***Paralectotypes***; same collection information except labelled with "*Cryphalus viburni* Stark. Typ. V.Stark det. 1932// Paralectotypus"; UFFE:34767 (ZIN).

Other material examined. Russia • 1 ♀; Primorskiy Kray, Laso Nature Reserve, Sukhoy River post; 15 Aug. 1990; M. Yu. Mandelshtam leg.; under bark of *Viburnum sargentii*; UFFE:31842 (FSCA) • 9 ♀♀, 3 ♂♂; same collection data; UFFE:34768 (ZIN).

Diagnosis. *Cryphalus viburni* can be identified by the size (1.40–1.70 mm), by the aciculate frons, by the antennal club with slightly recurved, almost straight sutures, by the short pronotal disc (1/4 of the length), by the entirely hair-like setae on the pronotal disc, by the hair-like interstrial bristles of approximately equal length over the elytra.

Cryphalus viburni can be distinguished from similar *Cryphalus scopiger* by the smaller size (*C. viburni*: 1.40–1.70 mm, versus *C. scopiger*: 1.70–1.95 mm), by the profile of the pronotum (*C. viburni*: more semi-circular, widest at base, versus *C. scopiger*: widest in line with summit), by the elytra sculpturing (*C. viburni*: striae barely impressed, versus *C. scopiger*: striae impressed), and by the interstrial ground vesiture at elytral apex of the females (*C. viburni*: similar to rest of elytra, versus *C. scopiger*: dense and elongated making a brush).

Cryphalus mangiferae and *C. paramangiferae* are somewhat similar but can be easily distinguished by the antennal sutures (*C. viburni*: almost straight, versus *C. mangiferae* and *C. paramangiferae*: procurved), and by the profile of the pronotum and the size of the pronotal disc (*C. viburni*: disc one quarter of pronotal length, pronotum widest at base, versus *C. mangiferae* and *C. paramangiferae*: disc one third of pronotal length, widest near in line of summit).

Female. Length 1.50–1.70 mm. Proportions variable, 2.06–2.36 as long as wide. Frons simple, convex, in the lower portion with aciculations converging at epistoma, and fine median keel, sometimes obscure, in the upper portion with sparsely set punctures, faintly shining. Antennal club with three slightly recurved, nearly straight sutures marked by setae (fourth suture only indistinctly feebly marked) at outer surface and three strongly procurved sutures at inner surface. Antennal funiculus with four funicular segments, total length short, less than half of length of club. Pronotal colour black, similar to head and elytra. Pronotal profile transverse (and not longitudinal or of equal length and width as stated by Krivolutskaya, 1996), 0.75–0.86 as long as wide, semicircular, widest at base, with parallel sides in posterior third and rather broadly rounded anteriorly, posterior angles nearly rectangular. Pronotal margin rounded, armed with four to six serrations at the anterior margin, rather widely spaced, central denticles larger than lateral. Pronotal declivity with approximately 50 asperities. Pronotal disc approximately one fourth of the pronotal length, sloping rather strongly from the summit. Pronotal vestiture short, hair-like, golden, on disc (without scale-like setae). Suture between pronotum and elytra sinuate, base of the pronotum is marked with fine carina. Scutellum V-shaped. Elytra variable in proportions, 1.43–1.57× as long as wide, 2.0–2.26× as long as pronotum, 1.07× wider than pronotum, parallel-sided for 3/4 of length, then broadly rounded toward apex. Striae not strongly impressed, interstriae only slightly convex, punctures in striae without flat bottom, touching neighbouring punctures, elytral surface only faintly shining. Interstrial bristles erect, hair-like, of even moderate length along elytra, with pointed apices. Interstrial ground vestiture scale-like arranged in three-four rows at each interstria consists of rather short blunt scales 1.5–2.0× as long as wide. Protibiae and protarsi with only straight, hair-like setae. Mesocoxae separated barely more than metacoxae. Proventriculus not examined.

Male. Length 1.45–1.70 mm. Similar to female, except elytra slightly stouter than in female. Aedeagus not studied. Proventriculus not studied.

Recorded plant hosts. Adoxaceae: *Viburnum sargentii* Koehne, *V. dilatatum* Thunb. (Yin et al. 1984 [unconfirmed]).

Distribution. Russia (Primorskiy Kray); China (Shaanxi (Tsai and Li 1963 [unconfirmed]); Shanxi (Alonso-Zarazaga et al. 2017 [unconfirmed])).

Suggested vernacular name. Russian: Калиновый крифал; Chinese: 荚蒾梢小蠹.

Remarks. Despite discovering that this species had been incorrectly reported from northern China, we expect it to be present. We were unable to corroborate records from Shaanxi, or records collected on *Viburnum dilatatum* as a host, initially mentioned by Yin et al. (1984) without citing any collections.

Conclusions

Cryphalus is remarkably diverse in East Asia, but most available literature does not allow for accurate species identification, particularly for taxa in tropical and subtropical broadleaf forests. This work changes number of valid species of *Cryphalus* to 254, based

on the taxa listed in Johnson et al. (2020); four additional taxa described here, and two taxa now recognized as synonyms. Thorough descriptions, redescriptions, photographs, and sequence data enable accurate and precise identification and further study of these minute bark beetles.

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Supplementary material 1

Summary of sequences used for the phylogeny of *Cryphalus* spp.

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Data type: molecular data

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RESEARCH ARTICLE

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Sequence and organisation of the mitochondrial genome of Japanese Grosbeak (*Eophona personata*), and the phylogenetic relationships of Fringillidae

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Abstract

Mitochondrial DNA is a useful molecular marker for phylogenetic and evolutionary analysis. In the current study, we determined the complete mitochondrial genome of *Eophona personata*, the Japanese Grosbeak, and the phylogenetic relationships of *E. personata* and 16 other species of the family Fringillidae based on the sequences of 12 mitochondrial protein-coding genes. The mitochondrial genome of *E. personata* consists of 16,771 base pairs, and contains 13 protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and one control region. Analysis of the base composition revealed an A+T bias, a positive AT skew and a negative GC skew. The mitochondrial gene order and arrangement in *E. personata* was similar to the typical avian mitochondrial gene arrangement. Phylogenetic analysis of 17 species of Fringillidae, based on Bayesian inference and Maximum Likelihood (ML) estimation, showed that the genera *Coccothraustes* and *Hesperiphona* are closely related to the genus *Eophona*, and further showed a sister-group relationship of *E. personata* and *E. migratoria*.

Keywords

Eophona personata, gene order, mitochondrial genome, phylogenetic analysis

Introduction

Eophona personata (Passeriformes: Fringillidae), commonly known as the Japanese Grosbeak, is a granivorous passerine with the adults reaching a size of ca. 23 cm. The species is mainly distributed in Far Eastern Asia including Eastern Siberia, Northeast China, North Korea, and Japan. Grosbeaks are migratory birds and move to South China during winter (Clement et al. 1993). Grosbeaks primarily feed on seeds, with preference for certain plants, and insects (Kominami 1987); for example, the seeds of *Celtis* and *Aphananthe* are favored during autumn and winter (Nimura 1993; Yoshikawa and Kikuzawa 2009).

The mitochondrial genome (hereafter mitogenome) is a useful molecular marker for phylogenetic analysis, and is widely used in the evolutionary analysis of a variety of species (Anderson et al. 1981; Boore 1999; Sun et al. 2016). The animal mitogenome is usually a short, closed, circular, double stranded molecule, and comprises of 37 genes: 13 protein-coding genes (PCGs; *ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5*, *ND6*, *COI*, *COII*, *COIII*, *ATP6*, *ATP8*, *CYTB*), two rRNA genes (12S rRNA and 16S rRNA), and 22 tRNA genes. Compared to nuclear DNA, the mitogenome is a more conserved molecule, and is maternally transmitted (Wolstenholme 1992; Boore 1999; da Fonseca et al. 2008).

With ca. 6000 species, passerines account for more than half of the total number of extant birds (Gill et al. 2020). Based on the analysis of hundreds of bird mitochondrial data, at least seven gene arrangements have been found (Zhou et al. 2014). Gene rearrangements in passerines are very common, and most reported passerines have the standard gene order consistent with chickens *Gallus gallus* (Desjardins and Morais 1990). In addition, Passeriformes have at least four gene arrangement (Harrison et al. 2004). The different rearrangements involve the initial tandem duplication, and the partial loss of the segment containing the control region (CR) (Caparroz et al. 2018).

The passerine family Fringillidae comprises ca. 50 genera and 230 species (Gill et al. 2020). The phylogenetic relationships between the species in the family Fringillidae and the superfamily Passeroidea are incompletely resolved (Van der Meij et al. 2005). Previous phylogenetic studies of Fringillidae have clarified several aspects of their relationships (Arnaiz-Villena et al. 2001; Van der Meij et al. 2005; Lerner et al. 2011; Zuccon et al. 2012; Tietze et al. 2013; Sangster et al. 2015; Zhao et al. 2016). The aim of this study is to determine the complete mitogenome of *E. personata* and provide a mitogenomic perspective on the phylogenetic relationships of *E. personata* and 16 other species of the family Fringillidae.

Materials and methods

Sample collection and DNA extraction

The specimen of *E. personata*, which had died due to poaching activities, was collected from Shenyang City, Liaoning Province, China, and was stored in the laboratory and

then frozen to -80 °C before further processing and analysis. All experiments involving animals were approved by the Qufu Normal University Institutional Animal Care and Use Committee (Permit number: QFNU2018-010) and executed in accordance with the Guide to Animal Experiments of the Ministry of Science and Technology (Beijing, China). DNA was extracted from muscle tissue using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

Cloning and sequencing of mitochondrial genome

Primers were designed based on mitogenome sequences of a sparrowhawk (*Accipiter nisus*, GenBank accession number KM360148) and Chinese Grosbeak *E. migratoria* (KX423959). MEGA5.1, Primer Premier 6.0, and the NCBI-Primer blast database (http://www.ncbi.nlm.nih.gov/BLAST) were used to determine the primers. All the amplified fragments were separated by gel electrophoresis and purified by Agarose Gel Extraction Kit (Qiagen). Purified fragments were cloned into PMD18-T vectors, and transformed into competent *E. coli* cells. Positive clones were identified by blue-white screening and sequenced. Bidirectional sequencing was conducted at Sangon Biotech (Shanghai) using the ABI 3730xl DNA Analyzer (PE Applied Biosystems, San Francisco, CA, USA).

Bioinformatics analysis and statistical procedures

The raw sequences were assembled using the software programs BioEdit (version 7.2. 5) and Chromas Pro 1.7.7 (Goodstadt and Ponting 2001). Genes were identified by aligning the identified sequences with the known sequence of the mitogenome of *E. migratoria*. The tRNA gene structure was predicted using Scan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE) and ARWEN (http://130.235.46.10/ARWEN/) (Lowe and Eddy 1997).

Phylogenetic analysis of 17 Fringillidae species (accession numbers are provided in Table 2) and *Phasianus colchicus* (NC_015526) was conducted using Bayesian inference and Maximum Likelihood (ML) estimation based on the sequences of 12 mitochondrial PCGs; *ND6* gene, which is encoded on the L-strand, was excluded from analysis. The sequences were aligned with ClustalX 1.81 (Thompson et al. 1997; Jeanmougin et al. 1998), and phylogenetic analysis was performed using MrBayes 3.1.2 and PAUP* 4.0 (Swofford 2001; Ronquist and Huelsenbeck 2003).

Based on the Akaike Information criterion (AIC), GTR+I+G was estimated as the best-fit substitution model using Modeltest 3.7 (Posada and Crandall 1998). Bootstrap tests were based on 1000 replicates (Felsenstein 1981; Wilgenbusch and Swofford 2003). For Bayesian analysis, Metropolis-coupled Markov chain Monte Carlo analysis was performed, with 4 chains run in parallel, for 2,000,000 generations, and the first 25% of each of the sampled 1000 generations were excluded as burn-in.

Following Sangster and Luksenburg (2020), we verified the identity and integrity of our mitogenome sequence of *E. personata* with reference sequences of multiple

protein-coding genes: NADH dehydrogenase subunit 2 (ND2, 1041 bp; n = 1), part of cytochrome oxidase subunit I (COX1, 698 bp; n = 6), and cytochrome *b* (CYTB, 1143 bp; n = 2). These markers are commonly used in avian systematics, and reference sequences were available for each marker.

Results

Comparison of mitogenomes of Japanese grosbeak and those of other species of Fringillidae

The complete mitochondrial genome of *E. personata* (KX812499, GenBank) was sequenced and a genome map was constructed (Fig. 1). The genome contains 13 PCGs, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and one control region (Table 1).

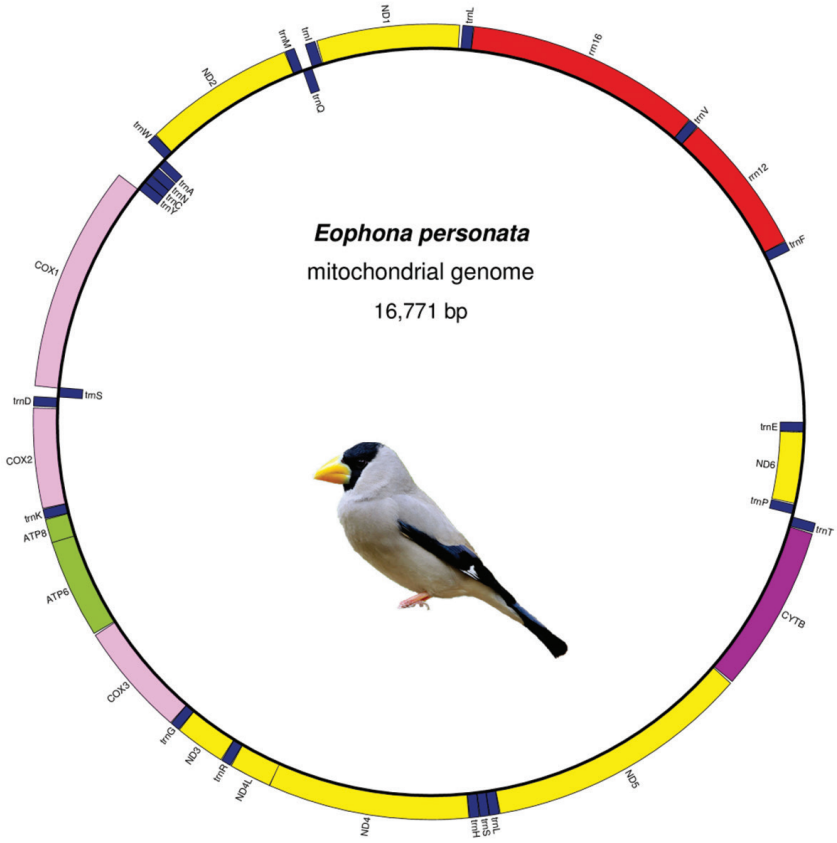

Figure 1. Circular map of the mitochondrial genome of *Eophona personata*. tRNAs are denoted as one-letter symbols according to IUPAC-IUB single-letter amino acid codes; L1 = UUR, L2 = CUN, S1 = UCN, S2 = AGY.

Table 1. Organization of the mitochondrial genome of *Eophona personata*.

Gene	Location (bp)	Size (bp)	Spacer (+) or overlap (–)	Strand	Start codon	Stop codon	Anticodon
D-loop	1–1187	1187		H	–	–	–
tRNA^{Phe}	1188–1255	68	0	H	–	–	GAA
12S rRNA	1256–2230	975	0	H	–	–	–
TRNA^{Val}	2231–2300	70	0	H	–	–	TAC
16S rRNA	2301–3904	1604	0	H	–	–	–
tRNA$^{Leu(UUR)}$	3905–3979	75	16	H	–	–	TAA
ND1	3996–4973	978	6	H	ATG	AGG	–
tRNA^{Ile}	4980–5053	74	3	H	–	–	GAT
tRNA^{Gln}	5057–5127	71	-1	L	–	–	TTG
tRNA^{Met}	5127–5195	69	0	H	–	–	CAT
ND2	5196–6236	1041	-1	H	ATG	TA-	–
tRNA^{Trp}	6236–6305	70	1	H	–	–	TCA
tRNA^{Ala}	6307–6375	69	1	L	–	–	TGC
tRNA^{Asn}	6384–6456	73	0	L	–	–	GTT
tRNA^{Cys}	6457–6523	67	-1	L	–	–	GCA
tRNA^{Tyr}	6523–6593	71	1	L	–	–	TGA
COX1	6595–8145	1551	-9	H	GTG	AGG	–
tRNA$^{Ser(UCN)}$	8137–8209	73	3	L	–	–	GTC
tRNA^{Asp}	8213–8281	69	8	H	–	–	GTC
COX2	8290–8973	684	1	H	ATG	TAA	–
tRNA^{Lys}	8975–9043	69	1	H	–	–	TTT
ATPase8	9045–9212	168	-10	H	ATG	TAA	–
ATPase6	9203–9886	684	7	H	ATG	TAA	–
COX3	9894–10677	784	0	H	ATG	T--	–
tRNA^{Gly}	10678–10746	69	0	H	–	–	TCC
ND3	10747–11097	351	1	H	ATG	TAA	
tRNA^{Arg}	11099–11168	70	1	H	–	–	TCG
ND4L	11170–11466	297	-7	H	ATG	TAA	–
ND4	11460–12837	1378	0	H	ATG	TAT	–
tRNA^{His}	12838–12907	70	0	H	–	–	GTG
tRNA$^{Ser(AGY)}$	12908–12973	66	-1	H	–	–	GCT
tRNA$^{Leu(CUN)}$	12973–13043	71	0	H	–	–	TAG
ND5	13044–14861	1818	8	H	ATG	AGA	–
CYTB	14870–16012	1143	5	H	ATG	TAA	–
tRNA^{Thr}	16018–16086	69	18	H	–	–	TGT
tRNA^{Pro}	16105–16174	70	6	L	–	–	TGG
ND6	16181–16699	519	-71	L	ATG	TAG	–
tRNA^{Glu}	16701–16771	71	1	L	–	–	TTC

The length of the complete mitogenome of *E. personata* is 16,771 bp, and is similar to that of other Fringillidae species (Table 2). The base composition of the genome is C (32.1%), A (30.7%), T (23.0%) and G (14.2%); the proportion of A+T (53.7%) is higher than G+C (46.3%), suggesting a strong A+T bias. The mitogenomes of 17 Fringillidae species showed a positive AT-skew and a negative GC-skew.

Sequence analysis of the 13 PCGs in the mitogenome of *E. personata* revealed that the base composition of the *ND6* gene was not consistent with the other genes, and the percentage of T and G is much higher than in the other genes, with a positive GC skew (Table 3), whereas the base composition and skewness are highly similar for the other genes. The encoded genes share the common start codon ATN, with ATG most

Table 2. Base composition (in percentages) of the mitochondrial genomes of 17 species of Fringillidae.

Species	Total length (bp)	T (%)	C (%)	A (%)	G (%)	A + T content (%)	AT-skew	GC-skew	Accession number
Eophona personata	16771	23.0	32.1	30.7	14.2	53.7	0.142	-0.386	KX812499
Eophona migratoria	16798	22.9	32.3	30.7	14.0	53.7	0.145	-0.397	KX423959
Oreomystis bairdi	16833	23.7	31.6	30.3	14.4	53.9	0.123	-0.373	KM078807
Paroreomyza montana	16832	23.5	31.5	30.8	14.2	54.4	0.134	-0.379	KM078771
Melamprosops phaeosoma	16840	24.2	31.0	30.5	14.3	54.7	0.114	-0.370	NC_025617
Acanthis flammea	16820	24.0	31.4	30.5	14.2	54.5	0.120	-0.378	NC_027285
Loxops coccineus	15589	24.2	31.9	30.1	13.8	54.3	0.108	-0.395	KM078785
Loxia curvirostra	16805	23.8	31.4	30.6	14.3	54.3	0.125	-0.375	KM078800
Carduelis spinus	16828	24.0	31.3	30.9	13.8	54.9	0.127	-0.388	HQ915866
Chloris sinica	16813	24.7	30.5	30.7	14.1	55.4	0.108	-0.369	HQ915865
Serinus canaria	16805	23.9	31.1	31.1	13.8	55.0	0.130	-0.384	KM078794
Haemorhous cassinii	16812	24.3	30.5	30.9	14.2	55.2	0.120	-0.364	KM078786
Coccothraustes coccothraustes	16823	23.9	31.0	30.9	14.3	54.8	0.127	-0.369	KM078789
Hemignathus parvus	16833	23.8	31.5	30.3	14.4	54.1	0.120	-0.371	KM078799
Fringilla montifringilla	16807	23.3	32.1	30.3	14.3	53.6	0.130	-0.382	JQ922259
Hesperiphona vespertina	16810	23.6	31.6	30.8	14.0	54.4	0.132	-0.387	KM078770
Crithagra dorsostriata	16804	24.2	30.8	31.1	13.8	55.4	0.125	-0.382	KM078798

Table 3. Base composition (in percentages) of the genes of *Eophona personata.*

Gene	Proportion of nucleotides				%A+T	AT skew	GC skew	%A+C	%G+T
	T	C	A	G					
ND1	25.3	33.3	27.3	14.1	52.6	0.039	-0.405	60.6	39.4
ND2	23.5	35.7	30.8	10.1	54.2	0.135	-0.559	66.4	33.6
COX1	23.3	32.7	27.5	16.5	50.8	0.081	-0.328	60.1	39.9
COX2	20.9	33.6	30.3	15.2	51.2	0.183	-0.377	63.9	36.1
ATP8	22.0	39.9	32.1	6.0	54.2	0.187	-0.740	72.0	28.0
ATP6	23.1	36.8	30.1	9.9	53.2	0.132	-0.575	67.0	33.0
COX3	24.0	33.3	27.6	15.2	51.5	0.069	-0.374	60.8	39.2
ND3	26.8	34.2	27.4	11.7	54.1	0.011	-0.491	61.5	38.5
ND4L	24.6	36.7	27.3	11.4	51.9	0.052	-0.524	64.0	36.0
ND4	23.1	35.6	30.5	10.9	53.6	0.138	-0.531	66.0	34.0
ND5	23.1	33.5	31.6	11.8	54.7	0.156	-0.480	65.1	34.9
CYTB	23.8	35.1	27.6	13.6	51.4	0.073	-0.442	62.6	37.4
ND6	38.9	9.6	10.4	41.0	49.3	-0.578	0.620	20.0	80.0
12S rRNA	20.0	27.7	31.4	20.9	51.4	0.222	-0.139	59.1	40.9
16S rRNA	21.4	24.2	34.7	19.7	56.1	0.236	-0.102	58.9	41.1
Total	23.6	31.9	29.3	15.2	52.9	0.108	-0.353	61.2	38.8

commonly observed. However, the start codon for COI is GTG. The non-coding regions include a control region (D-loop) and a few intergenic spacers. The control region is 1187 bp and is located between the $tRNA^{Glu}$ and $tRNA^{Phe}$.

Based on the tRNA gene sequences identified, secondary structures of the tRNAs were determined (Fig. 2).

Protein-coding genes and gene order

The 13 PCGs in the mitogenome of *E. personata* spans a length of 11,399 bp, and encode for six NADH dehydrogenase subunits, three cytochrome c oxidase subunits,

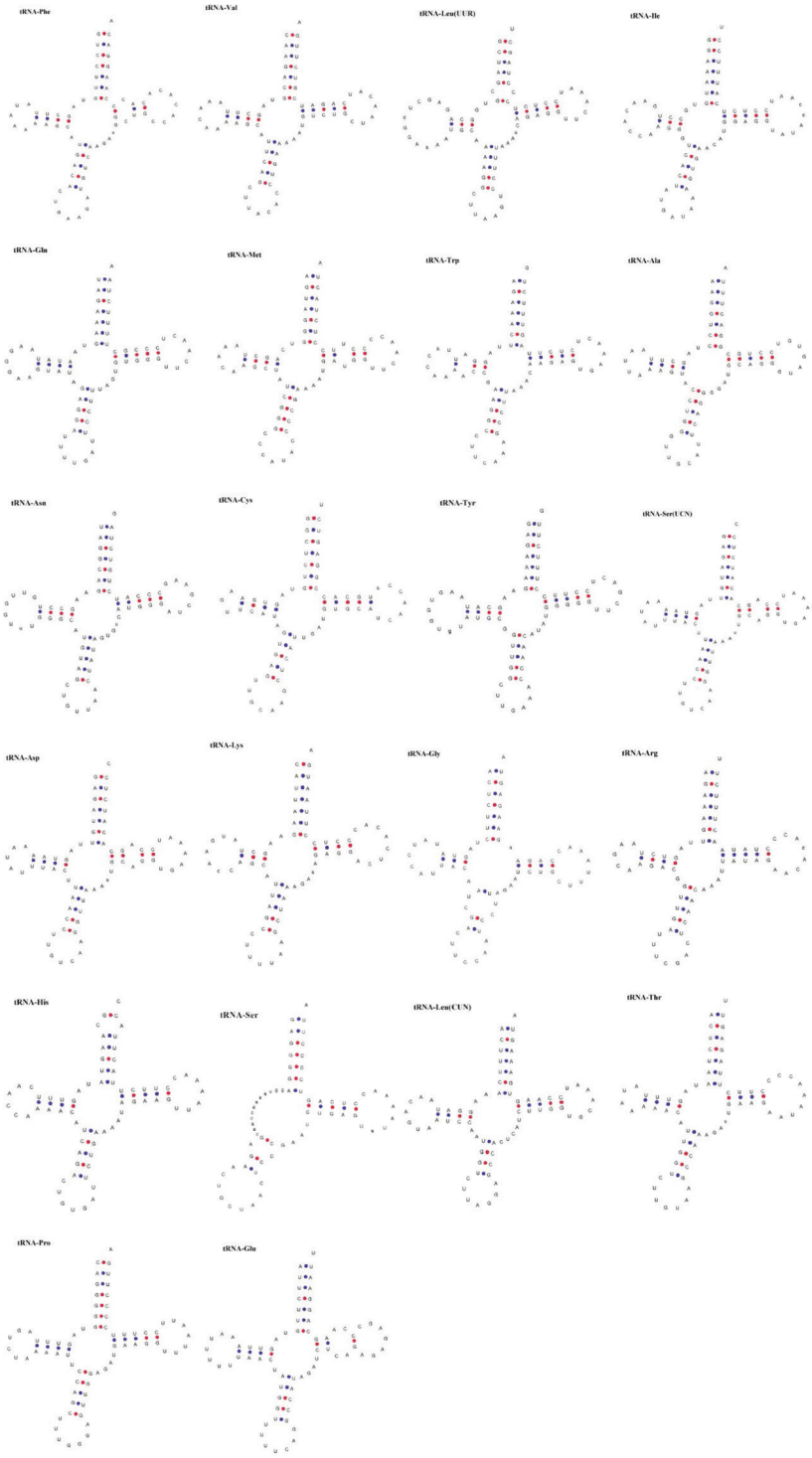

Figure 2. Predicted secondary structures for the 22 tRNAs in *Eophona personata.*

Table 4. Relative synonymous codon usage (RSCU) values for the 13 protein-coding genes in *Eophona personata*.

Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	41	0.56	UCU(S)	40	0.82
UUC(F)	105	1.44	UCC(S)	55	1.13
UUA(L)	32	0.37	UCA(S)	52	1.07
UUG(L)	8	0.09	UCG(S)	8	0.16
CUU(L)	83	0.96	CCU(P)	174	1.69
CUC(L)	162	1.88	CCC(P)	114	1.11
CUA(L)	192	2.23	CCA(P)	105	1.02
CUG(L)	40	0.46	CCG(P)	19	0.18
AUU(I)	80	0.74	ACU(T)	99	1.34
AUC(I)	154	1.43	ACC(T)	92	1.25
AUA(I)	89	0.83	ACA(T)	94	1.27
AUG(M)	42	1.00	ACG(T)	10	0.14
GUU(V)	36	1.01	GCU(A)	39	0.87
GUC(V)	42	1.17	GCC(A)	88	1.96
GUA(V)	50	1.40	GCA(A)	46	1.02
GUG(V)	15	0.41	GCG(A)	7	0.16
UAU(Y)	41	0.70	UGU(C)	17	0.76
UAC(Y)	76	1.30	UGC(C)	28	1.24
UAA(*)	17	0.59	UGA(*)	67	2.31
UAG(*)	3	0.10	UGG(W)	23	1.00
CAU(H)	97	0.93	CGU(R)	27	0.68
CAC(H)	111	1.07	CGC(R)	54	1.37
CAA(Q)	102	1.73	CGA(R)	40	1.01
CAG(Q)	16	0.27	CGG(R)	28	0.71
AAU(N)	109	0.98	AGU(S)	39	0.80
AAC(N)	113	1.02	AGC(S)	97	2.00
AAA(K)	89	1.73	AGA(R)	35	0.89
AAG(K)	14	0.27	AGG(R)	53	1.34
GAU(D)	19	0.60	GGU(G)	34	0.80
GAC(D)	44	1.40	GGC(G)	53	1.24
GAA(E)	49	1.78	GGA(G)	61	1.43
GAG(E)	6	0.22	GGG(G)	23	0.54

two ATPases and cytochrome b. The light-strand has nine genes, which includes eight tRNA genes and *ND6*, and the heavy-strand has 28 genes, which includes 14 tRNA genes, two rRNA genes and 12 protein-coding genes. Relative synonymous codon usage (RSCU) values for the 13 PCGs are shown in Table 4. There are 3798 codons in the 13 PCGs, and codons of leucine, proline, isoleucine, and threonine take a higher proportion (Fig. 3). The codons AAA-lysine, GAA-glutamic acid, AAC-asparagine, UUC-phenylalanine, and AAU-asparagine are AT-rich, and the codons CUC-leucine, CCU-proline, GCC-alanine, and AGC-serine are GC-rich.

The gene order and arrangement of the region located between *CYTB* and $tRNA^{Phe}$, is $tRNA^{Thr}$, $tRNA^{Pro}$, ND6, $tRNA^{Glu}$, control region, and $tRNA^{Phe}$, as shown in Fig. 4.

Phylogenetic analysis

Phylogenetic relationships of the 17 Fringillidae species, inferred from Bayesian and Maximum Likelihood analyses, recovered almost identical well-resolved topologies

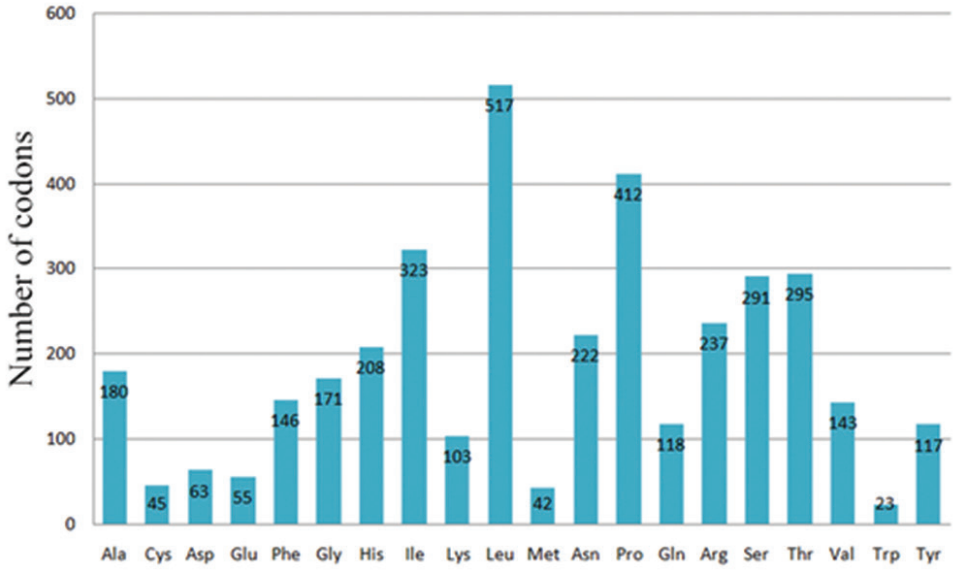

Figure 3. Codon distribution in the mitochondrial genome of *Eophona personata*.

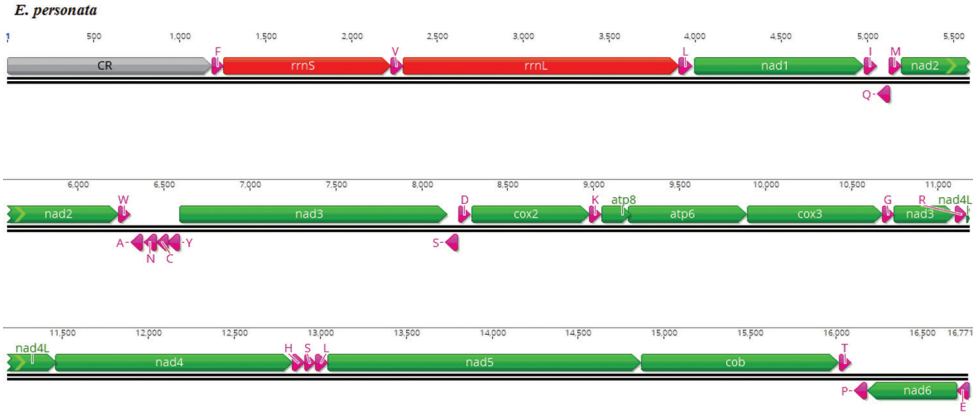

Figure 4. Mitochondrial gene order and arrangement in *Eophona personata*.

(Fig. 5). Four major clades can be distinguished: clade 1 consisted of the genus *Fringilla*; clade 2 comprised three genera of grosbeaks (*Coccothraustes*, *Eophona*, *Hesperiphona*); clade 3 comprised of five genera (*Acanthis*, *Loxia*, *Carduelis*, *Serinus*, and *Haemorhous*); and clade 4 consisted of three genera of honeycreepers (*Hemignathus*, *Loxops*, and *Paroreomyza*). Clades 3 and 4 were inferred as sister-groups, which together were sister to clade 2. Clade 1 was sister to all other cardeline finches.

The analysis recovered *C. coccothraustes* and *H. vespertina* as sister groups to *Eophona*, with strong support in both Bayesian and ML analysis (Fig. 5).

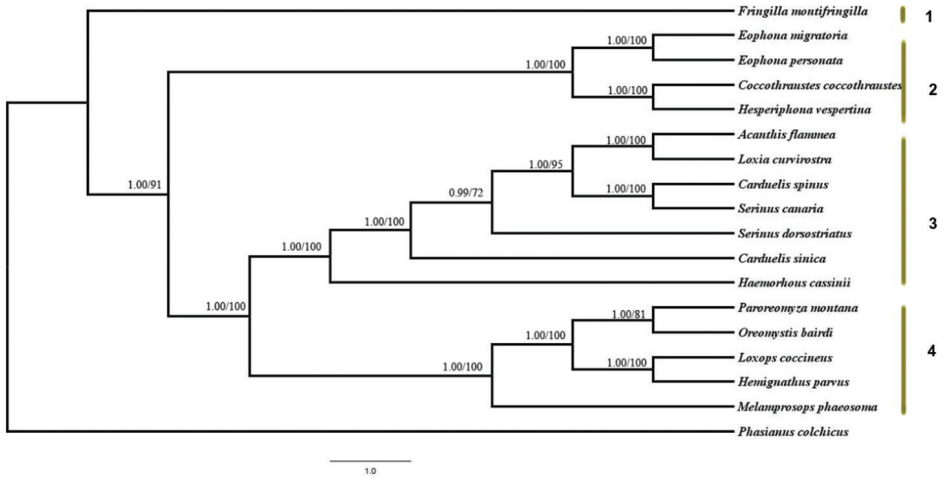

Figure 5. The phylogenetic tree generated for 17 species of Fringillidae. The values indicated at the nodes are Bayesian posterior probabilities (left) and ML bootstrap proportions (right).

No evidence for chimerism was found in comparisons of the ND2, COX1, and CYTB fragments with reference sequences on GenBank. Thus, in all cases the mitogenome of *E. personata* clustered with, and was very similar to, reference sequences of this species (data not shown).

Discussion

In this study, we obtained the complete mitogenome sequence of *E. personata*, and performed molecular phylogenetic analysis of 17 Fringillidae species based on the sequences of 12 mitochondrial PCGs. Our results revealed that the complete mitogenome of *E. personata* is 16,771 bp, and contains 13 protein-coding genes, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and one control region. Analysis of the base composition revealed an A+T bias, a positive AT skew and a negative GC skew. Phylogenetic analysis demonstrated that *Coccothraustes* is the closest genus to *Eophona* and that *E. personata* is the sister taxon of *E. migratoria*.

Our analysis showed that the mitogenome of *E. personata* is similar to that of other Fringillidae species. The genome structure was that of a typical vertebrate mitochondrial genome (Boore 1999). The A+T bias observed in the mitochondrial genome of *E. personata* was similar to that of other vertebrates (Yang et al. 2016), and the positive AT-skew value and a negative GC-skew value observed, is also consistent with the vertebrate mitogenome (Quinn and Wilson 1993). The gene order and arrangement in *E. personata* was similar to the typical arrangement seen in birds (Mindell et al. 1999; Haddrath and Baker 2001; Liu et al. 2013), including Passeriformes (Caparroz et al. 2018). It

is generally believed that the rearrangement of the mitochondrial genome represents a rare evolutionary event that can be used to construct the phylogenetic relationships of distantly related groups (Bensch 2000). In birds, two major types of gene order are found which differ by the number of control region copies (Singh et al. 2008). One of these is believed to be the ancestral gene order and the other is the remnant control region 2 gene order (Singh et al. 2008).

The phylogenetic relationships observed in this study are in accordance with previous research (Arnaiz-Villena et al. 2001; Zuccon et al. 2012). In the current study, the three grosbeak genera (*Coccothraustes*, *Eophona*, *Hesperiphona*; clade 2) clustered together and formed a well-defined clade. The grosbeak consists of a group of fairly large and stocky finches, feeding primarily on hard seeds. Our study corroborates a previous study based on the nuclear and mitochondrial sequences (Zuccon et al. 2012). A close phylogenetic relationship between the four genera (*Coccothraustes*, *Mycerobas*, *Hesperiphona*, *Eophona*) has been recognised previously (Vaurie 1959; Clement et al. 1993; Dickinson and Christidis 2015), and their close evolutionary relationships are widely accepted.

Our analysis showed that the genus *Fringilla* diverged very early within the family Fringillidae and was followed a deep divergence between the grosbeak clade and a clade formed by the other members Carduelinae. Our study as well as previous studies suggests that the analysis of phylogenetic relationships of passerines are more accurately resolved and better supported with complete mitogenomes than with short sequences of single genes (Van der Meij et al. 2005; Nguembock et al. 2009).

Conclusions

In this study, the complete mitogenome of *E. personata* was sequenced and analysed for the first time, and the phylogenetic analysis confirmed the taxonomic classification of *E. personata.* The results showed that the genera *Coccothraustes* and *Hesperiphona* have a close relationship with the genus *Eophona*, and this is consistent with the morphologicl similarity observed between them. Our analysis shows the phylogenetic relationship of *E. personata* as a sister group to *E. migratoria*, and the mitogenome was observed to be very similar between them.

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Reidentification of *Decapterus macarellus* and *D. macrosoma* (Carangidae) reveals inconsistencies with current morphological taxonomy in China

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Abstract

Decapterus macarellus and *D. macrosoma* are economically important pelagic fish species that are widely distributed in tropical and subtropical seas. The two species are often mistakenly identified due to their morphological similarities as described in the Chinese literature on fish identification. In this study, *D. macarellus* and *D. macrosoma* samples were collected in the Eastern Indian Ocean and the South China Sea and reidentified using morphological and DNA barcoding techniques. The characteristics that distinguish the two species primarily include the scute coverage of the straight portion of the lateral line (the most indicative characteristic for classification), the shape of the predorsal scaled area and its location relative to the middle axis of the eye, and the shapes of the posterior margin of the maxilla and the posterior margin of the operculum. The results revealed a large number of misidentified sequences among the homologous cytochrome oxidase (COI) sequences of the two species in the NCBI database and that the genus *Decapterus* may include cryptic species. In terms of genetic structure, the Sundaland has not blocked genetic exchange between *D. macarellus* populations in the South China Sea and the Eastern Indian Ocean, giving rise to a high level of genetic diversity. In this study, we made corrections to the Chinese classification standards for *D. macarellus* and *D. macrosoma* and the erroneous reference sequences in the NCBI database, thereby providing accurate reference points for the future exploration of cryptic species in the genus *Decapterus*.

Keywords
DNA barcoding, genetic diversity, mackerel, morphological characteristics, phylogeny, scad, species identification

Introduction

Fish species of the genus *Decapterus* in the family Carangidae are pelagic fish widely distributed in tropical and subtropical waters around the world and are generally of high economic value. Fishes of the genus *Decapterus* present one free finlet behind the second dorsal fin and the anal fin and varying degrees of scute coverage along the straight-line portion of the lateral line but no coverage along the curved portion of the lateral line. These characteristics make the fishes easily distinguishable from other species of the family Carangidae (Smith-Vaniz 1999). Currently, the genus *Decapterus* includes 11 species worldwide: *D. akaadsi* Abe, 1958, *D. koheru* (Hector, 1875), *D. kurroides* Bleeker, 1855, *D. macarellus* (Cuvier, 1833), *D. macrosoma* Bleeker, 1851, *D. maruadsi* (Temminck & Schlegel, 1843), *D. muroadsi* (Temminck & Schlegel, 1843), *D. punctatus* (Cuvier, 1829), *D. russelli* (Rüppell, 1830), *D. tabl* Berry, 1968, and *D. smithvanizi* Kimura, Katahira & Kuriiwa, 2013 (Kimura et al. 2013).

Decapterus macrosoma (shortfin scad) and *D. macarellus* (mackerel scad) are morphologically similar and thus often confused with each other. In Chinese literatures on fish morphological classification, the morphological descriptions of *D. macrosoma* and *D. macarellus* are largely incorrect (Zhu et al. 1962, 1963, 1979, 1985; Cheng and Zheng 1987; Meng et al. 1995); for example, "*D. macarellus* shows a convex posterior end of maxilla, and the majority of the rear straight-line portion the lateral line is covered with scutes" and "*D. macrosoma* shows a truncate posterior end of maxilla, and scutes cover the rear half of the straight-line portion of the lateral line". These descriptions contradict those from international studies, particularly those of type specimen morphology (Cuvier and Valenciennes 1833; Bleeker 1851; Nakabo 2013). Thus, in this study, samples of *D. macarellus* and *D. macrosoma* were collected from surveys of the fishery resources in the South China Sea and the Eastern Indian Ocean and were morphologically reidentified.

The mitochondrial cytochrome oxidase (COI) gene fragment varies little within species but significantly between species; this fragment can be amplified via polymerase chain reaction (PCR) using universal primers and standardized experimental procedures and is thus employed for DNA barcoding, which has been widely accepted and utilized (Hebert et al. 2003) for identifying species (Li et al. 2019a; Xu et al. 2019), discovering new species and new records (Li et al. 2018; Chao et al. 2019; Wu et al. 2020), identifying cryptic species (Cheng and Sha 2017; Delrieu-Trottin et al. 2018), identifying ichthyoplankton species (Hubert et al. 2015, Li et al. 2017), and detecting invasive species (Hernández-Triana et al. 2019), among other purposes. Therefore, in this study, we employed DNA barcoding to genetically compare *D. macarellus* and *D.*

macrosoma and then aligned the sequences with homologous sequences retrieved from GenBank for further analysis. The barrier formed by the Sundaland has caused the differentiation of various fish species, e.g., *Pampus chinensis* (Euphrasen, 1788) (Li et al. 2019b), between the Indian and Pacific Oceans. The question of whether the geographical barrier formed by the Sundaland has also driven species differentiation in the genus *Decapterus* will be addressed in this study based on the samples collected during surveys of the South China Sea and the Eastern Indian Ocean.

In summary, we aimed to reevaluate *D. macarellus* and *D. macrosoma* by combining morphological analysis with molecular genetics to discern the major diagnostic morphological characteristics and correct DNA barcoding for identification and to provide a timeline for the differentiation of the two species. The findings of this study can provide a scientific reference for the classification of fishes in China and the identification of Carangidae fishes and a theoretical basis for the protection, utilization, development and management of *Decapterus* species germplasm resources.

Materials and methods

Sample collection

Decapterus macarellus and *D. macrosoma* samples were collected from the South China Sea (10°N, 110°30'E) and the Eastern Indian Ocean (2°N, 88°E) in July and October 2019, respectively (Fig. 1); both species were collected from the South China Sea with light purse seining, whereas *D. macarellus* samples were collected from the Eastern Indian Ocean using lightnet lifting. Morphological identification of all samples was conducted with reference to Nakabo (2013) and Yamada et al. (2009). From the samples, 24 individuals of *D. macarellus* (A1~A24) and 21 individuals of *D. macrosoma* (B1~B21) from the South China Sea, in addition to 24 individuals of *D. macarellus* from the Eastern Indian Ocean, were randomly selected; the dorsal muscle was excised from each and preserved in 95% alcohol for use in subsequent molecular genetic analysis.

Morphological analysis

Using the methods of Kimura and Suzuki (1981) and Xu and Huang (1983), morphological measurements and description of the fish samples were conducted. The countable characteristics included spines and rays in the dorsal fin, rays in the pectoral fin, spines and rays in the pelvic fin, spines and rays in the anal fin, rays in the caudal fin, scutes, and vertebrae (counted from X-ray images), and the measurable characteristics included body length and fork length, which were performed using a Vernier caliper with an accuracy of 0.1 mm. The major morphological diagnostic characteristics included the location on the top of the head reached by the scaled area, the distribution of scutes in the straight-line portion of the lateral line, the morphological characteristics of the scutes, the shape of the posterior margin of the maxilla, and the shape of the posterior margin of the operculum.

Figure 1. *Decapterus macrosoma* (upper) and *D. macarellus* (lower).

Molecular analysis

Genomic DNA was extracted from specimens of both *Decapterus* species with a Qiagen DNeasy Kit and stored at 4 °C. Using universal primers for the mitochondrial COI gene fragment (F2: 5 '-TCGACTAATCATAAAGATATCGGCAC-3'; R2: 5'-ACTTCAGGGTGACCGAAGAATCAGAA-3') (Ward et al. 2005), the targeted fragment was amplified in a 25 μL PCR system consisting of 17.5 μL of ddH_2O, 0.15 μL of *Taq* DNA polymerase, 2.5 μL of dNTPs (2 mM), 2 μL of 10 × Taqbuffer (with Mg^{2+}), 1 μL each of the forward and reverse primers (2 mM), and 1 μL of the genomic DNA template. The following conditions were applied: 4 min of predenaturation at 94 °C, followed by 28 cycles of 94 °C for 45 sec, 50 °C for 40 sec, and 72 °C for 40 sec, with a final extension at 72 °C for 10 min. A negative control was included to detect DNA contamination. The PCR products (3 μL) were analyzed using 1.5% agarose gel electrophoresis (U = 5 V/cm) and were later submitted to Personal Biotechnology Co., Ltd., for purification and bidirectional sequencing.

To ensure the accuracy of the DNA barcoding for the two *Decapterus* species, we retrieved all homologous COI gene sequences of the two species from GenBank (Table 1) to facilitate subsequent comparative analyses. All the obtained sequences were processed and aligned using DNASTAR software (Madison, WI, USA) to ensure consistency. Using *Decapterus maruadsi* and *Trachurus japonicus* as outgroups, a neighbor-joining (NJ) tree of all the sequences was constructed based on the Kimura two-

Table 1. Information on haplotype, accession numbers, sequence similarity for the samples and sequences in this study.

	Haplotype	Number	Cited dataset from GenBank				Sequences in this study	
			Accession numbers	Scientific species name	sequence similarity (%)	Corrected species name	ID	Scientific species name
Group 1	Hap_5	63	HQ560948, HQ564377, HQ564442, JF493340, JF493341, JF493342, JF493343, JF493346, JX261016, JX261033, JX261126, JX261170, JX261203, JX261215, JX261216, JX261243, JX261268, JX261269, JX261389, JX261442, JX261499, JX261514, JX261515, JX261519, JX261629, KF841444, KP856776, KP856777, KP856778, KU943769, KU943771, KU943781, KY371382, KY371387, KY371390, KY371391, KY371392, KY371393, KY371394, KY371396, KY371397, KY371398, KY371399, KY371400, KY371401, MH085881, MH638661, MH638663	*D. macrosoma*	100	✓	B1, B2, B4, B5, B6, B7, B9, B10, B13, B14, B16, B17, B18, B19, B20	*D. macrosoma*
	Hap_6	6	JX261160, KY371395, MH638795	*D. macrosoma*	100	✓	B3, B11, B12	*D. macrosoma*
	Hap_7	2	JX260997	*D. macrosoma*	100	✓	B8	*D. macrosoma*
	Hap_8	1			99.8		B15	*D. macrosoma*
	Hap_9	1			99.8		B21	*D. macrosoma*
	Hap_20	1	EU514515	*D. macrosoma*	100	✓		
	Hap_21	1	EU514516	*D. macrosoma*	100	✓		
	Hap_24	1	HQ564441	*D. macrosoma*	100	✓		
	Hap_28	2	JF493344, JF493345	*D. macrosoma*	100	✓		
	Hap_32	1	JX261121	*D. macrosoma*	100	✓		
	Hap_33	4	JX261134, KC970467, KY371388, KY371389	*D. macrosoma*	100	✓		
	Hap_34	1	JX261441	*D. macrosoma*	100	✓		
	Hap_35	1	JX261596	*D. macrosoma*	100	✓		
	Hap_38	2	KP266782	*D. macrosoma*	100	✓	7HYS	*D.macrosoma*
	Hap_41	1	KU943770	*D. macrosoma*	100	✓		
	Hap_44	2	KY371383, KY371385	*D. macrosoma*	100	✓		
	Hap_45	2	KY371384, KY371386	*D. macrosoma*	100	✓		
	Hap_51	1	KY802095	*D. macrosoma*	100	✓		
	Hap_54	1	MF541319	*D. macrosoma*	100	✓		
	Hap_55	1	MF956638	*D. macrosoma*	100	✓		
	Hap_56	1	MF956639	*D. macrosoma*	100	✓		
	Hap_59	1	MH638662	*D. macrosoma*	100	✓		
Group 2	Hap_27	1	JF493339	*Decapterusmacarellus*	94.2	*Decapterus* sp. 2		
Group 3	Hap_63	1	MH980014	*Decapterusmacarellus*	96.4	*Decapterus* sp. 1		
Group 4	Hap_1	54	KM986880, KP266765, KU943796, KU943797, KU943798, KY371373, KY371374, KY371376, KY371377, KY371378, KY371380, KY371381, KY570721, KY570723, KY570729, KY570731, KY570733, MF414832, MF414849, MF414876, MH085883, MH085884, MH638676, MH638686, MH638719, MH638731	*D.macarellus*	100	✓	A15, A16, A17, A18, A19, A23, A24, C1, C5, C6, C7, C8, C9, C13, C17, C20, C21, C23, 1CTYS, A4, A10, A11, A12	*D. macarellus*

	Haplotype	Number	Cited dataset from GenBank				Sequences in this study	
			Accession numbers	Scientific species name	sequence similarity (%)	Corrected species name	ID	Scientific species name
Group 4			MH638732, MH638733, MH638755, MH638772, MH638781					
Group 4	Hap_2	1			99.8		A20	*D. macarellus*
	Hap_3	8	KY570726, KY570732, MF414875, MH638794, MN257556	*D. macarellus*	100	✓	A14 A21 C15	*D. macarellus*
	Hap_4	1			99.8		A22	*D. macarellus*
	Hap_10	1			99.8		C2	*D. macarellus*
	Hap_11	1			99.8		C3	*D. macarellus*
	Hap_12	1			99.8		C4	*D. macarellus*
	Hap_13	1			99.8		C10	*D. macarellus*
	Hap_14	2	MF541317	*D. macarellus*	100	✓	C11	*D. macarellus*
	Hap_15	2			99.8		C12 C18	*D. macarellus*
	Hap_16	3	KY371375	*D. macarellus*	100	✓	C14 C22	*D. macarellus*
	Hap_17	1			99.8		C16	*D. macarellus*
	Hap_18	1			99.8		C19	*D. macarellus*
	Hap_19	3	KY570727, MH638687	*D. macarellus*	100	✓	C24	*D. macarellus*
	Hap_23	1	HQ564302	*D. macarellus*	100	✓		
	Hap_25	1	JF493337	*D. macarellus*	100	✓		
	Hap_26	1	JF493338	*D. macarellus*	100	✓		
	Hap_36	1	KF009585	*D. macarellus*	100	✓		
	Hap_42	3	KY371372, MH638698	*D. macarellus*	100	✓	A9	*D. macarellus*
	Hap_43	2	KY371379, MH085882	*D. macarellus*	100	✓		
	Hap_46	1	KY570722	*D. macarellus*	100	✓		
	Hap_47	1	KY570724	*D. macarellus*	100	✓		
	Hap_48	1	KY570725	*D. macarellus*	100	✓		
	Hap_49	1	KY570728	*D. macarellus*	100	✓		
	Hap_50	2	KY570730, MH638739	*D. macarellus*	100	✓		
	Hap_52	2	MF414851, MH638756	*D. macarellus*	100	✓		
	Hap_53	1	MF414877	*D. macarellus*	100	✓		
	Hap_57	1	MH119969	*D. macarellus*	100	✓		
	Hap_58	1	MH119978	*D. macarellus*	100	✓		
	Hap_60	1	MH638714	*D. macarellus*	100	✓		
	Hap_61	1	MH638749	*D. macarellus*	100	✓		
	Hap_62	1	MH638771	*D. macarellus*	100	✓		
	Hap_64	1			99.8		17CTYS	*D. macarellus*
	Hap_65	1			99.8		A1	*D. macarellus*
	Hap_66	1			99.8		A2	*D. macarellus*
	Hap_67	1			99.6		A3	*D. macarellus*
	Hap_68	1			99.8		A5	*D. macarellus*
	Hap_69	1			99.8		A6	*D. macarellus*
	Hap_70	1			99.8		A7	*D. macarellus*
	Hap_71	1			99.8		A8	*D. macarellus*
	Hap_72	1			99.8		A13	*D. macarellus*
Group 5	Hap_40	2	KT326329, MF541318	*D. macrosoma*	100	*D. russelli*		
Group 6	Hap_31	1	JQ681500	*D. macarellus*	100	*D. maruadsi*		
	Hap_37	6	KT718513, KT718514, KT718515, KT718516, KT718519	*D. macarellus*	100	*D. maruadsi*	KP266752	*D. maruadsi*
Group 7	Hap_39	1			100		KP267655	*T. japonicus*
Group 8	Hap_22	1	EU514517	*D. macarellus*	100	*S. crumenophthalmus*		
	Hap_29	2	JQ431681, KJ202148	*D. macarellus*	100	*S. crumenophthalmus*		
	Hap_30	1	JQ431682	*D. macarellus*	100	*S. crumenophthalmus*		

Table 2. Comparison of countable and measurable characteristics of *D. macarellus* and *D. macrosoma*.

Parameters	*D. macrosoma*	*D. macarellus*	
	South China Sea (*N* = 50)	South China Sea (*N* = 50)	Eastern Indian Ocean (*N* = 50)
dorsal fin	VII-VIII, I-31-35+1	VIII, I-30-35+1	VII-VIII, I-30-36+1
pectoral fin	20-23	20-23	20-24
pelvic fin	I-5-6	I-5-6	I-5-6
anal fin	II, I-26-30+1	II, I-26-30+1	II, I-27-30+1
caudal fin	15-18	16-18	16-17
scute	24-38	25-36	24-38
vertebrae	23-26	23-25	24-26
body weight (g)	9.8-24.4	7.1-23.9	17.2-27.7
body length (mm)	92.1-119.3	20.6-114.3	108.2-127.3
fork length (mm)	104.3-128.4	29.3-125.1	114.5-134.6

parameter (K2P) model in MEGA 5.0 software (Tamura et al. 2011), and the genetic distances within and among groups were calculated. All the sequences were searched against the NCBI database using BLAST to validate the accuracy of the sequences of the two *Decapterus* species investigated in this study according to the following criteria: a pairwise sequence similarity ≥ 98% indicated the same species, a pairwise sequence similarity = 92~98% indicated the same genus, and a pairwise sequence similarity = 85~92% indicated the same family (Li et al. 2017).

Due to a lack of fossil records for fishes from the genus *Decapterus*, it is impossible to precisely determine the timing of their differentiation. In this study, the divergence time of investigated fishes was estimated based on a nucleotide site divergence rate of 1.2% per million years (Bermingham et al. 1997).

To determine whether the *Decapterus* species from the two sides of the Sundaland have differentiated, we assessed the genetic diversity and genetic structure of *D. macrosoma* and *D. macarellus* based on the acquired COI sequences. Specifically, diversity parameters and unrooted minimum spanning tree (MST) data were analyzed using ARLEQUIN software (Excoffier et al. 2005); the MST was constructed with the MINSPNET algorithm with manual correction.

Results

Morphological analysis

Based on the correct classification of *D. macarellus* and *D. macrosoma*, countable and measurable characteristics were determined for 50 individuals from each population (Table 2). The results revealed no significant variation in the countable characteristics between the South China Sea population and the Eastern Indian Ocean population for *D. macarellus*, as follows (populations combined): dorsal fin, VII–VIII, I-30~36, 1 finlet; pectoral fin, 20~24; pelvic fin, I-5~6; anal fin, II, I-26~30, 1 finlet; caudal fin, 16~18; scutes, 24~38; and vertebrae, 23~26. The countable characteristics

of *D. macrosoma* were as follows: dorsal fin, VII–VIII, I-31~35, 1 finlet; pectoral fin, 20~23; pelvic fin, I-5~6; anal fin II, I-26~30, 1 finlet; caudal fin, 15~18; scutes, 24~38; and vertebrae, 23~26. A comparison of the countable characteristics between the two species showed that most of the characteristics largely overlapped, making it impossible to distinguish these two species.

Combining the findings of previous studies (Zhu et al. 1962, 1985; Meng et al. 1995; Nakabo 2013) with observations of the morphological characteristics of the samples in this study, the major diagnostic characteristics of *D. macarellus* and *D. macrosoma* can be summarized as follows: (1) the straight-line portion of the lateral line of *D. macrosoma*, the majority (approximately 3/4) of which is covered with scutes in the rear end, begins below rays 13~14 of the second dorsal fin, and the scutes show no particular external characteristics; in contrast, the straight-line portion of the lateral line of *D. macarellus*, with the rear half covered with scutes, begins below rays 12~13 of the second dorsal fin, and the highest scute is approximately half the eye diameter; (2) The predorsal scales of *D. macrosoma* do not reach the middle axis of the eye, presenting an "m" shape, whereas the predorsal scaled area of *D. macarellus* reaches or extends past the middle axis of the eye, taking on a "∩" shape; (3) The posterior end of the maxilla of *D. macrosoma* is truncated, and the operculum has a straight posterior margin, whereas the posterior end of the maxilla of *D. macarellus* is convex and round, and the operculum has an oblique posterior margin.

Molecular analysis

The 652 bp COI gene fragments from both *D. macarellus* and *D. macrosoma* were amplified using the F2 and R2 primers, and *D. macarellus* exhibits a higher level of genetic diversity than that of *D. macrosoma*. The haplotype diversity (*h*) and the nucleotide diversity (π) were 0.862 ± 0.067 and 0.0037 ± 0.0023, respectively, for *D. macarellus* from the Eastern Indian Ocean; 0.797 ± 0.086 and 0.0030 ± 0.0019, respectively, for *D. macarellus* from the South China Sea; and 0.486 ± 0.124 and 0.0008 ± 0.0007, respectively, for *D. macrosoma* from the South China Sea. The MST constructed based on the COI sequences of the two fish species (Fig. 2) showed that the two species were distinct, with a significant mutation distance. However, the genetic structure did not correspond to the geological locations observed for individuals of *D. macarellus* in the South China Sea and the Eastern Indian Ocean, and there were only two shared haplotypes, one of which was clearly an ancestral haplotype; all other haplotypes were unique to the two seas.

After annotating and aligning all the sequences retrieved from GenBank and gained in this study, a 534 bp target fragment was obtained that hosted 142 mutation sites, including 24 single-nucleotide polymorphisms, 118 parsimony-informative sites, and no insertions/deletions. The A+T content was 51.7%, slightly higher than the G+C content, revealing an AT preference. The NJ tree was constructed using all studied sequences with *D. maruadsi* and *T. japonicus* as outgroups (Fig. 3). Eight groups were obtained, with genetic distance among groups ranging from 0.031 (between Groups 5 and 6) to 0.198 (between Groups 3 and 8) (Table 4) and genetic distance within

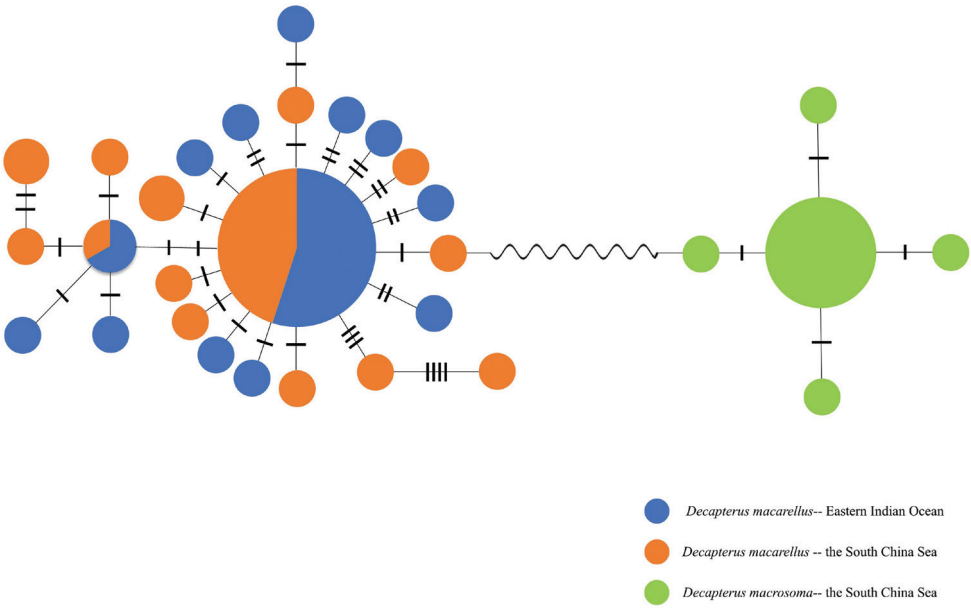

Figure 2. Minimum spanning tree for *D. macarellus* and *D. macrosoma* based on mitochondrial COI sequences.

groups of 0–0.009, consistent with the ten-fold rule between species and genera (Ward et al. 2005), which confirmed that each group is a valid species. After realignment, we found that Group 1 corresponded to *D. macrosoma*, Group 2 to *Decapterus* sp. 2, Group 3 to *Decapterus* sp. 1, Group 4 to *D. macarellus*, Group 5 to *D. russelli*, Group 6 to *D. maruadsi*, Group 7 to *T. japonicus*, and Group 8 to *Selar crumenophthalmus*, indicating that the most barcoding of *D. macarellus* and *D. macrosoma* was correct. Notably, for Groups 2 and 3, the highest similarity of the alignment with sequences from the GenBank database was below 95%, which enabled us to assign the species to the genus *Decapterus* but not to identify the species.

Based on a 1.2% nucleotide divergence rate per million years, we estimated the divergence time of the species (Table 4). The results showed that the genetic divergence time of the eight species was in the range of 2.58–16.50 million years, corresponding to the early Miocene Epoch and late Pliocene Epoch. The earliest differentiation appeared between *S. crumenophthalmus* and *Decapterus* sp. 1, and the latest differentiation appeared between *D. russelli* and *D. maruadsi*.

Discussion

Biodiversity is an important material basis and condition for human survival and sustainable development and usually encompasses species diversity, genetic diversity, ecosystem diversity, and landscape diversity. To study biodiversity, we must first accurately identify the existing species; only with this approach do follow-up studies

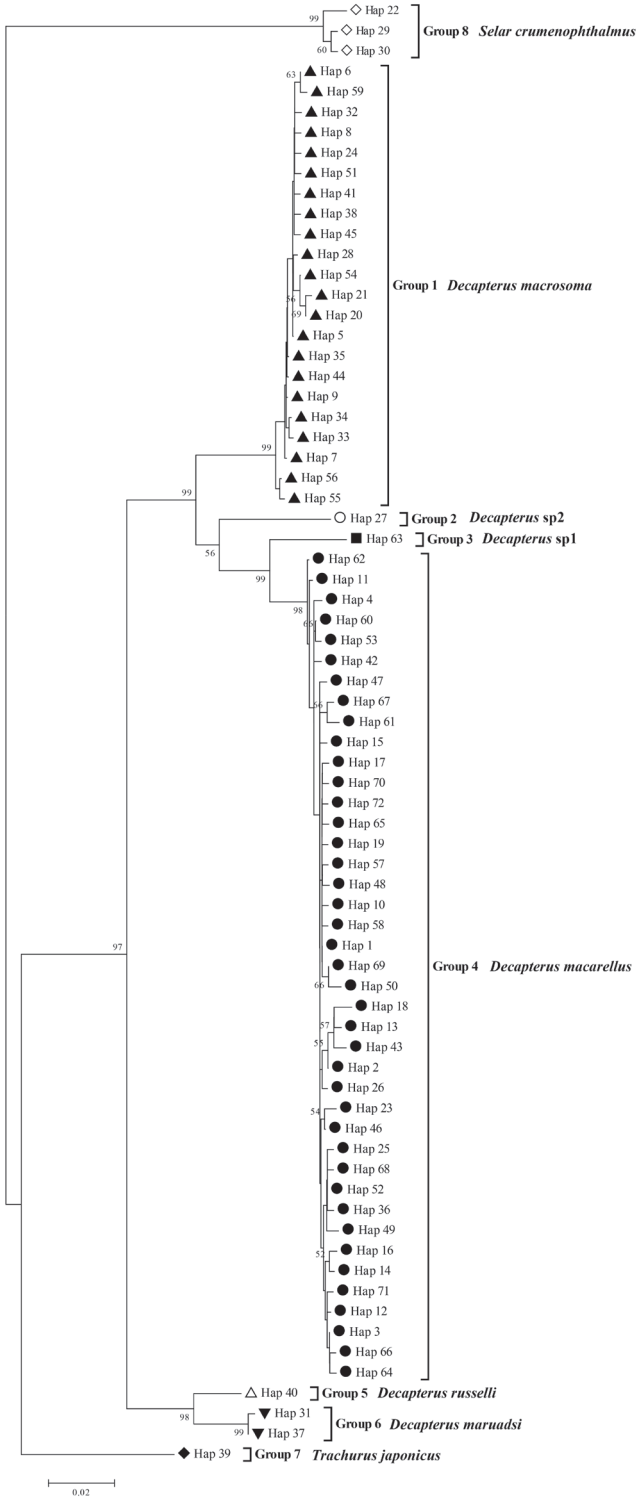

Figure 3. Neighbor-joining tree of detected species based on mitochondrial COI sequences.

Table 3. Comparison of major morphological diagnostic characteristics of *D. macarellus* and *D. macrosoma*.

	D. macarellus	***D. macrosoma***
straight-line portion of the lateral line covered with scutes	posterior end, approximately 1/2	majority in the rear, approximately 3/4
external morphological characteristics of scutes	the highest scute is approximately half the eye diameter	no particular external characteristics
whether the predorsal scaled area reaches the middle of the eye	reaching or extending past	not reaching
shape of the predorsal scales	"∩"	"m"
shape of the posterior end of the maxilla	convex and round	truncated
shape of the posterior margin of the operculum	oblique	straight

Table 4. Genetic distance of COI gene among (below the diagonal) and within (on the diagonal) groups, and the divergence time between groups (above the diagonal).

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
Decapterus macarellus	0.005	5.92	6.33	5.25	7.17	7.67	10.25	14.75
***Decapterus* sp. 2**	0.071	0	5.67	5.17	8.17	7.42	9.92	15.92
***Decapterus* sp. 1**	0.076	0.068	0	3.00	7.92	7.75	10.08	**16.50**
Decapterus macrosoma	0.063	0.062	0.036	0.007	7.50	7.58	11.58	16.00
Decapterus russelli	0.086	0.098	0.095	0.09	0	**2.58**	7.75	14.50
Decapterus maruadsi	0.092	0.089	0.093	0.091	**0.031**	0.002	8.17	14.67
Trachurus japonicus	0.123	0.119	0.121	0.139	0.093	0.098	0	12.33
Selar crumenophthalmus	0.177	0.191	**0.198**	0.192	0.174	0.176	0.148	0.009

Unit of divergence time: millions of years.

make sense. For example, both *D. macrosoma* and *D. macarellus* are economically important species in China, but due to historical reasons, the domestic literature on the identification of these two species has been confused, with the species descriptions from China contradictory to those from international literature. In this study, using samples collected in the Eastern Indian Ocean and the South China Sea, we re-examined the two *Decapterus* species from the perspectives of morphology and molecular genetics and provided their major morphological diagnostic characteristics and correct DNA barcoding.

The comparison of countable and measurable characteristics between the two species showed that most of the characteristics are identical or significantly overlapping, making it impossible to distinguish the two species, whereas some directly observable morphological characteristics allow differentiation of the two species (Cuvier and Valenciennes 1833; Bleeker 1851; Nakabo 2013) (Table 3). These characteristics include the scute coverage of the straight-line portion of the lateral line (the most indicative identification characteristic), the shape of predorsal scaled area and its relative location to the middle axis of the eye, and the shapes of the posterior end of the maxilla and the posterior margin of the operculum, among others, indicating that there are appropriate morphological characteristics that enable rapid and correct classification of the two *Decapterus* species. Therefore, correction of the relevant Chinese literature is needed, supporting the significance of the present study.

The DNA barcoding technique has been repeatedly applied for species identification and has successfully revealed the "cryptic biodiversity" in many taxa (Seidel et al. 2009). In this study, we employed DNA barcoding to reevaluate homologous sequences of *D. macrosoma* and *D. macarellus* and, regrettably, found many errors in

the GenBank database. Among the sequences submitted under a scientific name of *D. macrosoma* or *D. macarellus*, we detected seven valid species, including *D. russelli*, *D. maruadsi*, *S. crumenophthalmus*, *D. kurroides*, etc. Moreover, we were unable to identify *Decapterus* sp. 1 and *Decapterus* sp. 2 to species level, since the barcoding sequences of five of the reported 11 species in the genus *Decapterus* have not yet been submitted to the database. Therefore, it is not possible to determine the species level or exclude the possible presence of cryptic species.

We estimated the timing of divergence within the genus *Decapterus* to be in the early Miocene Epoch to the late Pliocene Epoch based on the COI nucleotide site divergence rate, which provides a rough timeline for the evolution of species in the family Carangidae. The species in Carangidae originated through differentiation via geographical isolation and adaptive evolution during the diffusion process (Cheng et al. 2011). These two evolutionary processes complemented and interacted with each other, such that the species in *Decapterus* gradually adapted to the surrounding environment and ultimately formed the current geographical distribution pattern.

Decapterus macarellus shows significantly higher genetic diversity than *D. macrosoma* and additional mutation characteristics, suggesting that it has higher adaptability, most likely related to its wider distribution. At the level of the COI gene, the genetic differentiation appeared in *P. chinensis* (Li et al. 2019b) was absent in *D. macarellus* from the South China Sea and the Eastern Indian Ocean, indicating that the Sundaland did not block genetic exchange, a result possibly related to the sensitivity of the molecular marker applied in this study and the long-distance migration of the species. We found a large number of unique haplotypes of *D. macarellus* in the two seas, and in the future, we will use more sensitive molecular markers to detect the genetic structure and adaptive evolution of this species in the two seas.

Currently, the shortage of experienced taxonomists capable of completing and updating the descriptions and cataloging work of biodiversity is a major challenge for the scientific community. Species classified by external morphological characteristics are referred to as morphospecies (Primack 2010). It is impossible to correctly classify *D. macrosoma* and *D. macarellus* in China based on morphological characteristics, however, no misidentified sequences corresponding to the morphological classification results were detected among the DNA barcoding data in the NCBI (among which a large number of sequences have been submitted by Chinese investigators from samples collected from various Chinese waters). This is most likely due to DNA barcoding technology maturation and streamlining, which enables investigators to readily obtain targeted sequences that can be aligned with referenced sequences in the database, allowing investigators to overlook the importance of morphology-based classification and instead only refer to data by others.

Initially, species classification primarily depended on the experience of the taxonomist and the accuracy of the literature. However, taxonomists do not necessarily have a background in genetics, whereas geneticists lack expertise in species identification and are unaware of the classification characteristics of the species, resulting in a rift between the two methods. Only by combining the two methods and using DNA barcoding technology as a new identification method enabling the disciplines

to complement each other is it possible to classify species rapidly and accurately based on correctly identified morphological characteristics. For example, by combining morphological characteristics and DNA barcoding technology, Li et al. (2019a) accurately classified the *Pampus* species of the world, proposed classification keys for *Pampus* species, and accurately described the distribution of seven *Pampus* species. Using the same strategy, Li et al. (2018) revealed that the originally described *Gymnothorax reticularis* is actually *G. minor*, which is widely distributed in China's coastal areas, whereas *G. reticularis* is not present in China and is only distributed from the Indian Ocean to the Red Sea. Chen et al. (2018) found that the originally described *Platyrhina tangi* is actually *P. sinensis*, which is present in the coastal area of Zhoushan, China. Therefore, only after correctly identifying a species is it possible to accurately determine the distribution and niche of the species, such that the accuracy of other, related studies can be ensured.

In summary, when identifying fish species, marine biologists need to understand the research status of different taxonomic categories of the fish at home and abroad to ensure the validity of morphological classification. The findings of this study have implications for the classification and evolution of fish species in the genus *Decapterus* and for the conservation of species diversity.

Conclusion

Decapterus macarellus and *D. macrosoma* in the Eastern Indian Ocean and the South China Sea waters were collected and reidentified using morphological and DNA barcoding techniques. The results showed that the morphological diagnostic characteristics of the two species primarily include the scute coverage of the straight portion of the lateral line (the most indicative characteristic for classification), the shape of the predorsal scaled area and its relative location to the middle axis of the eye, and the shapes of the posterior margin of the maxilla and the posterior margin of the operculum. Molecular analysis revealed that both the two species have high genetic diversity, and no genetic differentiation in *D. macarellus* from the South China Sea and the Eastern Indian Ocean was detected. By comparing the COI sequences obtained in this study and those homologous sequences downloaded from GenBank, we speculated that the genus *Decapterus* may include cryptic species and corrected a number of erroneous referenced sequences in the NCBI database.

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Two new *Leptobrachella* species (Anura, Megophryidae) from the Yunnan-Guizhou Plateau, southwestern China

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Abstract

Two new toad species of the genus *Leptobrachella* are described from the Yunnan-Guizhou Plateau of China, based on the combination of molecular and morphological data. The description of *Leptobrachella aspera* Wang, Lyu, Qi & Wang, **sp. nov.** from Huanglianshan Nature Reserve represents the thirteenth *Leptobrachella* species known from Yunnan Province, and the description of *Leptobrachella dorsospina* Wang, Lyu, Qi & Wang, **sp. nov.** from Yushe Forest Park represents the sixth *Leptobrachella* species known from Guizhou Province. These new discoveries further emphasize the extremely high diversity of the *Leptobrachella* toads in these regions.

Keywords

Leptobrachella aspera sp. nov., *Leptobrachella dorsospina* sp. nov., molecular phylogeny, morphology, taxonomy

Introduction

The generic classifications within the family Megophryidae Bonaparte, 1850 have always been controversial. For example, recent comprehensive approaches have produced different taxonomic schemes for the genus *Megophrys* sensu lato Kuhl and Van Hasselt 1822 (Chen et al. 2017; Mahony et al. 2017; Liu et al. 2018; Li et al. 2020b). The taxonomy of another group of megophrid toads are facing the same problem: Chen et al. (2018) presented the first well-resolved phylogenetic hypothesis for the genera *Leptolalax* Dubois, 1983 and *Leptobrachella* Bonaparte, 1850. They tended towards the most conservative "one-genus option" pending the acquisition of additional data by assigning *Leptolalax* as a junior synonym of *Leptobrachella*. Their results also rejected the hypothesis that *Leptolalax* consists of two subgenera as proposed by Delorme et al. (2006) and Dubois et al. (2010). In this context, the genus *Leptobrachella* currently contains 82 species widely distributed from southern China, west to northeastern India, through Indochina to the island of Borneo (Frost 2020). *Leptobrachella* is a species-rich genus of megophrid frogs, and a large number of new species have been discovered in recent years due to the application of integrative taxonomy incorporating detailed morphological, bioacoustic and molecular analyses (Rowley et al. 2016, 2017; Yang et al. 2016; Yuan et al. 2017; Eto et al. 2018; Nguyen et al. 2018; Wang et al. 2019; Chen et al. 2020; Luo et al. 2020; Qian et al. 2020).

During recent field surveys in the Yunnan-Guizhou Plateau of southwestern China, a number of megophrid specimens were collected from Yushe Forest Park in western Guizhou (Fig. 1, site 1) and Huanglianshan Nature Reserve in southern Yunnan (Fig. 1, site 2), respectively. Morphologically, all the specimens can be assigned to the genus "*Leptolalax*" (now a junior subjective synonym of *Leptobrachella*), based on the following characters: (1) small or moderate size, snout-vent length not greater than 60.0 mm, (2) rounded finger tips, the presence of an elevated inner palmar tubercle not continuous to the thumb, (3) presence of macroglands on body including supra-axillary, pectoral, femoral and ventrolateral glands, (4) vomerine teeth absent, (5) tubercles on eyelids present, and (6) anterior tip of snout with whitish vertical bar (Dubois 1983; Matsui 1997, 2006; Lathrop et al. 1998; Delorme et al. 2006; Das et al. 2010). Although their generic allocation is without doubt, some characters of these specimens do not correspond to the diagnoses of any recognized species. Subsequent molecular analysis further revealed that these specimens represent two distinct evolutionary lineages. Considering both the morphological differences and molecular divergences, these specimens are described herein as two new species.

Materials and methods

Sampling

For the molecular analyses, a total of 80 sequences (nine muscle tissue samples was sequenced and 71 sequences obtained from GenBank) were used, including five sequences of the undescribed species from Guizhou, four sequences of the undescribed species

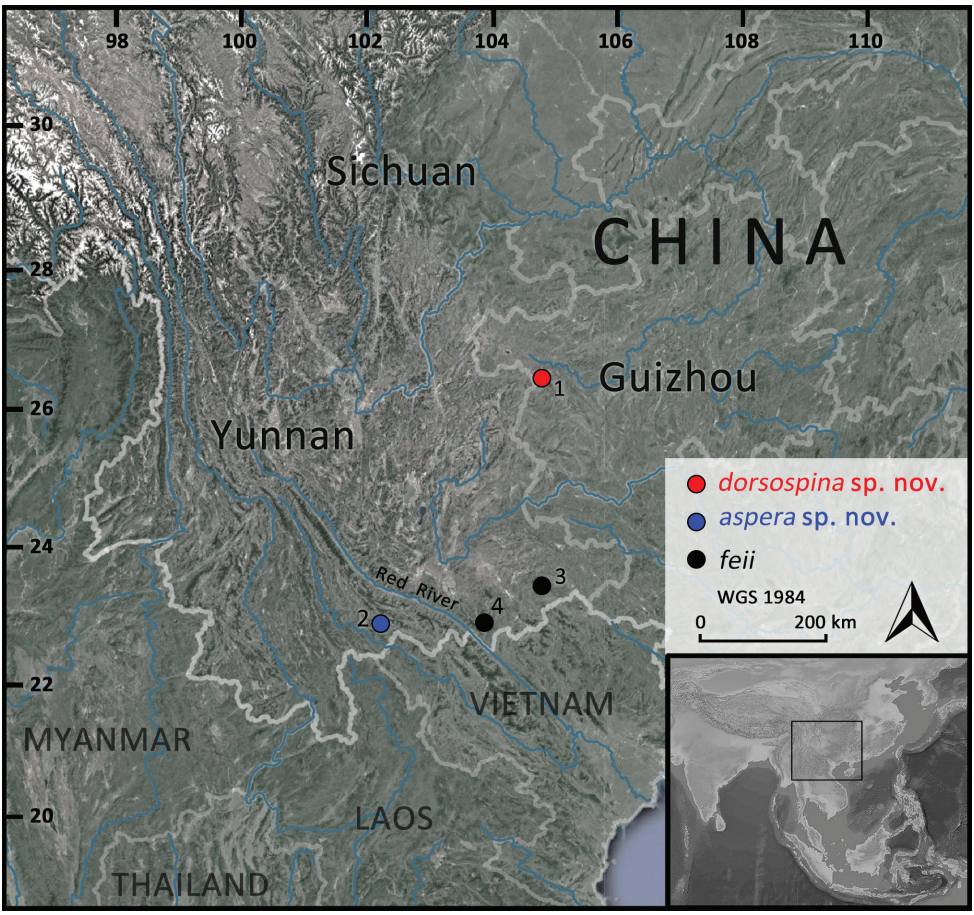

Figure 1. Collection sites. Site 1-Yushe Forest Park, Shuicheng County, Guizhou Province, the type locality of *Leptobrachella dorsospina* sp. nov.; site 2-Huanglianshan Nature Reserve, Lyuchun County, Yunnan Province, the type locality of *L. aspera* sp. nov.; site 3-Xiaoqiaogou Nature Reserve, Xichou County, Yunnan Province, the type locality of *L. feii*; and site 4-Daweishan Nature Reserve, Pingbian County, Yunnan Province, another distribution locality of *L. feii*.

from Yunnan, 69 sequences of 66 recognized congeners, and two out-group sequences of *Oreolalax rhodostigmatus* Hu & Fei, 1979 and *Leptobrachium tengchongensis* Yang & Huang, 2019, respectively (Table 1). Due to the presence of cryptic diversity within genus *Leptobrachella*, we chose sequences from type series or topotype specimens for molecular analysis if available to ensure the taxonomic identity of the species being studied.

DNA Extraction, PCR, and sequencing

DNA was extracted from muscle tissue using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. The mitochondrial gene 16S ribosomal RNA gene (16S rRNA) fragment from each sample was sequenced. Fragments were amplified using the primer

pairs L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3') (Simon et al. 1994). PCR amplifications were performed in a 20 μl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for five min; 35 cycles of denaturing at 95 °C for 40 s, annealing at 53 °C for 40 s and extending at 72 °C for one min; and a final extending step of 72 °C for 10 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer in Shanghai Majorbio Biopharm Technology Co., Ltd. All sequences have been deposited in GenBank (Table 1).

Phylogenetic analyses

Sequences were aligned in Clustal X 2.0 (Thompson et al. 1997) with default parameters. For GenBank sequences which lack information for part of the missing segments, we filled the blank sites with "N". The aligned data was trimmed allowing no gap positions and default parameters in Gblocks version 0.91b (Castresana 2000). lyWe ran Jmodeltest v2.1.2 (Darriba et al. 2012) with Akaike and Bayesian information criteria on the alignment and obtained the best-fitting nucleotide substitution model of GTR + I + G. Phylogenetic analysis was using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012). Two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1000 iterations. The first 25% of samples were discarded as burn-in. Convergence of the Markov Chain Monte Carlo simulations was assessed by PSRF ≤ 0.01 and ESS (effective sample size) value > 200 using Tracer 1.4 (http://tree.bio.ed.ac.uk/software/tracer/). Genetic distances among all *Leptobrachella* samples were calculated in MEGA 6 using the uncorrected *p*-distance model, with pairwise deletion of gaps and missing data.

Table 1. Collection localities, voucher data and GenBank numbers (16S rRNA) for all samples used in this study.

ID	Ingroup	Collection Locality	Voucher No.	GenBank No.
1	*Leptobrachella aspera* sp. nov.	Huanglianshan Nature Reserve, Lyuchun, Yunnan, China	SYS a007743	MW046199
2	*Leptobrachella aspera* sp. nov.	Huanglianshan Nature Reserve, Lyuchun, Yunnan, China	SYS a007744	MW046200
3	*Leptobrachella aspera* sp. nov.	Huanglianshan Nature Reserve, Lyuchun, Yunnan, China	SYS a007745	MW046201
4	*Leptobrachella aspera* sp. nov.	Huanglianshan Nature Reserve, Lyuchun, Yunnan, China	SYS a007746	MW046202
5	*Leptobrachella dorsospina* sp. nov.	Yushe Forest Park, Shuicheng, Guizhou, China	SYS a004961	MW046194
6	*Leptobrachella dorsospina* sp. nov.	Yushe Forest Park, Shuicheng, Guizhou, China	SYS a004962	MW046195
7	*Leptobrachella dorsospina* sp. nov.	Yushe Forest Park, Shuicheng, Guizhou, China	SYS a004973	MW046196
8	*Leptobrachella dorsospina* sp. nov.	Yushe Forest Park, Shuicheng, Guizhou, China	SYS a004974	MW046197
9	*Leptobrachella dorsospina* sp. nov.	Yushe Forest Park, Shuicheng, Guizhou, China	SYS a004975	MW046198
10	*Leptobrachella feii*	Xiaoqiaogou Nature Reserve, Yunnan, China	KIZ032625	MT302635
11	*Leptobrachella feii*	Xiaoqiaogou Nature Reserve, Yunnan, China	KIZ048894	MT302634
12	*Leptobrachella feii*	Xiaoqiaogou Nature Reserve, Yunnan, China	KIZ048972	MT302636
13	*Leptobrachella feii*	Xiaoqiaogou Nature Reserve, Yunnan, China	KIZ048973	MT302637
14	*Leptobrachella aerea*	U Bo, Quang Binh, Vietnam	ZFMK 86362	JN848409
15	*Leptobrachella alpina*	Huangcaoling, Jingdong, Yunnan, China	KIZ046816	MH055866
16	*Leptobrachella applebyi*	Ngoc Linh, Kon Tum, Vietnam	AMS R 173778	KR018108
17	*Leptobrachella arayai*	Mesilau, Sabah, Malaysia	BORNEENSIS 22931	AB847558
18	*Leptobrachella ardens*	Kon Ka Kinh, Gia Lai, Vietnam	AMS R 176463	KR018110

ID	Ingroup	Collection Locality	Voucher No.	GenBank No.
19	*Leptobrachella bidoupensis*	Hon Giao, Lam Dong, Vietnam	NCSM 77321	HQ902883
20	*Leptobrachella bijie*	Zhaozishan Nature Reserve, Guizhou, China	SYS a007320	MK414539
21	*Leptobrachella botsfordi*	Fansipan, Lao Cai, Vietnam	AMS R 176540	MH055953
22	*Leptobrachella bourreti*	Lao Cai, Vietnam	AMS R 177673	KR018124
23	*Leptobrachella chishuiensis*	Chishui, Guizhou Province, China	CIBCS20190518047	MT117053
24	*Leptobrachella crocea*	Kon Tum, Vietnam	AMS R 173740	MH055954
25	*Leptobrachella dringi*	Gunung Mulu National Park, Sarawak, Malaysia	NMBE1056372	KJ831298
26	*Leptobrachella eos*	Long Nai, Phongsaly, Laos	MNHN 2004.0274	JN848452
27	*Leptobrachella firthi*	Ngoc Linh Nature Reserve, Kon Tum, Vietnam	AMS R 176524	JQ739206
28	*Leptobrachella flaviglandulosa*	Xiaoqiaogou Nature Reserve, Yunnan, China	KIZ032626	MT302633
29	*Leptobrachella fritinniens*	Base Camp of Mulu NP, Sarawak, Malaysia	KUHE 55371	AB847557
30	*Leptobrachella fuliginosa*	Phetchaburi, Thailand	KUHE 20174	LC201987
31	*Leptobrachella gracilis*	Camp 1 of Gunung Mulu NP, Sarawak, Malaysia	NMBE1056364	KJ831300
32	*Leptobrachella hamidi*	Bukit Lanjak, Malaysia	KUHE 17545	AB969286
33	*Leptobrachella heteropus*	Larut, Malaysia	KUHE 15486	LC202005
34	*Leptobrachella isos*	Gia Lai, Vietnam	AMS R 176480	KT824769
35	*Leptobrachella kajangensis*	Tioman, Malaysia	LSUHC 4431	LC202001
36	*Leptobrachella kalonensis*	Song Luy, Binh Thuan, Vietnam	AMNH A191762	KR018115
37	*Leptobrachella kecil*	Cameron, Malaysia	KUHE 52440	LC202004
38	*Leptobrachella khasiorum*	Meghalaya, India	SDBDU 2009.329	KY022303
39	*Leptobrachella laui*	Tai Mo Shan, Hongkong, China	SYS a002057	KM014546
40	*Leptobrachella liui*	Guadun, Mt. Wuyi, Fujian, China	SYS a002479	MH605574
41	*Leptobrachella macrops*	Phu Yen, Vietnam	PYU DTD-508	MG787991
42	*Leptobrachella maculosa*	Phuoc Binh, Ninh Thuan, Vietnam	ZFMK 96600	KR018120
43	*Leptobrachella mangshanensis*	Mangshan Nature Reserve, Hunan, China	MSZTC201701	MG132196
44	*Leptobrachella maoershanensis*	Maoershan Nature Reserve, Guangxi, China	KIZ019385	KY986930
45	*Leptobrachella marmorata*	Annah Rais, Padawan, Malaysia	KUHE 53192	AB969287
46	*Leptobrachella maura*	Kinabalu, Malaysia	SP 21450	AB847559
47	*Leptobrachella melanoleuca*	Srat Thani, Thailand	KUHE 19719	LC201990
48	*Leptobrachella melica*	Virachey, Ratanakiri, Cambodia	MVZ 258197	HM133599
49	*Leptobrachella minima*	Changdao, Thailand	KUHE 23733	LC201980
50	*Leptobrachella nahangensis*	Na Hang Nature Reserve, Tuyen Quang, Vietnam	ROM 7035	MH055853
51	*Leptobrachella namdongensis*	Thanh Hoa, Vietnam	VNUF A.2017.95	MK965390
52	*Leptobrachella niveimontis*	Daxueshan Nature Reserve, Yunnan, China	KIZ015734	MT302618
53	*Leptobrachella nyx*	Malipo, Yunnan, China	ROM 35606	MH055814
54	*Leptobrachella oshanensis*	Mt. Emei, Sichuan, China	SYS a001830	KM014810
55	*Leptobrachella pallida*	Gia Rich, Lam Dong, Vietnam	UNS00510	KR018112
56	*Leptobrachella pelodytoides*	Tam Dao, Vinh Phu, Vietnam	MVZ 223642	AY236798
57	*Leptobrachella petrops*	Tuyen Quang, Vietnam	VNMN:2016 A.06	KY459998
58	*Leptobrachella picta*	Gunung Kinabalu National Park, Sabah, Malaysia	UNIMAS 8705	KJ831295
59	*Leptobrachella pluvialis*	Sa Pa, Lao Cai, Vietnam	MNHN: 1999.5675	JN848391
60	*Leptobrachella puhoatensis*	Nghe An, Vietnam	AMS R184852	KY849588
61	*Leptobrachella purpura*	Yingjiang, Yunnan, China	SYS a006531	MG520355
62	*Leptobrachella purpuraventra*	Wujing Nature Reserve, Guizhou, China	SYS a007277	MK414518
63	*Leptobrachella pyrrhops*	Loc Bac, Lam Dong, Vietnam	ZMMU ABV-00176	KP017576
64	*Leptobrachella rowleyae*	Son Tra, Da Nang, Vietnam	ITBCZ 4113	MG682549
65	*Leptobrachella sabahmontana*	Mahua, Crocker, Malaysia	BORNEENSIS 12454	AB847550
66	*Leptobrachella shangsiensis*	Shiwandashan, Guangxi, China	NHMG1401032	MK095460
67	*Leptobrachella sola*	Terengganu, Malaysia	KUHE 52342	LC202011
68	*Leptobrachella suiyangensis*	Suiyang, Guizhou, China	GZNU20180606002	MK829648
69	*Leptobrachella sungi*	Bac Giang, Vietnam	ZMMU-NAP-02269	MH055859
70	*Leptobrachella tadungensis*	Dak Nong, Vietnam	UNS00517	KR018122
71	*Leptobrachella tengchongensis*	Tengchong, Yunnan, China	SYS a004598	KU589209
72	*Leptobrachella tuberosa*	Kon Ka Kinh National Park, Gia Lai, Vietnam	ZMMU-NAP-02275	MH055959
73	*Leptobrachella ventripunctata*	Xishuangbanna, Yunnan, China	SYS a001768	KM014811
74	*Leptobrachella wuhuangmontis*	Mt. Wuhuang, Pubei, Guangxi, China	SYS a003485	MH605577
75	*Leptobrachella wulingensis*	Tianzishan Nature Reserve, Hunan, China	CSUFT 200	MT530317
76	*Leptobrachella yingjiangensis*	Yingjiang, Yunnan, China	SYS a006533	MG520350
77	*Leptobrachella yunkaiensis*	Yunkaishan Nature Reserve, Guangdong, China	SYS a004663	MH605584
78	*Leptobrachella zhangyapingi*	Chiang Mai, Thailand	KIZ07258	MH055864
79	*Leptobrachium tengchongense*	Tengchong, Yunnan, China	SYS a004603	KX066876
80	*Oreolalax rhodostigmatus*	Da Fang, Guizhou, China	CIB ZYCA746	EF397248

Morphometrics

Measurements followed Fei et al. (2009) and Rowley et al. (2013), and were taken with a digital caliper to the nearest 0.1 mm. These measurements were as follows:

SVL snout-vent length (from tip of snout to vent);
HDL head length (from tip of snout to rear of jaws);
HDW head width (head width at commissure of jaws);
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 upper eyelids);
IND internasal distance (distance between nares);
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 (distance from knee to heel);
ML manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
PL pes length (distance from tip of fourth toe to proximal edge of the inner metatarsal 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).

Sex was determined by the presence of internal vocal sac openings, and the presence of eggs in abdomen seen via external inspection.

All specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol for preservation, and deposited at the Museum of Biology, Sun Yat-sen University (**SYS**) and Chengdu Institute of Biology, the Chinese Academy of Sciences (**CIB**), China; tissue samples were preserved in 95% ethanol for molecular studies.

Comparative morphological data of *Leptobrachella* species were obtained from examination of museum specimens (see Appendix 1) and from the references listed in Table 2. Due to the high likelihood of undiagnosed diversity within the genus (Rowley et al. 2016; Yang et al. 2016), where available, we rely on examination of topotypic material and/or original species descriptions.

Results

The BI analyses are shown in Fig. 2 with Bayesian posterior probabilities (BPP) for major nodes > 0.90. Genetic distances among all *Leptobrachella* samples are given in the Suppl. material 1: Table S1. Comparative morphological data of all recognized *Leptobrachella* species occurring north of the Kra Isthmus are listed in Table 3.

Table 2. Data sources of the 82 currently known species of the genus *Leptobrachella*.

ID	*Leptobrachella* species	Literature
1	*L. aerea* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010)	Rowley et al. 2010c
2	*L. alpina* (Fei, Ye & Li, 1990)	Fei et al. 2009, 2016
3	*L. applebyi* (Rowley & Cao, 2009)	Rowley and Cao 2009
4	*L. arayai* (Matsui, 1997)	Matsui 1997
5	*L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
6	*L. baluensis* Smith, 1931	Dring 1983; Eto et al. 2016
7	*L. bijie* Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
8	*L. bidoupensis* (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
9	*L. bondangensis* Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
10	*L. botsfordi* (Rowley, Dau & Nguyen, 2013)	Rowley et al. 2013
11	*L. bourreti* (Dubois, 1983)	Ohler et al. 2011
12	*L. brevicrus* Dring, 1983	Dring 1983; Eto et al. 2015
13	*L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010a
14	*L. chishuiensis* Li, Liu, Wei & Wang, 2020	Li et al. 2020a
15	*L. dringi* (Dubois, 1987)	Inger et al. 1995; Matsui and Dehling 2012
16	*L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
17	*L. feii* Chen, Yuan & Che, 2020	Chen et al. 2020
18	*L. firthi* (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
19	*L. fritinniens* (Dehling & Matsui, 2013)	Dehling and Matsui 2013
20	*L. fuliginosa* (Matsui, 2006)	Matsui 2006
21	*L. flaviglandulosa* Chen, Wang & Che, 2020	Chen et al. 2020
22	*L. fusca* Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
23	*L. gracilis* (Günther, 1872)	Günther 1872; Dehling 2012b
24	*L. hamidi* (Matsui, 1997)	Matsui 1997
25	*L. heteropus* (Boulenger, 1900)	Boulenger 1900
26	*L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015a
27	*L. itiokai* Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
28	*L. juliandringi* Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
29	*L. kajangensis* (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
30	*L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
31	*L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
32	*L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
33	*L. lateralis* (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
34	*L. laui* (Sung, Yang & Wang, 2014)	Sung et al. 2014
35	*L. liui* (Fei & Ye, 1990)	Fei et al. 2009; Sung et al. 2014
36	*L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
37	*L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
38	*L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018)	Hou et al. 2018
39	*L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
40	*L. marmorata* (Matsui, Zainudin & Nishikawa, 2014)	Matsui et al. 2014b
41	*L. maura* (Inger, Lakim, Biun & Yambun, 1997)	Inger et al. 1997
42	*L. melanoleuca* (Matsui, 2006)	Matsui 2006
43	*L. melica* (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010b
44	*L. minima* (Taylor, 1962)	Taylor 1962; Ohler et al. 2011
45	*L. mjobergi* Smith, 1925	Eto et al. 2015
46	*L. nahangensis* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
47	*L. natunae* (Günther, 1895)	Günther 1895
48	*L. namdongensis* Hoang, Nguyen, Luu, Nguyen & Jiang, 2019	Hoang et al. 2019
49	*L. neangi* Stuart & Rowley, 2020	Stuart and Rowley 2020
50	*L. niveimontis* Chen, Poyarkov, Yuan & Che, 2020	Chen et al. 2020
51	*L. nokrekensis* (Mathew & Sen, 2010)	Mathew and Sen 2010
52	*L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
53	*L. oshanensis* (Liu, 1950)	Fei et al. 2009, 2016
54	*L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
55	*L. palmata* Inger & Stuebing, 1992	Inger and Stuebing 1992
56	*L. parva* Dring, 1983	Dring 1983
57	*L. pelodytoides* (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011

ID	*Leptobrachella* species	Literature
58	*L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017a
59	*L. picta* (Malkmus, 1992)	Malkmus 1992
60	*L. platycephala* (Dehling, 2012)	Dehling 2012a
61	*L. pluvialis* (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000, 2011
62	*L. puhoatensis* (Rowley, Dau & Cao, 2017)	Rowley et al. 2017b
63	*L. purpura* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
64	*L. purpuraventra* Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
65	*L. pyrrhops* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
66	*L. rowleyae* (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
67	*L. sabahmontana* (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
68	*L. serasanae* Dring, 1983	Dring 1983
69	*L. shangsiensis* Chen, Liao, Zhou & Mo, 2019	Chen et al. 2019
70	*L. sola* (Matsui, 2006)	Matsui 2006
71	*L. suiyangensis* Luo, Xiao, Gao & Zhou, 2020	Luo et al. 2020
72	*L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
73	*L. tadungensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
74	*L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
75	*L. tengchongensis* (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2016
76	*L. tuberosa* (Inger, Orlov & Darevsky, 1999)	Inger et al. 1999
77	*L. ventripunctata* (Fei, Ye & Li, 1990)	Fei et al. 2009, 2016
78	*L. wuhuangmontis* Wang, Yang & Wang, 2018	Wang et al. 2018
79	*L. wulingensis* Qian, Xia, Cao, Xiao & Yang, 2020	Qian et al. in publication
80	*L. yingjiangensis* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
81	*L. yunkaiensis* Wang, Li, Lyu & Wang, 2018	Wang et al. 2018
82	*L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

As shown by the phylogenetic result, *Leptobrachella* samples from Huanglianshan Nature Reserve are clustered in a distinct and robust monophyletic lineage with strong support (BPP 1.00). This lineage forms the sister taxon to *L. feii* occurring in Xiaoqiaogou Nature Reserve (BPP 1.00). The genetic distances between these two lineages are 3.0–3.4%, which is significantly larger than that among other recognized species (e.g., *p*-distance 2.6% between *L. liui* and *L. mangshanensis*). Detailed morphological examination also reveals a combination of characters that distinguish the specimens of the unnamed lineage from *L. feii* and other known congeners (see taxonomic comparison below). Therefore, based on the molecular and morphological differences, the population from Huanglianshan Nature Reserve is proposed as a new species, *Leptobrachella aspera* sp. nov.

Samples of the other unnamed lineage from Yushe Forest Park, cluster in another distinct and robust monophyletic lineage with strong support (BPP 1.00). This lineage is close to several species occurring in southwestern China, but its specific placement remains unresolved due to the insufficient support values. The smallest genetic distance between this lineage and another congener is 3.5% (vs. *L. purpuraventra*), which is significantly larger than that between other recognized species (e.g., *p*-distance 2.6% between *L. liui* and *L. mangshanensis*). Detailed morphological examination also reveals a combination of characteristics distinguishing the specimens of this lineage from all known congeners (see taxonomic comparison below). Therefore, based on the molecular and morphological differences, the population from Yushe Forest Park is proposed as a new species, *Leptobrachella dorsospina* sp. nov.

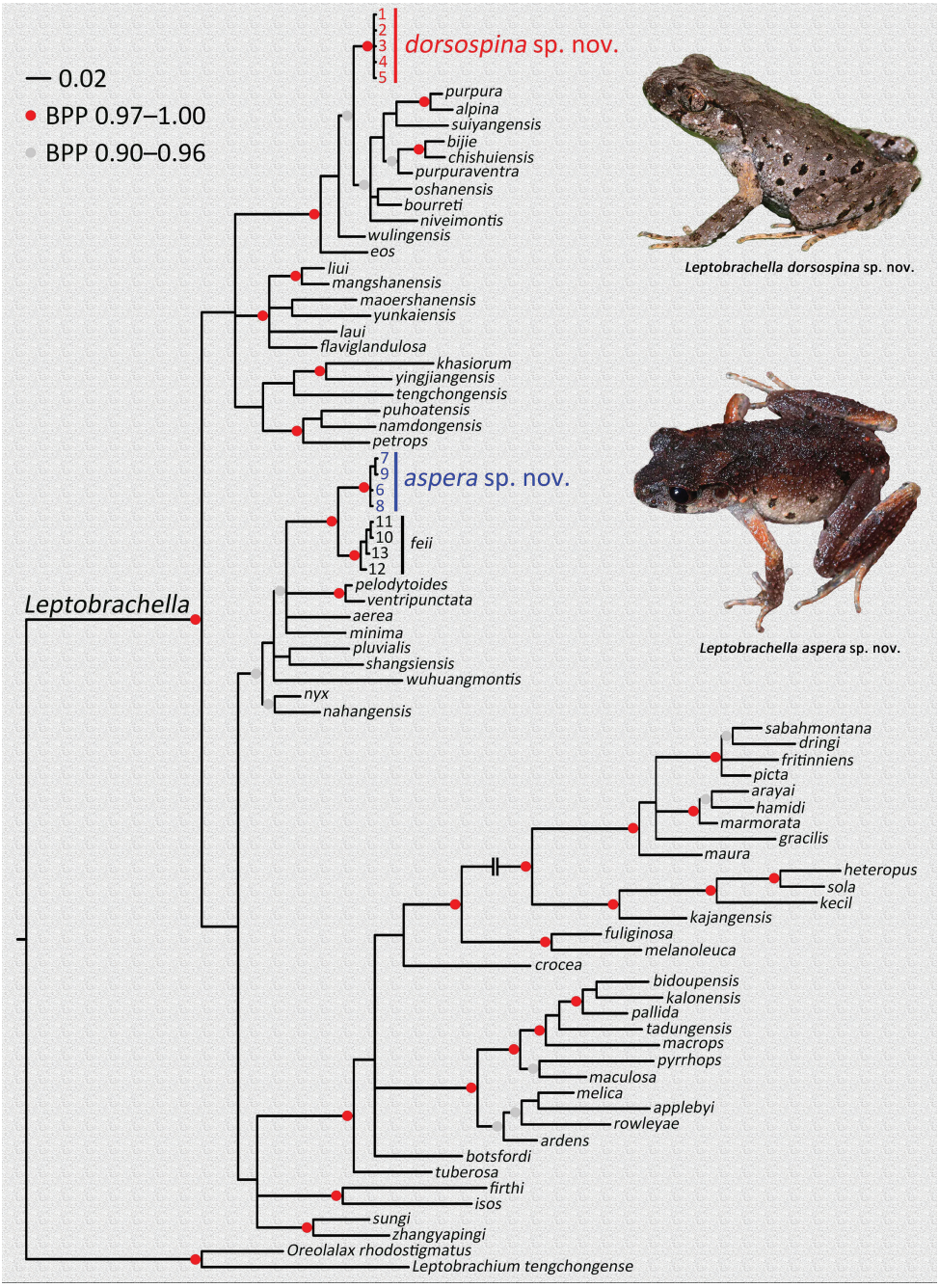

Figure 2. Bayesian Inference tree. The Bayesian posterior probabilities (BPP) > 0.90 were retained.

Table 3. Comparisons of selected diagnostic characters for the new species described herein and congeners occurring north of the Kra Isthmus (modified from Rowley et al. 2017; Wang et al. 2019; Chen et al. 2020).

***Leptobrachella* species**	**Male SVL (mm)**	**Black spots on flanks**	**Toe webbing**	**Toe fringes**	**Ventral coloration**	**Dorsal skin texture**
L. aspera sp. nov.	22.4	Present	Rudimentary	Narrow	Creamy white with distinct dark patches on chest and abdomen	Rough with dense conical granules, tubercles, and glandular folds
L. dorsospina sp. nov.	28.7–30.5	Present	Rudimentary	Narrow	Greyish white with black spots and orange pigmentations	Rough with dense conical granules, tubercles, glandular folds, and conical spines
L. aerea	25.1–28.9	Absent	Rudimentary	Wide	Near immaculate creamy white, brown speckles on margins	Finely tuberculate
L. alpina	24.0–26.4	Present	Rudimentary	Wide in males	Creamy-white with dark spots	Relatively smooth, some with small warts
L. applebyi	19.6–22.3	Present	Rudimentary	Absent	Reddish brown with white speckles	Smooth
L. ardens	21.3–24.7	Present	Absent	Absent	Reddish brown with white speckles	Smooth, finely shagreened
L. bidoupensis	18.5–25.4	Present	Rudimentary	Weak	Reddish brown with white speckles	Smooth
L. bijie	29.0–30.4	Present	Rudimentary	Narrow	White with distinct nebulous greyish speckles on chest and ventrolateral flanks	Shagreened and granular
L. botsfordi	29.1–32.6	Absent	Rudimentary	Narrow	Reddish brown with white speckles	Shagreened
L. bourreti	28.0–36.2	Present	Rudimentary	Weak	Creamy white	Relatively smooth, some with small warts
L. crocea	22.2–27.3	Absent	Rudimentary	Absent	Bright orange	Highly tuberculate
L. chishuiensis	30.8–33.4	Present	Rudimentary	Narrow	White with distinct nebulous greyish speckles on chest and ventrolateral flanks	Shagreened and granular
L. eos	33.1–34.7	Absent	Rudimentary	Wide	Creamy white	Shagreened
L. feii	21.5–22.8	Present	Rudimentary	Narrow	Creamy white with black blotches	Shagreened with small tubercles and ridge
L. firthi	26.4–29.2	Absent	Rudimentary	Wide in males	Creamy white	Shagreened with fine tubercles
L. flaviglandulosa	23.0–27.0	Present	Poorly developed	Narrow	Whitish with black speckles on margins	Shagreened with yellowish-brown tubercles
L. fuliginosa	28.2–30.0	Present	Rudimentary	Weak	White with brown dusting	Nearly smooth with few tubercles
L. isos	23.7–27.9	Absent	Rudimentary	Wide in males	Creamy white with white dusting on margins	Mostly smooth, females more tuberculate
L. kalonensis	25.8–30.6	Present	Absent	Absent	Pale, speckled brown	Smooth
L. khasiorum	24.5–27.3	Present	Rudimentary	Wide	Creamy white	Isolated, scattered tubercles
L. laui	24.8–26.7	Present	Rudimentary	Wide	Creamy white with dark brown dusting on margins	Round granular tubercles
L. liui	23.0–28.7	Present	Rudimentary	Wide	Creamy white with dark brown spots on chest and margins	Round granular tubercles with glandular folds
L. lateralis	26.9–28.3	Present	Rudimentary	Absent	Creamy white	Roughly granular
L. macrops	28.0–29.3	Present	Rudimentary	Absent	Greyish violet with white speckles	Roughly granular with larger tubercles
L. maculosa	24.2–26.6	Present	Absent	Absent	Brown with few white speckles	Mostly smooth
L. mangshanensis	22.2–27.8	Present	Rudimentary	Weak	White speckles on throat and belly	Nearly smooth
L. maoershanensis	25.2–30.4	Present	Rudimentary	Narrow	Creamy white chest and belly with irregular black spots	With longitudinal folds
L. melica	19.5–22.7	Present	Rudimentary	Absent	Reddish brown with white speckles	Smooth
L. minima	25.7–31.4	Present	Rudimentary	Absent	Creamy white	Smooth
L. nahangensis	40.8	Present	Rudimentary	Absent	Creamy white with light speckles on throat and chest	Smooth
L. niveimontis	22.5–23.6	Present	Rudimentary	Narrow	Marbling with black speckles	Relatively smooth with small tubercles
L. nokrekensis	26.0–33.0	Present	Rudimentary	Unknown	Creamy white	Tubercles and longitudinal folds
L. nyx	26.7–32.6	Present	Rudimentary	Absent	Creamy white with brown margins	Rounded tubercles
L. namdongensis	30.9	Present	Rudimentary	Absent	Immaculate white, chest and belly with dark specking on outer margins	Low, round tubercles, more dense in posterior part of the back

Leptobrachella species	Male SVL (mm)	Black spots on flanks	Toe webbing	Toe fringes	Ventral coloration	Dorsal skin texture
L. neangi	-	Present	Weak (in females)	Absent (in females)	Light purplish gray with dark brown mottling on throat	Small, irregular bumps and ridges
L. oshanensis	26.6–30.7	Present	Absent	Absent	Whitish with no markings or only small, light grey spots	Smooth with few glandular ridges
L. pallida	24.5–27.7	Absent	Absent	Absent	Reddish brown with white speckles	Tuberculate
L. pelodytoides	27.5–32.3	Present	Wide	Narrow	Whitish	Small, smooth warts
L. petrops	23.6–27.6	Absent	Absent	Narrow	Immaculate creamy white	Highly tuberculate
L. pluvialis	21.3–22.3	Present	Rudimentary	Absent	Dirty white with dark brown marbling	Smooth, flattened tubercles on flanks
L. puhoatensis	24.2–28.1	Present	Rudimentary	Narrow	Reddish brown with white dusting	With longitudinal skin ridges
L. purpura	25.0–27.5	Present	Rudimentary	Wide	Dull white with indistinct grey dusting	Shagreen with small tubercles
L. purpuraventra	27.3–29.8	Present	Rudimentary	Narrow	Grey purple with distinct nebulous greyish speckles on chest and ventrolateral flanks	Shagreened with granules
L. pyrrhops	30.8–34.3	Present	Rudimentary	Absent	Reddish brown with white speckles	Slightly shagreened
L. rowleyae	23.4–25.4	Present	Absent	Absent	Pinkish milk-white to light brown chest and belly with numerous white speckles	Smooth with numerous tiny tubercles
L. suiyangensis	28.7–29.7	Present	Rudimentary	Narrow	Yellowish creamy-white with marble texture chest and belly or with irregular light brown speckles	Shagreen with small granules
L. sungi	48.3–52.7	Absent or small	Wide	Weak	White	Granular
L. tadungensis	23.3–28.2	Present	Absent	Absent	Reddish brown with white speckles	Smooth
L. tamdil	32.3	Present	Wide	Wide	White	Weakly tuberculate
L. tengchongensis	23.9–26.0	Present	Rudimentary	Narrow	White with dark brown blotches	Shagreened with small tubercles
L. tuberosa	24.4–29.5	Absent	Rudimentary	Absent	White with small grey spots/streaks	Highly tuberculate
L. ventripunctata	25.5–28.0	Present	Rudimentary	Absent	Chest and belly with dark brown spots	Longitudinal skin ridges
L. wuhuangmontis	25.6–30.0	Present	Rudimentary	Narrow	Greyish white mixed by tiny white and black dots	Rough, scattered with dense conical tubercles
L. wulingensis	22.7–30.5	Present	Rudimentary	Narrow	Translucent creamy white, with distinct or indistinct brown speckles at margins	Shagreened with sparse large warts, some with longitudinal ridges
L. yingjiangensis	25.7–27.6	Present	Rudimentary	Wide	Creamy white with dark brown flecks on chest and margins	Shagreened with small tubercles
L. yunkaiensis	25.9–29.3	Present	Rudimentary	Wide	Belly pink with distinct or indistinct speckles	Shagreened with short skin ridges and raised warts
L. zhangyapingi	45.8–52.5	Absent	Rudimentary	Wide	Creamy-white with brown margins	Mostly smooth with distinct tubercles

Taxonomic accounts

***Leptobrachella aspera* Wang, Lyu, Qi & Wang, sp. nov.**
http://zoobank.org/4919B18E-B0D0-4329-90BF-8AC77280D263
Fig. 3

Type material. ***Holotype*.** SYS a007743, adult male, collected by Jian Wang, Yao Li and Yu-Long Li on 31 May 2019 from Huanglianshan Nature Reserve (22.89°N, 102.29°E; ca. 1930 m a.s.l.), Lyuchun County, Yunnan Province, China.

Paratypes (N = 3). Three adult females, SYS a007744–7745, SYS a007746/CIB116080, the same collection data as the holotype.

Diagnosis. (1) Small size (SVL 22.4 mm in a single adult male, 25.0–26.4 in three adult females), (2) dorsal skin rough, with dense conical granules, tubercles

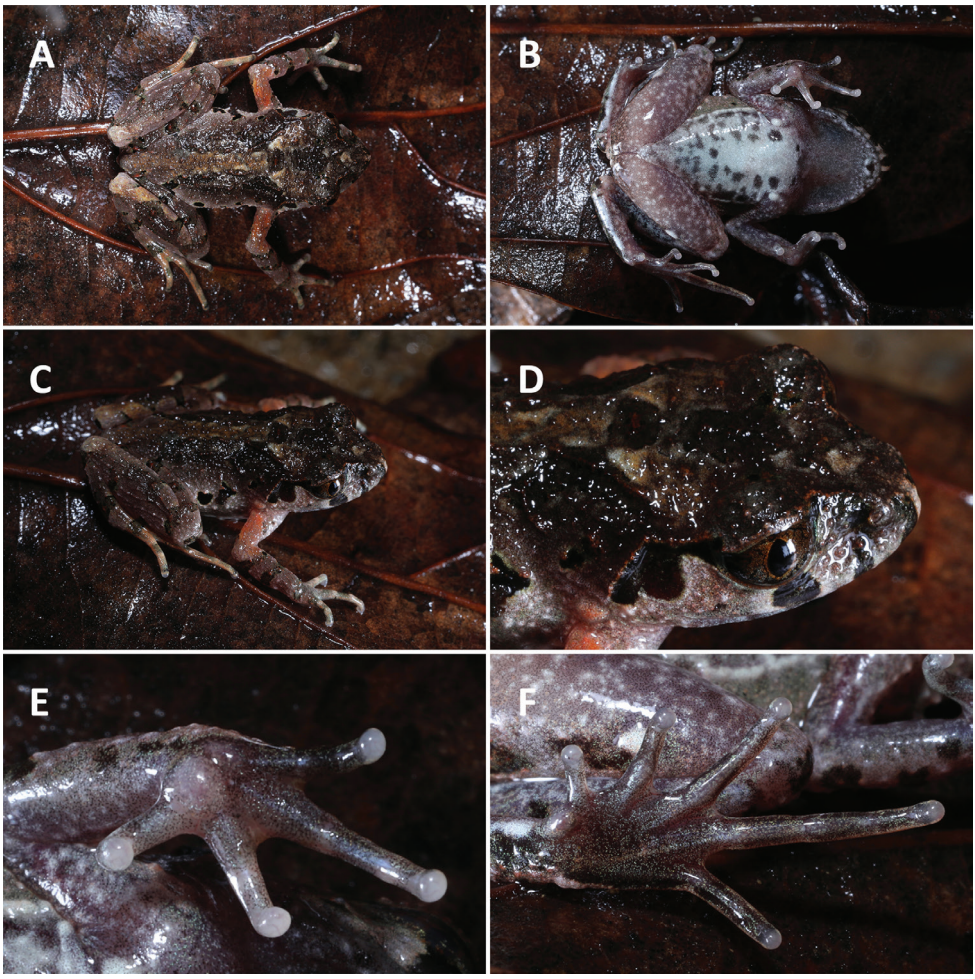

Figure 3. Morphological features in life. *Leptobrachella aspera* sp. nov., holotype SYS a007743.

and glandular folds, (3) iris bicolored, amber on upper half and silver on lower half, (4) tympanum distinctly discernible, distinct black supratympanic line present, (5) absence of webbing and lateral fringes on fingers, toes with rudimentary webbing and narrow lateral fringes both in males and females, (6) longitudinal ridges under toes not interrupted at the articulations, (7) relative finger lengths I < IV < II < III, relative toe length I < II < V < III < IV, (8) heels just meeting, tibia-tarsal articulation reaches the region between middle of eye to anterior corner of eye, (9) dorsum greyish brown to yellowish brown grounding, with small light orange granules and distinct darker brown markings scattered with irregular light orange or greyish white pigmentations, (10) flanks with several enlarged dark patches with light yellowish green margin, (11) ventral surface creamy white, with distinct regular dark patches on chest and abdomen.

Comparison. From the 26 known congeners of the genus *Leptobrachella* occurring south of the Kra Isthmus, the presence of supra-axillary and ventrolateral glands, can easily distinguish *L. aspera* sp. nov. from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana* and *L. sola*, all of which lack the supra-axillary and ventrolateral glands; and by the significantly larger body size, SVL 22.4 mm in a single male, *L. aspera* sp. nov. differs from the smaller *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. bondangensis* (17.8 mm in male), *L. fusca* (16.3 mm in male), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males), *L. mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in one adult male), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males), and *L. serasanae* (16.9 mm in female).

Leptobrachella aspera sp. nov. is recovered as a sister taxon to *L. feii* in the phylogenetic tree (Fig. 2). However, the new species can be distinguished from *L. feii* by the following morphological characters: head relatively short, HDL/SVL 0.33–0.35 (vs. head relatively long, HDL/SVL 0.38–0.43); distinct regular dark patches on skin of chest and abdomen (vs. irregular black blotches scattered on skin of chest and belly); color of upper half of iris amber (vs. color of upper half of iris lighter, golden orange); ventrolateral glands forming an non-continuous line (vs. ventrolateral glands forming a continuous line); relative finger lengths I < IV < II < III (vs. relative finger lengths II < I < IV < III); tibio-tarsal articulation of adpressed limb reaching the region between middle of eye to anterior corner of eye (vs. tibio-tarsal articulation of adpressed limb reaching beyond eye).

While *Leptobrachella pluvialis* is distributed in the same mountain range on the Vietnamese side and possesses similar body size (Ohler et al. 2000), it can be separated from *L. aspera* sp. nov. by the following characters: (1) smooth dorsal skin with flattened tubercles on flanks (vs. rough dorsal skin with dense conical granules in *L. aspera* sp. nov.), (2) absence of webbing on toes (vs. rudimentary webbing on toes in *L. aspera* sp. nov.), and (3) relatively longer hindlimbs, the tibia-tarsal articulation reaching to the nostril (vs. relatively shorter hindlimbs, the tibia-tarsal articulation reaching the region between middle of eye to anterior corner of eye in *L. aspera* sp. nov.).

For the remaining 54 members of the genus *Leptobrachella*, in having SVL 22.4 mm in a single male, *L. aspera* sp. nov. differs from the larger *L. aerea* (25.1–28.9 in males), *L. alpina* (24.0–28.9 mm in males), *L. bijie* (29.0–30.4 mm in males), *L. botsfordi* (29.1–32.6 mm in males), *L. bourreti* (28.0–36.2 mm in males), *L. chishuiensis* (30.8–33.4 in males), *L. eos* (33.1–34.7 mm in males), *L. firthi* (26.4–29.2 mm in males), *L. flaviglandulosa* (23.0–27.0 mm in males), *L. fuliginosa* (28.2–30.0 mm in males), *L. isos* (23.7–27.9 mm in males), *L. kalonensis* (25.8–30.6 mm in males), *L. khasiorum* (24.5–27.3 mm in males), *L. laui* (24.8–26.7 mm in males), *L. lateralis* (26.9–28.3 mm in males), *L. macrops* (28.0–29.3 mm in males), *L. maculosa* (24.2–26.6 mm in males), *L. minima* (25.7–31.4 mm in males), *L. nahangensis* (40.8 mm in male), *L. nokrekensis* (26.0–33.0 mm in males), *L. nyx* (26.7–32.6 mm in males), *L. neangi* (30.9 mm in male), *L. namdongensis* (30.9 mm in male), *L. oshanensis* (26.6–30.7 mm in males), *L. pallida*

(24.5–27.7 mm in males), *L. pelodytoides* (27.5–32.3 mm in males), *L. petrops* (23.6–27.6 mm in males), *L. puhoatensis* (24.2–28.1 mm in males), *L. purpura* (25.0–27.5 mm in males), *L. purpuraventra* (27.3–29.8 mm in males), *L. pyrrhops* (30.8–34.3 mm in males), *L. rowleyae* (23.4–25.4 mm in males), *L. suiyangensis* (28.7–29.7 mm in males), *L. sungi* (48.3–52.7 mm in males), *L. tadungensis* (23.3–28.2 mm in males), *L. tamdil* (32.3 mm in male), *L. tengchongensis* (23.9–26.0 mm in males), *L. tuberosa* (24.4–29.5 mm in males), *L. ventripunctata* (25.5–28.0 mm in males), *L. wuhuangmontis* (25.6–30.0 mm in males), *L. yingjiangensis* (25.7–27.6 mm in males), *L. yunkaiensis* (25.9–29.3 mm in males), and *L. zhangyapingi* (45.8–52.5 mm in males). By presence of black spots on flanks, the new species can be distinguished from *L. crocea*, versus absence of black spots on flanks; by rudimentary webbing on toes, the new species can be distinguished from *L. ardens*, versus absence of webbing on toes; by narrow lateral fringes on toes, the new species can be distinguished from *L. applebyi*, *L. ardens*, *L. crocea*, and L. melica, all having no lateral fringes on toes, and from *L. liui*, having wide lateral fringes on toes; by the creamy white ventral coloration and distinct regular dark patches on the chest and abdomen, the new species can be distinguished from *L. applebyi*, *L. ardens*, *L. bidoupensis*, and *L. melica*, all having reddish brown ventral coloration with white specks, from *L. crocea*, having bright orange ventral coloration, from *L. mangshanensis*, lacking dark skin patches on the chest and abdomen, from *L. liui*, having creamy white ventral coloration with dark brown spots on the chest and margins, and from *L. niveimontis*, having marbling ventral coloration with black speckling; by rough dorsal skin with dense conical granules, tubercles and glandular folds, the new species can be distinguished from *L. applebyi*, *L. ardens*, *L. bidoupensis*, *L. mangshanensis*, *L. melica*, and *L. niveimontis*, all having smooth dorsal skin.

Description of holotype. Adult male. Body size small, SVL 22.4 mm. Head length slightly larger than head width, HDW/HDL 0.99; snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance greater than interorbital distance, IND/IOD 1.07; pineal ocellus absent; pupil vertical; snout length longer than eye diameter, SNT/EYE 1.26; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye and larger than tympanum-eye distance, TMP/EYE 0.52 and TEY/TMP 0.44; upper margin of tympanum in contact with supratympanic ridge; distinct black supratympanic line present; vomerine teeth absent; vocal sac openings slit-like, paired, located posterolaterally on floor of mouth in close proximity to the margins of the mandible; tongue deeply notched posteriorly; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths I < IV < II < III; nuptial pad absent; subarticular tubercles absent; large, rounded inner palmar tubercle distinctly separated from small, rounded outer palmar tubercle; webbing and lateral fringes on fingers absent. Tips of toes rounded, slightly swollen; relative toe length I < II < V < III < IV; subarticular tubercles absent; distinct longitudinal dermal ridges present under the 3rd to 5th toes, not interrupted; large, oval inner metatarsal tubercle

present, outer metatarsal tubercle absent; toes webbing rudimentary; narrow lateral fringes present on all toes. Tibia 47% of snout-vent length; tibiotarsal articulation reaching to anterior corner of eye; heels slightly overlapping when thighs are appressed at right angles with respect to body.

Dorsal skin rough, with dense conical granules, tubercles and glandular folds; ventral skin smooth; sparse tiny tubercles present on surface of chest; pectoral gland and femoral gland oval; the size of pectoral glands almost equal to tips of fingers and femoral glands; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary glands raised. Ventrolateral glands distinctly visible, raised, forming an incomplete line.

Coloration of holotype in life. Dorsum greyish brown with small light orange granules, distinct darker brown markings scattered with irregular light orange and greyish white pigmentations. A dark brown inverted triangular pattern between the anterior corners of the eyes in connection with a dark brown W-shaped marking in the interorbital region, which is also connected to a W-shaped marking between the axillae. Tympanum dark brown. Small light orange granules present on dorsum of body and limbs; a dark brown blotch under the eye; transverse dark brown bars present on dorsal surface of limbs and digits; distinct dark brown patches with light yellowish green margin on flanks from groin to axilla; elbow and upper arms with distinct coppery orange coloration.

Ventral surface of throat, chest, and belly creamy white; presence of distinct nebulous greyish speckles present on throat, and distinct dark patches on chest and abdomen; ventral surface of limbs greyish purple, scattered with greyish white spots and small patches. Supra-axillary gland coppery orange; femoral, pectoral, and ventrolateral glands greyish white. Iris bicolored, amber on upper half and silver on lower half.

Coloration of holotype in preservative (Fig. 4A). Dorsum of body and limbs dark brown; transverse bars on limbs become more distinct; dark brown patterns, markings and spots on the back become indistinct, orange pigmentations become dark brown, greyish white pigmentations become dark grey. Ventral surface of limbs and surface of throat light brown, surface of abdomen greyish white, nebulous speckles on throat absent, dark patches on chest, abdomen and flanks become more distinct, light yellowish green margin of patches on flanks absent. Supra-axillary, femoral, pectoral, and ventrolateral glands greyish white.

Variation. Measurements and body proportions are listed in Table 4. Nonsexual characters of all the female paratypes (SYS a007744, 7745, 7746) match the overall characters of the holotype except that: the dorsum is greyish brown in the holotype SYS a007743 (vs. yellowish brown in the paratypes); the size of the pectoral glands are almost equal to the tips of the fingers and the femoral glands (vs. the size of the pectoral glands are larger than the tips of fingers and the femoral glands in the paratypes); the tibia-tarsal articulation reaches forward to the anterior corner of the eye in the holotype (vs. the tibia-tarsal articulation reaches forward to the middle of the eye in the paratypes SYS a007745, 7746); the ventral skin of the thighs smooth (vs. the ventral skin of the thighs rough with dense raised tubercles in the paratypes).

Figure 4. Morphological features in preserved specimens of **A** *Leptobrachella aspera* sp. nov., holotype SYS a007743 **B** *Leptobrachella dorsospina* sp. nov., holotype SYS a004974. Ellipse selected region showing the tiny spines on dorsal skin.

Table 4. Measurements and body proportions of *Leptobrachella aspera* sp. nov.

Voucher	SYS a 007743	SYS a 007744	SYS a 007745	SYS a 007746
Sex	Male	Female	Female	Female
SVL	22.4	25.3	25.0	26.4
HDL	8.1	9.5	9.5	9.6
HDW	8.0	9.3	9.2	9.0
SNT	3.7	3.8	3.8	3.4
IND	2.5	2.3	2.7	2.7
IOD	2.3	2.5	2.5	2.5
EYE	2.9	3.2	3.2	3.1
TMP	1.5	1.8	1.9	1.6
TEY	0.7	1.0	1.0	0.8
ML	5.9	7.0	6.6	6.3
LAHL	11.2	13.5	12.7	12.6
PL	10.1	11.7	10.2	11.1
TIB	10.6	12.4	11.9	11.9
HLL	34.4	41.5	40.4	39.1
HDL/SVL	0.36	0.37	0.38	0.36
HDW/SVL	0.36	0.37	0.37	0.34
HDW/HDL	0.99	0.98	0.97	0.94
SNT/HDL	0.16	0.15	0.15	0.13
IND/HDW	0.31	0.25	0.29	0.30
IOD/HDW	0.29	0.27	0.27	0.28
IND/IOD	1.07	0.91	1.08	1.09
EYE/HDL	0.36	0.34	0.34	0.32
TMP/EYE	0.52	0.56	0.60	0.51
ML/SVL	0.26	0.28	0.26	0.24
LAHL/SVL	0.50	0.53	0.51	0.48
PL/SVL	0.45	0.46	0.41	0.42
TIB/SVL	0.47	0.49	0.48	0.45
HLL/SVL	1.53	1.64	1.61	1.48

Etymology. The specific epithet, *aspera*, is a Latin adjective which means rough, in reference to the dorsal skin texture of the new species. According to its type locality, we suggest its English common name as "Huanglianshan Leaf Litter Toad", and the Chinese name "Huang Lian Shan Zhang Tu Chan (黄连山掌突蟾)".

Distribution and habits. Currently, *Leptobrachella aspera* sp. nov. is known only from its type locality Huanglianshan Nature Reserve, near the border between China and Vietnam. The new species was found along a drainage ditch of a mountainous road. The road was surrounded by broad-leaved forest at an altitude ca. 1930 m and not close to any hillstreams. Males were not heard calling during the field survey from 31 May to 1 June 2019.

Leptobrachella dorsospina Wang, Lyu, Qi & Wang, sp. nov.

http://zoobank.org/B0EA8FA8-0193-43BF-AA93-6D010467CF84
Fig. 5

Type material. ***Holotype*.** SYS a004974, adult male, collected by Zhi-Tong Lyu and Run-Lin Li on 21 June 2016 from Yushe Forest Park (26.47°N, 104.80°E; ca. 2100 m a.s.l.), Shuicheng District, Liupanshui City, Guizhou Province, China.

Paratypes (N = 6). An adult male, SYS a004977, and five adult females, SYS a004961/CIB116081, SYS a 004962, SYS a004973, 4975, 4976, collected by Zhi-Tong Lyu and Run-Lin Li on 20–21 June 2016 from the same locality as the holotype.

Diagnosis. (1) Small size (SVL 28.7–30.5 mm in two adult males, 32.1–39.8 mm in five adult females), (2) dorsal skin rough, with dense conical granules, tubercles, glandular folds and conical spines, (3) iris bicolored, light orange on upper half and silver on lower half, (4) tympanum distinctly discernible, distinct black supratympanic line present, (5) absence of webbing and lateral fringes on fingers, toes with rudimentary webbing and narrow lateral fringes both in males and females, (6) longitudinal ridges under toes interrupted at the articulations, (7) relative finger lengths II = IV < I < III, relative toe length I < II < V < III < IV, (8) heels slightly overlapping, tibia-tarsal articulation reaches forward to the posterior corners of eyes, (9) dorsum greyish brown to dark brown grounding, with distinct darker brown markings and scattered with irregular light greyish brown pigmentations and yellowish brown spots, (10) flanks with several enlarged dark patches positioned longitudinally in two rows, (11) ventral surface greyish white with black spots and orange pigmentations.

Comparison. Compared with the 26 known congeners of the genus *Leptobrachella* occurring south of the Kra Isthmus, *L. dorsospina* sp. nov. can be easily distinguished by the presence of supra-axillary and ventrolateral glands, from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana* and *L. sola*, all of which are lacking the supra-axillary and ventrolateral glands; and by the significantly larger body size, SVL 28.7–30.5 mm in two adult male, *L. dorsospina* sp. nov. differs from the smaller *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. bondangensis* (17.8 mm in male), *L. fusca* (16.3 mm in male), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males), *L. mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in one adult male), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males), and *L. serasanae* (16.9 mm in female).

Leptobrachella dorsospina sp. nov. can be easily distinguished from *Leptobrachella aspera* sp. nov. by having distinctly larger body size, SVL 28.7–30.5 mm in males, 32.1–39.8 mm in females (vs. SVL 22.4 mm in male, 25.0–26.4 in females); conical spines on dorsal skin present (vs. absent); black spots on flanks in one row (vs. black spots on flanks in two rows); ventral skin greyish white with black spots and orange pigmentations (vs. ventral skin creamy white with distinct dark patches on chest and abdomen); longitudinal ridges under toes interrupted at the articulations (longitudinal ridges under toes not interrupted at the articulations).

For the remaining 56 members of the genus *Leptobrachella*, in having SVL 28.7–30.5 mm in two males, *L. dorsospina* sp. nov. differs from the larger *L. eos* (33.1–34.7 mm in males), *L. nahangensis* (40.8 mm in male), *L. sungi* (48.3–52.7 mm in males), *L. tamdil* (32.3 mm in male), and *L. zhangyapingi* (45.8–52.5 mm in males); and from the smaller *L. alpina* (24.0–26.4 mm in males), *L. applebyi* (19.6–22.3 mm in males), *L. ardens* (21.3–24.7 mm in males), *L. bidoupensis* (18.5–25.4 mm in males), *L. crocea* (22.2–27.3 mm in males), *L. feii* (21.5–22.8 mm in males), *L. flaviglandulosa* (23.0–27.0 mm in males), *L. isos* (23.7–27.9 mm in males), *L. khasiorum* (24.5–

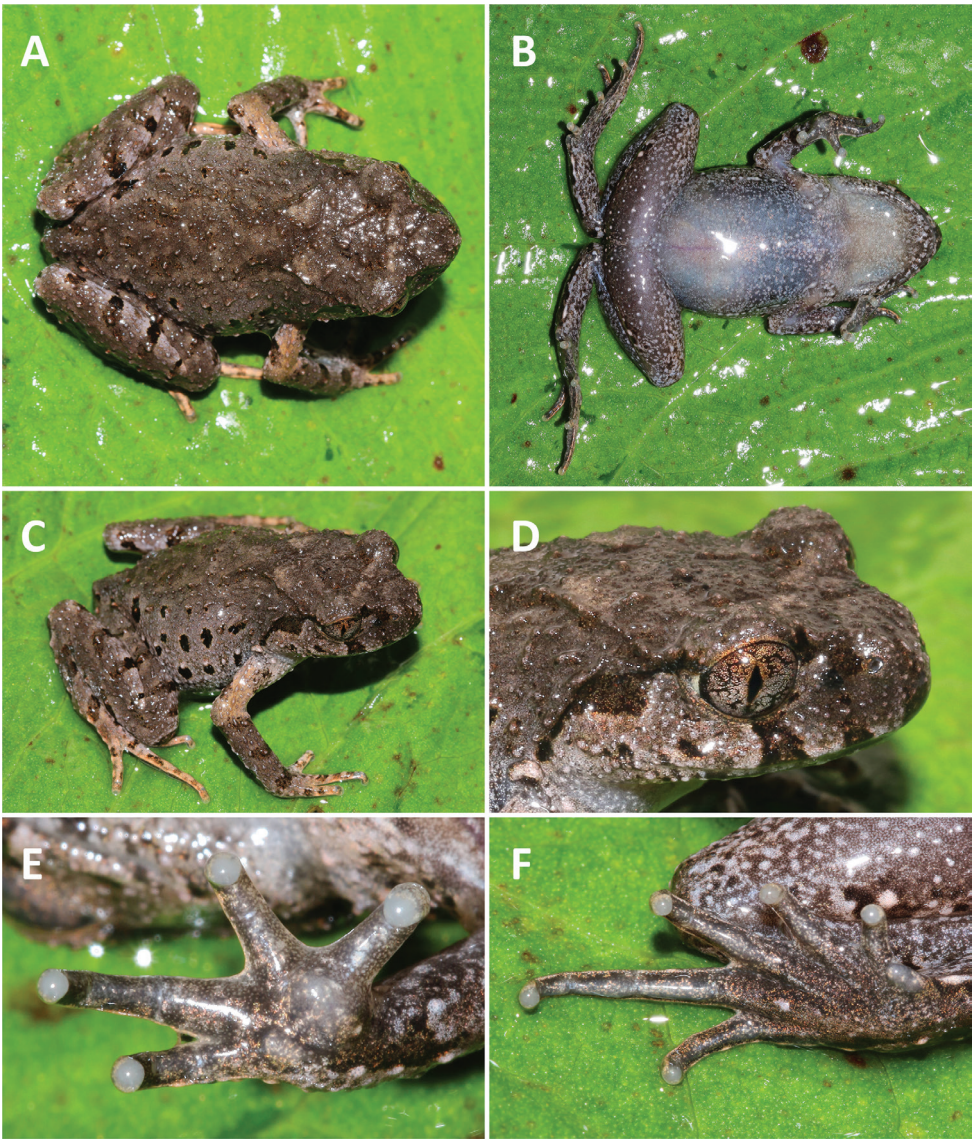

Figure 5. Morphological features in life. *Leptobrachella dorsospina* sp. nov., holotype SYS a004974.

27.3 mm in males), *L. laui* (24.8–26.7 mm in males), *L. maculosa* (24.2–26.6 mm in males), *L. mangshanensis* (22.2–27.8 mm in males), *L. melica* (19.5–22.7 mm in males), *L. niveimontis* (22.5–23.6 mm in males), *L. pallida* (24.5–27.7 mm in males), *L. petrops* (23.6–27.6 mm in males), *L. pluvialis* (21.3–22.3 mm in males), *L. puhoatensis* (24.2–28.1 mm in males), *L. purpura* (25.0–27.5 mm in males), *L. rowleyae* (23.4–25.4 mm in males), *L. tadungensis* (23.3–28.2 mm in males), *L. tengchongensis* (23.9–26.0 mm in males), *L. ventripunctata* (25.5–28.0 mm in males), and *L. yingjiangensis* (25.7–27.6 mm in males). By having black spots on the flanks, *L. dorsospina* sp. nov. can be distinguished from *L. aerea*, *L. botsfordi*, *L. firthi*, and *L. tuberosa*, all of which

lack black spots on the flanks. By having rough dorsal skin with conical spines, the new species can be distinguished from *L. bijie*, *L. chishuiensis*, *L. liui*, *L. maoershanensis*, *L. pyrrhops*, *L. purpuraventra*, *L. suiyangensis*, *L. wuhuangmontis*, *L. wulingensis*, and *L. yunkaiensis* (dorsal skin lacking spines); and from *L. bourreti*, *L. fuliginosa*, *L. kalonensis*, *L. minima*, *L. oshanensis*, and *L. pelodytoides* (dorsal skin smooth). By having narrow lateral fringes on the toes, the new species can be distinguished from *L. lateralis*, *L. macrops*, *L. nyx*, *L. pyrrhops*, *L. namdongensis* and *L. neangi*, all of which lack lateral fringes on the toes. The new species can be separated from the remaining *L. nokrekensis* by having greyish white ventral coloration with black patches and orange pigmentations (vs. creamy white), and having dense short glandular folds on the dorsal surface (vs. only a few glandular folds on the dorsal surface).

Description of holotype. Adult male. Body size rather small, SVL 30.5 mm. Head length slightly larger than head width, HDW/HDL 0.99; snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance smaller than interorbital distance, IND/IOD 0.91; pineal ocellus absent; vertical pupil; snout length larger than eye diameter, SNT/EYE 1.29; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye and larger than tympanum-eye distance, TMP/EYE 0.43 and TEY/TMP 0.50; upper margin of tympanum in contact with supratympanic ridge; distinct black supratympanic line present; vomerine teeth absent; vocal sac openings slit-like, paired, located posterolaterally on floor of mouth in close proximity to the margins of the mandible; tongue deeply notched posteriorly; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths II = IV < I < III; nuptial pad absent; subarticular tubercles absent; large, rounded inner palmar tubercle distinctly separated from small, rounded outer palmar tubercle; absence of webbing and lateral fringes on fingers. Tips of toes rounded, slightly swollen; relative toe length I < II < V < III < IV; subarticular tubercles absent; distinct longitudinal dermal ridges present under the 3rd to 5th toes, interrupted; large, oval inner metatarsal tubercle present, outer metatarsal tubercle absent; toes webbing rudimentary; narrow lateral fringes present on all toes. Tibia 44% of snout-vent length; tibiotarsal articulation reaches to posterior corner of eye; heels slightly overlapping when thighs are appressed at right angles with respect to body.

Dorsal skin rough, with dense conical granules, tubercles, glandular folds and conical spines; ventral skin smooth; pectoral gland and femoral gland oval; the size of pectoral glands almost equal to tips of fingers and femoral glands; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary glands raised. Ventrolateral glands distinctly visible, raised, forming an incomplete line.

Coloration of holotype in life. Dorsum greyish brown with distinct darker brown markings on sides and scattered with irregular light greyish brown pigmentations and yellowish brown spots. An indistinct, darker brown inverted triangular pattern between anterior corners of the eyes, connected to an indistinct dark brown W-shaped marking between the axillae. Dense translucent spines present on dorsal skin of body

and limbs. Upper 2/3 of the tympanum dark brown, lower 1/3 light orange, scattered with tiny coppery orange spots. Small greyish white and light brown granules present on the dorsum of the body and limbs; a dark brown vertical bar under the eye; transverse dark brown bars on the dorsal surface of the limbs and digits; distinct dark brown patches on the flanks, from groin to axilla; elbow and upper arms with distinct light orange coloration.

Ventral surface of throat, chest, and belly greyish white; throat with light brown speckles, chest, and abdomen with distinct dark patches; ventral surface of limbs dark grey, scattered with greyish white spots and small patches. Supra-axillary gland light orange; femoral, pectoral, and ventrolateral glands greyish white. Iris bicolored, light orange on upper half and silver on lower half.

Coloration of holotype in preservative (Fig. 4B). Dorsum of body and limbs dark brown; transverse bars on limbs, dark brown patterns, markings, and spots on back become indistinct, light greyish brown pigmentations and yellowish spots absent. Translucent spines on dorsal skin of body and limbs become grey. Ventral surface of limbs and surface of throat light brown, surface of abdomen greyish white, dark patches on chest, abdomen and flanks become more distinct. Supra-axillary, femoral, pectoral, and ventrolateral glands greyish white.

Variations. Measurements and body proportions are listed in Table 5. All the female paratypes match the overall characters of the holotype except that: the dorsum

Table 5. Measurements, and body proportions of *Leptobrachella dorsospina* sp. nov.

Voucher	SYS a004977	SYS a004974	SYS a004961	SYS a004962	SYS a004973	SYS a004975	SYS a004976
Sex	Male	Male	Female	Female	Female	Female	Female
SVL	28.7	30.5	36.1	37.3	39.8	32.1	33.8
HDL	10.3	10.8	12.8	12.4	12.9	11.3	12.0
HDW	10.6	10.7	11.9	12.9	13.2	11.8	12.2
SNT	4.5	4.2	5.1	5.4	5.8	5.0	4.8
IND	3.1	3.2	3.6	3.9	4.0	3.7	3.4
IOD	3.4	2.9	3.5	3.4	3.3	3.0	2.9
EYE	3.5	3.7	3.9	3.7	4.3	4.2	3.8
TMP	1.7	1.6	2.3	2.3	2.6	2.1	2.1
TEY	1.1	0.8	1.3	1.4	1.5	1.2	1.1
ML	7.4	7.3	8.8	7.7	9.1	7.8	7.6
LAHL	14.1	14.2	17.1	16.8	17.5	16.2	15.9
PL	12.1	12.8	14.9	14.5	15.5	13.9	13.6
TIB	13.5	13.4	15.5	16.3	16.6	14.9	14.5
HLL	41.7	42.7	49.1	49.9	52.9	46.8	48.0
HDL/SVL	0.36	0.35	0.35	0.33	0.32	0.35	0.36
HDW/SVL	0.37	0.35	0.33	0.35	0.33	0.37	0.36
HDW/HDL	1.03	0.99	0.93	1.04	1.02	1.04	1.02
SNT/HDL	0.44	0.39	0.40	0.44	0.45	0.44	0.40
IND/HDW	0.29	0.30	0.30	0.30	0.30	0.31	0.28
IOD/HDW	0.32	0.27	0.29	0.26	0.25	0.25	0.24
EYE/HDL	0.34	0.34	0.30	0.30	0.33	0.37	0.32
TMP/EYE	0.49	0.43	0.59	0.62	0.60	0.50	0.55
ML/SVL	0.26	0.24	0.24	0.21	0.23	0.24	0.22
LAHL/SVL	0.49	0.47	0.47	0.45	0.44	0.50	0.47
PL/SVL	0.42	0.42	0.41	0.39	0.39	0.43	0.40
TIB/SVL	0.47	0.44	0.43	0.44	0.42	0.46	0.43
HLL/SVL	1.45	1.40	1.36	1.34	1.33	1.46	1.42

is greyish brown in the holotype SYS a004974 (vs. dark brown in the paratypes SYS a004961, 4962), and black spots on the ventral skin are more dense and distinct in the paratypes SYS a004961, 4962.

Etymology. The specific epithet, *dorsospina*, is in reference to the conical spines on the dorsal surface of body in the new species. According to its type locality, we suggest its English common name as "Shuicheng Leaf Litter Toad", and the Chinese name "Shui Cheng Zhang Tu Chan (水城掌突蟾)".

Distribution and habits. Currently, *Leptobrachella dorsospina* sp. nov. is known only from its type locality, Yushe Forest Park, which is near the border between Guizhou and Yunnan. The new species was found on the surface of fallen leaves by the clear-water rocky hill-stream in well-preserved montane evergreen broadleaf forest (ca. 2100 m a.s.l.). Males were not heard calling.

Discussion

In the phylogenetic tree, the *Leptobrachella pelodytoides* (voucher number: MVZ 223642) sample from Tam Dao, northern Vietnam is clustered together with the topotypic *L. ventripunctata* (voucher number: SYS a001768) sample from Xishuangbanna, Yunnan, China, with a genetic divergence of only 1.5% (Fig. 2, Suppl. material 1: Table S1), which is of a intraspecific level. In addition, the type locality of *L. pelodytoides* is Thao [= Thamo], Kayah State, Myanmar, which is geographically distant from northern Vietnam with a distance over 900 km. Considering the above, we recommend that the specimen MVZ 223642 be reappraised as *L. ventripunctata.*

Yunnan and Guizhou are both largely within the species-rich Dian freshwater zoogeographical dominion (Huang et al 2020). Spanning the Indo-Burma Hotspot and the Mountains of Southwest China Hotspot (Tordoff et al. 2012), Yunnan Province has for long been considered as one of the most biodiverse regions in China and its flora and fauna have attracted much attention. However, Guizhou Province, which also shares the Yunnan-Guizhou Plateau, remains relatively neglected; knowledge of biodiversity levels and patterns are seriously lacking. In recent years, large numbers of discoveries have been made from Guizhou, dramatically raising the number of frog species known from the region (Zhang et al. 2017; Li et al. 2018a, b, 2019a, b, 2020a; Lyu et al. 2019; Wang et al. 2019; Luo et al. 2020; Wei et al. 2020). Further comprehensive surveys are urgently needed to determine the true diversity of the amphibians of Guizhou Province.

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Appendix 1

Specimens examined

***Leptobrachella alpina* (n = 6):** China: Yunnan Province: Jingdong County: Mt. Wuliang: CIB 24353 (holotype), CIB 24354; SYS a 003927.

***Leptobrachella bijie* (n = 8):** China: Guizhou: Bijie City: SYS a007313–7320.

***Leptobrachella laui* (n = 26):** China: Hong Kong: SYS a002057 (holotype), SYS a002058; China: Guangdong Province: Shenzhen City: SYSa 001505–1507, 1515–1521, 3471–3472, 5644–5645.

***Leptobrachella liui* (n = 18):** China: Fujian Province: Mt. Wuyi: CIB 24355 (holotype), CIB 24356, SYS a001571–1578, 1595–1599, 2478–2479, 5925–5826.

***Leptobrachella mangshanensis* (n = 5):** China: Guangdong: Nanling Nature Reserve: SYS a002827–2830, 5754.

***Leptobrachella purpuraventra* (n = 15):** China: Guizhou: Bijie City: SYS a007277–7284, 7300–7306.

***Leptobrachella tengchongensis* (n = 6):** China: Yunnan Province: Baoshan City: Mt. Gaoligong: SYS a004600 (holotype), 4596–4599, 4601–4602.

***Leptobrachella wuhuangmontis* (n = 12):** China: Guangxi Province: Pubei County: Mt. Wuhuang: SYS a003500/CIB107274, SYS a000578, 0580–0581, 3485–3489, 3499, 3504–3506.

***Leptobrachella yunkaiensis* (n = 8):** China: Guangdong Province: Maoming City: Dawuling Forest Station: SYS a004664/CIB107272, SYS a004663, 4665–4669, 4690.

Supplementary material 1

Table S1. Pairwise distances based on 16S gene among all sample used in this study

Authors: Jian Wang, Zhi-Tong Lyu, Shuo Qi, Zhao-Chi Zeng, Wen-Xiang Zhang, Long-Shan Lu, Ying-Yong Wang

Data type: phylogenetic

Explanation note: Genetic distances among all *Leptobrachella* samples.

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Phylogeny and biogeography of Sumatra´s cloud forest lizards of the genus *Dendragama* and status of *Acanthosaura schneideri*

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Abstract

Lizards of the genus *Dendragama* are endemic to the highland cloud forests of Sumatra's Barisan Mountain Range in western Indonesia, and recent studies have uncovered widespread diversity within the genus. Here, a suite of morphological characters and mitochondrial DNA are used to compare three geographically isolated populations of *D. boulengeri* from (1) Mount Kerinci in Jambi province, (2) Mount Marapi of west Sumatra, and (3) the Karo Highlands of north Sumatra. Additional phylogeographic analyses with two recently described sister species, *D. australis* and *D. dioidema* were conducted. Five genetically distinct clades of *Dendragama*, all distributed allopatrically of one another were identified and some are suspected to inhabit small distributions. Morphological and genetic data confirm the Karo Highlands population *D. schneideri* (previously *Acanthosaura schneideri* Ahl, 1926) should be revalidated from the synonymy of *D. boulengeri*. *Dendragama schneideri* is endemic to montane forests of the Karo Highlands surrounding Lake Toba in Sumatra Utara province. Pairwise genetic distances of 6–11% separate *D. schneideri* from

congeners. Two distinct clades of *D. boulengeri* from Mount Kerinci and Mount Marapi were identified, which are 5.0% genetically distant from one another. Using morphological characters, we provide the first key for distinguishing between species of *Dendragama*. Based on biogeographic patterns and levels of genetic variation it is suspected that at least 18 other isolated cloud forest locations may hold new species or divergent populations of *Dendragama* but lack survey work. Collectively, these comparisons among populations of montane lizards further elucidate the complex biogeographic history of Sumatra's montane forest species and the first phylogeny of the genus *Dendragama*.

Keywords
Barisan Range, biodiversity, Indonesia, IUCN, Pacific Ring of Fire, phylogeography, Toba eruption

Introduction

Uncovering tropical diversity is essential for conservation initiatives and understanding complex ecological and evolutionary processes (Ladle and Whittaker 2011). However, many regions and taxonomic groups across the globe remain largely unstudied, and for a multitude of reasons, Indonesia is particularly under-represented in this regard (O'Connell et al. 2018). Sumatra's Barisan Mountains are especially interesting because the entirety of the range is a volcanically active strip of the "Pacific Ring of Fire" which runs along the edge of Sumatra's west coast.

Volcanic activity and other historical biogeographic pressures throughout the Barisan Range have led to the development of a fascinating array of biodiversity (Demos et al. 2016), including a whole suite of montane forest species that live in high elevation cloud forest (temperate moist forest typically between 1,300 and 2,800 m of elevation in the tropics). However, Sumatra's cloud forest biodiversity has remained largely unexplored and the pressures that have driven the development of that diversity are not yet fully understood (O'Connell et al. 2018). Reptilian diversity is no exception in this regard despite the fact that Sumatra is known to harbor some of the most evolutionarily unique lineages of reptiles, including enigmatic species like the Modigliani's nose-horned lizard which was recently rediscovered (Putra et al. 2020).

Interestingly, despite the lack of herpetofaunal survey work throughout the region, Sumatra is already considered the most draconine (family Agamidae) diverse island in southeast Asia (Harvey et al. 2014). Given the complex geologic history, the presence of several isolated montane forest islands, and the wealth of diversity that has been discovered in nearby regions, it is clear that montane agamid lizards remain under studied and are an excellent group for better understanding the biogeographic history of the region (Harvey et al. 2014).

There has been considerable uncertainty regarding the taxonomic status of *Dendragama boulengeri*, which was considered to belong to a monotypic genus until only recently (Harvey et al. 2017). Early studies reported *D. boulengeri* from isolated montane forests of Jambi, west Sumatra (Sumatra Barat) and north Sumatra (Sumatra Utara) provinces (Doria 1874). However, Harvey et al. (2017) recently described two new species, *D. australis* from south Sumatra and *D. dioidema* from Aceh Province. They also provide

a thorough redescription of *D. boulengeri*. In their paper, *D. boulengeri* is redescribed as a species distributed throughout much of Central Sumatra, including the type locality (Mount Singgalang in Sumatra Barat) and nearby Mount Marapi (paralectotype locality).

Previously, a population of *Dendragama* was described as *Acanthosaura schneideri* (Ahl 1926) from Sumatra Utara Province. The species was mentioned infrequently thereafter. In an unpublished dissertation, Moody (1980) conducted a thorough family-wide review of the Agamidae and split the genus *Calotes* into four genera: *Bronchocela*, *Calotes*, *Dendragama*, and *Pseudocalotes*. However, there is no mention of *A. schneideri* in his work. Only later did Manthey and Grossmann (1997) transfer *A. schneideri* to the synonymy of *D. boulengeri*. To date, there has been no phylogenetic analyses of *Dendragama* to confirm the status of the population from Sumatra Utara in relation to other populations.

We examined a series of *Dendragama* collected by MBH and ENS from their herpetofaunal inventory conducted between 2012 and 2014 throughout the Barisan Mountain Range of Sumatra. These samples include specimens from various isolated mountain peaks across much of central, northern, and southern Sumatra. Using an integrative analysis, we investigate species boundaries among these populations and biogeographic patterns revealed by the phylogenetic analyses.

Materials and methods

Biological inventory

A thorough herpetofaunal survey was conducted across the highland forests of Sumatra's Barisan Mountain Range between 2012 and 2014. An international team of collaborators systematically targeted mountains based on geographic isolation from one another. We predominantly collected specimens at night. In total, we collected 195 specimens of *Dendragama* from localities throughout the highlands of Sumatra between 1231–2253 m elevation. We recorded GPS coordinates and ecological data on site. Animals were euthanized following appropriate IACUC protocols and DNA samples were taken for future identification prior to preservation in 10% formalin. Photographs were taken before and after euthanizing.

Counts and measurements

We scored 32 different morphological characters for each specimen from the three populations of *D. boulengeri*. To avoid systematic errors introduced by separate observers, K. Shaney collected all mensural and meristic characters. Sex was determined by examining the gonads. We examined 19 *D. boulengeri* collected from Mount Marapi, west Sumatra (Marapi population), nine specimens from Mount Kerinci and Mount Tujuh, Jambi (Kerinci population), and 15 specimens from various mountains across the Karo Highlands of Sumatra Utara, collected near the type locality of *A. schneideri* (Karo population; Fig. 1).

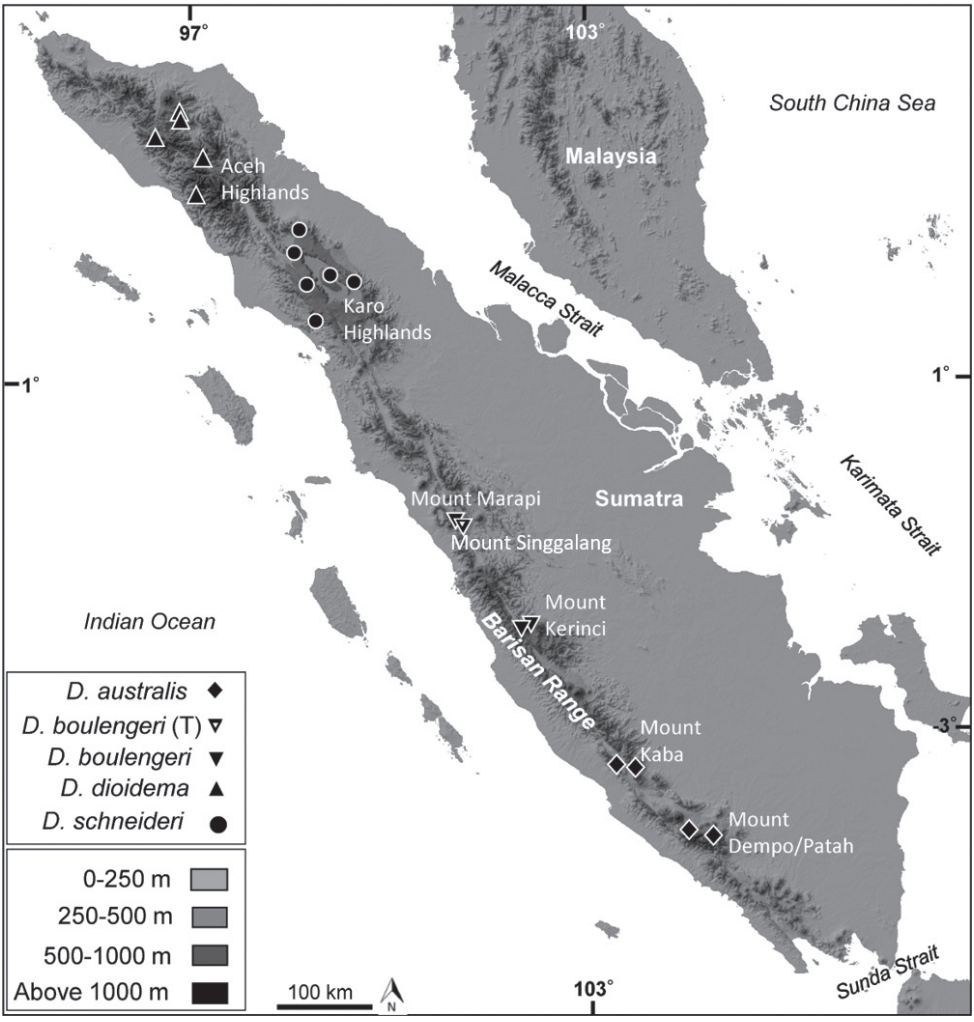

Figure 1. Collection localities of specimens of *Dendragama* used for comparisons in this study. (T) designates type locality and nearby paralectotype locality (Marapi). White dots represent hypothesized potential locations for new species or divergent populations. Dotted white lines show major biogeographic breaks.

Because measurements and scores are often done differently depending upon the study, some of the morphological characters used here require further comment (Harvey et al. 2014). We measured eye–nostril distance from the anterior ocular angle (bony edge of the orbit) to the tip of the snout. We consider the last supralabial to be lower and more elongate than the supralabials in front of it, and the last infralabial is positioned directly below the center of the last supralabial. We counted gular scales beginning immediately behind the mental (or the first pair of infralabials when in medial contact) until the transverse crease where the gulars meet the pectoral region. From the crease, we counted ventral scales to the last scale on the anterior edge of the cloacal opening. We counted nasal–rostral scales as the number of scales between the nasal and rostral. Postrostral scales were counted as the number of scales contacting

the rostral which were not supralabials. We counted canthals as the number of scales between the nasal scale and the first supraocular scale. The number of scales between the supralabials and the first canthal touching the nasal scale are described here as the "scales between first canthal and supralabial". Circumorbital scales include a canthal and the postciliary modified scale. We counted postmentals (or chin shields) if they contacted the infralabials. The number of midbody scales around the body included the ventrals and a scale of the dorsal crest. We counted lamellae on the fourth digit of the hands and feet, starting from the interdigital skin at the base of the digit and extending to the claw (i.e., including the elongate ungual scale). We counted nuchal crest scales from the first projecting scale to the last enlarged scale before the pectoral gap. Only projecting scales were counted and small flat vertebrals and paravertebrals that interrupt the crest were excluded.

To the nearest 0.1 mm with digital calipers, we measured snout-vent length (SVL) from the tip of the snout to the anterior lip of the vent and tail length by straightening the tail along the edge of a ruler and measuring from the posterior edge of the vent to the tip of the tail. We measured head length from skin covering the posterior edge of the mandible to the tip of the snout, trunk length as the distance from the axilla to the groin, and hand and foot length from the proximal margin of the palm or sole to the tip of the claw on the fourth digit. We measured the brachium as the length of the entire humerus and the antibrachium from skin covering the proximal end of the ulna (antebracheal fold) to the base of the palm. We measured the shank as the length of the tibia. The proximal and distal ends were determined with the elbow or leg flexed 90°. We measured internarial distance as the distance between the upper edge of each nostril, bony orbit, and tympanum width from the anterior to the posterior edge of each. Additional specimens examined from sister taxa and outgroups are provided in Suppl. material 1.

Statistical analyses

Using our mensural and meristic data we compared the Karo, Kerinci, and Marapi populations of *Dendragama boulengeri*. For meristic characters, we compared means between the three populations using Tukey's test after confirming assumptions of normality (using the Shapiro-Wilk test) and homoscedasticity (using Levene's test). *Dendragama boulengeri* populations from the Karo population represent the group that we hypothesize to be a distinct species, *D. schneideri*; however, we will continue to refer to this as the "Karo population" until the results section. Thus, for clarity between the text, figures, and tables, when we refer to *D. boulengeri* (Karo population) or *D. schneideri* we are referring to the same population.

When making comparisons among populations, we analyzed males only for head width and head length, because we found these traits to be sexually dimorphic in a preliminary study of our series from Marapi. To investigate sexual dimorphism and to compare mensural characters among populations, we used analysis of covariance treating SVL as a covariate. To avoid inflation of the type I error rate in our morphometric comparisons, we performed three additional calculations. First, we made Bonferroni corrections to the probability scores for the tests among populations (Glover and

Mitchell 2016). Second, having identified several apparent morphometric differences in among populations, we then verified the difference by rerunning the analysis using a different measurement as covariate in each apparently different trait: eye-nostril distance as a covariate for comparisons among thigh lengths, length of brachium for comparisons among hand length and length of shank for comparisons among foot length. Third, as a final validation of these results, M. B. Harvey measured SVL and tail length of a separate sample of nine *Dendragama* housed in the MZB and compared them to his own measurements for specimens from the type locality and Marapi.

DNA extraction and amplification

We digested tissue in 100 μL of lysis buffer, then added 5 μL of proteinase K (20 mg/ml) and incubated at 55 °C for 1–6 hours. After incubation, we added 1.8 μL of serapure beads (Rohland and Reich 2012) for every 1 μL of digested sample. DNA extraction was carried out following the same methods that are used in PCR cleaning protocols described in AMPure magnetic beads literature (Agencourt, Bioscience, Beverly, MA, USA).

Phylogenetic Analyses – We extracted genomic DNA from 17 specimens of Sumatran *Dendragama*, including: *D. boulengeri* from Mount Marapi (Marapi population), *D. boulengeri* from Mount Kerinci (Kerinci population), *D. boulengeri* representing the Karo Highlands population (Mount Sibuatan, Mount Pangururan and Vicinity of Tele), *D. dioidema* (Mount Kaba, Mount Patah and Mount Dempo) and *D. australis* (Berni Terlong and Takengan). It's important to note that individuals from the Karo Highlands were not collected precisely from the type locality of *A. schneideri* but were found in nearby geographic locations. We then combined new sequences from these specimens with sequences already published by Shaney et al. (2016) and Harvey et al. (2014, 2017) on GenBank. The published sequences include the outgroup taxa *Bronchocela cristatella* (Kuhl, 1820), *Lophocalotes ludekingi* (Bleeker, 1860), and *Pseudocalotes tympanistriga* (Gray 1831) see Suppl. material 2.

ND4 provided sufficient data for us to generate a phylogenetic hypothesis of *Dendragama*. We sequenced a fragment of the NADH dehydrogenase subunit 4 (ND4) gene using the forward primer "ND4" (CACCTATGACTACCAAAAGCTCATGTAGAAGC) and reverse primer "LEU" (CATTACTTTTACTTGGATTTGCACCA). The ND4 thermal cycle profile consisted of an initial denaturation at 94 °C for three minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, a 50 °C annealing phase for 45 seconds and a 72 °C extension for one minute, followed by a 72 °C extension for seven minutes, then a holding phase at 4 °C.

All sequences were aligned using the Geneious aligner implemented within Geneious v. 6.1.8 (Kearse et al. 2012). ND4 sequences range in length from 616 to 934 bp. We identified the most likely model of evolution for each codon position using Bayesian information criteria implemented in PartitionFinder (Lanfear et al. 2012). We partitioned codon positions using GTR+ Γ. We conducted maximum likelihood analyses using raxmlGUI (Sylvestro et al. 2012). We utilized the thorough bootstrapping

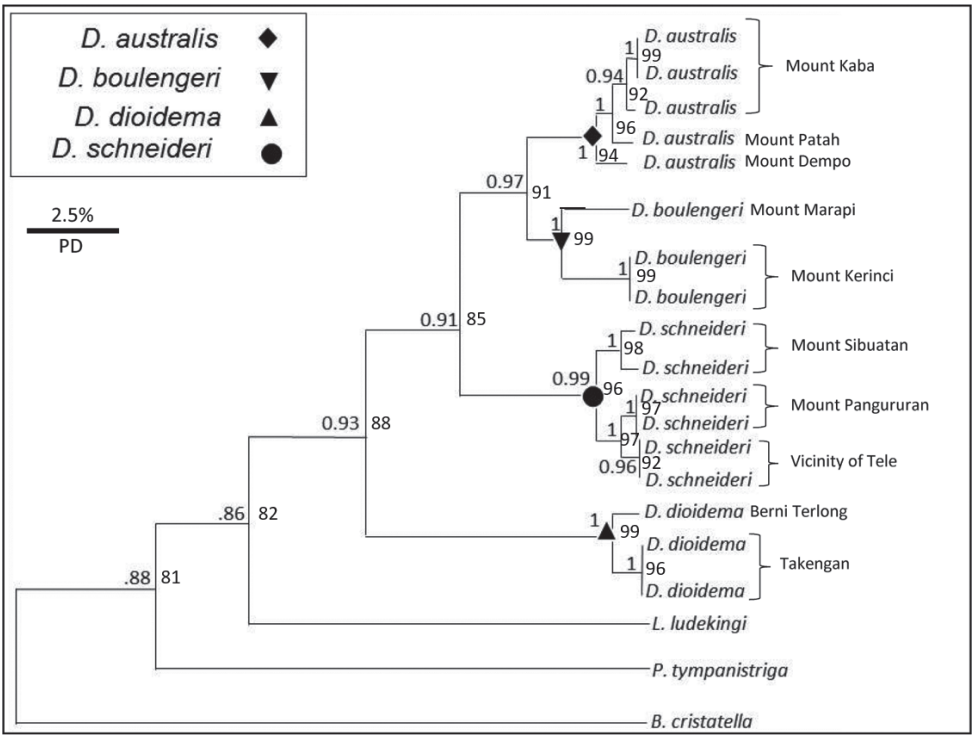

Figure 2. Bayesian tree of *Dendragama* and other agamid taxa included in our analyses. PD = Pairwise Distance. Mountains associated with *Dendragama* population sampling are noted. *Bronchocela* abbreviated as B, *Lophocalotes* as L, and *Pseudocalotes* as P.

setting, sampling over 10 runs of 10,000 repetitions. We carried out Bayesian phylogenetic analysis using MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003). We used four independent runs (nruns = 4) and four chains (three heated chains and one cold chain) for 10 million generations, sampling every 100 generations. We discarded the first 25% of samples as burn-in (Rambaut et al. 2014). We confirmed adequate mixing and assessed the appropriate amount of burn-in and convergence by inspecting the trace files in the program TRACER v1.6 (Rambaut et al. 2014). We conducted UPGMA analyses and calculated uncorrected pairwise distances using Mega 5.1 (Tamura et al. 2011).

Results

Phylogenetics and biogeography

Both our Maximum Likelihood and Bayesian Analyses revealed the same relationships within *Dendragama*, the only difference being that the ML tree returned slightly lower

Table 1. Uncorrected pairwise genetic distances (ranges) for ND4 sequences between populations of *Dendragama* (including 5 species described by Harvey et al. (date), *Lophocalotes ludekingi* and *Pseudocalotes tympanistriga*. For *Dendragama*, M = Marapi population, K = Kerinci population, Ka = Karo population.

Species	*D. boulengeri* (M)	*D. boulengeri* (K)	*D. schneideri* (Ka)	*D. australis*	*D. dioidema*	*L. ludekingi*
D. boulengeri (K)	5%					
D. schneideri (Ka)	6.0–7.0%	7.0–8.0%				
D. australis	6.0–6.4%	6.0–6.4%	7.0–8.0%			
D. dioidema	10.7–12.1%	9.0–10.1%	9.0–10.1%	10.7–11.7%		
L. ludekingi	16–17%	16–17%	16–17%	16–17%	16–17%	
P. tympanistriga	19–20%	19–20%	19–20%	19–20%	19–20%	19–20%

support values (Fig. 2). Both analyses found *Lophocalotes* to be sister to *Dendragama*, followed by insular *Pseudocalotes*. We refer to *Pseudocalotes* from Java and Sumatra as "insular" because they are not closely related to *Pseudocalotes* from mainland Asia (Harvey et al. 2014).

Within *Dendragama* five clades have strong nodal support (posterior probabilities between 0.98 and 1.00): *D. australis* (Mount Dempo, Kaba and Patah region), *D. boulengeri* (Mount Kerinci), *D. boulengeri* (Mount Marapi), *D. dioidema* (Aceh Highlands) and *D. boulengeri* (Karo Highlands). For clarity, the Karo Highlands populations will be discussed in the redescription section of *D. schneideri*. Each of these clades is restricted to geographically isolated locations. Dispersal between populations is highly unlikely to occur given that all *Dendragama* rely on cloud forest habitat which is not continuous between any of these populations.

Dendragama schneideri from the Karo highlands represent a genetically distinct group from central Sumatra and includes all specimens from Mount Sibuatan, Mount Pangururan and the Vicinity of Tele (Fig. 2). *Dendragama boulengeri* populations from Mount Marapi (paralectotype locality of *D. boulengeri*) and *D. boulengeri* populations from Mount Kerinci are geographically isolated by elevation and breaks in montane cloud forest patches. Based on biogeographic patterns, there are 18 distinct localities that might hold new species of *Dendragama*.

Genetic distances

The ND4 gene is 5.0–12.1% distant (Table 1) among *D. australis*, *D. dioidema* and the three populations of *Dendragama boulengeri*. At the lower extreme, 5.0% distance separates the Marapi and Kerinci populations of *D. boulengeri*. While *D. boulengeri* from the Karo population are 6.0–8.0% divergent from Marapi and Kerinci populations.

Dendragama dioidema is the most distant from species. Its ND4 gene is 10.7–11.7% distant from *D. australis*, and 10.7–12.1% from populations of *D. boulengeri*. In contrast, ND4 sequences of *D. australis* (south Sumatra) are 6.0–6.4% distant from populations of *D. boulengeri.*

The Karo population of *Dendragama boulengeri* (Now *D. schneideri*) has diverged by 6.0–8.0% from the Marapi and Kerinci populations and by 6.0–11.9% from *D. australis* and *D. dioidema*.

Table 2. Measurements of *D. schneideri* and *D. boulengeri* populations. Ranges are followed by average ± standard deviation in parentheses.

Measurement	*D. boulengeri* (Marapi population, n = 19)	*D. boulengeri* (Kerinci population, n = 9)	*D. schneideri* (Karo population, n = 15)
Flank/Pectoral Width	2.58–4.21% (3.54 ± 0.43)	2.57–4.99% (3.20 ± 0.92)	2.41–3.41% (3.03 ± 0.32)
Thigh/Shank Length	1.02–1.55% (1.31 ± 0.15)	1.46–1.67% (1.55 ± 0.08)	1.26–1.70% (1.53 ± 0.12)
Brachium/Anti. Length	0.90–1.29% (1.11 ± 0.08)	0.96–1.40% (1.13 ± 0.14)	0.93–1.21% (1.10 ± 0.08)
Snout Vent/Tail Length	1.96–2.43% (2.22 ± 0.43)	2.02–2.23% (2.11 ± 0.92)	2.02–2.46% (2.08 ± 0.32)
Head Length/Head Width	1.47–1.77% (2.0 ± 0.29)	1.29–1.66% (1.48 ± 0.14)	1.26–1.83% (1.62 ± 0.18)
Max. Snout–Vent Length	78.13 mm	80.56 mm	79.2 mm
Nasal to Rostral Scales	1–2, 1 (95%), 2 (5%)	1 (100%)	1 (100%)
Nasal to Sup. Scales	0–2, 0 (58%), 1 (37%), 2 (5%)	0 (100%)	0 (75%), 1 (25%)
Post Rostral Scales	5 (100%)	5 (100%)	5–6, 5 (91%), 6 (9%)
Canthals (Nasal to Sup.)	5–7, 5 (74%), 6 (21%), 5%)	5–6, 5 (83%), (17%)	5–7, 5 (45%), 6 (45%), 7 (9%)
Loreal Scales	5–6, 5 (89%), 6 (11%)	6–7, 6 (50%), 7 (50%)	6–7, 6 (73%), 7 (27%)
Scales Canth. and Sup.	2–4, 2 (5%), 3 (90%), 4 (5%)	2–3, 2 (17%), 3 (83%)	2–3, 2(37%), 9 (63%)
Circumorbital Scales	13–16, 13 (37%), 14 (53%), 15 (5%), 16 (5%)	11–13, 11 (17%), 12 (66%), 13 (17%)	13–15, 13 (73%), 14 (18%), 15 (9%)
Scales Nuch. and Dor.	8–10, 8 (47%), 9 (21%), 10 (26%), 11 (5%)	6–9, 6 (17%), 7 (33%), 8 (33%), 9 (17%)	5–9, 5 (9%), 6 (9%), 7 (36%), 8 (18%), 9 (27%)
Scales up at Midbody	20–24 (21.21 ± 1.27)	20–25 (23.66 ± 1.9)	13–19 (16 ± 1.95)
Midbody Scales	77–84 (79.57 ± 1.89)	75–89 (84.16 ± 4.99)	59–68 (62.36 ± 2.8)
Gular Scales	35–43 (38.95 ± 2.01)	34–42 (37.89 ± 2.97)	32–44 (36.81 ± 3.51)
Ventral Scales	52–63 (57.89 ± 3.71)	56–68 (62.16 ± 3.97)	48–59 (52.45 ± 3.14)
Sub. Lamellae of Toe IV	27–36 (30.42 ± 2.38)	25–31 (28.5 ± 2.58)	25–32 (28.09 ± 2.02)
Sub. Lamellae of Finger IV	24–31 (27.42 ± 1.95)	22–24 (23.16 ± 0.75)	22–26 (24.27 ± 1.19)
Supralabials	9–10, 9 (58%), 10 (42%)	8–10, 8 (50%), 9 (33%), 10 (17%)	9–10, 9 (91%), 10 (9%)
Infralabials	8–11, 8 (21%), 9 (53%), 10 (21%), 11 (5%)	8–9, 8 (67%), 9 (33%)	8–9, 8 (45%), 9 (55%)

Morphology

A suite of meristic characters distinguishes the Karo population of *D. boulengeri* from the other two populations. Specimens from Karo have statistically significant differences in several characters (Tukey Test), including fewer scales around midbody, fewer ventral scales, and large heterogeneous scales along the flanks (Table 2). The Marapi population of *D. boulengeri* has more scales between the nuchal and dorsal crest and subdigital lamellae than the other two populations. Finally, *D. boulengeri* specimens from Kerinci have fewer circumorbitals (11–13) than specimens from Marapi and Karo (usually 15) (Tables 2 and 3). We did not find interpopulation differences for the other meristic characters (P > 0.05). Fig. 3 provides a visual of some of the relationships among characters.

Male *Dendragama boulengeri* from Marapi have wider ($F_{1,16}$ = 9.08, P = 0.008) heads than females and width of their heads increases faster during ontogeny ($F_{\text{equal slopes}}$ = 6.50, P = 0.022). Although just not significant if 0.05 is chosen as the type I error rate, male *D. boulengeri* from Marapi also have longer heads (P = 0.072) than females. With small samples sizes from Karo and Kerinci, we lacked sufficient statistical power to confirm sexual dimorphism in head size (P > 0.2). Nonetheless, males from Karo appear to follow the same growth trajectory. We could not demonstrate sexual dimorphism in our meristic characters or in tail length, eye–nostril length, pectoral width, or length of the body (P > 0.26).

Table 3. Results of Tukey's Tests. The three *Dendragama boulengeri* populations (Karo Highlands, Kerinci, and Marapi) were compared and statistically significant results show that the Karo Highlands population is morphologically distinct from Kerinci and Marapi populations. The Karo Highlands population should be referred to as *D. schneideri*.

Character	Tukey's *Q*, probability	
	Marapi	Kerinci
Circumorbitals		
Karo	NS	5.00, 0.003
Kerinci	8.21, 0.000	
Scales between nuchal and dorsal		
Karo	4.16, 0.015	NS
Kerinci	4.92, 0.004	
Dorsals pointing upward		
Karo	10.89, 0.000	13.44, 0.000
Kerinci	NS	
Scales around midbody		
Karo	21.3, 0.000	20.8, 0.000
Kerinci	NS	
Ventral scales		
Karo	4.40, 0.010	6.04, 0.000
Kerinci	NS	
Lamellae under toe 4		
Karo	3.89, 0.024	NS
Kerinci	3.82, 0.027	
Lamellae under finger 4		
Karo	7.81, 0.000	NS
Kerinci	10.2, 0.000	
Character	**ANCOVA *F*, Bonferroni corrected probability**	
	Marapi	**Kerinci**
Tail length		
Karo	NS	15.57, 0.002
Kerinci	6.77, 0.046	
Hand length		
Karo	NS	11.25, 0.009
Kerinci	11.61, 0.007	
Foot length		
Karo	NS	10.51, 0.012
Kerinci	17.8, 0.001	
Orbit		
Karo	Nonparallel (18.94, $P < 0.001$)	NS
Kerinci	8.67, 0.021	
Thigh length		
Karo	NS	7.91, 0.031
Kerinci	NS	

Dendrama boulengeri specimens from Kerinci have relatively shorter tails, hands, and feet than specimens from the other two *D. boulengeri* populations. They also have shorter thighs than specimens from Karo and a smaller orbit than specimens from Marapi. Small specimens from Karo have a relatively smaller orbit than small specimens from Marapi; however, orbits are about the same size for larger specimens from the two populations. Our limited data suggests a different growth trajectory for the orbit at Karo vis-à-vis Marapi, but having violated the assumption of parallel regression lines, we do not report a probability for this comparison between Karo and Marapi. As detailed in

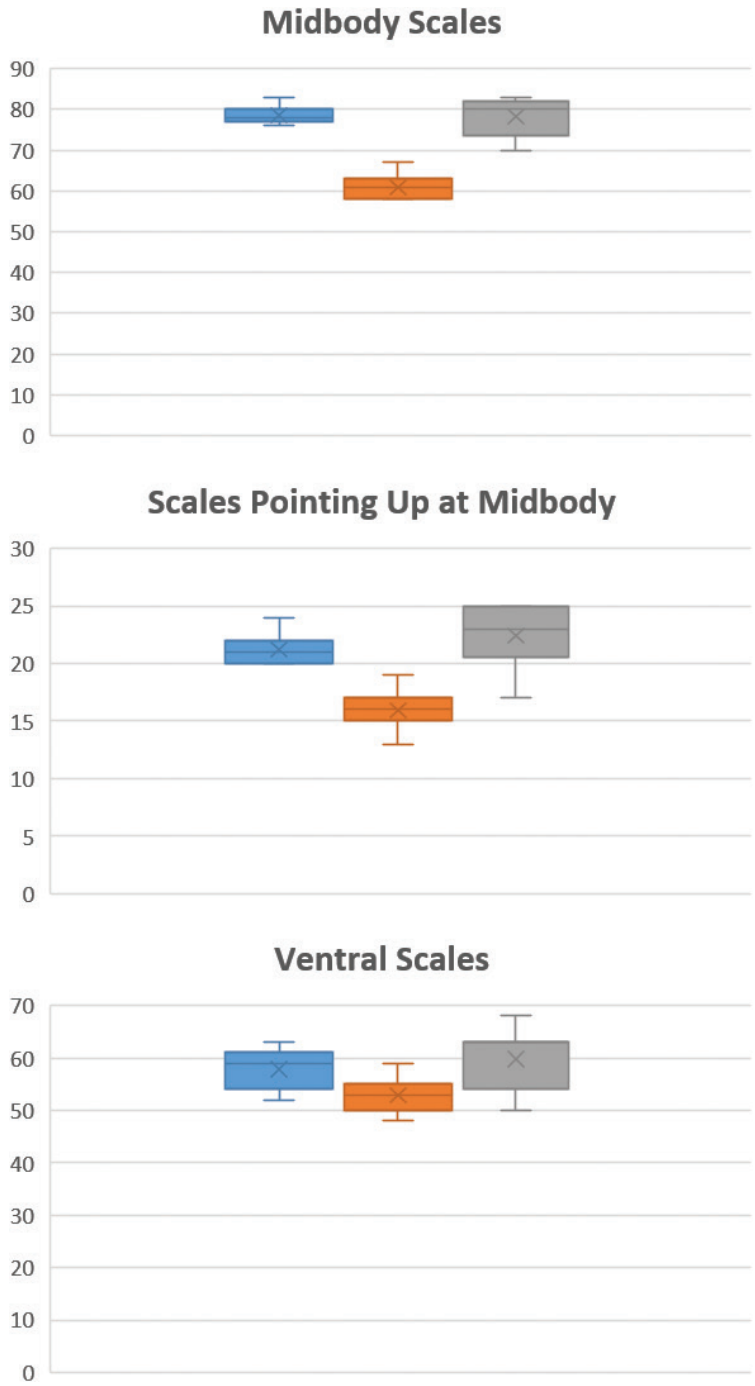

Figure 3. Box-and-whisker plots of three statistically significant different characters compared: **A** midbody scale counts, **B** scales pointing up at midbody, and **C** ventral scales. Scales abundance is provided on the Y axis and the three species are provided in different colors: blue for *D. boulengeri* (Marapi), orange for *D. schneideri*, and grey for *D. boulengeri* (Kerinci).

the methods, we confirmed each of these morphometric differences by treating other measurements as covariates. Moreover, a separate sample of nine *Dendragama* from Kerinci had relatively shorter tails ($F_{1,27}$ = 7.75, P = 0.010) than the sample of *D. boulengeri* from both Marapi and the type locality described by Harvey et al. (2017). We did not find differences among populations for eye–nostril distance, pectoral width, length of body, length of shank, length of brachium, length of antebrachium, or internarial distance (P > 0.12). Tables 2 and 3 provide statistics for the three populations compared.

In addition, *Dendragama* from the Marapi and Kerinci populations have a bright yellow buccal epithelium and tongue, whereas lizards from the Karo population have a pink to red buccal epithelium and tongue. Along their lower flanks, lizards from Karo have numerous distinctly enlarged tubercular scales. In contrast, specimens from the other populations lack these scales.

Species delineation

Our analysis revealed numerous differences between the Karo population on the one hand and the Marapi and Kerinci populations on the other. Numerous different means and high genetic divergence is evidence of an interruption in gene flow among these populations, but is of only limited diagnostic value. However, we also identified four fixed characters that distinguish the Karo population from the other two. Unlike these populations (characters in parentheses), the Karo specimens have pink to red buccal epithelia (yellow, Fig 4), numerous enlarged tubercles on the lower flank (scales of lower flanks homogenous or with few slightly enlarged scales), 59–68 scales around midbody (77–89), and 13–19 dorsals pointing upward and backward at midbody (20–25). Restricted to highland areas above 1,200 m, inhospitable lowlands isolate the Karo population from all other populations and species of *Dendragama*. Direct comparison of Ahl' (1926) type of *Acanthosaura schneideri* to the Karo specimens reveals that they are the same species. Accordingly, our analyses support the removal of *A. schneideri* Ahl, 1926 from the synonymy of *Dendragama boulengeri* Doria, 1888. Hereafter the Karo population should be recognized as a distinct species *Dendragama schneideri*.

Redescription of *Dendragama schneideri*

Acanthosaura schneideri Ahl 1926: 186, Simbolon, Battaker-Hochebene, Sumatra.
Dendragama boulengeri: Werner 1900, Schenkel 1901, De Rooij 1915 (in part): 119, Wermuth 1967, Häupl 1994, Manthey and Grossmen 1997 (in part): 166–167, Denzer et al. 1997, Hallermann 2000.

Holotype. An adult male (ZMB 15664, Fig 5) from "Simbolon, Battaker-Hochebene, Sumatra (= Simbolon, Sumatra Utara Province, Indonesia.)". Approximate GPS locality 3.005538°N, 98.923650°E.

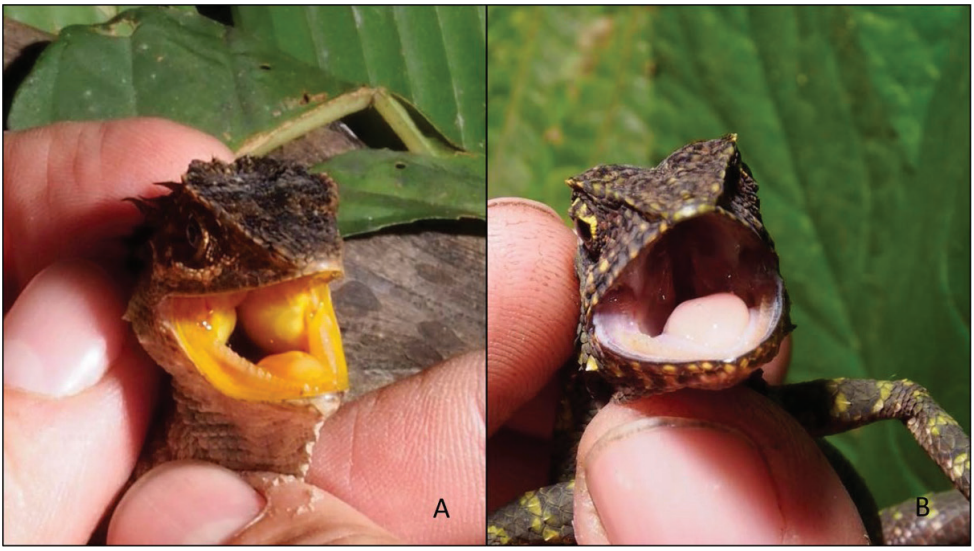

Figure 4. The bright orange mouth of *Dendragama boulengeri* is shown in **A**, whereas the pink mouth of *D. schneideri* is shown in **B** (photographs by ENS).

Referred material. All specimens were collected in Sumatra Utara near the type locality. Four specimens (UTA 62872, 2.91032°N, 98.4516°E; UTA 62873, 2.91329°N, 98.46091°E; UTA 62874, 2.9121°N, 98.46222°E; MZB 14126, 2.91189°N, 98.46538°E) from Mount Sibuatan, 1595–1883 m. Two specimens (UTA 62863, 3.2143°N, 98.49955°E; MZB 14127, 3.2143°N, 98.49955°E) from Sibayak, 1550 m. Two specimens (UTA 62865, 3.22576°N, 98.51974°E; UTA 62866, 3.20637°N, 98.51974°E) from the vicinity of Peceran, 1530–1727 m. One specimen (UTA 62870, 2.5911°N, 99.93921°E) from Mount Pangulubao, 1258 m. One specimen (UTA 62871, 2.1706°N, 98.63612°E) from an unnamed road near Onan Ganjang, 1231 m. One specimen (MZB 12098, 2.56103°N, 98.59106°E) from the vicinity of Tele, 1768 m.

Diagnosis. A species reaching at least 201 mm in total length (SVL) and distinguished from congeners by the following characters: (1) midbody scales 58–67; (2) dorsal scales heterogeneous across flanks (Fig 6); (3) strongly keeled white/yellow scales randomly distributed along flanks (more numerous and distinct in females); (4) ventral scales 48–59; (5) banding pattern along flanks often muddled, but typically vertical when present; (6) mouth and tongue pink to red in life; (7) narrow, vertical black stripes across dorsal crest, limbs, digits and most of tail; (8) female color in life dark brown, yellow and black with amber coloration on underside, while males green and lacking amber coloration along ventral surface; (9) dorsal and nuchal crest clearly separated by 5–9 dorsal scales; (10) dorsal crest serrate, extending to base of the tail, comprised of 23–31 projecting, triangular scales; (11) a series of 3–4 enlarged tubercles present along the chin of males and females; (12)

Figure 5. A, C holotype of *D. schneideri* from two different angles (photographs by MBH) **D** specimen MZB 12098 from the same angles (photographs by ENS).

A series of 12–18 strongly keeled, white/yellow femoral spines present (combined count on both sides).

Description and variation. The description is based on the 19 referred specimens. Where appropriate we provide character state frequencies or means ± standard deviation in parentheses. When available and not subject to interobserver biases, we also provide data gathered by MBH for the holotype in brackets.

Flank/pectoral width 2.41–3.41 (3.03 ± 0.32); thigh/shank length 1.26–1.70 (1.53 ± 0.12); brachium/antibrachium length 0.93–1.21 (1.10 ± 0.08); SVL/tail length 2.02–2.46 (2.24 ± 0.11); head length/head width 1.26–1.83 (1.62 ± 0.18); snout–vent length 61.35–79.2 mm (68.82 ± 5.59) (74 mm, tail length 145 mm).

Supralabials smooth, nine (91%) or 10 (9%); infralabials smooth eight (45%) or nine (55%); supraocular scales five (82%) or six (18%); postrostrals small, five (91%) or six (9%) [5]; scales between nasal and rostral one (100%); nasal separated from supralabials by small lorilabials (75%) or contacting first supralabial (25%); canthals from nasal to supraocular five (45%), six (45%), or seven (9%) [5]; loreal scales six (73%) or seven (27%), scales between first canthal and supralabials two (37%) or three (63%); circumorbitals 13–15, usually 11 (73%); postmentals contacting infralabials one (9%) or two (91%); first pair of postmentals in medial contact (66%) or separated by one gular (34%) [1].

Nuchal crest clearly separated from dorsal crest and gap between crests spanning 5–9 scales; dorsal crest serrate, continuous down to tail; scales on dorsum, large and heterogeneous, with series of enlarged strongly keeled, yellow/white scales

Figure 6. A flank of male *Dendragama boulengeri* (MZB 9825) and its thin, horizontal banding patterns, thick vertical bands along dorsal crest, small homogenous scales, and lack of enlarged, keeled scales **B** flank of male *D. schneideri* (UTA 62868) and its lack of horizontal banding along the flanks, thin vertical bands along dorsal crest, large heterogeneous scales, and enlarged, strongly keeled scales dispersed across the flanks (photographs by ENS).

in row below dorsal crest; all other scales along dorsum and flank smooth to feebly keeled; scales along flank consistent with dorsum, with more enlarged strongly keeled scales in vertical rows along sides; midbody scales 58–67 (61.36 ± 2.8) [61], gulars smooth 32–44 (36.81 ± 3.51) [30]; ventral scales 48–59 (52.45 ± 3.14) [52], ventrals keeled from chest to lower abdomen before transitioning to smooth scales near precloacal area; precloacal scale width small 0.75–1.4 (1.02 ± 0.22); scales along limbs strongly keeled, with continuation of keeled scales down to fingers on both hands and feet; subdigital lamellae on finger IV 22–26 (24.27 ± 1.19)

[23]; subdigital lamellae on toe IV 25–32 (28.09 ± 2.02) [27]; dorsal crest scales 23–31 (26.63 ± 2.69) [28].

Coloration in life. There is distinct sexual dichromatism in this species and coloration changes in all *Dendragama* in response to rough handling. Females of *Dendragama schneideri* are typically shades of dark brown, green, and black with vertical black and yellow bands running along the extent of the dorsal crest. Bands extend almost to the end of the tail; tail bands 14–18 (12.2 ± 0.83), and enlarged green, yellow or white, strongly keeled scales are present intermittently along the flanks. Black and yellow/green bands also extend along all limbs, hands, and feet. A black spot is present under the base of the nuchal crest as in other species of *Dendragama*. The throat has amber and brown coloration, which may or may not be broken up by small lateral brown lines. Brown and amber coloration extends along the lower flanks and all the way to the end of the tail. Yellow and black lines radiate around the eyes and across much of the face. Yellow, green or white enlarged tubercles are present below the eye and ear, and the mouth is pink to red.

Males may also be brown but are typically much lighter in coloration. They are often bright green and yellow with incomplete stripes of black scales, which zigzag vertically along the flanks. Black bands extend along the length of the dorsal crest and throughout the extent of the tail. Bands also cross the arms, legs, hands, and feet. A black prescapular blotch is present under the base of the nuchal crest, but may be less pronounced in some specimens. The venter is much lighter than in females, with a white or cream gular region, with some brown shading along the ventral side. Darker individuals may have some brown shading along the gular region as well. Green or yellow and black stripes radiate out from the eyes and, as with females, the mouth is pink to red.

Etymology. The name "*schneideri*" honors Gustav Schneider (17 January 1867–14 April 1948).

Standard English name. Schneider's Tree Agamid

Distribution and natural history. *Dendragama schneideri* occurs in high elevation, montane forest in north Sumatra's Bukit Barisan Mountain Range (Figs 1, 7). Our sampling encompasses Lake Toba (where the Toba blast occurred 71.6 kya) and the surrounding Karo Highlands. We hypothesize that the northern latitudinal limit of *D. schneideri* occurs at a break in the Barisan Range where elevation drops down into valley floors below 1000 m and does so continuously from the eastern to western edge of the range. That break in topography would certainly prohibit dispersal between montane sky islands today. Yet, maybe more importantly in terms of maintaining population differentiation, elevational ranges that drop below that point would have even prohibited dispersal during periods of glacial maxima during the Pleistocene epoch when montane forests retreated downslope 300–500 m (Hall 2009). The break occurs approximately at 3.196889°N 98.102583°E and we only found populations of *D. dioidema* north of that point. We hypothesize that the southern latitudinal limit of *D. schneideri* occurs approximately near the border of Sumatra Utara and Bengkulu. Similar to the northern limitation, there is a continuous elevational break that drops below 1000 m that extends across the width of the Barisan Range at the southern end of their range.

Figure 7. A, B highland cloud forest habitat of *Dendragama schneideri* (Photographs by ENS). Photographs taken at Mount Kerinci, Sumatra Barat Province illustrating similar habitats to cloud forests of the Karo Highlands where *D. schneideri* is found.

All the referred specimens were found sleeping in low vegetation 0.7–2.5 m above ground and between 1200–2800 m. All were found in montane cloud forest habitat and in higher elevations some individuals were found on moss covered vegetation in stunted forests where temperatures were slightly lower. Because the forests they inhabit receive high levels of precipitation relatively evenly distributed throughout cloud forests, *D. schneideri* are not dependent upon water bodies. Although some individuals were located along slopes near the edges of streams, those encounters did not seem to occur in any higher frequency than individuals being found in other areas away from bodies of water.

Many of the individuals collected were found along the edges of roadways and thus it seems they survive well along the edges of disturbed habitat, although they seem to be cloud forest obligates and require the presence of montane forest in some amount in order to persist in fragmented habitat.

Virtually nothing is known about the home range sizes and movement patterns of *D. schneideri*; however, given that they are relatively small arboreal lizards and live in cooler temperature habitats we would expect that they don't move long distances throughout the year and do not have large home ranges.

We documented distinct sexual dichromatism. Because the males tend to be more brightly colored, we suspect they use their brightly colored dewlaps to display during mating season. It is also likely they use their displays to defend territories, as do nearly all other brightly colored lizards (Sinervo and Lively 1996; Steffen and Guyer 2014). They may also use their colors when competing with other individuals. Although little is known about the life cycles of *D. schneideri* specifically, Harvey et al. (2017) suggest that other species of *Dendragama* reach maturity at around 60 mm, females lay 2–4 ovoid eggs and probably produce multiple clutches each year. This may also be true for *D. schneideri* given their other similarities in life histories. Little is known about the diet of *D. schneideri*; however, they are suspected to be insectivores.

Key to the species of *Dendragama*

We present a key to the species of *Dendragama* based on morphology and color pattern. Fig. 8 illustrates all four species. High supratemporal ridges enclosing a depressed parietal region, a row of white to yellow sublabial tubercles, and a visible tympanum immediately distinguish species of *Dendragama* from all other Sumatran agamids.

1 Gular scales *large*, 15–30, ventrals smooth to feebly keeled **2**
– Gular scales *small*, 32–42 ..... **3**
2 *Small* midbody scales, 61–94, mouth and tongue orange to yellow; short white sublabial stripe extending from below the eye to below (or just behind) the ear; brown band on neck, no large black prescapular blotch absent; proximal half of tail with 8–13 dark brown or green bands ..... ***D. australis***
– Midbody scales *moderate* in size, 57–77; mouth and tongue pink to red; no distinctive prescapular blotch present; proximal half of tail with 6–10 dark brown or green bands; *no* white sublabial stripe, however one or two white or pale yellow spots *present* ..... ***D. dioidema***
3 *Large* midbody scales, 59–68, ventrals strongly keeled, upper dorsals 13–19 directed upward and backward; mouth and tongue pink to red; enlarged tubercles present on lower flanks ..... ***D. schneideri***
– *Small* midbody, 74–88, ventrals heavily keeled, upper 20–25 dorsals directed upward and backward; mouth and tongue yellow; few weakly keeled scales along lower flanks, enlarged tubercles absent ..... ***D. boulengeri***

Discussion

Morphological and molecular data presented here show the clear distinction between *D. boulengeri* and *D. schneideri*. *Dendragama schneideri* is geographically isolated from other species and only distributed throughout the Karo Highlands. It occurs in high elevation cloud forest allopatric from *D. australis*, *D. boulengeri*, and *D. dioidema*. Based on the lack of biological inventory in other parts of the Barisan Range, it is likely that other undescribed species of *Dendragama* may occur across the region.

Biogeography

Using biogeographic patterns of genetic variation among *Dendragama* we identified at least 18 mountains that are likely candidates for new species. Moving from north to south, we estimate the break between the *D. schneideri* and *D. dioidema* lineages probably occurs where low elevation valleys run between Mount Sinabung and Mount Sibuatan slightly to the west of the Aceh-Sumatra Utara provincial border. For reference, the point 3.196889°N 98.102583°E is approximately along the line where this break occurs. We suggest that area as a likely break because that seems to be where

Figure 8. Male representatives currently recognized *Dendragama* **A** *D. boulengeri* ENS 19656 **B** *D. australis* ENS 18556 **C** *D. dioidema* ENS 19433 **D** *D. schneideri* UTA 62868 (photographs by ENS).

topography of the Barisan Range drops to its lowest point between the distributions of those groups. Among *D. dioidema* lineages there is distinct genetic variation between populations in the Leuser Mountains and the Boundahara Mountains, which are divided by a low valley running north and south, which is paralleled by the Blangkejeren-Kutacane Road.

Based on Shaney et al. (2020) breaks in species boundaries of *Dendragama* seem to occur consistently wherever elevation dips to 650–700 m (or lower) between mountains based on glacial periods during the Pleistocene. Thus, we hypothesize the following mountains will hold new *D. dioidema* sister lineages (Table 4).

Further south in latitude, the Karo highlands surrounding the Toba eruption site consist of topography that is continuously connected by higher elevation pieces of terrain and thus, there are likely few new sister species of *D. schneideri* throughout the Karo highlands between the latitudes 3.196889°N, 98.102583°E and 1.418139°N, 99.243389°E. However, there is distinct population subdivision among the populations that we sampled.

The break between populations of *D. schneideri* and *D. boulengeri* likely occurs around the point 1.418139N, 99.243389E which we hypothesize based on the dramatic drop-off in elevation that cuts through the entire width of the Barisan Range

Table 4. Estimated geographic limitations of species boundaries and hypothesized locations where new species of *Dendragama* are presumed to be found based on biogeographic patterns. PNS = potential location of new species, SB = species boundary. Locations ordered from northern to southern latitude.

Feature	Coordinates	Locality
PNS	5.445944, 95.662639	Cot seulawah Agam
PNS	5.042222, 95.634722	Gunung Hulumasen
PNS	5.371194, 95.348500	Aceh Besar Regency
PNS	4.811722, 96.828694	Mount Bur ni Geureudong
PNS	4.921167, 96.350250	The mountains around Gunung Peuet Sagoe
PNS	4.636124, 97.411502	East Aceh Regency
SB	3.196889, 98.102583	Break in *D. dioidema* and *D. schneideri*
SB	1.418139, 99.243389	The break between *D. schneideri* and *D. boulengeri*
PNS	0.995028, 99.379694	South Tapanuli Regency
PNS	0.968111, 99.651917	Padang Lawas Regency
PNS	0.743472, 100.232889	Rokan Hulu Regency
PNS	0.060503, 99.984076	Mount Talakmau
PNS	0.209722, 100.299139	Pasaman Regency,
PNS	-0.341389, 100.678278	Mount Sago
PNS	-0.399639, 100.334917	Mount Singgalong
PNS	-2.503333, 101.874083	Mount Masurai
PNS	-3.397250, 102.347028	Bukit Daun
SB	-3.489034, 102.535034	Break between *D. boulengeri* and *D. australis*
PNS	-3.510812, 102.625556	Mount Kaba
PNS	-3.618861, 102.913278	Empat Lawang Regency
PNS	-3.893361, 103.259111	Lahat Regency
SB	-4.460662, 103.430325	Southern Limit of *Dendragama*

throughout the area. South of that point, the Barisan's topography becomes more variable in terms of having lower elevation valleys between mountains in a larger number of locations. Thus, we hypothesize the following nine mountains will have new sister species of *D. boulengeri*, including the mountains of the south Sapanuli Regency, Padang Lawas Regency, Rokan Hulu Regency, Mount Talakmau, Muaro Sungai Lolo of Pasaman Regency, Mount Sago, Mount Masurai, Bukit Daun, and Air Duku area of Rejang Lebong Regency. Not all of these ranges had clear names on the topographic maps, but for reference we provide GPS coordinates to the center point of the ranges in (Table 4).

The southern limitation of *D. boulengeri* populations probably occurs near the town of Curup where the mountains drop down very low in elevation, again separating *D. boulengeri* populations (and likely sister species) from *D. australis* populations. Among *D. australis* we hypothesize the following three mountains will hold new closely related species: Mount Kaba, Unknown strip of mountain in Empat Lawang Regency, Mountains in Sukabumi of Lahat Regency. The southern limitation of *D. australis* is not precisely known although we did not collect *Dendragama* further south in Latitude than Gunung Patah at -4.460662, 103.430325. We did collect *Pseudocalotes cybelidermus* and *P. guttalineatus* extensively throughout mountains further south, including Gunung Pesagi and montane forest above Ngarip and *P. rhammanotus* from Danau Ranau in Lampung Province. The absence of *Dendragama* from locations further south suggests populations of *D. australis* probably do not occur much further south than Gunung Patah.

We hypothesize that each mountain probably has a new species, not just the possibility of one or two new species among all mountains mentioned. Based on Shaney et al. (2020) findings regarding the elevational shifts of Pleistocene cloud forests, it is likely that all of the 18 mountains mentioned remained isolated even during periods of glacial maxima. This latter point is crucial because Pleistocene connectivity is what would have facilitated gene flow historically and sympatric distributions contemporarily (which is not how they are distributed). Similar patterns have been seen in among peninsular Malaysia's herpetofauna as well (Loredo et al. 2013; Grismer et al. 2017). It is unlikely that *Dendragama* were able to disperse among any of the isolated mountains for millions of years, allowing for a whole array of species to diverge. Using these biogeographic patterns to identify likely locations for new species of *Dendragama* is certainly interesting on its own; however, perhaps more importantly, those same locations are probably where the distributions of many obligate cloud forest groups with limited dispersal (e.g. insects, rodents, amphibians) change in Sumatra. Thus, those mountains would be excellent candidates for other taxonomists conducting biological inventory throughout the region as well.

Additionally, a comparative biogeographic analysis among *Dendragama* and other agamid lizard groups would be a fascinating and informative study to better understand Sumatra's complex biological history. Grismer et al. (2016) provided a biogeographic study of draconid lizards of southeast Asia that yielded fascinating information; however, species of draconids from Sumatra remain underrepresented in biogeographic analyses (Inger and Voris 2001; Cannon et al. 2009). Furthermore, genetic splits between agamid lizard species seem quite old, and divergence dating of Sumatran agamid phylogenies may corroborate estimated geologic events from before the Pleistocene such as those put forth by (Voris 2000).

Phylogenetics

Phylogenetic analyses uncovered five distinct clades of *Dendragama*, among which are *D. australis*, *D. dioidema*, *D. schneideri* and two distinct clades of *D. boulengeri*, the first clade of *D. boulengeri* from Mount Kerinci and the second from near the type locality, Mount Marapi. These two clades are 5.0% pairwise genetically distant, which in many cases would constitute distinct species designations, if accompanied by readily identifiable morphological variation between populations. For example, Bradley and Baker (2009) show that sister species of mammals with readily distinguishable morphological characteristics typically had greater than 5.0% genetic variation in Cytochrome B sequences upon genetic evaluation later. *Dendragama boulengeri* populations examined in this study are on the edge of that genetic cutoff. Although means of some meristic and mensural characters differ between the Kerinci and Marapi populations of *D. boulengeri*, we did not find any fixed differences. Although geographically isolated from one another, we continue to recognize these two populations as *D. boulengeri*. However, we suspect that further studies may indeed identify these populations as distinct species.

These phylogenetic analyses further elucidate some of the patterns associated with cloud forest agamid lizard distributions in Sumatra. It seems that very few species have overlapping distributions and in contrast species, within *Dendragama*, species are distributed in a site-specific endemic pattern. Each species is distributed allopatrically with relatively small geographic ranges that match natural breaks in cloud forest habitat due to elevational changes. Furthermore, *Lophocalotes* is sister to the *Dendragama* group followed by insular *Pseudocalotes*, supporting Harvey et al. (2017).

Conservation

The conservation status of species of *Dendragama* has not yet been assessed by the IUCN (http://www.iucnredlist.org/), particularly because of the lack of population data. However, it is clear that *Dendragama* inhabits isolated cloud forest patches and Sumatra's rapid rates of deforestation are causing some of those patches to decline in size. Between 1900 and 2019 Sumatra's lowland forests were nearly wiped out for agriculture and timber, and because of forest fires (Poor et al. 2019). Deforestation is extending into highland areas that encompass remaining patches of *D. schneideri* habitat. Many forested areas have already been diminished beyond macroecological tipping points (Nowosad et al. 2019) and the ecological ramifications could cascade throughout the remaining cloud forest habitat.

Conclusions

The information from this paper contributes data on populations of *D. boulengeri* and *D. schneideri*, which we believe have small distributions, but are found in high abundance within those ranges. We estimated the latitudinal and longitudinal limits of *D. boulengeri* and *D. schneideri* (Fig. 1); however, we hypothesize that inventories of the rest of Sumatra's mountains would yield an array of new species and would show that the true distributional limits of all *Dendragama* species are actually small. These data may be used towards conducting IUCN Red List status assessments in the future; however additional information is needed to provide proper assessments. Regardless of their current conservation status, it is clear that rapidly increasing anthropogenic pressures throughout Sumatra are likely to have a significant impact on all species of *Dendragama*. Shaney et al. (2016) provide examples of how cryptic Indonesian lineages may be lost before being described and cryptic species may be overharvested due to poor taxonomic evaluation. Thus, continuation of biological inventory will be important in agamid lizard discovery and conservation of Sumatra's montane forest diversity in the near future. Given the rapid discovery of herpetofaunal diversity across Sumatra's highlands (Iskandar 2006; Kurniati et al. 2009) it is likely that an array of new agamid lizard species remains undiscovered throughout the region (Shaney et al. 2016).

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Supplementary material 1

List of additional specimens examined for morphological work, museum IDs and species names

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Data type: specimens examined

Link: https://doi.org/10.3897/zookeys.995.49355.suppl1

Supplementary material 2

Specimens included in phylogenetic assessment and GenBank accession numbers

Authors: Kyle J. Shaney, Michael B. Harvey, Amir Hamidy, Nia Kurniawan, Eric N. Smith

Data type: genbank data

Link: https://doi.org/10.3897/zookeys.995.49355.suppl2