RESEARCH ARTICLE



A new species of Denisiphantes Tu, Li & Rollard, 2005 (Araneae, Linyphiidae) from Yunnan, China

Guchun Zhou¹, Muhammad Irfan^{2,3}, Xianjin Peng²

I 1College of life Sciences, National Navel Orange Engineering Research Center, Gannan Normal University, Ganzhou 341000, Jiangxi, China 2 College of Life Sciences, Hunan Normal University, Changsha, Hunan Province, 410081, China 3 Key Laboratory of Eco-environments in Three Gorges Reservoir Region (Ministry of Education), School of Life Sciences, Southwest University, Chongqing 400715, China

Corresponding authors: Xianjin Peng (xjpeng@126.com); Muhammad Irfan (irfanuos94@yahoo.com)

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Abstract

Denisiphantes arcuatus **sp. nov.** ($\mathcal{F}^{\mathbb{Q}}$) is described from Yunnan, China. Detailed descriptions of somatic features, genitalic characters, photos of habitus of the new species as well as of copulatory organs of *Denisiphantes denisi* (Schenkel, 1963) ($\mathcal{F}^{\mathbb{Q}}$) are presented. A distribution map is also provided.

Keywords

Copulatory organ, Micronetinae, Southeast Asia, sheet-web spiders, taxonomy

Introduction

Li (2020) listed 403 Chinese linyphiid species from 162 genera, of which 120 species from 44 genera belong to Micronetinae (Li and Lin 2016). Spider collections made in the Yunnan Province between 2002–2006 revealed a new Linyphiidae species herein described. The new species belong in the monotypic

genus, *Denisiphantes* Tu, Li & Rollard, 2005. The type species, *Denisiphantes denisi* (Schenkel, 1963), was described based on a female specimen from Ganzu Province and it also occurs in Qinghai Province (Hu 2001; Tu, Li and Rollard 2005). The male was later described by Zhu and Li (1983). Color photos of both species are presented (Figs 1–7) as well as a detailed morphological description, a diagnosis, and a locality map (Fig. 8).

Material and methods

Specimens were collected by hand collecting and beating shrubs and were kept in 75% ethanol. After dissection, the epigyna were cleared in trypsin enzyme solution before examination and photography. The left male palps were used for description and illustration. Specimens were examined and measured with a Leica M205C stereomicroscope. Photos were taken with a digital Leica MC170 HD camera mounted on a Leica M205C and were stacked by Helicon Focus software (3.10.). The map (Fig. 8) was created using ArcMap 10.2, and then modified using Adobe Photoshop CS2 Extended. Leg measurements are given in the following order: total length (femur, patella + tibia, metatarsus, tarsus). All measurements are given in millimeters (mm). The terminology used in text and figure legends follows Tu et al. (2005). The type specimens are deposited at the College of Life Sciences, Hunan Normal University (**HNU**), Changsha, China.

Abbreviations

AER	anterior eye row	PH	pit hook on suprategulum
ALC	anterior projection of lamel-	PLE	posterior lateral eye
	la characteristica	PLC	posterior projection of la-
ALE	anterior lateral eye		mella characteristica
AME	anterior median eye	PME	posterior median eye
AME-ALE	the distance between AME	PME-PLE	distance between PME and
	and ALE		PLE
AME-AME	the distance between AMEs	PME-PME	distance between PMEs
ATA	anterior terminal apophysis	PMP	posterior median plate
EG	entrance groove	PTA	posterior terminal apophysis
EP	embolus proper	R	radix
MM	median membrane	S	spermatheca
PC	paracymbium	ST	subtegulum
PCA	proximal cymbial apophysis	Т	tegulum
PER	posterior eye row	TH	thumb of embolus

Taxonomy

Order Araneae Clerck, 1757 Family Linyphiidae Blackwall, 1859 Subfamily Micronetinae Hull, 1920 Genus *Denisiphantes* Tu, Li & Rollard, 2005

Denisiphantes arcuatus sp. nov. http://zoobank.org/E5E1CCE9-291E-4137-9B74-26EF67FB9308 Figures 1–4

Material examined. *Holotype*: male, CHINA, Yunnan Province, Nujiang Prefecture, Sanjiang Township, Lushui County, Nu Jiang, 25.72964°N, 98.87180°E, alt. 790 m, 26.VI.2000, leg. D. H. Kavanaugh, Charles Griswold & Heng–mei Yan (HNU–00– Lan–1). *Paratypes*: 2 females, collected together with the holotype (HNU–00–Lan–2-3).

Diagnosis. This new species resembles Denisiphantes denisi (Schenkel, 1963) in having a similar long ridge-shaped proximal cymbial apophysis of the cymbium in the male palp and a broad scape, almost hexagonal in shape in the epigynum, but can be distinguished by the following characters: (1) Lower margin of distal arm of the paracymbium with a projection, wider than long with a blunt end and covering the posterior margin of the tibia in retrolateral view in the new species (Figs 1A-D, 2A, B), whereas the projection of the lower margin of the distal arm in D. deni is longer than wide with a pointed end and not covering the posterior margin of the tibia (Fig. 5A, B); (2) Anterior projection of the lamella characteristica (ALC) leaf-shaped with pointed end, protruding above the tegulum and the posterior projection of the lamella characteristica (PLC) as long as the ALC with pointed end (Figs 1A–D, 2A, B), whereas ALC short, almost equal to the suprategulum (ST) with pointed end and PLC long, almost touching the anterior terminal apophysis (ATA) in D. denisi (Fig. 5A, B); (3) Anterior terminal apophysis (ATA) strongly curved with blunt end and pointing towards ALC in new species (Figs 1A–D, 2A, B), whereas slightly curved with pointed end and pointing towards the tegulum in ventral view in D. denisi (Fig. 5A, B); (4) Posterior terminal apophysis (PTA) triangular in the new species (Figs 1A, 2A), whereas it is tongue-shaped in D. denisi (Tu et al. 2005, figs 16, 17; Fig. 5A, B). (5) Female posterior median plate (PMP) tetragonal shape in the new species (Fig. 3D), whereas it is hexagonal in *D. denisi* (Fig. 6C).

Description. Male (holotype, HNU–00–Lan–1): Total length: 2.57. Carapace 1.06 long, 0.97 wide, cephalic region slightly elevated, brown, fovea, cervical and radial grooves distinct. Clypeus 0.35 high. Sternum wider than long, brown, with spine-like setae; labium wider than long; maxillae long, distal-end broad with scopulae. Chelicerae with 5 retromarginal teeth, promarginal teeth absent. Eye region narrow, AER recurved, PER straight, slightly wider than AER. Eye sizes and interdistances: AME 0.06, ALE 0.08, PME 0.09, PLE 0.08, AME–AME 0.03, PME–PME 0.05, AME–ALE, 0.08, PME–PLE 0.07, AME–PME 0.06, ALE–ALE 0.40, PLE–



Figure 1. *Denisiphantes arcuatus* sp. nov., male holotype palp **A** prolateral view **B** retrolateral view **C** ventral view **D** dorsal view.



Figure 2. Denisiphantes arcuatus sp. nov., male holotype palp. A prolateral view B retrolateral view.

PLE 0.43, ALE-PLE contiguous. Length of legs: I 5.89 (1.48, 1.91, 1.53, 0.97), II 5.03 (1.31, 1.57, 1.31, 0.84), III 3.9 (1.12, 1.19, 0.97, 0.62), IV 5.32 (1.49, 1.61, 1.38, 0.84). Leg formula (longest to shortest legs): I-IV-II-III. TmI 0.66 and TmIV 0.48. Tibial spine formula: 2–2–2. Abdomen 1.51 long, 0.94 wide, oval, light grey, with distinct pattern on dorsal surface from proximal end to base of spinnerets and extending laterally, ventral side green. Palp (Figs 1A-D, 2A, B): patella short, with long dorsal spine; tibia conic, with two retrolateral and one dorsal trichobothria; paracymbium U-shaped, basal part with several setae, lower margin of distal arm with a projection, wider than long with a blunt end, covering the posterior margin of tibia in retrolateral view; cymbium rather long, ridge-shaped, proximal to cymbial apophysis (PCA). Pit hook curved with a pointed end. Embolic division: Radix long, sclerotized; anterior projection of lamella characteristica (ALC) leaf-shaped with a pointed end, protruding above tegulum; posterior projection of lamella characteristica (PLC) as long as ALC with a pointed end; terminal apophysis sclerotized, dark, strongly curved with a blunt end, pointing towards anterior projection of lamella characteristica (ALC). Embolus short, tip bifurcated; apical margin of thumb (TH) serrated, median membrane simple.



Figure 3. *Denisiphantes arcuatus* sp. nov., female paratype **A**, **C** epigynum, ventral view **B** epigynum, lateral view **D** vulva, dorsal view **E** vulva, anterior view.

Female (paratype HNU–00–Lan–2): Total length: 3.16. Carapace 1.26 long, 0.92 wide; cephalic region slightly elevated, dark brown; fovea, cervical and radial grooves distinct. Clypeus 0.31 high. Sternum wider than long, brown, with spine-like setae; labium wider than long; maxillae long, distal-end broad with scopulae. Chelicerae with 4 promarginal and 3 retromarginal teeth. Eye region narrow, AER recurved, PER straight, slightly wider than AER. Eye sizes and interdistances: AME 0.07, ALE 0.09, PME 0.08, PLE 0.08, AME–AME 0.02, PME–PME 0.05, AME–ALE, 0.06, PME–PLE 0.06, AME–PME 0.06, ALE–ALE 0.39, PLE–PLE 0.41,



Figure 4. *Denisiphantes arcuatus* sp. nov., male holotype (**A**, **B**) and female paratype (**C–D**) **A**, **C** habitus, dorsal view **B**, **D** habitus, ventral view.



Figure 5. Denisiphantes denisi (Schenkel, 1963) A palp, ventral view B palp, retrolateral view.

ALE–PLE contiguous. Length of legs: I 5.55 (1.49, 1.82, 1.39, 0.85), II 4.8 (1.32, 1.55, 1.21, 0.72), III 3.83 (1.09, 1.27, 0.92, 0.55), IV 5.18 (1.46, 1.63, 1.33, 0.76). Leg formula I–IV–II–III. TmI 0.58 and TmIV 0.43. Tibial spine formula: 2–2–2–2. Abdomen 1.90 long, 1.16 wide; oval, light grey, dorsally with distinct pattern from proximal end to base of spinnerets and extending laterally, ventral side green. Epigynum (Fig. 3A–E): broad, posteriorly pointing scape almost hexagonal-shaped, stretcher and lateral pockets absent; posterior median plate (PMP) conspicuously large, tetragonal-shaped, covering most of dorsal side of scape; spermathecae elliptical.

Etymology. The species name comes from the Latin adjective "*arcuatus*" meaning "curved" and refers to the curved anterior terminal apophysis in the male palp.

Distribution. Known only from the type locality in Yunnan, China (Fig. 8).

Denisiphantes denisi (Schenkel, 1963)

Figures 5-7

Lepthyphantes denisi Schenkel, 1963: 118, fig. 70a–c. Lepthyphantes denisi Zhu & Li, 1983: 146, fig. 3d–f. Lepthyphantes denisi Hu, 2001: 503, fig. 334.1–4.



Figure 6. *Denisiphantes denisi* (Schenkel, 1963) **A, B** epigynum, ventral view **C** vulva, dorsal view **D** vulva, anterior view.

Denisiphantes denisi Tu, Li & Rollard, 2005: 652, figs 11–25. *Denisiphantes denisi* Tanasevitch, 2006: 303, figs 76, 77.

Material examined. 3Å3Q, CHINA, **Yunnan Province,** Tengchong County, Beihai Township, 15.2 km NE of Tengchong at Qinghai (lake), 25.13408°N, 98.57144°E,



Figure 7. *Denisiphantes denisi* (Schenkel, 1963), male (**A–C**) and female (**D–F**) **A**, **D** habitus, dorsal view **B**, **E** habitus, lateral view **C**, **F** habitus, ventral view.

alt. 1842 m, 07 June 2006, D. H. Kavanaugh, R. L. Brett & Da–zhi Dong (HNU– DHK–2006–060); 1^Q, **Guizhou Province**, Dafang County, Xingshu Township, Cuisuba, 27°23'N, 105°52'E, alt. 1750 m, 15 August 2020, Zhang Mao (HNU–



Figure 8. Distribution of Denisiphantes arcuatus sp. nov. and Denisiphantes denisi (Schenkel, 1963).

202008–W–5–10); 1♀, Dafang County, Xingshu Township, Cuisuba, 27°23'N, 105°52'E, alt. 1750 m, 17 August 2020, Zhang Mao (HNU–202008–S–3–20). **Distribution.** China (Gansu, Guizhou, Qinghai and Yunnan, Fig. 8).

Discussion

Denisiphantes was described as a monotypic genus based on the material collected from Qinghai and also reported from Gansu and Guizhou. The new species described here is

reported from Yunnan. Considering the vast distribution of the type species (Fig. 8), it is possible that there are still several species of this genus that need to be explored from Yunnan and across the adjacent areas to understand the distribution and origin of this genus.

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References

- Hu JL (2001) Spiders in Qinghai-Tibet Plateau of China. Henan Science and Technology Publishing House, 658 pp.
- Li SQ (2020) Spider taxonomy for an advanced China. Zoological Systematics 45(2): 73–77.
- Li SQ, Lin YC (2016) Species Catalogue of China (Vol. 2) Animals Invertebrates (I): Arachnida: Araneae. Science Press, Beijing, 549 pp.
- Schenkel E (1963) Ostasiatische Spinnen aus dem Muséum d'Histoire naturelle de Paris. Mémoires du Muséum National d'Histoire Naturelle de Paris (A, Zool.) 25: 1–481.
- Tanasevitch AV (2006) On some Linyphiidae of China, mainly from Taibai Shan, Qinling Mountains, Shaanxi Province (Arachnida: Araneae). Zootaxa 1325: 277–311. https://doi. org/10.11646/zootaxa.1325.1.18
- Tu LH, Li SQ, Rollard C (2005) A review of six linyphild spiders described from China by Dr E. Schenkel (Araneae: Linyphildae). Revue Suisse de Zoologie 112: 647–660. https://doi. org/10.5962/bhl.part.80318
- Zhu CD, Li ZS (1983) Three new species of spiders of the genus *Lepthyphantes* and description of the male spider of *L. denisi* Schenkel (Araneae: Linyphiidae). Journal of the Bethune Medical University 9(suppl.) 144–147.

RESEARCH ARTICLE



A new species and first record of the genus Procerobaetis Kaltenbach & Gattolliat, 2020 (Ephemeroptera, Baetidae) from Thailand

Chanaporn Suttinun¹, Thomas Kaltenbach^{2,3}, Jean-Luc Gattolliat^{2,3}, Boonsatien Boonsoong¹

Animal Systematics and Ecology Speciality Research Unit (ASESRU), Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand 2 Museum of Zoology, Palais de Rumine, Place Riponne 6, CH-1005 Lausanne, Switzerland 3 University of Lausanne (UNIL), Department of Ecology and Evolution, CH-1015 Lausanne, Switzerland

Corresponding author: Boonsatien Boonsoong (fscibtb@ku.ac.th)

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Abstract

The genus *Procerobaetis* Kaltenbach & Gattolliat, 2020 is reported for the first time from Thailand, and *Procerobaetis totuspinosus* **sp. nov.** is described as a new species based on larvae. It can be easily distinguished from other known *Procerobaetis* species by the presence of triangular spines at the posterior margin of tergites VI–IX. COI sequences were obtained from all known species. The genetic distances (Kimura 2-parameter) between the new species and the other species are between 20% and 23%. The morphological characters of the new species and its closely related species are discussed; larval key to all species of the genus *Procerobaetis* is also provided.

Keywords

Mayfly, taxonomy, Southeast Asia

Introduction

The family Baetidae is the most diverse mayfly family at the species level, accounting for about 30% of all mayfly species worldwide (Barber-James et al. 2008; Jacobus et al. 2019). Approximately nine genera and 13 species have been recorded from Thailand (Müller-Liebenau and Heard 1979; Thomas 1992; Sites et al. 2001; Boonsoong et al. 2004; Kluge 2016; Kluge and Novikova 2017; Sutthinun et al. 2018; Kluge et al. 2020; Kluge and Suttinun 2020). In the past decade, knowledge about the diversity of Baetidae in Thailand has continued to increase, and more taxa have been described, including *Procloeon* Bengtsson, 1915 (Tungpairojwong and Bae 2015; Kluge 2016), *Anafroptilum* Kluge, 2012 (Kluge and Novikova 2017), *Platybaetis* Müller-Liebenau, 1980 (Sutthinun et al. 2018), *Centroptella* Braasch & Soldán, 1980 (Kluge et al. 2020), *Indocloeon* Müller-Liebenau, 1982 (Kluge and Suttinun 2020), as well as the genus *Cymbalcloeon* Suttinun, Gattolliat & Boonsoong, 2020, which is endemic to this country (Suttinun et al. 2020).

Recently, the genus *Procerobaetis* Kaltenbach & Gattolliat, 2020 of Baetidae was discovered from Southeast Asia. It presently contains three species distributed in Indonesia: Sumatra (*P. leptobranchius* and *P. petersorum*) and the Philippines (*P. freitagi*), as described by Kaltenbach et al. (2020). Here, we describe a new species of *Procerobaetis* from Thailand based on material collected from the northern part of the country. This is one of the results of the first mass survey of the family Baetidae in Thailand. Materials were collected from May 2017 to June 2020 in 105 localities from all parts of Thailand, mainly from the southern and the western parts. As the family Baetidae is still poorly known in Thailand, our study will help to improve this situation. The latest new genus of this family was also described based on the same collection campaign (Suttinun et al. 2020).

Materials and methods

The specimens were collected from streams and wadeable rivers from the northern part of Thailand. They were subsequentely preserved in 95% ethanol. Larval dissection was performed in Cellosolve, with subsequent mounting on slides with Euparal. Measurements (given in mm) and photographs were taken using a Visionary LK System (Dun, Inc., USA). All drawings were made with the aid of a camera lucida attached to a compound microscope. For scanning electron microscopy (SEM), specimens (head, legs, tergites, paraproct, caudal firmaments) were dried in a critical point drier (CPD7501) and coated with gold (Sputter Coater SC7620). The SEM photographs were observed with a FEI Quanta 450 SEM. Final plates were prepared with Adobe Photoshop CC 2020.

The DNA was extracted using non-destructive methods, allowing subsequent morphological analysis (see Vuataz et al. 2011 for details). The COI (658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1) were amplified using

Species	Locality	GenBank Accession Number (GenSeq Nomenclature)
P. totuspinosus sp. nov.	Chiang Mai, Thailand	MW549043 (genseq-2 COI)
P. leptobranchius	Sumatra, Indonesia	MN453816, MN453817
P. petersorum	Sumatra, Indonesia	MN453818, MN453814
Procerobaetis sp. C	Sumatra, Indonesia	MN453815
P. freitagi	Mindoro, Philippines	MN453819

Table 1. Sequenced specimens of the genus *Procerobaetis* (in bold new sequence).

the primers LCO1490 and HCO2198 (Folmer et al. 1994). The polymerase chain reaction (PCR) conditions and procedure were performed as described by Kaltenbach et al. (2020). Sequencing was done with Sanger's method (Sanger et al. 1977). The genetic distances between species was performed using Kimura-2-parameter distances (K2P, Kimura 1980), calculated with the program MEGA X (Kumar et al. 2018). The GenBank accession numbers are given in Table 1.

The distribution map was generated with the software SimpleMappr (Shorthouse 2010).

The material is deposited in the collection of the Zoological Museum at Kasetsart University in Bangkok, Thailand (**ZMKU**) and at the Museum of Zoology in Lausanne, Switzerland (**MZL**).

Taxonomy

Order Ephemeroptera Hyatt & Arms, 1891 Family Baetidae Leach, 1815 Genus *Procerobaetis* Kaltenbach & Gattolliat, 2020

Procerobaetis totuspinosus Suttinun, Kaltenbach & Boonsoong, sp. nov. http://zoobank.org/964C6E8F-8417-4F94-ABE1-40D28F07CD4D Figures 1–7

Materials examined. *Holotype.* 1 larva on slide, deposited in ZMKU, Thailand, Chiang Mai, Mae Chaem district, Mae Chaem wadeable river, 18°30'45.3"N, 98°21'23.8"E, 475 m, 16.02.2020, B. Boonsoong leg.

Paratypes. 1 larva on slide, deposited in ZMKU, same data as holotype; 1 larva in alcohol, GBIFCH00673237, deposited in MZL, same data as holotype.

Other materials. 6 larvae in alcohol, deposited in ZMKU, same data as holotype. 2 larvae in alcohol, deposited in ZMKU, 1 larva in alcohol, GBIFCH00673238, deposited in MZL, Thailand, Chiang Mai, Mae Rim district, Mae Sa stream, 18°54'39.1"N, 98°55'33.6"E, 355 m, 15.02.2020, B. Boonsoong leg.

Description. Larva (Figs 1–7). Body length 4.5–4.6 mm.

Colouration (Fig. 7). Head, thorax and abdomen dorsally brown, head and thorax with bright, median, dorsal suture. Head, thorax and abdomen ventrally light brown. Legs whitish. Caudal filaments whitish.



Figure 1. *Procerobaetis totuspinosus* sp. nov., larval morphology **A** labrum **B** right mandible **C** right prostheca **D** left mandible **E** left prostheca **F** hypopharynx **G** maxilla **H** labium. Scale bar: 0.1 mm.



Figure 2. *Procerobaetis totuspinosus* sp. nov., larval morphology **A** antenna **B** gill I **C** gill II **D** gill III **E** gill IV **F** gill V **G** gill VI **H** gill VII **I** paraproct. Scale bars: 0.1 mm.

Head. *Antenna* (Figs 2A, 6A, B) approximately 3–4× as long as head length; flagellum with lanceolate spines at apex of each segment, longer at inner lateral margin, increasing in length distally in segment VII–XI and decreasing thereafter.



Figure 3. Procerobaetis totuspinosus sp. nov., SEMs of legs A foreleg B middle leg C hind leg.

Labrum (Fig. 1A). Rectangular, length 0.6× maximum width. Distal margin with medial emargination and a small process. Dorsally with many long, stout, simple setae scattered over area, erratically distributed, not arranged in an arc. Ventrally with a marginal row composed of five lateral long, feathered setae and ten anterolateral medial



Figure 4. *Procerobaetis totuspinosus* sp. nov., SEMs of foreleg **A** setae at dorsal margin of femur apex **B** setae near ventral margin of femur **C** setae at ventral margin of tibia apex and proximal tarsus **D** fore claw.

long, bifid, pectinate setae; ventral surface with ca five short, spine-like setae near lateral and anterolateral margins, increasing in length distally.

Right mandible (Fig. 1B, C). Outer and inner sets of denticles with 4 + 1 + 3 denticles. Prostheca stout, arched, shorter than half of canines. Margin between prostheca and mola straight, with a row of medium, stout setae. Setae at apex of mola present.

Left mandible (Fig. 1D, E). Outer and inner sets of denticles with 4 + 3 denticles. Prostheca stout, apically broad, reduced comb-shaped structure. Subtriangular process long and slender, above level of area between prostheca and mola. Denticles of mola apically constricted. Setae at apex of mola absent.

Both mandibles with lateral margins almost straight. Basal half with fine, simple setae scattered over dorsal surface.

Hypopharyns (Fig. 1F). Lingua equal to superlingua, longer than broad, with medial tuft of long, stout setae. Superlingua distally almost straight, lateral margin rounded, with fine, long, simple setae along laterodistal margin.

Maxilla (Fig. 1G). Galea-lacinia with two simple, robust apical setae under crown. Medially with one pectinate, spine-like seta and a row of five long, simple setae increasing in length. Maxillary palp $1.8 \times$ as long as length of galea-lacinia with segment I shorter than galea-lacinia; palp segment II $0.5 \times$ length of segment I, palp segment III $1.4 \times$ length of segment II; setae on maxillary palp fine, simple, scattered over surface of segments I, II and III; apex of last segment rounded.



Figure 5. *Procerobaetis totuspinosus* sp. nov., SEMs of tergites **A** tergites V–IX **B** enlargement of tergites VIII–IX.



Figure 6. *Procerobaetis totuspinosus* sp. nov., SEMs of larval morphology **A** antenna **B** closer view detail of antenna showing details of lanceolate spines **C** paraproct **D** caudal filaments middle section.

Labium (Fig. 1H). Glossae basally broad, narrowing toward apex, shorter than paraglossae; inner margin with eight spine-like setae; apex with two long and one medium, robust, pectinate setae; outer margin with six spine-like setae, increasing in length distally; ventral surface with medium, fine, simple, scattered setae. Paraglossae subrectangular, apically curved inward; apex rounded, with two rows of long, robust, simple setae; ventrally two medium, simple setae in anteromedial area; dorsally with a row of four or five long, spine-like setae near inner margin. Labial palp with segment I 0.85× length of segments II and III combined, ventrally scattered with short, fine, simple setae; segment II with very small distomedial expansion, ventrally with scattered short, fine, simple setae, dorsally with a row of four long, spine-like setae, dorsally with a row of four long, spine-like setae, dorsally with a row of four long, spine-like setae, dorsally with a row of four long, spine-like setae, dorsally with a row of four long, spine-like setae; segment III subquadrangular, apex rounded, ventral surface with an arc of five stout, spine-like setae on anteromedially, covered with short spine-like, simple setae and short, fine, simple setae. Mentum distally scattered with fine, simple setae.



Figure 7. *Procerobaetis totuspinosus* sp. nov., habitus, larvae **A** dorsal view, male **B** lateral view, male **C** dorsal view, female **D** lateral view, female. Scale bars: 1 mm.

Thorax. *Foreleg* (Figs 3A, 4). Ratio of foreleg segments 1.4:1.0:0.9:0.3. *Femur.* Length 3.7–4.1× maximum width; dorsal margin with a row of six curved, spine-like setae; length of setae 0.23× maximum width of femur; apex rounded, with one pair of spine-like setae (Fig. 4A); many stout, lanceolate, laterally pectinate setae scattered along ventral margin (Fig. 4B); femoral patch absent. *Tibia.* Dorsal margin with a row of fine, simple setae; ventral margin with a row of curved, laterally pectinate, spine-like setae, on apex three longer, laterally pectinate setae; patellotibial suture present on basal 1/3 area. *Tarsus.* Dorsal margin with a row of fine, simple setae (Fig. 4C); ventral margin with a row of curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on proximal area with some curved, laterally pectinate, spine-like setae, on spine setae (Fig. 4D) elongate, slender, apically pointed, with one row of six or seven larger denticles and many minute denticles, ventral margin at apex straight, with many stripes.

Middle leg (Fig. 3B). As foreleg, but dorsal margin of femur slightly concave.

Hind leg (Fig. 3C). As foreleg, but dorsal margin of femur slightly concave.

Abdomen. *Tergites* (Fig. 5). Surface with scattered scales, U-shaped scale bases and micropores. Posterior margin of tergites I–V bare, tergites VI–VIII with triangular spines, tergite IX (Fig. 5B) with triangular spines absent in middle part.

Gills (Fig. 2B–H). Present on segments I–VII; elongate with very long, extended points; margin with very small denticles intercalating fine, simple setae; tracheae limited to main trunk. Gill I (Fig. 2B) as long as length of segments II and III combined, gill II (Fig. 2C) as long as length of segments III and 2/3 of IV combined, gill III (Fig. 2D) as long as length of segments IV and V combined, gill IV (Fig. 2E) as long as length of segments V and VI combined, gill V (Fig. 2F) as long as length of segments VI and VII combined, gill VI (Fig. 2G) as long as length of segments VI and VII combined, gill VI (Fig. 2G) as long as length of segments VII and VIII combined, gill VII (Fig. 2H) as long as length of segments VIII–X combined.

Paraproct (Figs 2I, 6C). Posterior margin with nine or ten stout spines; surface scattered with scales and U-shaped scale bases; posterolateral extension (cercotractor) with nine medium, blunt, marginal spines.

Caudal filaments (Fig. 6D). Cerci ca $0.4 \times$ body length, median caudal filament ca $0.8 \times$ length of cerci.

Diagnostic characters. Larva. The main diagnostic characters are: i) the posterior margin of tergites VI–VIII entirely with triangular spines; ii) the maxillary palp is shorter than in other species, with segment I shorter than galea-lacinia; and iii) all gills present extended points as in *P. freitagi*, while in *P. leptobranchius* and *P. petersorum* only gills I and II are apically strongly produced.

Winged stages. Unknown.

Etymology. *Procerobaetis totuspinosus* sp. nov. is a combination of *totus* (derived from the Latin word meaning entire, whole, complete) and *spina* (meaning spine), in reference to the remarkable complete row of triangular spines at the posterior margin of tergites VI–VIII.



Figure 8. Habitats of *Procerobaetis totuspinosus* sp. nov. larva **A** Mae Sa stream **B** Mae Chaem wadeable river **C** submerged woods with bottom sand and gravel **D** riverbank with vegetation and root.

Table 2. Genetic distances (COI) between sequenced specimens, using the Kimura 2-parameter.

		1	2	3	4
1	Procerobaetis leptobranchius				
2	Procerobaetis petersorum	0.13			
3	Procerobaetis sp. C	0.16	0.16		
4	Procerobaetis freitagi	0.20	0.19	0.20	
5	Procerobaetis totuspinosus sp. nov.	0.22	0.20	0.23	0.20

Distribution. Chiang Mai province

Biological aspects. The specimens were collected in a stream and a wadeable river (Fig. 8) at a range of altitudes (355–475 m a.s.l.). Mae Sa stream is located mostly in residential areas with a partly closed canopy; the substrate was dominated by pebble and gravel, with few patches of leaf litter or dead wood (Fig. 8A). Mae Chaem wadeable river is kind of the submontane type, bordered by farmland and residential areas; its substrate was dominated by sand (Fig. 8B). The larvae of the new species were found in the littoral zone, which was characterised by submerged wood, a sand and gravel bottom (Fig. 8C), and vegetation and roots along the riverbank (Fig. 8D).

Molecular analysis. The Kimura 2-parameter (K2P) analysis revealed interspecific distances (COI) between the new species and the other species of 20–23%. In addition, the interspecific genetic distances of known *Procerobaetis* species varied between 13–20% (Table 2).



Figure 9. Distribution of genus Procerobaetis: Procerobaetis totuspinosus sp. nov. (star); P. leptobranchius (square); P. petersorum (hexagonal); P. freitagi (circle).

Discussion

The genus *Procerobaetis* was established in 2020 by Kaltenbach & Gattolliat. This small genus comprises three species: *Procerobaetis leptobranchius*, *P. petersorum* and *P. freitagi*. It shares with *Nigrobaetis* Kazlauskas in Novikova & Kluge, 1987 and *Alainites* Waltz & McCafferty, 1994 important morphological characters such as: i) body laterally compressed with hypognathous mouthparts, ii) shape of glossae and paraglossae iii) shape of segments II and III of the labial palp (Müller-Liebenau 1984; Kang et al. 1994; Waltz et al. 1994; Gattolliat 2004; Gattolliat et al. 2012; Zrelli et al. 2012). The genus *Procerobaetis* is characterized by very slender, elongate gills with pronounced points and a very long, extended apex at least in gills I and II (Kaltenbach et al. 2020). The relationship of this genus with other closely related genera and non-closely related genera was stated by Kaltenbach et al. 2020.

Procerobaetis totuspinosus sp.nov. belongs to the genus *Procerobaetis* based on characters defined by Kaltenbach et al. (2020), and it mainly differs from the three previously known species by the triangular spines on the posterior margin of tergites VI–IX. Both *P. lepto-branchius* and *P. petersorum* have triangular spines on the posterior margin of tergite IX only, while *P. freitagi* has triangular spines present on tergites VIII–IX (Kaltenbach et al. 2020). A comparison between this new species and other known *Procerobaetis* species indicates a close morphological similarity between *Procerobaetis totuspinosus* sp. nov. and *P. freitagi* (from the Philippines) in terms of the gill apex shape, the number of medial simple setae of

the maxilla, the absence of stout setae on the dorsal margin of the mid tibia and the shape of the ventral margin at the apex of the claw on all legs. However, the new species can be easily distinguished by the presence of triangular spines on tergites VI–IX and a shorter maxillary palp. In addition to the material of the new species described here, we collected a single specimen from a another, distant locality in southern Thailand (Ai Krading stream, Waeng district Narathiwat province). This larva obviously belongs to an undescribed species; however, the lack of sufficient material does not allow a formal description of the new species.

The molecular analysis clearly supports *P. totuspinosus* sp. nov. as a valid species. It is clearly differentiated from other species, as the genetic distances between species range from 20% to 23% (K2P; Table 2); this is much higher than 3.5% which is generally considered as the maximum value for intraspecific divergence (Hebert et al. 2003; Zhou et al. 2010). These results are in line with the genetic distances measured between the three previous *Procerobaetis* species (Kaltenbach et al. 2020). However, this result was based on a single specimen, distances between different populations of the new species obviously remain unknown.

The discovery of *Procerobaetis* in Thailand is the first report of the genus from continental Asia, as all other specimens were collected on islands. The new type locality is situated between Sumatra and the northern Philippines (Fig. 9).

Larvae of *Procerobaetis* should not be overlooked, as they are easily recognisable both in the field and in the lab. However, *Procerobaetis* remains local and rare in Thailand, as in Sumatra and the Philippines, probably due to precise ecological requirements. We may expect a broader distribution including poorly known areas such as continental Malaysia, Laos, Cambodia and Vietnam. Despite intensive field sampling in Brunei and North Kalimantan, allowing recent improvements in the knowledge of the mayfly fauna of Borneo, the genus is still not recorded from this island.

Key to species of the genus Procerobaetis

- 1 Triangular spines on posterior margin present only on tergite IX, apex of gills III– VII pointed (Kaltenbach et al. 2020: 10, fig. 3B–H)......**2**
- 2 Dorsal margin of femur (middle and hind legs) slightly concaveP. leptobranchius
- Dorsal margin of femur (middle and hind legs) almost straight...... P. petersorum
- 3 Triangular spines on posterior margin present on tergites VIII-IX P. freitagi

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References

- Barber-James HM, Gattolliat JL, Sartori M, Hubbard MD (2008) Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. Hydrobiologia 595: 339–350. https://doi. org/10.1007/s10750-007-9028-y
- Boonsoong B, Thomas A, Sangpradub N (2004) *Gratia narumonae* n.sp., a new mayfly from Thailand (Ephemeroptera, Baetidae). Ephemera 4(1): 1–9.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
- Gattolliat JL (2004) First records of the genus *Nigrobaetis* Novikova & Kluge (Ephemeroptera: Baetidae) from Madagascar and La Réunion with observations on Afrotropical biogeography. Revue Suisse de Zoologie 111(3): 657–669. https://doi.org/10.5962/bhl.part.80259
- Gattolliat JL (2012) Two new genera of Baetidae (Ephemeroptera) from Borneo (East Kalimantan, Indonesia). International Journal of Limnology 48: 187–199. https://doi. org/10.1051/limn/2012012
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of The Royal Society B 270: 313–321. https://doi.org/10.1098/ rspb.2002.2218
- Jacobus LM, Macadam CR, Sartori M (2019) Mayflies (Ephemeroptera) and their contributions to ecosystem services. Insects 10(6): e170. https://doi.org/10.3390/insects10060170
- Kaltenbach T, Garces JM, Gattolliat JL (2020) A new genus of Baetidae (Insecta, Ephemeroptera) from Southeast Asia. European Journal of Taxonomy 612: 1–32. https://doi. org/10.5852/ejt.2020.612
- Kang CH, Chang HC, Yang CT (1994) A revision of the genus *Baetis* in Taiwan (Ephemeroptera, Baetidae). Journal of Taiwan Museum 47(2): 9–44. [In Chinese]
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581
- Kluge NJ (2016) A new subgenus Oculogaster subgen. n. for viviparous representatives of Procloeon s. l., with discussion about status of the generic name Austrocloeon Barnard 1932 and the species name africanum Esben-Petersen 1913 [Cloeon] (Ephemeroptera, Baetidae). Zootaxa 4107(4): 491–516. https://doi.org/10.11646/zootaxa.4107.4.2
- Kluge NJ, Novikova EA (2017) Occurrence of *Anafroptilum* Kluge 2012 (Ephemeroptera: Baetidae) in Oriental Region. Zootaxa 4282: 453–472. https://doi.org/10.11646/zootaxa.4282.3.2
- Kluge NJ, Godunko RJ, Svitok M (2020) Nomenclatural changes in *Centroptella* Braasch & Soldán, 1980 (Ephemeroptera, Baetidae). ZooKeys 914: 81–125. https://doi.org/10.3897/ zookeys.914.46652

- Kluge NJ, Suttinun C (2020) Review of the Oriental genus *Indocloeon* Müller-Liebenau 1982 (Ephemeroptera: Baetidae) with descriptions of two new species. Zootaxa 4779: 451–484. https://doi.org/10.11646/zootaxa.4779.4.1
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547– 1549. https://doi.org/10.1093/molbev/msy096
- Müller-Liebenau I, Heard WH (1979) Symbiocloeon: a new genus of Baetidae from Thailand (Insecta, Ephemeroptera). In: Pasternak K, Sowa R (Eds) Proceedings of the 2nd International Conference on Ephemeroptera. Pan'stwowe Wydawnictwo Naukowe, Warzawa–Kraków, 57–65.
- Müller-Liebenau I (1984) New genera and species of the family Baetidae from West-Malaysia (River Gombak) (Insecta: Ephemeroptera). Spixiana 7: 253–284.
- Novikova EA, Kluge NJ (1987) Systematics of the genus *Baetis* (Ephemeroptera, Baetidae), with descriptions of new species from Middle Asia. Vestnik Zoologii 1987 (4): 8–19. [In Russian]
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74: 5463–5467. https://doi.org/10.1073/ pnas.74.12.5463
- Sites RW, Wang T, Permkam S, Hubbard MD (2001) The mayfly genera (Ephemeroptera) of Southern Thailand. Natural History Bulletin of the Siam Society 49: 243–268.
- Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. https://www.simplemappr.net [accessed 5 Nov. 2020]
- Sutthinun C, Gattolliat JL, Boonsoong B (2018) A new species of *Platybaetis* Müller-Liebenau, 1980 (Ephemeroptera: Baetidae) from Thailand, with description of the imago of *Platybaetis bishopi* Müller-Liebenau, 1980. Zootaxa 4378(1): 85–97. https://doi.org/10.11646/zootaxa.4378.1.5
- Suttinun C, Gattolliat JL, Boonsoong B (2020) Cymbalcloeon gen. nov., an incredible new mayfly genus (Ephemeroptera: Baetidae) from Thailand. PLoS ONE 15(10): e0240635. https://doi.org/10.1371/journal.pone.0240635
- Thomas A (1992) *Gratia sororculaenadinae* n. gen., n. sp., Ephéméroptère nouveau de Thaïlande (Ephemeroptera, Baetidae). Bulletin de la Société d'Histoire Naturelle de Toulouse 128: 47–51.
- Tungpairojwong N, Bae YJ (2015) Three new species of *Procloeon* (Ephemeroptera: Baetidae) from Thailand. Animal Systematics, Evolution and Diversity 31(1): 22–30. https://doi. org/10.5635/ASED.2015.31.1.022
- Vuataz L, Sartori M, Wagner A, Monaghan MT (2011) Toward a DNA taxonomy of Alpine *Rhithrogena* (Ephemeroptera: Heptagenidae) using a mixed Yule-coalescent analysis of mitochondrial and nuclear DNA. PLoS ONE 6: 1–11. https://doi.org/10.1371/journal.pone.0019728
- Waltz RD, McCafferty WP, Thomas A (1994) Systematics of *Alainites* n. gen., *Diphetor, Indo-baetis, Nigrobaetis* n. stat., and *Takobia* n. stat. (Ephemeroptera, Baetidae). Bulletin de la Societé d'Histoire naturelle de Toulouse 130: 33–36.
- Zhou X, Jacobus LM, DeWalt RE, Adamowicz SJ, Hebert PDN (2010) Ephemeroptera, Plecoptera, and Trichoptera fauna of Churchill (Manitoba, Canada): insights into biodiversity patterns from DNA barcoding. Journal of the North American Benthological Society 29(3): 814–837. https://doi.org/10.1899/09-121.1
- Zrelli S, Gattolliat JL, Boumaïza M, Thomas A (2012) First record of *Alainites sadati* Thomas, 1994 (Ephemeroptera: Baetidae) in Tunisia, description of the larval stage and ecology. Zootaxa 3497(1): 60–68. https://doi.org/10.11646/zootaxa.3497.1.6

RESEARCH ARTICLE



Cercopidae spittle-bugs (Hemiptera, Cicadomorpha) of Madagascar: a new species of Bourgoinrana and revision of the Locris species

Emilien Bouteille¹, Maxime Le Cesne¹, Adeline Soulier-Perkins¹

I Mécanismes adaptatifs et évolution (MECADEV), Muséum national d'histoire naturelle, CNRS, 57 rue Cuvier, CP 50, 75005 Paris, France

Corresponding author: Adeline Soulier (emilien.bouteille@edu.mnhn.fr)

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Abstract

The *Locris* species and subspecies from Madagascar are revised and a new combination is proposed: *Locris johannae* var. *nigrolimbata* Lallemand, 1910 to *L. nigrolimbata* **comb. nov**. Illustrations and description of male terminalia are given for the first time for the three *Locris* species and an identification key is provided. A new species of the Malagasy endemic genus *Bourgoinnana* Soulier-Perkins, 2012 is described: *B. beondrokaensis* Le Cesne & Soulier-Perkins **sp. nov**. An updated identification key to the species of *Bourgoinnana* is provided.

Keywords

Afrotropical, Cercopoidea, key, male genitalia, taxonomy

Introduction

Hemiptera are one of the most diverse groups of insects (Bartlett et al. 2018). They are mainly distinguished by a biting-sucking mouth apparatus. Many of them are phytophagous, but some feed on other liquids such as from animals or fungi. Despite their great diversity, some families, such as the Cercopidae Leach (1815), remain little studied.

Hamilton (2014) removed two genera: *Ambonga* Melichar, 1915 and *Pseudomachaerota* Melichar, 1915 from the Malagasy Cercopidae, leaving this family with nine genera known for the island. Six of these genera are endemic: *Alluaudensia* Lallemand, 1920, *Amberana* Distant, 1908, *Bourgoinrana* Soulier-Perkins, 2012, *Nesaulax* Jacobi, 1917, *Paramioscarta* Lallemand, 1949 and *Pogonorhinella* Schmidt, 1910. The remaining three genera *Literna* Stål, 1866, *Locris* Stål, 1866 and *Rhinaulax* Amyot & Serville, 1843 are present as well on the African continent. *Locris* is one of the largest genera of Cercopidae, with 87 known species, according to COOL (Soulier-Perkins 2020). It is widespread throughout tropical and South Africa as well as in Madagascar. A revision of Malagasy *Locris* species is presented here with male genitalia drawings and photos of habitus, and a new species of *Bourgoinrana* is described and the key to the species updated.

Material and methods

The abdomen of each specimen examined was cut off and cleared for one hour in hot (85 °C) 10% KOH. Dissections and cleaning of genital structures were performed in distilled water. If needed, a few drops of blue paragon for dying the ectodermic genital ducts were added for a few minutes. Observations were done in glycerol using a Leica microscope (MZ16). Drawings were produced using a camera lucida attached to the microscope and finalised with ILLUSTRATOR CS6 (Adobe Inc. 2012). Photos of the habitus were taken using a Canon EOS 6D with a Macro Lens Canon EF 100 mm f/2.8, viewed on computer with the software Canon EOS utility and then assembled with the software Helicon Focus 6. Terms used for the male genitalia are those of Soulier-Perkins and Kunz (2012). Qgis 3.10 (2020) was used to draw the distribution map.

Abbreviations

CAS	California Academy of Sciences, San Francisco, USA;
MIIZ	Muzeum i Instytut Zoologii, Warsaw, Poland;
MNHN	Muséum national d'Histoire naturelle, Paris, France;
MRAC	Musée royal de l'Afrique central, Tervuren, Belgium;
RIScNB	Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium.

Taxonomy

Locris Stål, 1866

Type species. Locris rubra (Fabricius, 1794)

The genus *Locris* Stål, 1866 was largely studied by Lallemand (1949) on the basis of morphological characters that are not all completely consistent in all species according to our observations. These characters are as follows: postclypeus rounded with a medio-longitudinal carina and transverse ridges, when observed laterally; it can be, either rounded (*L. rubra* (Fabricius, 1794)), angular (*L. vestigans* Jacobi, 1904) or protruding (*L. schmidti* Jacobi, 1910). Rostrum is short and barely extends to base of median trochanters. Antennae are short and their length equals diameter of eyes. Vertex is broader than long and its length equals half width of pronotum. Ocelli are much closer to each other than to compound eyes and are medium to small in size, except in *L. atra* Lallemand, 1923 where they are very large (Lallemand 1949). Pronotum is large, with a usually straight posterior margin but weakly indented in some species like *L. maculata* (Fabricius, 1781) according to Lallemand (1949) and may have a more or less distinct carina in middle. Scutellum is as long as wide and has three dimples, two small ones on anterior margin and one large centred. Tegmina are about 3 times as long as wide, cubital and median veins are fused from base to middle of tegmen, apical veins network is relatively dense. A spine is present on posterior tibiae. In genitalia, males have thin subgenital plates that look like a filament (Lallemand 1949) curved up or downward; they are wide and sometimes silky at base.

In describing the genus *Locris* Lallemand (1949) listed a series of exceptions in order to include more species in the genus. As a result, our view is that now the homogeneity of the genus and its taxonomic unity is questionable. However, the aim of our work here is not to revise the entire genus (which is present in Madagascar and the whole of Africa except for the northern countries Egypt, Tunisia, Libya, Algeria and Morocco) but to provide a clear identification for the few species and subspecies of *Locris* present in Madagascar.

Locris bipunctata (Signoret, 1860)

Figures 1–3

Monecphora bipunctata Signoret, 1860: 182 (original description) Locris bipunctata: Stål 1866: 60 (transfer) Locris bipunctata var. atra Lallemand, 1950: 94

Note. When Lallemand (1950) described *Locris bipunctata atra*, he described it as a variety, which according to the article 45.6.4 of the International Code of Zoological Nomenclature (ICZN 1999) should now be considered as a subspecies. Therefore, *Locris bipunctata* now contains two subspecies, easily distinguished from each other by the colouration of their tegmina, red for *L. b. bipunctata* (Fig. 1) and nearly completely black for *L. b. atra* (Fig. 2), the latter appearing as a strong melanisation. Geographically, the two subspecies are clearly separated (Fig. 3E) but from the material examined, no difference can be observed in the male terminalia between the two subspecies. For this reason, we decided to keep them as subspecies and illustrate here only the male terminalia of the paratype specimen of the subspecies *L. bipunctata atra* (Fig. 3A–D).

Distribution. Madagascar (Fig. 3E), *L. bipunctata bipunctata* in northern Madagascar, *L. bipunctata atra* in western Madagascar.



Figure I. Locris bipunctata bipunctata (Signoret), male A frontal view B labels C dorsal view D lateral view.



Figure 2. *Locris bipunctata atra* (Lallemand), male paratype **A** frontal view **B** labels **C** dorsal view **D** lateral view.

Description of the male terminalia (Fig. 3A–D). Pygofer (Fig. 3A), in lateral view almost trapezoidal with posterior margin being the longest side and slightly cut out in its first dorsal third. Aedeagus consists of two parts, first basal part, representing 2/3 of



Figure 3. *Locris bipunctata atra* (Lallemand), male terminalia of the paratype, in lateral view (**A–D**) and *Locris bipunctata* (Signoret), distribution map (**E**) **A** pygofer, anal tube, aedeagus, left paramere and left subgenital plate **B** aedeagus **C** left paramere **D** left subgenital plate **E** black dots: occurrences for *L. bipunctata atra*, white dots: occurrences for *L. bipunctata bipunctata*. Scale bars: 1 mm.

total length, tubular and almost same thickness over its entire length, curving regularly down then dorsally, and bearing on its apical dorsal part a small hump directed dorsoanteriorly; second part, hanging from first, narrowing in its middle then developing posteriorly a pair of pointed processes and finishing in a smooth and flattened apex slightly digit shaped ventro-posteriorly (Fig. 3B). Parameres smoothly widening from pygofer's attachment and finishing posteriorly by a curved spine (Fig. 3C). Subgenital plates large at base, narrowing quickly in a long and fine structure curving gently dorsally on its apical half (Fig. 3D).

Body length: 7.5–10 mm.

Material examined. Nominotypical subspecies-10 males. [Ambabalame, Madagascar], [Coll. Mus. Congo, Coll. V. Lallemand], [3], [R. Det., 6664 H], [Muséum Paris, MNHN (EH) 24628]; [Madagascar], [Lavaudon], [Collectic E de Bergev], [Muséum Paris], [Muséum Paris, MNHN (EH) 24115], [Muséum Paris, MNHN (EH) 24625]; [Madagascar Nord, distr. d'Ambanja, N. de Beangona-Ambevy, Vallée d'Antremade, 400 m, II-1964, P. Soga], [Museum Paris]; [Museum Paris, Sainte-Marie-de-Madagascar, Coll. Noualhier, 1898]; [Museum Paris, Madagascar, Baie d'Antongil, A. Mocquerys, Coll. Noualhier, 1898], [Muséum Paris, MNHN (EH), 24720]; [Museum Paris, Madagascar, Baie d'Antongil, A. Mocquerys, Coll. Noualhier, 1898], [Locris bipunctata Sign.], [Muséum Paris, MNHN (EH), 24722]; [Madagascar Nord, distr. d'Ambanja, N. de Beangona-Ambevy, Vallée d'Antremabe, 400, II -1964, P. Songa], [Muséum Paris], [Muséum Paris, MNHN (EH), 24719]; [Coll. Mus. Tervuren, Madagascar: Fampanambo, 1962, J. Vadon], [Locris bipunctata Signoret, H. Synave det., 1965], [Locris bipunctata Sign 33], [Muséum Paris, MNHN (EH), 24721]; [Madagascar Amber-Geb.], [Locris vicina Sign. d Edm. Schmidt determ. 1911], [Miz Pan Warszawa 12/1945, 2487]; [Madagascar Amber-Geb.], [Locris vicina Sign. & Edm. Schmidt determ. 1911], [970], [Miz Pan Warszawa. 12/1945, 2488]; and [Madagascar Amber-Geb.], [Locris vicina Sign. \mathcal{J} Edm. Schmidt determ. 1911], [Miz Pan Warszawa. 12/1945, 2489].

Subspecies *atra – Paratype* (male). [Entre Ambakaka et Bekopaka sur la Manambolo, Inst. Scient. Madagascar, VII – 49, RP], [Muséum Paris], [Paratype], [Muséum Paris MNHN (EH) 1474], [Locris bipunctata v. atra Lall.], [Muséum Paris MNHN (EH) 24634] - 5 males. [CASENT3004533], [Madagascar: Mahajanga, Prov: Parc National Tsingy de Bemaraha, 3.4 km 93°E, Bekopaka, Tombeau Vazimba, Elev 50 m, 6–10 Nov. 2001], [19°8'31"S, 44°49'41"E, coll: Fischer, Griswold et al., California Acad. Of Sciences malaise trap, in tropical dry forest, coll. Code: BLF4233], [8], [Locris bipunctata atra Lallemand, 1950, A. Soulier-Perkins det 2018]; [Madagascar Ouest, S-P. Antsalova Antsingy, Rés. Nat. 9, A. Peyrieras, I-1975], [Museum Paris]; [Madagascar Lambomakandro, Tuléar], [Museum Paris, 1935, B. Catala], [H. Synave det., 1979, Locris bipunctata var. atra Lall.]; [Andobo 190 m, forêt Antsingy, det. Antsalova, II – 57, P. Guiv], [Institut Scientifique, Madagascar], [Muséum Paris, MNHN (EH), 24718]; and [Madagascar, Lambomakandro, Tuléar], [Muséum Paris, 1935, R. Catala], [H. Synave., 1979, Locris bipunctata var. atra Stall.], [Muséum Paris, MNHN (EH), 24717]; 2 females. [Q], [Madagascar, province de Toliara, massif du Makay, 159 m, 21°40'29.4"S, 44°59'36.2"E], [Muséum Paris, ft Ambalamanga, rv Mangoky, 18-I-2011, A. Soulier-Perkins rec.], [Locris bipunctata atra Lallemand, 1950, A. Soulier-Perkins det. 2020], [Muséum Paris, MNHN (EH), 24759]; [2], [Madagascar, province de Toliara, massif du Makay, 159 m, 21°40'29.4"S, 44°59'36.2"E], [Muséum Paris, Près rivière, PL, 20-I-2011, A. Soulier-Perkins rec.], [Locris bipunctata atra Lallemand, 1950, A. Soulier-Perkins det. 2020], [Muséum Paris, MNHN (EH), 24760].

Locris nigrolimbata (Lallemand, 1910) comb. nov.

Figures 4-5

Locris johannae var. nigrolimbata Lallemand, 1910: 47 (description)

Note. When Lallemand (1910) described the species *Locris johannae* (from the southwest bank of Lake Tanganyika) he described as well a variety *nigrolimbata* from Madagascar. According to article 45.6.4 of the International Code of Zoological Nomenclature (ICZN 1999), *Locris johannae nigrolimbata* should be regarded as a subspecies (Fig. 4). This subspecies supposedly differs by the colouration of the tegmina. *Locris j. johannae* has completely red tegmina while *L. j. nigrolimbata* bears some black at their apex (Lallemand 1910). Our studies of material from Tanzania and Kenya, showed that the specimens with the black apex tegmina have the same male genitalia as the holotype of *L. j. nigrolimbata* while the specimens with the entire red tegmina have different male genitalia. This led us to consider *L. j. nigrolimbata* as a valid species and here change its rank to *L. nigrolimbata* comb. nov. However, we remain for now with only one specimen of this species from Madagascar, the other specimens were collected on the African continent.

Distribution. Madagascar and eastern Africa

Description of the male terminalia (Fig. 5). Pygofer (Fig. 5A), in lateral view, dorsal margin straight and perpendicular to anterior margin, posterior margin generally convex. Aedeagus consist of two parts, first basal part, representing 3/4 of total length with a base elbow shaped before widening ventrally then curving up dorsally and narrowing into a tubular structure almost of same thickness to its regular rounded dorsal apex; second part, hanging from first, its width is regular for most of its length with apex in shape of a swan, neck oriented posteriorly (Fig. 5B). Parameres prolonged apically by two little structures, inner one curved into a spine and external one rounded (Fig. 5C). Subgenital plates wide at base then abruptly narrowing in a long filament shape curved downward at its apex (Fig. 5D).

Body length: 8–10.5 mm.

Material examined. *Holotype* (male). [Holotypus], [Madagascar], [Coll. Mus. Congo, Coll. V. Lallemand], [R. DET. 6664 z z], [Locris johannae ssp. nigrolimbata Lall.], [H. Synave det.. 195 L. johannae f. nigrolimbata Lall.], [Holotype ♂ L. johannae ssp. nigrolimbata Lall. Michel Boulard det. 1989] – 4 males. [Afrique orient. Anglaise Voï Alluaud & Jeannel Mars 1911 . 600 m . St. 60], [Coll. Mus. Congo Coll. V. Lallemand], [R. Det. 6665]; [Afr. Or. Angl. (Wa-Kikuyu), Fort-Hall Alluaud & Jeannel Janv. 1912 – 1330 m – St. 80], [Coll. Mus. Congo Coll. V. Lallemand], [R. Det. 6665]; [♂], [Nairobi B. E. A.], [G. Babault, avril 1923], [Locris johannae ssn. nigrolimbata], [Muséum Paris, MNHN (EH), 24629]; and [Tanzanie: Mts Uluguru, Kimboza ofr. Héliophile, alt. 600M 24-30/VII/71], [Coll. Mus. Tervuren, Mission Mts. Uluguru, L. Berger, N. Leleup, J. Debecker V/VIII/71], [H. Synave det. 1957, Locris johannae Lall.], [Muséum Paris, MNHN (EH), 24635].
COLL. MUS. CONGO Madagase HOLOTYPUS R. DET. 6664 ZZ Coll. V. Lallemand H. Synave det. 195 L. Johannas gvolinobata Louis Johannae 10p. higrolineata (31). Holotype d 6 12421142 Michel Boulard det. 1959 1mm А В



Figure 4. *Locris nigrolimbata* (Lallemand), male holotype **A** frontal view **B** labels **C** dorsal view **D** lateral view.



Figure 5. *Locris nigrolimbata* (Lallemand), male terminalia, in lateral view **A** pygofer, anal tube, aedeagus, left paramere and left subgenital plate **B** aedeagus **C** left paramere in latero-dorsal view **D** left subgenital plate. Scale bars: 1 mm.

Locris vicina (Signoret, 1860)

Figures 6–7

Monecphora vicina Signoret, 1860: 182 (original description) *Locris vicina*: Stål 1866: 60 (transfer).

Distribution. Madagascar (Fig. 7E)

Description of the male terminalia (Fig. 7A–D). Pygofer (Fig. 7A) in lateral view, dorsal and anterior margins perpendicular, posterior margin S-shaped and making an acute angle with the ventral margin. Aedeagus consists of two parts, first basal part, representing a small 2/3 of total length with a very regular tubular shape almost curving as half a circle, its dorsal apical part bearing two little bumps oriented dorso-anteriorly; second part is hanging from first, it is widening smoothly, two processes are pointing posteriorly at mid-length, apex bulbous and covered in a dense padding (Fig. 7B), genital duct passes through aedeagus and open in the centre of padded apex (Fig. 7B). Parameres with a dorsal margin S-shaped giving it a cup shape prolonged apically by a spine curved downward (Fig. 7C). Subgenital plates wide at base and narrowing abruptly in a long filament structure curving abruptly dorsally on last third (Fig. 7D).

Body length: 10.5–12.5 mm.

Material examined. MNHN Collection–1 female $[\mathcal{Q}]$, [Madagascar. Tam.], [Soanierana-Ivongo, 8 . XI . 57, F. Keiser], [R. I. Sc. N. B., I. G. 23.285], [Muséum Paris, MNHN (EH), 24723]; -10 males. [20/III/2006, 633 m, parc de Zombitse Leobondro, brd rivière, 22°40.460'S, 44°51.633'E], [Muséum Paris, Madagascar 2006, rég. Atsimo-andrefana, A. Soulier-Perkins réc.], [Locris vicina (Signoret, 1860), A. Soulier-Perkins det. 2007], [], [Muséum Paris, MNHN (EH), 24659]; [], [Museum Paris Madagascar, Antanambé, Mocquerys, Coll.Noualhier 1898]; [3], [Museum Paris, Madagascar, catat 1844-91]; [Madagascar Nord, distr. d'Ambanja, N. de Beangona-Ambevy, Vallée d'Antremabe, 400 m, II – 1964, P. Sogal, [Muéum Paris], [Muséum Paris, MNHN (EH), 24724]; [Fort. Dauphin], [Muséum Paris, 1933, A. Seyrig], [Muséum Paris, MNHN (EH), 24725]; [Maeyatanana, Madagscar], [Collection le Moult, Naturaliste. Paris], [R. Mus. Hist. Nat., Belg. I. G. 12.595], [Muséum Paris, MNHN (EH), 24726]; [Madagascar, province de Mahajanga, P N Tsingy de Namoroka, 16°28'08"S, 45°20'52"E], [Muséum Paris, 09-IX-2012, Th. Bourgoin rec.], [Muséum Paris, MNHN (EH), 24727]; [21/XI/2005, région lac Alaotra, bord riv. Mavolava, 17°40.357'S, 47°54.289'E], [904 m, entre Ambakireny et Morano-Chrome], [Muséum Paris, Madagascar 2005, Bourgoin, Ouvrard, Attié, Soulier-Perkins], [Locris vicina (Signoret, 1860), A. Soulier-Perkins det. 2007], [[]], [Muséum Paris, MNHN (EH), 24728]; [Madagascar Mahajanga, P.N. Tsingy de Namoroka, wetland near village, 16°23'50"S, 45°17'12"E], [Muséum Paris, 29-X-2016, PL., T. Bourgoin, G. Kunz & A. Soulier-Perkins rec.], [Muséum Paris, MNHN (EH), 24729] - RIScNB [Ampijoro, Tsaramandroso], [Institut scientifique, Madagascar], [H. Synave det., 1957, Locris vicina Signoret], [R. I. Sc. N. B., I. G. 21.002]; MIIZ collection - 5 males [Madagasckar, Ambodimanga, Hammerstem S., I. 1906], [Locris bipunctata



Figure 6. Locris vicina (Signoret, 1860), male A frontal view B labels C dorsal view D lateral view.

Sign. ♂ Edm. Schmidt determ. 1909], [Miz Pan Warszawa. 12/1945, 2490]; [Madagaskar, Ambodimanga, Hammerstem S., I. 1906], [*Locris bipunctata* Sign. ♂ Edm. Schmidt determ. 1909], [Miz Pan Warszawa. 12/1945, 2491]; [Madagaskar, Ambodimanga, Hammerstem S., I - II. 1906], [*Locris bipunctata* Sign. ♂ Edm. Schmidt



Figure 7. *Locris vicina* (Signoret, 1860), male terminalia, in lateral view and distribution map **A** pygofer, anal tube, aedeagus, left paramere and left subgenital plate **B** aedeagus **C** left paramere **D** left subgenital **E** distribution map. Scale bars: 1 mm.

determ. 1909], [Miz Pan Warszawa. 12/1945, 2492]; [Madagaskar, Ambodimanga, Hammerstem S., I - II. 1906], [*Locris bipunctata* Sign. d Edm. Schmidt determ. 1909], [Miz Pan Warszawa. 12/1945, 2493]; and [Madagaskar, Ambodimanga, Hammerstem S., I - II. 1906], [*Locris bipunctata* Sign. d Edm. Schmidt determ. 1909], [Miz Pan Warszawa. 12/1945, 2494].

Casent collection – 7 males [CASENT8107240], [Madagascar: Majunga, Ampijoroa National Park, 160 km N of Maevatanana, on RN 04, elev 43 m, 25 Jan-7 Feb 2005], [16°19.16'S, 46°48.80'E, California Acad of Sciences, coll: M. Irwin, R. Harin'Hala, malaise trap – in deciduous forest, MA-25-42]; [CASENT8107233], [Madagascar: Majunga, Ampijoroa National Park, 160 km N of Maevatanan, on RN 04, elev 43 m, 2–9 November 2003], [16°19.16'S, 46°48.80'E, California Acad of Sciences, coll: M. Irwin, R. Harin'Hala, malaise trap - in deciduous forest, MA-25-22]; [CASENT8107236], [Madagascar: Majunga, Ampijoroa National Park, 160 km N of Maevatanan, on RN 04, elev 43 m, 10–21 January 2004], [16°19.16'S, 46°48.80'E, California Acad of Sciences, coll: M. Irwin, R. Harin'Hala, malaise trap – in deciduous forest, MA-25-29]; [CASENT8077112], [Perinet, Madagascar, 14 Jul. 1966, Liusnan], [Locris vicina, det. Penny, 99]; [CASENT8077114], [Madagascar, Hellville, Nossi-Be Isl., XI – 18 – 1959], [E. S. Ross, Collector], [Locris vicina]; [CASENT3002067], [Madagscar: Province, d'Antsiranana, Montagne des Français. 7.2 km 142°SE, Antsiranana (=Diego Suarez), Elev 180 m, 22–28 Feb 2001], [12°19'22°S, 49°20'17° E, colls: Fisher. Griswold et al. Calif. Academy of Sciences, malaise trap, in tropical dry forest. Code: BLF3130]; and [CASENT3008146], [Madagascar: Fianarantsoa, Province, Parc National d'Isalo, 9.1 km 354°N Ranohira, elev 725 m, 27-31 Jan 2003, 22°28'54"S, 045°27'42"E], [coll. Fisher, Griswold et al. California Acad. Of Sciences, collected at light-gallery forest, collection code: BLF7304].

Identification key to the species and subspecies of Locris from Madagascar

e2	Pronotum bearing two red dots in mide	1
iddle3	- Pronotum not bearing two red dots in 1	_
<i>bipunctata bipunctata</i> (Signoret)	2. Tegmina completely red (Fig. 1) <i>Locr</i>	2
n (Fig. 2)	- Tegmina black except for red anal marg	_
Locris bipunctata atra Lallemand		
	Tegmina completely red (Fig. 6)	3
	- Tegmina red with black apex (Fig. 4)	_
<i>rolimbata</i> (Lallemand) comb. nov.	Locris ni	

Bourgoinrana Soulier-Perkins, 2012

Type species. Amberana perinetana Synave, 1957.

Bourgoinrana beondrokaensis Le Cesne & Soulier-Perkins, sp. nov.

http://zoobank.org/75FDC14D-6616-43C9-AE85-EA7135771F4A Figures 8–9

Diagnosis. Uniformly coloured brownish with smokey yellowish tegmina compared to the similar *B. sandrangatensis* which has a red head, thorax and base of tegmina. It

also differs from this species by the length of its subgenital plates, 1.18 times longer than its pygofer height compared to 1.48 for *B. sandrangatensis*.

Distribution. Mount Beondroka in the natural reserve of Marojejy, Madagascar (Fig. 9E).

Description. Total length of male holotype 7.9 mm (tegmina included), paratypes 8.4 and 8.6 mm. Flattened ventro-dorsally. Head in dorsal view, 1.6 times wider between eyes than long in midline, anterior and posterior margins gently and regularly curved. Ocelli very close to each other with distance between eye and ocellus 9 times greater than between ocelli, located close to head posterior margin. Pronotum slightly convex, 1.8 times wider than long in midline, posterior margin wave-shaped, concave in middle. Tegmina 3.8 times longer than wide, M and CuA with a common stem at base and forking around 1/3 of tegmen length, ScP+R(+MA) forking after mid-length of tegmen, M and CuA both forking in apical third of tegmen. Metatibia bearing 1 lateral spine at 2/3 of its length.

Description of the male terminalia (Fig. 9A–D). Pygofer height 1.6 mm (Fig. 9A) in lateral view, dorsal margin almost straight, posterior margin almost straight 2/3 of its dorsal length then curving strongly anteriorly before having a final straight section, ventral margin straight and shorter than dorsal margin, anterior margin S-shaped. Subgenital plates 1.18 longer than pygofer height, gently and regularly curving dorsally with a rounded apex pointing dorso-posteriorly, thickness gradually diminishing from base to apex with a slight constriction before apex. Parameres in lateral view with a small hump in the middle of dorsal margin ending in a narrow elongate finger-shape apex slightly curved inward (Fig. 9C, D), ventral margin ending in a finger-shape apex shorter and less narrow than dorsal one; in dorsal view, internal margin cut out in two small extension facing each other (Fig. 9C). Lateral plates present with dorsal margin straight, making a rounded acute angle with the postero-ventral margin. Aedeagus long, shaped as a circumvented tube with a thumb-shaped extension on its dorsal margin, oriented dorso-anteriorly, small constriction before thumb extension; apical part bifid, prolonging ventral margin a lateral toothed extension folding posteriorly with apex pointing antero-ventrally and prolonging dorsal margin a thin ending in a lanceolate shape pointing anteriorly (Fig. 9B).

Colouration. Generally yellowish brown, head darker between eyes along posterior margin, pedicel of antennae dark brown. Tegmina smokey yellow and translucent. Abdomen red and legs yellowish with darker tarsal segments, lateral metatibial spine black (Fig. 8).

Etymology. The species is named after the type locality, Beondroka.

Type locality. Madagascar Est, Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga

Type material. *Holotype* (male), pinned: [Madagascar Est, Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga], [3], [Holotype], [Museum Paris, MNHN(EH) 24730]. *Paratypes:* 1 male: [Madagascar Est, Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga], [Museum Paris], [Paratype], [Museum Paris, MNHN(EH) 24731] 5 females: [Madagascar Est, Marojejy, Rés. Nat. Int. XII,



Figure 8. *Bourgoinrana beondrokaensis* Le Cesne and Soulier-Perkins, sp. nov., male A frontal view B labels C dorsal view D lateral view.



Figure 9. *Bourgoinrana beondrokaensis* Le Cesne and Soulier-Perkins sp. nov., male terminalia and distribution map **A** pygofer, anal tube, left lateral plate and left subgenital plate **B** aedeagus **C** left paramere in lateral view **D** left paramere, in dorsal view **E** distribution map. Scale bars = 0.5 mm.

Beondroka 1200 m, XII.1960, P. Soga], $[\bigcirc]$, [Paratype], [Museum Paris, MNHN(EH) 24732]; [Madagascar Est, Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga], $[\bigcirc]$, [Paratype], [Museum Paris, MNHN(EH) 24734]; [Madagascar Est,

Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga], [Q], [Paratype], [Museum Paris, MNHN(EH) 24735]; [Madagascar Est, Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga], [Q], [Paratype], [Museum Paris, MNHN(EH) 24736]; and [Madagascar Est, Marojejy, Rés. Nat. Int. XII, Beondroka 1200 m, XII.1960, P. Soga], [Museum Paris], [Paratype], [Museum Paris, MNHN(EH) 24733]

Identification key to the species of Bourgoinrana

1	Pronotum uniformly coloured
_	Pronotum with an anterior transverse yellowish band B. perinetana (Synave)
2	Tegmina completely red
_	Tegmina mostly smokey yellowish translucent
3	Head, thorax and base of tegmina redB. sandrangatensis (Synave)
_	Head and thorax ochre-brown and tegmina entirely smokey yellowish

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References

Adobe Inc (2012) Adobe Illustrator. https://adobe.com/products/illustrator

- Amyot CJB, Serville JG (1843) Deuxième partie. Homoptères. Homoptera Latr. Histoire Naturelle des insectes. Hemiptères 1843: 1–676.
- Bartlett CR, Deitz LL, Dmitriev DA, Sanborn AF, Soulier-Perkins A, Wallace MS (2018) Chapter 19: The diversity of the true Hoppers (Hemiptera: Auchenorrhyncha). In: Foottit RG, Adler PH (Eds) Insect Biodiversity: Science and Society, II. John Wiley & Sons Ltd, 501–590. https://doi.org/10.1002/9781118945582.ch19
- Distant WL (1908) Rhynchotal notes xlv. Annals and Magazine of Natural History. London (Ser. 8) 2: 309–323. https://doi.org/10.1080/00222930808692488
- Fabricius JC (1781) Ryngota. In: Fabricius JC (Ed.) 1781 Species insectorum exhibentes eorum differentias specificas, synonyma auctorum, loca natalia, metamorphosin adiectis observationibus, descriptionibus, 2: 313–331. https://doi.org/10.5962/bhl.title.36509
- Fabricius JC (1794) Ryngota. In: Fabricius JC (Ed.) 1794 Entomologia systematica emendata et aucta. Secundum classas, ordines, genera, species adjectis synonimis, locis, observationibus, descriptionibus, 4. Hafniae, impensis C.G. Proft, 472 pp.
- QGIS.org (2020) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.org

- Hamilton KGA (2014) The old-world Zygonini tr. nov. (Hemiptera, Cercopoidea, Clastopteridae), with new taxa from the related Machaerotinae. Zootaxa 3768(4): 437–459. https:// doi.org/10.11646/zootaxa.3768.4.3
- ICZN (1999) International Code of Zoological Nomenclature (4th edn). The International Trust for Zoological Nomenclature, London, 306 pp.
- Jacobi A (1904) Homopteren aus Nordost-Afrika, gesammelt von Oscar Neumann. Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Thiere, Jena 19: 761–782.
- Jacobi A (1910) Neue ikaden von Ostafrica. Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin. Berlin 1910: 299–305.
- Jacobi A (1917) Die Zikadenfauna Madagascars und der Comoren. In: Jacobi A (Ed.) Voeltzkow's Reise in Ostafrika, 3: 519–552.
- Lallemand V (1910) Diagnoses de Cercopides africains nouveaux. Annales de la Société entomologique de Belgique 54: 45–48.
- Lallemand V (1920) Cercopides nouveaux de Madagascar. Annales de la Société Entomologique de France 88: 280–288.
- Lallemand V (1923) Notes sur les Cercopides africains. Annals and Magazine of Natural History, London (Ser. 9) 11: 230–235. https://doi.org/10.1080/00222932308632845
- Lallemand V (1949) Revision des Cercopinae (Hemiptera Homoptera) Première partie. Mémoires de l'Institut Royal des Sciences Naturelles de Belgique (Ser. 2) 32: 1–193.
- Lallemand V (1950) Contribution à l'étude des Homoptères de Madagascar. Mémoires de l'Institut des Sciences de Madagascar (Ser. A) 4: 83–96.
- Leach WE (1815) Entomology. The Edinburgh Encyclopedia 9: 57–172.
- Melichar L (1915) Neue Cercopidenarten. Verhandlungen der Kaiserlich-Königlichen Zoologisch-botanischen Gesellschaft in Wien, Wien 65: 1–16.
- QGIS Development Team (2020) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org
- Schmidt E (1910) Zur Kenntnis indoaustralischer und südamerikanischer Cercopiden. (Hemiptera-Homoptera). Entomologische Zeitung. Herausgegeben von dem entomologischen Vereine zu Stettin, Stettin 71: 324–340.
- Signoret V (1860) Faune des Hémiptères de Madagascar. 1^{ère} partie. Homoptères. Annales de la société entomologique de France (3)8: 177–206.
- Soulier-Perkins A (2020) COOL: Cercopoidea Organised On Line. https://hemipetra-database.org/cool/ [Accessed 8 march 2020]
- Soulier-Perkins A, Kunz G (2012) Revision of the malagassy endemic genus Amberana Distant (Hemiptera, Cercopidae) with description of one new genus. Zootaxa 3156: 1–42. https:// doi.org/10.11646/zootaxa.3156.1.1
- Stål C (1866) Hemiptera Homoptera Latr. Hemiptera Africana 4: 1–27.
- Synave H (1957) Contribution à l'étude des Cercopidae de Madagascar (Hemiptera, Homoptera). Le Naturaliste Malgache 9: 133–140.

RESEARCH ARTICLE



Two new species of the leafhopper genus Calodia Nielson (Hemiptera, Cicadellidae, Coelidiinae) from China, with a checklist and key to Chinese species

Xian-Yi Wang¹, Zi-Zhong Li¹, Ren-huai Dai¹

Institute of Entomology, Guizhou University; Guizhou Provincial Key Laboratory for Agricultural Pest Management of the Mountainous Region, Guiyang 550025, China

Corresponding author: Ren-huai Dai (dmolbio@126.com)

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Abstract

Two new species of the leafhopper genus *Calodia* Nielson are described and illustrated: *C. quadrimacula* **sp. nov.** from Guizhou and Yunnan Provinces and *C. zuoae* **sp. nov.** from Yunnan Province, China. A checklist along with distribution and a key to species based on male genitalia of the genus *Calodia* from China are provided. *Olidiana nigritibiana* (Li), **comb. nov.** (earlier in the genus *Calodia*) is proposed. At present, this genus comprises 45 known species worldwide, of which 19 species are recorded from China.

Keywords

Distribution, identification key, morphology, new combination, Olidiana, taxonomy

Introduction

The genus *Calodia* (Hemiptera, Cicadellidae, Coelidiinae) was described by Nielson (1982) with *Calodia multipectinata* as the type species. *Calodia* is a relatively small genus of leafhoppers widely distributed throughout Asia and also the Pacific (Indonesia and the Philippines). In recent taxonomic studies on Coelidiinae, Nielson (2015)

revised the classification of Coelidiini by reassigning the species to several new genera and dealt with six new species of *Calodia*, provided a revised key to species of *Calodia* and also an updated catalogue of the species; of these, two species were from China. Nielson (2015) also resurrected *Lodiana nigritibiana* Li, 1988 and placed it in the genus *Calodia*. Li and Fan (2017) and Viraktamath and Meshram (2019) described new species of the genus from China and India, respectively. So far, there has been a total of 17 species from China.

In this paper, two new species of *Calodia* from China are described together with a checklist to Chinese species of the genus and a key for their separation. *Lodiana nigritibiana* Li, 1988 was resurrected from synonymy and transferred to the genus *Calodia* by Nielson (2015: 13, 83), however, examination of the aedeagus of this species shows that it has one apical process which is bifurcate apically, a character of a number of species of *Olidiana*. Therefore, *Olidiana nigritibiana* (Li), comb. nov. is proposed here.

Materials and methods

All specimens described in this study were collected by sweep net. Morphological terminology follows mainly Nielson (2015). Other methods follow Fan et al. (2014). Habitus photographs were obtained using a Keyence VHX-6000 system. Illustrations of male genitalia were drawn with Adobe Illustrator CS6 software. These images were combined using the photomerge command in Adobe Photoshop CS6 software.

The type specimens of the new species and other materials examined are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (GUGC), under the following accession numbers: *C. quadrimacula* sp. nov.: #CCW9043; *C. zuoae* sp. nov.: #CCW9065.

Taxonomy

Genus Calodia Nielson, 1982

Calodia Nielson, 1982: 14.

Type species. *Calodia multipectinata* Nielson, 1982.

Diagnosis. The species of the genus *Calodia* can be recognized among the genera of Coelidiini by the aedeagus having two medial or apical to subapical processes glabrous or with multi-spinose or multi-setose secondary processes (Nielson 2015).

Checklist of Calodia species from China

Calodia ailaoshanensis Li & Fan

Calodia ailaoshanensis Li & Fan, 2017: 37, fig. 7. Distribution: China (Yunnan).

Calodia apicalis Li

Calodia apicalis Li, 1989: 3, figs 20–24; Li and Wang 1991: 114, fig. 58; Zhang 1994: 123; Fan et al. 2014: 98; Nielson 2015: 5; Li and Fan 2017: 39, fig. 8. Distribution: China (Guizhou).

Calodia bispinea Li & Fan

Calodia bispinea Li & Fan, 2017: 41, fig. 9.

Distribution: China (Yunnan).

Calodia curveprocessa Li & Fan

Calodia curveprocessa Li & Fan, 2017: 43, fig. 10.

Distribution: China (Yunnan).

Calodia expenda Li & Fan

Calodia expenda Li & Fan, 2017: 45, fig. 11.

Distribution: China (Yunnan).

Calodia forkstyla Li & Fan

Calodia forkstyla Li & Fan, 2017: 47, fig. 12.

Distribution: China (Yunnan).

Calodia fusca (Melichar)

Jassus fusca Melichar, 1903;179.

- Jassus pauperculus Spangberg, 1878: 35. Synonymised by Nielson (1982).
- Tettigonia frontalis Kirby, 1891: 171. Synonymised by Nielson (1982).
- Calodia fusca (Melichar): Nielson 2015: 1982: 156, figs 498–503; Zhang 1994: 123, fig. 121.

Distribution. China.

Calodia guttivena (Walker)

Coelidia guttivena Walker, 1857: 99.

Jassus guttivena (Walker), Distant 1908: 149.

Calodia guttivena (Walker), Nielson 1982: 160, figs 522, 523; Li and Wang 1991: 273; Zhang 1994: 120, fig. 118; Fan et al. 2014: 98; Nielson 2015: 6 Distribution: China (Fujian), Malaysia, Thailand.

Calodia harpagota Zhang

Calodia harpagota Zhang, 1994: 125, fig. 124; Fan et al. 2014: 98; Nielson 2015: 5; Li and Fan 2017: 49, fig. 13.

Distribution: China (Shaanxi, Yunnan).

Calodia lii Zhang

Calodia lii Zhang, 1994: 123, fig. 120; Fan et al. 2014: 98; Nielson 2015: 5; Li and Fan 2017: 51, fig. 14.

Distribution: China (Tibet).

Calodia longilamina (Zhang)

Lodiana longilamina Zhang, 1994: 88 fig. 83.

Calodia longilamina (Zhang), Nielson 2015: 7.

Distribution: China (Yunnan).

Calodia longispina Li & Wang

Calodia longispina Li & Wang, 1991: 116, fig. 60; Zhang 1994: 123; Fan et al. 2014: 98; Nielson 2015: 5; Li and Fan 2017: 51, fig. 15.



Figures 1–13. *Calodia* species, male genitalia 1 *C. guttivena* (Walker), aedeagus and dorsal connective, dorsal view 2 *C. ostenta* (Distant), aedeagus and dorsal connective, dorsal view 3 *C. curveprocessa* Li & Fan, pygofer side, lateral view 4 *C. ailaoshanensis* Li & Fan, pygofer side, lateral view 5 *C. patricia* Jacobi, male subgential plate, ventral view 6 *C. bispinea* Li & Fan, male subgential plate, ventral view 7 *C. apicalis* Li, style, lateral view 8 *C. lii* Zhang, aedeagus and dorsal connective, dorsal view 9 *C. longilamina* (Zhang), style, lateral view 10 *C. longispina* Li & Wang, style, lateral view 11 *C. scutopunctata* (Zhang), style, lateral view 12 *C. expenda* Li & Fan, style, lateral view 13 *C. harpagota* Zhang, aedeagus and dorsal connective, dorsal view. Scale bars: 0.5 mm.

53

Distribution: China (Guizhou).
<i>Calodia vincula</i> Nielson
<i>Calodia vincula</i> Nielson, 2015: 9, 12, Pl. 1C, figs 28 – 32.
Distribution: China (Kouy Tchéou).
Calodia ostenta (Distant)
Jassus ostentus Distant, 1918: 49.
Coelidia ostenta (Distant), Metcalf 1964: 68.
Jassus pauperculus Spangberg, 1878: 35; Ge 1966: 78. Synonymised by Nielson (1982).
Coelidia <i>paupercula</i> (Spangberg), Metcalf 1964: 50; Ge 1988: 129. <i>Tettigonia frontalis</i> Kirby, 1891: 169. Synonymised by Nielson (1982). <i>Coelidia frontalis</i> (Kirby), Metcalf 1964: 50.
273; Zhang 1994: 119, fig. 117; Fan et al. 2014: 98; Nielson 2015: 6.
Distribution: China (Tibet, Yunnan), India, Sri Lanka.
Calodia patricia (Jacobi)
Jassus patricius Jacobi, 1944: 49.
<i>Coelidia patricia</i> (Jacobi), Metcalf 1964: 69.
Jassus ochraceus Jacobi, 1944: 50. Synonymised by Nielson (1982).
<i>Coelidia ochracea</i> (Jacobi), Metcalf 1964: 63.
Calodia flavinota Cai & Kuoh, 1993; 219; Nielson 2015: 84. Synonymised by Nielson (2015).
<i>Calodia paricia</i> (Jacobi), Nielson 1982: 144; Li and Wang 1991: 273; Zhang 1994: 112, fig. 109; Fan et al. 2014: 97; Nielson 2015: 7. Synonymised by Nielson (1982).
Calodia anadrimacula sp. pox
Distribution: China (Cuizhou Vunnan)
Calodia scutopunctata (Thang)
Lodiana scutopunctata Zhang, 1994: 83, fig. 78. Olidiana scutopunctata (Zhang, 1994) McKamey 2006: 506. Calodia scutopunctata (Zhang, 1994) Nielson 2015:14; Li and Fan 2017: 55, fig. 16. Distribution: China (Shaanxi, Yunnan).
Calodia sichuanensis Nielson
<i>Calodia sichuanensis</i> Nielson 2015: 9, plate 1B, figs 20 – 27. Distribution. China (Sichuan).
Calodia zuoae sp. nov.
Distribution: China (Yunnan).
Key to species of Calodia (males) from China
1 Aedeagal shaft with two short processes

_	Aedeagal shaft with two long processes	
2	Processes of aedeagal shaft bifurcate apically (Fig. 1)	C. guttivena
_	Processes of aedeagal shaft not bifurcate apically (Fig. 2)	C. ostenta

3	Pygofer side with narrowed at apex and produced posteriorly4
_	Pygofer side not narrowed and produced posteriorly5
4	Pygofer apex strongly sinuate (Fig. 3) C. curveprocessa
_	Pygofer apex not sinuate (Fig. 4) C. ailaoshanensis
5	Subgenital plate with apical process
_	Subgenital plate without apical process7
6	Subgenital plate (Fig. 5) with one tiny process at apex C. patricia
_	Subgenital plate with one apical and one subapical processes (Fig. 6)C. bispinea
7	Aedeagal shaft processes with secondary spines and arise close to apex (Fig. 8)8
_	Aedeagal shaft with either one or both the processes glabrous (Fig. 13)10
8	Style apophysis more than 5 times as long as basal width and longitudinally ru-
	gose (Fig. 7)
_	Style at most 3 times longer than basal width (Fig. 10)
9	Aedeagal shaft proximal process at most 11/2 times as long as distal process, with
	secondary spines before apex sparse, longer than width of process (Fig. 9)C. lii
_	Aedeagal shaft proximal process twice as long as distal process, with secondary
	processes before apex dense and shorter than width of process (Fig. 30)
	<i>C. zuoae</i> sp. nov.
10	Aedeagal shaft processes glabrous (Li and Fan 2017, plate 17, fig. 7)
	C. longispina
_	One of the aedeagal shaft processes with secondary spines (Fig. 13)
11	Aedeagal shaft with dorsal margin before apical group of teeth and base of distal
	process smooth in lateral view (Zhang 1994, fig. 121)
_	Aedeagal shaft with dorsal margin before apical group of teeth and base of distal
	process dentate in lateral view (Figs 13, 22)
12	Aedeagal shaft processes almost equal in length (Zhang 1994, fig. 121) C. fusca
_	Aedeagal shaft with proximal process more than 3 times as long as distal spine-
	like process (Nielson 2015, fig. 30) C. vincula
13	Style apophysis either bifid (Fig. 20) or bilobed (Li and Fan 2017, plate 14, fig.
	8)
_	Style apophysis neither bifid nor bilobed (Figs 9, 11, 12)16
14	Style apophysis with bilobed apex (Fig. 20)
_	Style apophysis deeply bifid (Li and Fan 2017, plate 14, fig. 8) C. forkstyla
15	Style apophysis with a subapical spur (Fig. 20) <i>C. quadripunctula</i> sp. nov.
_	Style apophysis without subapical spur (Li and Fan 2017, plate 15, fig. 8)
16	Style apophysis tapered towards apex (Figs 9, 11, 12)17
_	Style apophysis not tapered towards apex (Figs 11, 12)18
17	Aedeagal shaft distal process with lateral margin serrate (Zhang 1994, fig. 83)
	C. longilamina
_	Aedeagal shaft distal process with lateral margin smooth (Nielson 2015, fig. 30)
	C. sichanensis

- 18 Style apophysis almost of uniform width throughout (Fig. 11).... C. scutopunctata
- Style apophysis widened near apex (Fig. 12)......C. expanda

Calodia quadrimacula sp. nov.

http://zoobank.org/8C89F5F1-C4DC-4FA5-92ED-6AC3C1D9268F Figs 14–23

Type material. *Holotype*, \Im , CHINA: Guizhou Province, Bijie City, Weining County, Caohai Reserve, 3 July 2017, coll. Caohai expedition team (GUGC). *Paratype*, 3 \Im , 6 \Im , 6 \Im same information as holotype. 2 \Im , CHINA: Yunnan Province, Yuxi City, Xinping County, 21 July 2018, coll. Xian-yi Wang (GUGC).

Diagnosis. The new species is similar to *C. harpagota* Zhang, 1994, but differs in having the style apophysis with a subapical spur and the aedeagal shaft with angular projection on the ventral margin in lateral view and with two slender subapical processes.

Description. Middle-sized species. Body length (including tegmina): male, 7.2–7.8 mm, female, 7.9–8.4 mm.

Coloration. Ground color brown. Crown yellow with two pairs of brown spots medially, ocelli black (Fig. 14). Face (Fig. 16) with clypeus and clypellus black; area between laterofrontal sutures and eye yellow. Pronotum (Fig. 14) dark brown, with yellow markings. Mesonotum (Fig. 14) with basal triangles and one round spot on either side of median line anterior to scutoscutellar suture, black. Venation black, with numerous, small, brown spots. Legs (Fig. 15) dark brown to black.

Morphology. Head narrower than pronotum, anterior margin broadly obtuse; crown as wide as diameter of one eye, produced anteriorly beyond eyes; ocelli on anterior margin of crown; coronal suture extending to level of ocelli (Fig. 14); clypeus (Fig. 16) flat, narrow, laterally expanded under antennal sockets, apex constricted, base inflated longitudinally, apically with lateral margins expanded. Pronotum (Fig. 14) surface bullate. Mesonotum (Figs 14, 15) nearly as long as pronotum.

Male genitalia. Pygofer with caudal lobe (Fig. 17) broadly triangular in lateral view. Subgenital plate (Fig. 19) long with base slightly broad, sparsely setose apically. Style (Figs 20, 21) well developed, base expanded, apex bilobed, with a subapical short spur. Connective (Fig. 18) Y-shaped with stem short. Aedeagal shaft (Figs 22–23) sinuated, ventral margin in lateral view with angular projection slightly distad of half length, curved apically in lateral view, apex with group of spines, with two subapical retrose processes arising on same side, distal process about twice as long as proximal one, with outer margin, serrate proximal process glabrous; gonopore large, subapical, situated laterally more proximal than proximal process.

Etymology. The new species name is derived from the words "*quadri*" and "*macu-la*", referring to the scutellum with four black plaques.

Distribution. China (Guizhou, Yunnan).



Figures 14–23. *Calodia quadrimacula* sp. nov. 14 adult, dorsal view 15 adult, lateral view 16 face 17 male pygofer side, lateral view 18 connective, dorsal view 19 male subgential plate, ventral view 20 style, dorsal view (Yunnan) 21 style, dorsal view (Guizhou) 22 aedeagus and dorsal connective, dorsal view 23 aedeagus and dorsal connective, lateral view. Scale bars: 1 mm (14–16); 0.5 mm (17–23).

Calodia zuoae sp. nov.

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http://zoobank.org/681D84A8-5E50-4166-9235-9961CB491DC1
Figs 24–31
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Type material. *Holotype*, ♂, CHINA: Yunnan Province, Lushui County, Pianma town, Mt. Gaoligong, 26 May 2019, coll. Qin Zuo (GUGC). *Paratype*, ♂, same information as holotype.

Diagnosis. The new species is similar to *C. lii* Zhang, 1994, but differs in the structure of aedeagal shaft processes and the aedeagal shaft.

Description. Moderately large species. Body length (including tegmina): male, 8.8–9.4 mm.

Coloration. Ground color blackish. Head with crown brown; clypellus with median narrow yellowish stripe; area between lateral frontal sutures and eyes ochraceous (Figs 24, 26). Forewing with numerous, small, ivory to yellow spots.

Morphology. Head, narrower than pronotum, anterior margin broadly rounded; crown broad, slightly broader than width of one eye, slightly produced beyond anterior margin of eyes, eyes about ²/₃ width of pronotum (Fig. 24); clypeus wide and short, without middle longitudinal ridge; clypellus slender, apex wider (Fig. 26). Pronotum large, nearly twice as long medially as crown wider than long (Fig. 24). Scutellum large, nearly twice as long medially as pronotum.

Male genitalia. Pygofer in lateral view triangulate, with small lobe apically (Fig. 27). Subgenital plate nearly rectangular, apex rounded (Fig. 27). Style simple, without process (Fig. 29). Connective Y-shaped, stem short (Fig. 28). Aedeagal shaft asymmetrical, slender, narrowly tubular in dorsal view, with two large processes arising almost near apex, shorter process about half as long as longer process, with numerous apical fine setae, longer process extending to midlength of shaft with numerous fine setae on outer margin proximad of midlength; gonopore large, near apex, situated laterally (Figs 30, 31).

Etymology. The new species is named after Ms Qin Zuo who collected the holotype.

Remarks. The new species closely resembles *C. lii* but differs in the structure of aedeagal shaft processes, i.e., aedeagal shaft processes have finer and denser setae in *C. zuoae* compared to sparse and elongate secondary spines in *C. lii*; the shorter process has setae confined to apex of the process in *C. zuoae* and in *C. lii* the spines on the shorter process are along entire lateral margin; the setae on longer process in *C. zuoae* are confined to an area proximad of the midlength on the outer margin of the process and in *C. lii* the sparse spines are found in the distal ³/₄ length and they are on both margins of the process in the distal ¹/₃.

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Figures 24–31. *Calodia zuoae* sp. nov. **24** adult, dorsal view **25** adult, lateral view **26** face **27** male pygofer side and subgential plate, lateral view **28** connective, dorsal view **29** style, dorsal view **30** aedeagus and dorsal connective, dorsal view **31** aedeagus and dorsal connective, lateral view. Scale bars: 1 mm (**24–26**); 0.5 mm (**27–31**).

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References

- Cai P, Kuoh CL (1993) Two new species of Coelidiidae from China (Homoptera: Cicadelloidea). Journal of Anhui Agricultural University 20(3): 218–221.
- Distant WL (1908) The fauna of British Indian including Ceylon and Burma. Rhynchota 4: 157–419.
- Distant WL (1918) The fauna of British India, including Ceylon and Burma, Rhynchota– Homoptera. Part 7. Taylor & Francis, London, 210 pp.
- Fan ZH, Li ZZ, Chen XS (2014) A new species of the leafhopper genus *Calodia* Nielson, 1982 (Hemiptera, Cicadellidae, Coelidiinae) from China, with a key to Chinese species. ZooKeys 466: 95–102. https://doi.org/10.3897/zookeys.466.8680
- Ge ZL (1988) Homoptera: Cicadelliodea. In: Chen SX, Huan FS (Eds) Insects of the Nnamjagbarwa Mountains Region (Vol. 1). Beijing, Science Press, 127–130.
- Jacobi A (1944) Die Zikadenfauna der Provinz Fukien in Südchina and ihre tiergeographischen Beziehungen. Mitteilungen der Münchener Entomologischen Gesel lschaft München 34: 5–66.
- Kirby WF (1891) Catalogue of the described Hemiptera, Heteroptera, and Homoptera of Ceylon, based on the collection formed (chiefly at Pundalaya) by Mr. E. Ernest Green. Zoological Journal of the Linnean Society 24: 72–176. https://doi.org/10.1111/j.1096-3642.1891. tb02479.x
- Li ZZ (1988) Three new species of Fanjingshan mountain leafhoppers from Guizhou, China. Guizhou Science S1: 87–91.
- Li ZZ (1989) Five new species of Coelidiidae from Guizhou Province (Homoptera: Cicadelloidea). Journal Agricultural College 1: 1–5.
- Li ZZ, Wang LM (1991) Agricultural and Forestry Insect Fauna in Guizhou (Vol. 4). Guizhou Science and Technology Publishing House, Guiyang, China, 304 pp.
- Li ZZ, Fan ZH (2017) Coelidiinae from China (Hemiptera: Cicadellidae). Guizhou Science and Technology Press, Guiyang, 443 pp. [In Chinese]
- Matcacalf ZP (1964) General catalogue of the Homoptera-Fascicle VI, Cicadelloidea-part 11, Coelidiidae. Agriculture Research Service United States Department of Agriculture Washington, 182 pp.
- McKamey SH (2006) Further new genus-group names in the Cicadellidae (Hemiptera). Proceedings of the Entomological Society of Washington 108(3): 502–510.
- Melichar L (1903) Homopteren-Fauna von Ceylon. J. Klinkhardt. Leipzig, 248 pp.
- Nielson MW (1982) A revision of the subfamily Coelidiinae (Homoptera: Cicadellidae) IV. Tribe Coelidiini. Pacific Insects Monograph 38: 1–318.
- Nielson MW (2015) A revision of the tribe Coelidiini of the Oriental, Palearctic and Australian biogeographical regions (Hemiptera: Cicadellidae: Coelidiinae). Insecta Mundi 0410: 1–202.

- Spangberg J (1878) Species Jassi generis Homopterorum descripsit. Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar 35(8): 3–40.
- Viraktamath CA, Meshram NM (2019) Leafhopper tribe Coelidiini (Hemiptera: Cicadellidae: Coelidiinae) of the Indian subcontinent. Zootaxa 4653(1): 1–91. https://doi. org/10.11646/zootaxa.4653.1.1
- Walker F (1851) List of the specimens of homopterous Insects in the collection of the British Museum 3: 637–907.
- Walker F (1857) Catalogue of the homopterous insects collected at Sarawak, Borneo, by Mr. A. R. Wallace, with descriptions of new species. Journal of the Proceedings of the Linnean Society of London Zoology 1(4): 141–75. https://doi.org/10.1111/j.1096-3642.1857. tb00966.x
- Zhang YL (1994) Taxonomic study of Chinese Coelidiinae (Homoptera: Cicadellidae). Henan Science and Technology Press, Zhengzhou, 151 pp. [In Chinese]

RESEARCH ARTICLE



Two new species of Neotrichoporoides Girault (Hymenoptera, Eulophidae) from China and a key to Chinese species

Wen-Jian Li¹, Cheng-De Li¹

School of Forestry, Northeast Forestry University, Harbin, 150040, China

Corresponding author: Cheng-De Li (lichengde0608@sina.com)

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Abstract

Seven species of *Neotrichoporoides* Girault from China are reviewed, including two new species: *N. basi-flavus* **sp. nov.**, *N. flavothorax* **sp. nov.** and two new country record species: *N. cavigena* Graham, 1991, *N. szelenyii* (Erdös, 1951). New distributional data for *N. mediterraneus* Graham, 1986, *N. nyemitawus* (Rohwer, 1921) and *N. viridimaculatus* (Fullaway, 1955) are provided and a key to Chinese species is given based on females.

Keywords

Chalcidoidea, parasitoids, taxonomy, Tetrastichinae

Introduction

The genus *Neotrichoporoides* (Eulophidae: Tetrastichinae) was erected by Girault (1913) with *N. uniguttatus* Girault as type species. Currently the genus contains 73 valid species (Noyes 2019). It is distributed widely and especially diverse in Asia, Africa and Australia (Graham 1987), but only four species were known from China: *N. mediterraneus* Graham, 1986, *N. dubius* (Girault, 1913), *N. nyemitawus* (Rohwer, 1921), and *N. viridimaculatus* (Fullaway, 1955) (Zhu and Huang 2001, 2002; Zhang et al. 2007). Most species of the genus are parasitoids of Diptera in stems of grasses (Graham 1987; LaSalle 1994).

Neotrichoporoides can be recognized by the following combination of characteristics (Graham 1987): malar sulcus usually foveate below eyes; antenna of female with four discoid anelli (only three discoid anelli were found in *N. basiflavus* sp. nov.), funicular segments usually elongate; mesosoma with pronotum conical, propodeum usually much longer than dorsellum and strongly reticulate, spiracles small; fore wing with MV 5.5–9.5 × as long as STV, the latter very short; external surface of metacoxae sometimes strongly reticulate; body usually with distinct metallic reflections on dark parts or mainly yellow without metallic reflections.

In the present paper, we add four more species, including two new species and two new country record species to the Chinese fauna. A key to Chinese species is given based on females.

Materials and methods

Specimens were collected by sweeping, yellow pan trapping and malaise trapping, and were dissected and mounted dorsally in Canada balsam following the method described by Noyes (1982) or glued to triangular cards. Photographs were taken with a digital CCD camera attached to an Olympus BX51 compound microscope and a Aosvi HK-830 microscope. Most measurements were made from slide-mounted specimens using an eye-piece reticle with an Olympus CX21 microscope. In the descriptions below, measurements/ratio in brackets after measurement/ratio ranges refer to the measurement/ratio of the holotype. Terminology follows the Hymenoptera Anatomy Consortium (2020), and the following abbreviations are used:

F1–4	(flagellomeres 1–4),	POL	(minimum distance between	lat-
MV	(marginal vein),		eral ocelli),	
OOL	(minimum distance between lat-	STV	(stigmal vein),	
	eral ocellus and eye margin),	SMV	(submarginal vein).	
OD	(largest diameter of a lateral ocellus),			

All the specimens listed below were deposited in the insect collections at Northeast Forestry University (**NEFU**), Harbin, China.

Taxonomy

Key to the Chinese species of Neotrichoporoides Girault (females)

N. dubius was excluded from the key because of its insufficient original description.

1	Mesosoma with combination of yellow and green/black parts (Figs 32, 35)2
_	Mesosoma completely green to black (Figs 33, 34) 3

2	Malar sulcus with a subtriangular fovea, extending $0.4-0.5 \times$ the length of malar space (Fig. 11); F1 1.4-1.5 × as long as pedicel (Fig. 12); propodeum
	completely yellow (Fig. 13)
_	Malar sulcus with a small fovea, extending $0.2 \times$ the length of malar space; F1
	$2.4-2.5 \times as$ long as pedicel; propodeum completely green (Fig. 35)
3	Propodeum 2.0-2.5 × as long as dorsellum; midlobe of mesoscutum with
	two rows of adnotaular setae on each side; externo-dorsal surface of meta-
	coxae with distinct reticulation4
_	Propodeum 1.5 × as long as dorsellum; midlobe of mesoscutum with only
	one row of adnotaular setae on each side (Fig. 19); externo-dorsal surface of
	metacoxae without distinct reticulation (Fig. 22)N. cavigena Graham
4	Antenna with F1 1.4–1.6 × as long as pedicel5
_	Antenna with F1 2.0–2.4 × as long as pedicel6
5	Antennal clava $3.5-3.7 \times as$ long as broad (Fig. 2); lower half of face yellow
	and basal 1/3 of gaster yellowish (Fig. 31)
_	Antennal clava 2.8-3.3 × as long as broad (Fig. 30); face and gaster com-
	pletely green N. mediterraneus Graham
6	Antenna with F1 5.2–5.5 × as long as broad (Fig. 29); lower half of face yel-
	lowN. nyemitawus (Rohwer)
_	Antenna with F1 4.0–4.5 \times as long as broad (Fig. 24); lower half of face
	green

Neotrichoporoides basiflavus sp. nov.

http://zoobank.org/E14AED3B-638F-4437-8BBB-B1BE84418D5C Figures 1–10, 31

Type material. *Holotype*, female [on slide], CHINA, Hainan Province, Haikou City, Hainan University, 27–29.VI.2019, Yu-Ting Jiang, by yellow pan trapping. Deposited in NEFU.

Paratypes. 6 females, 4 males: [2 females and 2 males on slides, 1 male on card], CHINA, Hainan Province, Haikou City, same data as holotype; [2 females on slides, 1 male and 2 females on cards], CHINA, Shandong Province, Qingdao City, Mt. Xiaozhu, 18– 20.V.2014, Guo-Hao Zu, Si-Zhu Liu, by yellow pan trapping. All deposited in NEFU.

Diagnosis. Female. Body metallic green with lower half of face yellow and basal 1/3 of gaster yellowish; antenna with three discoid anelli, F1 1.4–1.5 × as long as pedicel; midlobe of mesoscutum with two rows of adnotaular setae; fore wing $2.7-2.8 \times$ as long as broad, speculum closed posteriorly; SMV with five setae on dorsal surface. **Male.** Antenna with scape shorter than an eye, reaching above vertex, $4 \times$ as long as broad; ventral plaque 0.63 × as long as scape.

Among the species recorded from China, *N. basiflavus* is similar to *N. mediterraneus* in F1 1.4–1.6 × as long as pedicel, but can be separated from it by the following



Figures 1–6. Neotrichoporoides basiflavus sp. nov., holotype, female 1 head, frontal view 2 antenna, lateral view 3 mesosoma, dorsal view 4 fore and hind wings, dorsal view 5 metasoma, ventral view 6 legs, lateral view, from left to right: fore, mid, and hind legs. Scale bars: 100 μ m.

combination of characteristics: lower half of face yellow and basal 1/3 of gaster yellowish (vs. green); antennal clava $3.5-3.7 \times as$ long as broad (vs. $2.8-3.3 \times$); fore wing with speculum closed posteriorly (vs. open posteriorly). The new species is also similar to the extralimital species *N. beonus* Narendran in base of gaster yellow, but can be separated from it by following characteristics: pronotum $0.3-0.5 \times as$ long as mesoscutum (vs. $0.93 \times$); fore wing $2.7-2.8 \times as$ long as broad (vs. $3.7 \times$), SMV with five setae on dorsal surface (vs. six), speculum closed posteriorly (vs. open posteriorly).

Description. Female. *Body* length 1.7–2.3 mm (1.8 mm), dark green to green with metallic reflections (Fig. 31). Upper half of face green with metallic reflections, lower half of face yellow, mandibles bronze. Antenna with radicle yellowish, scape mainly yellowish, dark brown along dorsal edge, pedicel with dorsal half dark brown, ventral half yellowish brown, flagellum dark brown. Mesosoma dark green to green with metallic reflections. Wings hyaline, venation yellowish brown. Legs mainly yellow with dorsal half of mesocoxae, and base of metacoxae concolorous with metallic reflections, with ca. basal 1/3 yellowish, sometimes with a green spot on lateral sides of basal tergite, ovipositor sheaths with third valvula black.

Head (Fig. 1) in dorsal view, $2.3-2.6 \times (2.6 \times)$ as broad as long, and as broad as mesosoma; POL equal to OOL, OOL $2.8-3.0 \times (2.9 \times)$ OD. Vertex with setae shorter than OD. Eyes separated by $1.2-1.3 \times (1.2 \times)$ their length. Malar space ca. $0.5 \times$ as long as eye, malar sulcus with a subtriangular fovea below eyes, extending ca. $0.5 \times$ the length of malar space; mouth opening $1.5 \times$ as wide as malar space. Clypeus with lower margin bidentate. Mandibles tridentate. Facial depression deep. Torulus with lower margin above the level of ventral margin of eyes. Antenna (Fig. 2) with scape $3.7-4.1 \times (3.7 \times)$ as long as broad, shorter than eye length and not reaching the level of vertex; pedicel $2.3-2.4 \times (2.4 \times)$ as long as broad; with three discoid anelli; F1 $3.6-4.3 \times (3.7 \times)$ as long as broad and $1.4-1.5 \times (1.4 \times)$ as long as pedicel, F2 and F3 $3.1-3.2 \times (3.2 \times)$ and $2.3-2.6 \times (2.3 \times)$ as long as broad respectively; clava $3.5-3.7 \times (3.6 \times)$ as long as broad, sa broad as broad respectively; clava $3.5-3.7 \times (3.6 \times)$ as long as broad as F3, $0.7 \times$ as long as F2 and F3 combined, sensilla numerous, slender, setae on funicle and clava short and dense.

Mesosoma (Fig. 3) $1.7-1.9 \times (1.7 \times)$ as long as broad. Pronotum subconical, $0.3-0.5 \times (0.3 \times)$ as long as mesoscutum. Midlobe of mesoscutum $1.2 \times$ as broad as long, without median line, with fine reticulation and with two rows of adnotaular setae, four or five setae in outer row and two or three setae in inner row. Scutellum ca. as broad as long; anterior pair of setae distinctly situated before the middle of scutellum, submedian grooves and sublateral grooves distinct, distance between submedian grooves, enclosing a space ca. $2.4 \times$ as long as broad. Reticulation on scutellum similar to that on mesoscutum. Dorsellum $2.5-3.1 \times (2.9 \times)$ as broad as long. Propodeum ca. $2.5 \times$ as long as dorsellum medially; with distinct reticulation, median carina distinct and narrow; spiracles small, circular, separated from metanotum by ca. their own diameter; callus with four or five setae arranged irregularly. Fore wing (Fig. 4) $2.7-2.8 \times (2.75 \times)$ as long as broad, SMV with five setae on dorsal surface; costal cell $0.8 \times$ as long as MV;



Figures 7–10. *Neotrichoporoides basiflavus* sp. nov., paratype, male **7** head, frontal view **8** antenna, lateral view **9** metasoma, ventral view **10** fore and hind wings, dorsal view. Scale bars: 100 μm.

MV 7.3–8.8 × (8.7 ×) as long as STV with 12–15 setae on its anterior margin; STV short with a long uncus; speculum small, closed posteriorly, subcubital line of setae not reaching to speculum. Hind wing (Fig. 4) 5.0–5.5 × (5.2 ×) as long as broad. Legs (Fig. 6) with metacoxae stout, ca. 1.5 × as long as broad, externo-dorsal surface with distinct reticulation, metafemora $3.3-3.4 \times (3.4 \times)$ as long as broad; spur of metatibia ca. 0.6 × as long as length of metabasitarsus.

Gaster (Fig. 5) lanceolate, slightly depressed dorsally, $2.2-2.5 \times (2.5 \times)$ as long as broad and $1.2-1.5 \times (1.4 \times)$ as long as head and mesosoma combined; petiole transverse; first sternite with a 'V' shaped carina and several thin longitudinal carinae; the longest cercal seta 2 × as long as the second longest. Ovipositor originates from ca. basal third of gaster, and is ca. $0.7 \times$ as long as gaster, reaching to, or slightly exserted at, apex of gaster; tip of hypopygium situated at the middle of gaster.

Male. Similar to female. Head (Fig. 7) as shown. Antenna (Fig. 8) with scape shorter than an eye, reaching above vertex, $4 \times$ as long as broad; ventral plaque 0.60– 0.65 × as long as scape; pedicel 1.8 × as long as broad; flagellum slightly broader than

pedicel, tapering slightly distally, F1 shortest, $2.1 \times as long as broad and <math>1.6 \times as long$ as pedicel, F2–F4 subequal in length, $3.0 \times as long as broad;$ clava as broad as funicle, $8.5-9.0 \times as long as broad,$ all three segments subequal in length and distinctly separated, terminal spine long, ca. $0.33 \times as long as the third segment;$ funicular segments with whorled long setae, the longest seta on each funicular segment $1.0-1.4 \times as long$ as length of next funicular segment. Fore wing (Fig. 10) with costal cell $0.8 \times as long$ as MV, MV 7.0–8.0 × as long as STV. Gaster (Fig. 9) $2.0-2.5 \times as long$ as broad, $1.0-1.2 \times as long$ as mesosoma; genitalia ca. $2.0 \times as long$ as broad.

Host. Unknown.

Distribution. China (Shandong, Hainan).

Etymology. From the Latin *basis* (base), and *flavus* (yellow), and refers to the yellowish basal part of gaster.

Neotrichoporoides flavothorax sp. nov.

http://zoobank.org/C6AC84DD-B3CB-4002-A8B5-6D0CA984E2B7 Figures 11–16, 32

Type material. *Holotype*, female [on slide], CHINA, Shandong Province, Qingdao City, Mt. Xiaozhu, 18–20.V.2014, Guo-Hao Zu, Si-Zhu Liu, by yellow pan trapping. Deposited in NEFU.

Paratypes. 2 females: [1 female on slide], same data as holotype; [1 female on slide], CHINA, Hainan Province, Wan Ning City, Shuangxi Village, 17–19.IV.2019, Yu-Ting Jiang, by yellow pan trapping. All deposited in NEFU.

Diagnosis. Female. Body mainly yellow with green or black markings (Fig. 32); F1 4.2 × as long as broad, $1.4-1.5 \times$ as long as pedicel; mid lobe of mesoscutum with three adnotaular setae in one row; propodeum $2.0-2.3 \times$ as long as dorsellum; fore wing with MV 9.5 × as long as STV, speculum closed posteriorly.

Among the species recorded from China, *N. flavothorax* is similar to *N. viridi-maculatus* (Fullaway) in having similar combination of yellow and green/black parts on mesosoma, but can be separated from *N. viridimaculatus* by the following characteristics: propodeum completely yellow (vs. completely green); malar sulcus with a subtriangular fovea, extending $0.4-0.5 \times$ the length of malar space (vs. small, $0.2 \times$); F1 1.4–1.5 × as long as pedicel (vs. 2.4–2.5 ×). The new species is also similar to the extralimital species *N. dispersus* Graham in having similar combination of yellow and green/black parts on mesosoma, but can be separated by the following characteristics: propodeum completely yellow (vs. partly green); F1 1.4–1.5 × as long as pedicel (vs. 2.4–2.5 ×).

Description. Female. *Body* length 1.9–2.3 mm (1.9 mm). Head with upper half of face and posterior upper part of gena green with metallic reflections, lower half of face yellow; vertex with subtriangular ocelli area and occiput black, mandibles bronze; antenna with radicle, scape and pedicel yellow, flagellum brown. Mesosoma mainly yellow (Fig. 32), with pronotum, anterior middle part of mid lobe of mesoscutum



Figures 11–16. *Neotrichoporoides flavothorax* sp. nov., holotype, female **11** head, frontal view **12** antenna, lateral view **13** mesosoma, dorsal view **14** fore and hind wings, dorsal view **15** metasoma, ventral view **16** legs, lateral view, from left to right: fore, mid, and hind legs. Scale bars: 100 μm.

black, scutellum green with metallic reflections; legs mainly yellow except tarsomere 4 of all legs dark brown; wings hyaline, venation yellowish brown. Gaster mainly dark brown with basal 1/3 yellow and a yellow spot on the terminal part of gaster, ovipositor sheaths with 1/3 valvula black.

Head (Fig. 11) in dorsal view, 2.3 × as broad as long, 1.0–1.1 × as broad as mesosoma. POL 1.2–1.3 × (1.3 ×) OOL, OOL 2.5 × OD. Eyes separated by 1.2 × their length. Malar space ca. 0.6 × as long as eye, malar sulcus with a subtriangular fovea below eyes, extending 0.4–0.5 × (0.4 ×) the length of malar space; mouth opening 1.5 × as wide as malar space. Clypeus with lower margin bidentate. Mandibles tridentate. Facial depression shallow. Torulus with lower margins above the level of ventral margin of eyes. Antenna (Fig. 12) with scape 4.5–5.0 × (5.0 ×) as long as broad, slightly shorter than eye length and reaching above the level of vertex; pedicel 2.6× as long as broad; with four discoid anelli; F1 4.2 × as long as broad, 1.4–1.5 × (1.5 ×) as long as pedicel, F2 and F3 3.2–3.3 × (3.3 ×) and 2.3–2.4 × (2.3 ×) as long as broad respectively; clava 3.8–4.0 × (3.8 ×) as long as broad, 0.8 × as long as F2 and F3 combined, indistinctly segmented and pointed at apex, sensilla numerous, slender; setae on flagellum short and dense.

Mesosoma (Fig. 13) $1.9 \times as$ long as broad. Pronotum subconical, $0.3-0.4 \times (0.4 \times)$ as long as mesoscutum. Mid lobe of mesoscutum ca. as broad as long, without median line, with extremely fine reticulation and three adnotaular setae in one row. Scutellum as broad as long; anterior pair of setae situated distinctly before the middle of scutellum, submedian grooves superficial and sublateral grooves distinct, distance between submedian grooves greater than distance between submedian groove and sublateral groove, enclosing a space ca. $2.2 \times$ as long as broad. Reticulation on scutellum similar to that on mesoscutum. Dorsellum ca. 2.5 × as broad as long, without reticulation, posterior edge slightly curved. Propodeum $2.0-2.3 \times as$ long as dorsellum medially, with distinct reticulation, median carina distinct and narrow; spiracles small, circular, separated from anterior margin of propodeum by ca. their own diameter; callus with three setae. Fore wing (Fig. 14) 2.8 × as long as broad, SMV with five setae on dorsal surface; costal cell 0.62 × as long as MV; MV 9.5 × as long as STV; STV with a long uncus; speculum small, closed posteriorly. Hind wing 6.2 × as long as broad, pointed. Legs (Fig. 16) with metacoxae stout, ca. $1.4 \times$ as long as broad, externo-dorsal surface with fine reticulation, metafemora $3.6 \times$ as long as broad; spur of metatibia $0.7 \times$ as long as length of metabasitarsus.

Gaster (Fig. 15) lanceolate, not depressed dorsally, $3.0 \times$ as long as broad and $1.4 \times$ as long as head and mesosoma combined; petiole transverse; the longest cercal seta $2 \times$ as long as the second longest. Ovipositor ca. $0.9 \times$ as long as gaster and slightly exserted at apex of gaster; tip of hypopygium situated at ca. basal 1/3 of gaster.

Male. Unknown.

Host. Unknown.

Distribution. China (Shandong, Hainan).

Etymology. From the Latin *flavus* (yellow), and refers to the mainly yellow thorax of the species.

Neotrichoporoides cavigena Graham, 1987

Figures 17-22

Neotrichoporoides cavigena Graham, 1987: 70.

Material examined. 2 females: [2 females on slides], CHINA, Beijing, Mt. Baihua, 1.V.2012, Guo-Hao Zu, Jiang Liu, by sweeping. All deposited in NEFU.

Diagnosis. Female. Head (Fig. 17) with malar fovea large and deep, extending ca. half the length of malar space; antenna (Fig. 18) with scape ca. $3.3 \times as$ long as broad, shorter than an eye, not reaching above the level of vertex; pedicel $2.35 \times as$ long as broad; F1–F3: $3.0 \times, 2.8 \times, 2.4 \times as$ long as broad respectively; clava ca. $3.0 \times as$ long as broad, indistinctly segmented. Midlobe of mesoscutum (Fig. 19) with four adnotaular setae in one row; scutellum with submedian grooves distinct, distance between submedian grooves subequal to the distance between submedian groove and sublateral groove, enclosing a space ca. $3.5 \times as$ long as broad; propodeum medially $1.5 \times as$ long as dorsellum. Wings (Fig. 20) and legs (Fig. 22) as shown in figures. Gaster (Fig. 21) ca. $1.8 \times as$ long as broad. **Male.** Unknown for Chinese material.

Host. Unknown.

Distribution. China (Beijing) [new record], Bulgaria, France, Czech Republic (Graham 1987), Slovakia (Kalina 1989), Russia (Yegorenkova and Kostjukov 2006), Turkey (Sakaltaş and Gençer 2005).

Comments. This species can be distinguished by the narrow space, ca. $3.5 \times as$ long as broad, enclosed by submedian grooves on the scutellum. For a more detailed description, see Graham (1987).

Neotrichoporoides szelenyii (Erdös, 1951)

Figures 23-28, 33

Geniocerus szelenyii Erdös, 1951: 232. Lectotype designated by Graham 1987: 69. *Aprostocetus szelenyii*: Graham, 1961: 50.

Tetrastichus szelenyi: Bouček, 1965: 212 (misspelling).

Tetrastichus szelenyii: Domenichini, 1966b: 50.

Neotrichoporoides szelenyii: Graham, 1987: 68.

Neotrichoporoides szelynii: Yefremova, 2008: 358 (misspelling).

Material examined. 8 females: [2 females on slides], CHINA, Hainan Province, Haikou City, Hainan University, 27–29.VI.2019, Yu-Ting Jiang, by yellow pan trapping; [2 females on slides], Hainan Province, Chengmai County, Jinjiang Town, 24–26. IV.2019, Yu-Ting Jiang, by yellow pan trapping; [4 females on cards], Shanghai City, Songjiang District, Yexie Town, 11–20.IX.2011, Zhen Yang, by malaise trapping. All deposited in NEFU.

Diagnosis. Female. Antenna (Fig. 24) with scape $0.9-1.0 \times$ as long as an eye, F1 4.0-4.5 × as long as broad, ca. 2 × as long as pedicel and 0.9 × as long as clava; F2



Figures 17–22. *N. cavigena*, female **17** head, frontal view **18** antenna, lateral view **19** mesosoma, dorsal view **20** fore and hind wings, dorsal view **21** metasoma, ventral view **22** legs, lateral view, from left to right: fore, mid, and hind legs. Scale bars: 100 µm.

 $2.8-3.3 \times as \ long \ as \ broad; F3 \ 2.0-2.6 \times as \ long \ as \ broad; \ clava \ 3.6-4.0 \times as \ long \ as \ broad.$ Propodeum (Fig. 25) medially $2.5 \times as \ long \ as \ dorsellum$. Fore wing (Fig. 26) $2.7-2.8 \times as \ long \ as \ broad, \ SMV \ with \ five \ to \ seven \ setae \ on \ dorsal \ surface; \ costal \ cell \ 0.8 \times as \ long \ as \ MV, \ MV \ 8.0-8.8 \times as \ long \ as \ STV; \ speculum \ open \ posteriorly. \ Gaster$



Figures 23–28. *N. szelenyii*, female **23** head, frontal view **24** antenna, lateral view **25** mesosoma, dorsal view **26** fore and hind wings, dorsal view **27** metasoma, ventral view **28** legs, lateral view, from left to right: fore, mid, and hind legs. Scale bars: 100 µm.


Figures 29, 30. Females **29** *N. nyemitawus*, antenna, lateral view **30** *N. mediterraneus*, antenna, lateral view. Scale bars: 100 µm.

(Fig. 27) $2.3-2.5 \times as$ long as broad and $1.1-1.3 \times as$ long as head and mesosoma combined. Head (Fig. 23) and legs (Fig. 28) as shown in figures. **Male.** Unknown for Chinese material.

Host. Unknown.

Distribution. China (Hainan, Shanghai) [new record], Azerbaijan, Hungary, Portugal (Graham 1987), Italy, Greece, Bulgaria (Boyadzhiev 1999), Czechoslovakia, Moldova (Bouček 1965), Iran (Hesami et al. 2010), Romania (Hansson 2016), Turkey (Sakaltaş and Gençer 2005), Saudi Arabia (OILB 1971), United Arab Emirates (Yefremova 2008).

Comments. This species is similar to *N. mediterraneus*, but can be distinguished using characters in couplet 6 in the key.

Neotrichoporoides mediterraneus Graham, 1986

Figure 30

Neotrichoporoides mediterraneus Graham, 1986: 6.

Material examined. 2 females: [1 female on slide], Henan Province, Xinyang City, Mt. Wusheling, 7.VIII.2015, Hui Geng, Yan Gao, by sweeping; [1 female on slide], Guangxi Province, Fangchenggang City, Mt. Shiwandashan, 25.VII.2019, Jun Wu, Jun-Jie Fan, by sweeping. All deposited in NEFU.

Diagnosis. Female. Antenna (Fig. 30) with scape 0.8–0.9 × as long as an eye, F1 3.0–4.0 × as long as broad, 1.4–1.6 × as long as pedicel; F2 3.0–3.7 × as long as broad;

F3 2.0–2.6 × as long as broad; clava 2.8–3.3 × as long as broad. Fore wing 2.7–2.8 × as long as broad, SMV with five setae on dorsal surface, MV 8.0–9.3 × as long as STV; speculum open posteriorly. Gaster 2.4 × as long as broad and 1.2 × as long as head and mesosoma combined. **Male.** Unknown for Chinese material.

Host. Unknown.

Distribution. China (Guangxi (Zhu and Huang 2002), Henan [New record]), Bulgaria, Czech Republic, Slovakia (Boyadzhiev 1999), Spain, France, Italy (Graham 1987), Romania (Hansson 2016), Russia (Yegorenkova and Kostjukov 2006), Turkey (Sakaltaş and Gençer 2005), India (Graham 1987), Australia (Bouček 1988), Canary Islands, Madeira (Graham 1987).

Comments. According to Graham (1986), *N. mediterraneus* is quite similar to *N. szelenyii*. For a more detailed description, see Graham (1986).

Neotrichoporoides nyemitawus (Rohwer, 1921)

Figures 29, 34

Tetrastichus nyemitawus Rohwer, 1921: 131.

Tetrastichus agarwali Shafee, Fatma & Kishore, 1984: 393. [Synonymized by Hayat and Shahi 2004: 308].

Neotrichoporoides nyemitawus: Graham, 1987: 68.

Material examined. 3 females: [1 female on slide], Henan Province, Xinyang City, Mt. Wusheling, 7.VIII.2015, Hui Geng, Yan Gao, by sweeping; [1 female on slide], Zhejiang Province, Jinhua City, Xishan Village, 25–27.VI.2019, by yellow pan trapping; [1 female on card], Yunnan Province, Tengchong City, Guanpojiao, Xiang-Xiang Jin, Guo-Hao Zu, Chao Zhang, by sweeping. All deposited in NEFU.

Diagnosis. Female. Antenna (Fig. 29) with scape ca. as long as an eye, reaching well above the level of vertex; F1 5.2–5.5 × as long as broad, $2.2-2.4 \times$ as long as pedicel; F2 4.1 × as long as broad; F3 3.0–3.1 × as long as broad; clava 4.2–4.7 × as long as broad, distinctly segmented. Fore wing 3.0 × as long as broad, SMV with five to seven setae on dorsal surface, MV 8.0–9.3 × as long as STV. Gaster (Fig. 34) 2.6–3.0 × as long as broad and 1.2–1.3 × as long as head and mesosoma combined. **Male.** Unknown for Chinese material.

Hosts. Unknown from China. Non-Chinese records include Atherigona naqvii (Husain & Khan, 1986), A. conigera, A. soccata (Graham, 1987), A. hyalinipennis (Sileshi, 1997), A. varia (Raodeo, Tikar & Chundurwar, 1972) (Diptera: Anthomyiidae).

Distribution. China (Gansu, Jiangsu (Zhang et al. 2007), Zhejiang (Zhu and Huang 2001), Guangxi (Zhu and Huang 2002), Henan, Yunnan [new records]), Thailand, India, Kenya (Graham 1987), Ethiopia (Sileshi 1997), Burkina Faso (Zongo, Vincent and Stewart 1993).

Comments. This species can be distinguished by its distinctly segmented clava that is $4.2-4.7 \times as$ long as broad, and the yellow lower half of face.



Figures 31–35. Females, dorsal view 31 Neotrichoporoides basiflavus sp. nov. 32 Neotrichoporoides flavothorax sp. nov. 33 N. szelenyii 34 N. nyemitawus 35 N. viridimaculatus

Neotrichoporoides viridimaculatus (Fullaway, 1955) Figure 35

Burksia viridimaculata Fullaway, 1955: 410.
Tetrastichus viridimaculatus: Domenichini, 1966a: 140.
Tetrastichus bicolor Saraswat, 1975: 2. [Synonymised by Hayat and Shahi 2004: 309].
Tetrastichus saraswati Husain & Khan, 1986: 242. [Synonymised by Hayat and Shahi 2004: 309].

Neotrichoporoides viridimaculatus: Graham, 1987: 67.

Material examined. 6 females, 2 males: [1 female on slide], Henan Province, Xinyang City, Mt. Wusheling, 8.VIII.2015, Hui Geng, Yan Gao, by sweeping; [1 female on card], Guangxi Province, Fangchenggang City, Mt. Shiwandashan, 25.VII.2019, Jun Wu, Jun-Jie Fan, by sweeping; [4 females, 2 males on cards], Shanghai City, Songjiang District, Yexie Town, 11–20.IX.2011, Zhen Yang, by malaise trapping. All deposited in NEFU.

Diagnosis. Female. Malar sulcus with a small fovea, extending $0.2 \times$ the length of malar space; antenna with scape ca. as long as an eye; F1 2.4–2.5 × as long as pedicel; scutellum without submedian grooves; propodeum medially $1.5-2.0 \times$ as long as dorsellum; body (Fig. 35) with characteristic green markings on midlobe of mesoscutum and scutellum which form broad longitudinal stripes, propodeum completely green. **Male.** Scutellum without submedian grooves.

Host. Unknown.

Distribution. China (Gansu (Zhang et al. 2007), Zhejiang (Zhu and Huang 2001), Guangxi, Henan, Shanghai [New records]), Bulgaria, France, Hungary, Czechoslovakia, Italy, Madeira, Portugal (Graham 1987), Sweden (Hedqvist 2003), Turkey (Sakaltaş and Gençer 2005), Russia (Yegorenkova and Kostjukov 2006), India (Narendran et al. 2006), South Africa (Yegorenkova and Yefremova 2010), USA (LaSalle 1994), Hawaii (Graham 1987), Cuba (De Santis 1979), Bermuda (De Santis and Fidalgo 1994), Argentina (Graham 1987), Colombia (Domenichini 1966b).

Comments. This species is similar to *Neotrichoporoides flavothorax* sp. nov., but can be distinguished using characters in couplet 2 in the key.

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References

- Boyadzhiev PS (1999) New species Eulophidae (Hymenoptera: Chalcidoidea) to the fauna of Bulgaria II. Plovdivski Universitet "Paisij Khilendarski" Nauchni Trudove Biologiya Animalia 35(6): 63–66.
- Bouček Z (1965) A review of the Chalcidoid fauna of the Moldavian SSR, with descriptions of new species (Hymenoptera). Sborník Faunistickych Prací Entomologického Oddeleni Národního Musea v Praze 11: 5–37.
- Bouček Z (1988) Australasian Chalcidoidea (Hymenoptera). A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species. CAB International, Wallingford, Oxon, U.K., Cambrian News Ltd; Aberystwyth, Wales, 832 pp.
- De Santis L (1979) Catálogo de los Himénopteros Calcidoideos de América al sur de los Estados Unidos. Publicación Especial Comisión de Investigaciones Científicas Provincia de Buenos Aires, 488 pp.
- De Santis L, Fidalgo P (1994) Catalogo de Himenopteros Calcidoideos. Serie de la Academia Nacional de Agronomia y Veterinaria 13: 1–154.
- Domenichini G (1966a) I Tetrastichinae (Hymenoptera Eulophidae) palearctici ed i loro ospiti. Bollettino di Zoologia agraria e di Bachicoltura (II) 6: 61–205.
- Domenichini G (1966b) Hym. Eulophidae. Palaearctic Tetrastichinae. Index of Entomophagous Insects 1. Le François, Paris, 101 pp.
- Erdös J (1951) Eulophidae novae. Acta Biologica. Academiae Scientiarum Hungaricae 2(1–3): 169–237.
- Fullaway DT (1955) Description of a new genus and species of parasitic wasp (Hymenoptera: Eulophidae). Proceedings of the Hawaiian Entomological Society 15: 409–410.
- Girault AA (1913) Australian Hymenoptera Chalcidoidea IV. Memoirs of the Queensland Museum 2: 140–296. https://www.biodiversitylibrary.org/page/39666066
- Graham MWR de V (1961) The genus *Aprostocetus* Westwood sensu lato (Hym., Eulophidae) notes on the synonymy of European species. Entomologist's Monthly Magazine 97: 34–64.
- Graham MWR de V (1986) Four new species of Eulophidae (Insecta, Hymenoptera) from Madeira and Europe. Bocagiana 95: 1–9.
- Graham MWR de V (1987) A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae), with a revision of certain genera. Bulletin of the British Museum (Natural History) (Entomology) 55(1): 1–392.
- Hayat M, Shahi MH (2004) Taxonomic notes on Indian Eulophidae (Hymenoptera: Chalcidoidea) – 1. On the types of some Tetrastichinae. Oriental Insects 38: 303–314. https:// doi.org/10.1080/00305316.2004.10417396
- Hansson C (2016) New records of Eulophidae (Hymenoptera: Chalcidoidea) from Romania, including two new species. Travaux du Muséum d'Histoire Naturelle 'Grigore Antipa', Bucuresti 59(1): 53–72. https://doi.org/10.1515/travmu-2016-0017
- Hesami S, Ebrahimi E, Ostovan H, Shojai M, Kamali K, Yefremova Z, Yegorenkova E (2010) Contribution to the study of Eulophidae (Hymenoptera: Chalcidoidea) of Fars province of

Iran: I-subfamilies Entedoninae and Tetrastichinae. Munis Entomology & Zoology 5(1): 148–157.

- Hedqvist KJ (2003) Katalog över svenska Chalcidoidea. Entomologisk Tidskrift 124(1–2): 73–133.
- Hymenoptera Anatomy Consortium (2020) Hymenoptera Anatomy Ontology Portal. http://glossary.hymao.org [Accessed on 20 Dec 2020.]
- Husain T, Khan MY (1986) Family Eulophidae. In: Subba Rao BR, Hayat M (Eds) The Chalcidoidea (Insecta: Hymenoptera) of India and the adjacent countries.) Oriental Insects 20: 211–245. https://doi.org/10.1080/00305316.1986.10433730
- Kalina V (1989) Checklist of Czechoslovak Insects III (Hymenoptera). Chalcidoidea. Acta Faunistica Entomologica Musei Nationalis Pragae 19: 97–127.
- LaSalle J (1994) North American genera of Tetrastichinae (Hymenoptera: Eulophidae). Journal of Natural History 28: 109–236. https://doi.org/10.1080/00222939400770091
- Noyes JS (1982) Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). Journal of Natural History 16: 315–334. https://doi.org/10.1080/00222938200770261
- Noyes JS (2019) Universal Chalcidoidea Database. http://www.nhm.ac.uk/chalcidoids [Accessed on 2020.12]
- Narendran TC, Girish Kumar P, Santhosh S, Jilcy MC (2006) A revision of *Neotrichoporoides* Girault (Hymenoptera: Eulophidae) from India. Oriental Insects 40: 1–21. https://doi.or g/10.1080/00305316.2006.10417452
- OILB (1971) Liste d'identification des entomophages 8. OILB, Geneva, 64 pp.
- Raodeo AK, Tikar DT, Chundurwar RD (1972) Records of natural parasites of sorghum shoot fly, *Atherigona varia soccata* Rondani. Current Science 41(11): 430–431.
- Rohwer SA (1921) Description of new chalcidoid flies from Coimbatore, south India. Annals and Magazine of Natural History (9)7:123–135. https://doi.org/10.1080/00222932108632493
- Saraswat GG (1975) On some *Tetrastichus* (Hymenoptera: Chalcidoidea) from India. Memoirs of the School of Entomology, St. John's College, Agra 4: 2–5.
- Sakaltaş E, Gençer L (2005) Contribution to the knowledge of the Tetrastichinae (Hymenoptera: Eulophidae) from Ankara, Turkey, with some new records. Acta Phytopathologica et Entomologica Hungarica 40 (3–4): 383–390. https://doi.org/10.1556/APhyt.40.2005.3-4.19
- Sileshi G (1997) Biology of the tef shootfly, *Atherigona hyalinipennis* van Emden in eastern Ethiopia. Insect Science and its Application 17(3–4): 349–355. https://doi.org/10.1017/ S1742758400019172
- Shafee SA, Fatma A, Kishore P (1984) Descriptions of two new species of *Tetrastichus* Haliday (Hymenoptera: Eulophidae) from India. Journal of the Bombay Natural History Society 80(2): 393–396.
- Yefremova Z (2008) Order Hymenoptera, family Eulophidae. Arthropod fauna of the UAE 1: 345–360.
- Yegorenkova EN, Kostjukov VV (2006) New species of the genus *Neotrichoporoides* Girault, 1913 (Hymenoptera: Eulophidae, Tetrastichinae) from Ul'yanovsk Province of Russia. Russian Entomological Journal 15(4): 421–422.

- Yegorenkova EN, Yefremova ZA (2010) Notes on some taxa of subfamily Tetrastichinae (Hymenoptera, Chalcidoidea, Eulophidae) from South Africa with description of a new species. Trudy Russkogo Entomologicheskogo Obshestva 80(2): 56–63.
- Zhu CD, Huang DW (2001) A taxonomic study on Eulophidae from Zhejiang, China (Hymenoptera: Chalcidoidea). Acta Zootaxonomica Sinica 26(4): 533–547.
- Zhu CD, Huang DW (2002) A taxonomic study on Eulophidae from Guangxi, China (Hymenoptera: Chalcidoidea). Acta Zootaxonomica Sinica 27(3): 583–607.
- Zhang YZ, Ding L, Huang HR, Zhu CD (2007) Eulophidae fauna (Hymenoptera, Chalcidoidea) from south Gansu and Qinling mountain areas, China. Acta Zootaxonomica Sinica 32(1): 6–16.
- Zongo JO, Vincent C, Stewart RK (1993) Effects of intercropping sorghum-cowpea on natural enemies of the sorghum shoot fly, *Atherigona soccata* (Diptera: Muscidae), in Burkina Faso. Biological Agriculture and Horticulture 9(3): 201–213. https://doi.org/10.1080/014487 65.1993.9754636

RESEARCH ARTICLE



A review of Copelatus diving beetles from the Solomon Islands, reporting the discovery of six new species (Coleoptera, Dytiscidae, Copelatinae)

Jiří Hájek¹, Helena Shaverdo², Lars Hendrich³, Michael Balke^{3,4}

l Department of Entomology, National Museum, Cirkusová 1740, CZ-193 00, Prague 9 – Horní Počernice, Czech Republic 2 Naturhistorisches Museum Wien, Burgring 7, 1010, Vienna, Austria 3 SNSB-Zoologische Staatssammlung München, Münchhausenstraße 21, D-81247, Munich, Germany 4 GeoBioCenter, Ludwig-Maximilians-University, Munich, Germany

Corresponding author: Jiří Hájek (jiri.hajek@nm.cz)

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Abstract

The first account of the genus *Copelatus* Erichson, 1832 in the Solomon Islands is provided, reporting 10 species for the Archipelago. Six of these are new to science: *C. baranensis* **sp. nov.**, *C. laevipennis* **sp. nov.**, *C. urceolus* **sp. nov.**, and *C. variistriatus* **sp. nov.** from Guadalcanal and *C. bougainvillensis* **sp. nov.**, and *C. kietensis* **sp. nov.** from Bougainville. *Copelatus tulagicus* Guignot, 1942, described from Tulaghi Island of the Solomons, is recorded from Guadalcanal and Santa Isabel for the first time. The widely distributed Australasian *C. portior* Guignot, 1956 is reported from the Solomon Islands (Guadalcanal and Ontong Java Atoll) for the first time. Two species from Guadalcanal remain unidentified since they are so far known only from a limited number of females.

Keywords

Australian Region, new records, new species, Solomon Islands Archipelago, Taxonomy

Introduction

The circumtropical genus *Copelatus* Erichson, 1832 (Copelatinae) represents the most speciose genus of the family Dytiscidae, with more than 450 described species to date (Nilsson and Hájek 2020). *Copelatus* species inhabit a large variety of both running and stagnant waters, mainly in forested areas of the tropics, and have also been recorded in South America from water tanks in bromeliad plants (Balke et al. 2008) and on Madagascar from wet leaf litter on tropical forest floors (Ranarilalatiana and Bergsten 2019). Recently, one troglomorphic species from Brazil was described from a cave (Caetano et al. 2013).

Most species of *Copelatus* are characterised by longitudinal elytral striae, the number of which has been used to assemble species groups (Sharp 1882; Guignot 1961; Guéorguiev 1968), although this character does not always delineate monophyletic units (Balke et al. 2004; Hájek et al. 2010, 2018). In fact, elytral striation can vary within species and even within sex, and thus the use of this character contributes to confusion in the current classification of *Copelatus* (see, e.g., Hájek et al. 2018; Manuel et al. 2018).

To understand the diversity, distributional patterns, and origin of *Copelatus* in the Australian Region, we have initiated a taxonomic research initiative, starting with the Australian (s. str.) taxa (Hendrich et al. 2019). The present second part addresses the Solomon Islands fauna. Despite of the considerable diversity of *Copelatus* in the Australian Region, only a single species, *Copelatus apicalis* J. Balfour-Browne, 1939 (= *C. tulagicus* Guignot, 1942), has been described from the Solomon Islands to date. However, a short visit by the first author on Guadalcanal, recent collections made by parataxonomists (Kinikoto Mailautoka, Aloysisus Posman) on Guadalcanal and Bougainville respectively, and older material deposited in the Natural History Museum, London, revealed the presence of nine more species in the islands. The material contains six species new to science, which are described here; two other species known only from few female specimens remain unidentified to species level.

Materials and methods

Exact label data are cited for the type material. Authors' remarks are found in square brackets: [p] – preceding data are printed, [hw] – preceding data handwritten. Separate label lines are indicated by a slash (/), separate labels by a double slash (//). The specimens included in this study are deposited in the following institutional collections:

NHMUK	Natural History Museum [former British Museum (Natural History)],
	London, Great Britain (Christine Taylor);
LHCM	Lars Hendrich collection, Munich, Germany (property of NHMW);
NHMW	Naturhistorisches Museum Wien, Vienna, Austria (Manfred Jäch);
NMPC	Národní muzeum, Prague, Czech Republic (Jiří Hájek);

ZSMG Zoologische Staatssammlung München, Munich, Germany (part of Staatliche Naturwissenschaftliche Sammlungen Bayern, SNSB) (Michael Balke, Lars Hendrich).

Specimens were examined using an Olympus SZX12 and Leica M205 C stereomicroscopes; measurements were taken with an ocular graticule. Photographs were taken with a Canon EOS 550D digital camera using Canon's MP-E 65 mm macro lens. Images of the same specimen/structure at different focal planes were combined using Helicon Focus 6.0.3 software. Male genitalia were studied and figured in wet condition. For photography, they were treated with lactic acid; due to that, parts of their apexes less pressed together as in the freshly prepared condition. Aedeagus images were captured by Harald Schillhammer (Vienna, Austria) with a Nikon D4 (in combination with a Novoflex bellows and a Mitutoyo 10/0.25 Apo ELWD) tethered to a PC and controlled with Nikon Camera Control Pro. Resulting image stacks were treated with Zerene Stacker and then post-processed in Adobe Photoshop CS 5.

The species descriptions are provided in the alphabetic order. The descriptive style partly follows Hendrich et al. (2019). The following abbreviations were used in the descriptions:

TL	total length, measured from clypeal margin to apex of elytra;
TL-h	total length minus head length, measured from anterior margin of prono-
	tum to apex of elytra;
MW	maximum width of body measured at right angle to TL.

Taxonomy

Copelatus baranensis sp. nov.

http://zoobank.org/4A94D800-F6FB-47A6-A534-33E556BE1775 Figures 1, 15

Type locality. Solomon Islands, Guadalcanal, Barana Village area, Mount Austine, 09°28.0'S, 159°58.4'E.

Type material. *Holotype*: ♂, labelled: "Solomon Islands, GUADALCANAL / Mt. Austine – BARANA vill. env. / (secondary forest, gardens, stream) / 09°28.0'S, 159°58.4'E; 280 m / Jiří Hájek leg., 23.xi.–8.xii.2013 [p] // HOLOTYPE ♂ / *COPELATUS* / *baranensis* sp. nov. / Hájek, Shaverdo, Hendrich & Balke det. 2020 [red label, p]" (NMPC).

Paratypes: 1 3, 3 9, same data as holotype (NMPC); 14 33, 17 99, labelled: "Solomon Islands, GUADALCANAL / ca. 3.5 km SE of Barana vill. / (drying up stream in shaded gorge) / 09°29.8'S, 159°59.5'E; 190 m / Jiří Hájek leg., 24.xi.-14. xii.2013 [p]" (NHMUK, NHMW, NMPC, ZSMG); 6 33, 6 99, labelled: "Solomon Islands, GUADALCANAL / ca 4.5 km S of Barana vill., forest / nr. "Japanese camp" & Moka river / 09°30.3'S, 159°58.9'E; 275 m / Jiří Hájek leg., 5.–6.xii.2013 [p]"



Figures 1, 2. Habitus of *Copelatus* 1 *C. baranensis* sp. nov. (holotype; TL: 5.9 mm) 2 *C. bougainvillensis* sp. nov. (holotype; TL: 5.2 mm).

(NMPC); 1 \bigcirc , labelled: "Solomon Islands, GUADALCANAL I. / Honiara reg. Barana vill. Env. / 100–300 m XI–XII.2018 / St. Jakl leg." (LHCM). All paratypes with the respective printed red label.

Description of male holotype. *Habitus*: Elongate, oblong-oval, almost parallelsided, broadest in mid-length of elytra; body moderately convex in lateral view. Body outline continuous, with only indistinct discontinuity between pronotum and elytra. Dorsal surface shiny (Fig. 1).

Colouration: Body colour brown blackish; head, sides of pronotum and appendages paler, ferruginous; base of elytra with irregular transverse testaceous band reaching neither suture nor lateral margin of elytra, band comb-like shaped due to dark colouration of elytral striae; appendages testaceous; ventral part brown blackish. **Head:** Moderately broad, ca. 0.64× width of pronotum, trapezoidal. Anterior margin of clypeus indistinctly concave. Antenna with antennomeres long and slender. Reticulation consisting of moderately deeply impressed polygonal isodiametric meshes. Punctation double, consisting of coarse setigerous punctures, and very small punctures spread sparsely on surface; row of coarse punctures present around inner margin of eyes, several punctures present at frontal level of eyes, and anterolaterally to eyes in fronto-clypeal depressions.

Pronotum: Transverse (width/length ratio = 2.54), broadest between posterior angles, lateral margins moderately curved. Sides with lateral beading very thin and indistinct. Reticulation similar to that of head. Punctation similar to that of head; rows of coarse setigerous punctures present along anterior margin, laterally in longitudinal depression close to sides, several punctures present also in basolateral depressions along basal margin. Disc of pronotum laterally with long, irregularly distributed longitudinal strioles; several strioles present also in depressions close to posterior angles. Centre of disc with medial longitudinal smooth line.

Elytra: Base of elytra as broad as pronotal base; lateral margins of elytra subparallel in basal two thirds, curved in apical third. Eleven longitudinal discal striae present on each elytron: stria 1 short, beginning at a fourth of elytral length and ending approximately before apical fifth of elytral length; striae 2 and 3 absent at base; striae 4–6 beginning at base; stria 7 fragmented basally; stria 8 complete; stria 9 absent at base and ending at apical fifth of elytra; stria 10 fragmented into several small strioles, hardly perceptible; stria 11 fragmented apically; all odd striae generally shortened apically. Submarginal stria absent. Surface reticulation similar to that of head and pronotum, meshes slightly smaller and less impressed. Punctation double, consisting of row of coarse setigerous punctures along elytral striae 4, 6, 8 and along lateral margin of elytra, and very fine sparsely distributed punctures.

Legs: Protibia simple, slightly broadened anteriorly, club shaped. Pro- and meso-tarsomeres 1–3 distinctly broadened, with adhesive setae on their ventral side.

Ventral side: Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered laterally; reticulation consisting of shallow, hardly perceptible polygonal meshes. Metaventrite with microsculpture consisting of polygonal meshes; lateral parts of metaventrite ("metasternal wings") tongue-shaped, slender. Metacoxal lines well impressed, nearly complete, absent only close to metaventrite. Metacoxal plates covered with long, deep longitudinal strioles; reticulation consisting of elongated, longitudinal polygonal meshes. Metacoxal processes rounded and indistinctly incised at posterior margin. Abdominal ventrites I and II with longitudinal strioles; ventrites III and IV with oblique strioles laterally. Tuft of setae present antero-medially on ventrites I and II, oblique on ventrite III and transverse on ventrites IV–VI. Punctation consisting of fine, sparsely distributed punctures; punctures coarser and denser laterally on apical ventrite.

Genitalia: Median lobe of aedeagus (Fig. 15) sickle-shaped, with obtuse apex and distinct rugose surface sculpture visible in lateral view (Fig. 15A, B); consisting of dorsal and ventral sclerites; dorsal sclerite divided into two parts of different shape in apical half, left part slightly shorter than right one, with lateral margin distinctly curved and apex more or less rounded (Fig. 15C); ventral sclerite with left part strongly sclerotised, its apex in shape of a small, weak hook visible in lateral left view (Fig. 15B), right part membranous; apexes of dorsal and ventral sclerites more or less pressed together.

Lateral lobes (parameres) of narrow triangular form, with almost straight setigerous dorsal margin; setae numerous, dense, and strong distally, and distinctly less numerous, weaker, and sparser basally (Fig. 15D).

Female. Identical to male in habitus. Pro- and mesotarsomeres not broadened, without adhesive setae.

Variability. The specimens of the type series vary in extent of the basal testaceous band on elytra. There is also a variation in number and position of longitudinal strioles on pronotum. The highest variability is however, in elytral striation: from complete eleven striae to largely fragmented striae 1, 2, 4, 6, 7, 9, 10; in rare case, stria 1 completely absent; in several specimens, submarginal stria (or several short striae) present at the level of apical fifth of elytral length, sometimes only on one side.

Measurements. TL: 5.4–6.2 mm (mean value: 5.7 ± 0.2 mm); holotype: 5.9 mm. TL-h: 4.9–5.7 mm (mean value: 5.2 ± 0.2 mm); holotype: 5.4 mm. MW: 2.4–2.9 mm (mean value: 2.6 ± 0.1 mm); holotype: 2.6 mm.

Differential diagnosis. Based on the presence of 11 dorsal elytral striae and absence of submarginal stria, the new species can be classified within *C. nigrolineatus* species group. This group contain worldwide only five species: two in Neotropics, one in southern China, one in India, and one species in Australia (Nilsson and Hájek 2020). On the other hand, presence of a submarginal stria in some specimens indicates affiliation of the new species to the *C. trilobatus* species group. The *C. trilobatus* species group includes up to now 24 species occurring in tropics of all continents; two species of the group are occurring in the Papua New Guinea, one species in Fiji, and three species are known from Australia (Nilsson and Hájek 2020).

Based on shape of the male genitalia, *C. baranensis* sp. nov. is not similar to any of the species currently included in the *C. nigrolineatus* group. On the other hand, it is most likely very close to *C. bougainvillensis* sp. nov., *C. kietensis* sp. nov., and to the numerous undescribed species of the *C. trilobatus* species group from New Guinea (H. Shaverdo et al., in preparation). For separation of *C. baranensis* sp. nov., *C. bougainvillensis* sp. nov., see under the two latter species.

Etymology. The new species is named after Barana Village, in the vicinity of which the new species was collected. In this way, the first author would like to thank the people of Barana for their company and help with collecting during the 2013 trip. The specific epithet is an adjective in the nominative singular.

Distribution. The species is known so far only from the small area around Barana Village, south from Honiara City, northern Guadalcanal.

Habitat. At the type locality, the species was collected in small shaded pools of a forest stream (Figs 26, 27). At the other places, the specimens were collected in puddles/pools with muddy bottom made by a temporary forest stream (Fig. 28).

Copelatus bougainvillensis sp. nov.

http://zoobank.org/D01977C9-500E-4752-8DE4-1D9D7652C6C5 Figures 2, 16

Type locality. Papua New Guinea: Autonomous Region of Bougainville, Kieta.

Type material. *Holotype:* ∂, labelled: "Papua New Guinea: Bougainville, / Kieta, 620 m, 16.vi.2008, 06.13.035S / 155.30.401E, Posman, (PNG179a) [p] // HOLO-TYPE ∂ / *COPELATUS / bougainvillensis* sp. nov. / Hájek, Shaverdo, Hendrich & Balke det. 2020 [red label, p]" (ZSMG).

Paratypes: 20 $\Diamond \Diamond$, 15 $\bigcirc \bigcirc$, same data as holotype (NHMUK, NHMW, NMPC, ZSMG).

Description of male holotype. *Habitus*: Elongate, oblong-oval, broadest before mid-length of elytra; body moderately convex in lateral view. Body outline continuous, with only indistinct discontinuity between pronotum and elytra. Dorsal surface shiny (Fig. 2).

Colouration: Body colour ferruginous; head on vertex, disc of pronotum, elytra along striae, and metaventrite darkened, brown blackish.

Head: Moderately broad, ca. $0.64 \times$ width of pronotum, trapezoidal. Anterior margin of clypeus indistinctly concave. Antenna with antennomeres long and slender. Reticulation consisting of moderately deeply impressed polygonal isodiametric meshes. Punctation double, consisting of coarse setigerous punctures, and very small punctures spread sparsely on surface; row of coarse punctures present around inner margin of eyes, several punctures present at frontal level of eyes, and anterolaterally to eyes in fronto-clypeal depressions.

Pronotum: Transverse (width/length ratio = 2.50), broadest between posterior angles; lateral margins almost straight in basal two thirds, slightly curved in anterior third. Sides with lateral beading very thin but distinct, except for anterior angles. Reticulation similar to that of head. Punctation similar to that of head; rows of coarse setigerous punctures present along anterior margin, laterally in longitudinal depression close to sides, several punctures present also in basolateral depressions along basal margin. Disc of pronotum laterally with few short, irregularly distributed longitudinal strioles; several strioles present also in depressions close to posterior angles. Centre of disc with medial longitudinal smooth line.

Elytra: Base of elytra as broad as pronotal base; lateral margins of elytra slightly diverging in basal third, distinctly narrowing in apical half. Eleven discal and a submarginal longitudinal striae present on each elytron: stria 1 beginning posterior from scutellum; striae 2, 4, 6, 8 complete; striae 3, 5, 7, 9, 10 absent at base; stria 9 and 10 somewhat fragmented basally; all odd striae generally shortened apically. Submarginal stria present only as few short striolae in two thirds of elytral length. Surface reticulation similar to that of head and pronotum, meshes slightly smaller and less impressed. Punctation double, consisting of row of coarse setigerous punctures along elytral striae 4, 6, 8 and along lateral margin of elytra, and very fine sparsely distributed punctures.

Legs: Protibia simple, slightly broadened anteriorly, club shaped. Pro- and mesotarsomeres 1–3 distinctly broadened, with adhesive setae on their ventral side. Anterior protarsal claw broad basally, slightly constricted in apical third, strongly curved.

Ventral side: Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered laterally; reticulation consisting of shallow, hardly perceptible polygonal meshes. Metaventrite with microsculpture consisting of polygonal meshes; lateral parts of metaventrite ("metasternal wings") tongue-shaped, slender. Metacoxal lines well impressed, nearly complete, absent only close to metaventrite. Metacoxal plates covered with long, deep longitudinal strioles; reticulation consisting of elongated, longitudinal polygonal meshes. Metacoxal processes rounded and indistinctly incised at posterior margin. Abdominal ventrites I–II with longitudinal strioles; ventrites III and IV with oblique strioles laterally. Tuft of setae present antero-medially on ventrites III–V; ventrite VI with setigerous punctures laterally on either side. Abdominal reticulation consisting of elongate polygonal meshes, longitudinal on ventrites I and II, oblique on ventrite III and transverse on ventrites IV–VI. Punctation consisting of fine, sparsely distributed punctures; punctures coarser and denser laterally on apical ventrite.

Genitalia: Median lobe of aedeagus (Fig. 16) sickle-shaped, with pointed apex and distinct rugose surface sculpture visible in lateral view (Fig. 16A, B); consisting of dorsal and ventral sclerites; dorsal sclerite divided into two parts of different shape in apical half, left part with lateral margin slightly curved and apex pointed (Fig. 16C); ventral sclerite with left part strongly sclerotised, its apex in shape of a small hook visible in lateral left view (Fig. 16B), right part membranous; apexes of dorsal and ventral sclerites elongate, more or less pressed together.

Lateral lobes (parameres) of narrow triangular form, with almost straight setigerous dorsal margin; setae numerous, dense, and strong distally, and distinctly less numerous, weaker, and sparser basally (Fig. 16D).

Female. Identical to male in habitus. Pro- and mesotarsomeres not broadened, without adhesive setae; protarsal claws simple.

Variability. The specimens of the type series vary in extent of infuscation of the pronotal disc and elytra; in some specimens, the pronotum is largely brown blackish with ferruginous sides, and the centre of elytral disc is almost uniformly brown blackish. There is also a variation in number and position of longitudinal strioles on pronotum. Finally, there is a little variability in elytral striation: striae 2–9 may all beginning at the base of elytra, but also the odd striae 3, 5, 7, 9 may be fragmented at the base of elytra.

Measurements. TL: 5.2–5.7 mm (mean value: 5.5 ± 0.1 mm); holotype: 5.2 mm. TL-h: 4.7–5.2 mm (mean value: 5.0 ± 0.1 mm); holotype: 4.7 mm. MW: 2.4–2.7 mm (mean value: 2.6 ± 0.1 mm); holotype: 2.5 mm.

Differential diagnosis. Based on the presence of eleven dorsal striae and a submarginal stria, the new species can be classified within *C. trilobatus* species group, see under *C. baranensis* sp. nov. *Copelatus bougainvillensis* sp. nov. differs from all species of the *C. trilobatus* group by combination of small body length, dorsal surface colouration and the shape of the male genitalia.

On the other hand, in general appearance and structure of the male genitalia, *Copelatus bougainvillensis* sp. nov. is very similar (and probably closely related) to *C. baranensis* sp. nov. It can be distinguished from the latter species by habitus with pronotal sides almost straight in basal two thirds, and elytra diverging in basal third (not subparallel), and by median lobe apically more elongate and more slender, with apex differently shaped (cf. Figs 15, 16). For differentiation from sympatric *C. kietensis* sp. nov., see below.

Etymology. The species is named after the Bougainville Island where it was collected. The specific epithet is an adjective in the nominative singular.

Distribution. The species is to date only known from the type locality on the eastern coast of the Bougainville Island.

Habitat. Unknown.

Copelatus kietensis sp. nov.

http://zoobank.org/FF97573A-ECD1-4807-9BF5-4A3599B0927B Figures 3, 17

Type locality. Papua New Guinea: Autonomous Region of Bougainville, Kieta.

Type material. *Holotype:* ∂, labelled: "Papua New Guinea: Bougainville, / Kieta, 520 m, 12.vi.2008, 06.12.955S / 155.29.775E, Posman, (PNG180) [p] // HOLO-TYPE ∂ / *COPELATUS / kietensis* sp. nov. / Hájek, Shaverdo, Hendrich & Balke det. 2020 [red label, p]" (ZSMG).

Description of male holotype. *Habitus*: Elongate, oblong-oval, broadest before mid-length of elytra; body moderately convex in lateral view. Body outline continuous, with only indistinct discontinuity between pronotum and elytra. Dorsal surface shiny (Fig. 3).

Colouration: Head including appendages ferruginous, darkened along eyes; pronotum black with ferruginous anterior angles; scutellar shield ferruginous translucent; elytra black with large irregular subapical testaceous spots; legs ferruginous, hind legs somewhat darker than preceding ones; ventral part of head and prosternum ferruginous, metaventrite, metacoxa and abdominal ventrites darker, blackish brown.

Head: Moderately broad, ca. $0.60 \times$ width of pronotum, trapezoidal. Anterior margin of clypeus indistinctly concave. Antenna with antennomeres long and slender. Reticulation consisting of moderately deeply impressed polygonal isodiametric meshes. Punctation double, consisting of coarse setigerous punctures, and very small punctures spread sparsely on surface; row of coarse punctures present around inner



Figures 3, 4. Habitus of *Copelatus* 3 *C. kietensis* sp. nov. (holotype; TL: 6.3 mm) 4 *C. laevipennis* sp. nov. (holotype; TL: 6.9 mm).

margin of eyes, several punctures present at frontal level of eyes, and anterolaterally to eyes in fronto-clypeal depressions.

Pronotum: Transverse (width/length ratio = 2.79), broadest between posterior angles, lateral margins moderately curved. Sides with lateral beading very thin but distinct, except for anterior angles. Reticulation similar to that of head. Punctation similar to that of head; rows of coarse setigerous punctures present along anterior margin, laterally in longitudinal depression close to sides, several punctures present also in basolateral depressions along basal margin. Disc of pronotum laterally with short, irregularly, and sparsely distributed longitudinal strioles; several strioles present also in depressions close to posterior angles. Centre of disc with superficial medial longitudinal groove.

Elytra: Base of elytra as broad as pronotal base; lateral margins of elytra slightly diverging in basal third, distinctly narrowing in apical half. Eleven discal and a submarginal longitudinal striae present on each elytron: stria 1 shallow, largely fragmented in basal fourth of elytral length and ending approximately before apical fourth of elytral length; striae 2 and 3 shallow, absent at base; striae 4–9 complete; stria 10 absent at base; stria 11 ending at apical fifth of elytra; all odd striae generally shortened apically. Submarginal stria shallow, starting at approximately elytral mid-length. Surface reticulation

similar to that of head and pronotum, meshes slightly smaller and less impressed. Punctation double, consisting of row of coarse setigerous punctures along elytral striae 4, 6, 8, and along lateral margin of elytra, and very fine sparsely distributed punctures.

Legs: Protibia simple, slightly broadened anteriorly, club shaped. Pro- and mesotarsomeres 1–3 distinctly broadened, with adhesive setae on their ventral side. Anterior protarsal claw broad basally, strongly curved in apical third.

Ventral side: Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered laterally; reticulation consisting of shallow, hardly perceptible polygonal meshes. Metaventrite with microsculpture consisting of polygonal meshes; lateral parts of metaventrite ("metasternal wings") tongue-shaped, slender. Metacoxal lines well impressed, nearly complete, absent only close to metaventrite. Metacoxal plates covered with long, deep longitudinal strioles; reticulation consisting of elongated, longitudinal polygonal meshes. Metacoxal processes rounded and indistinctly incised at posterior margin. Abdominal ventrites I and II with longitudinal strioles; ventrites III and IV with oblique strioles laterally. Tuft of setae present antero-medially on ventrites I and II, oblique on ventrite III and transverse on ventrites IV–VI. Punctation consisting of fine, sparsely distributed punctures; punctures coarser and denser laterally on apical ventrite.

Genitalia: Median lobe of aedeagus (Fig. 17) sickle-shaped, with pointed apex and distinct rugose surface sculpture visible in lateral view (Fig. 17A, B); consisting of dorsal and ventral sclerites; dorsal sclerite divided into two parts of different shape in apical half, left part with lateral margin slightly curved and apex broadly pointed (Fig. 17C); ventral sclerite with left part strongly sclerotised, its apex in shape of a small hook visible in lateral left view (Fig. 17B), right part less sclerotised; apexes of dorsal and ventral sclerites elongate, more or less pressed together.

Lateral lobes (parameres) of narrow triangular form, with almost straight setigerous dorsal margin; setae numerous, dense, and strong distally, and distinctly less numerous, weaker, and sparser basally (Fig. 17D).

Female. Unknown.

Measurements. TL: 6.3 mm. TL-h: 5.7 mm. MW: 3.1 mm.

Differential diagnosis. Based on the presence of 11 dorsal striae + a submarginal stria, the new species can be classified within *C. trilobatus* species group, see under *C. baranensis* sp. nov. *Copelatus kietensis* sp. nov. differs from all species of the *C. trilobatus* group by combination of body length, dorsal surface colouration and the shape of the male genitalia. In particular, *C. kietensis* sp. nov. differs from the sympatric *C. bougain-villensis* sp. nov. in larger body size, broader and more oval habitus, dark colouration of the elytra with testaceous subapical spots, as well as in structure and setation of the male genitalia (cf. Figs 16, 17).

Etymology. The species is named after Kieta, a port town located on the eastern coast of Bougainville Island, where it was collected. The specific epithet is an adjective in the nominative singular.

Distribution. The species is known so far only from the type locality on the eastern coast of the Bougainville Island.

Habitat. Unknown.

Copelatus laevipennis sp. nov.

http://zoobank.org/507C2AEA-9E85-4957-A8BC-F3442EC83D7A Figures 4, 18, 23

Type locality. Solomon Islands, Guadalcanal, 0.5 km N of Mbaole Village, 09°37.69'S, 160°06.69'E.

Type material. *Holotype:* 3, labelled: "Salomonen: C-Guadalcanal, / 0.5 km N Mbaole, 2799 feet / S 09°37.69 E 160°06.69E / 2007 K. Mailautoka leg. [p] // DNA / M. Balke / 2907 [green label, p] // HOLOTYPE 3 / *COPELATUS / laevipennis* sp. nov. / Hájek, Shaverdo, Hendrich & Balke det. 2020 [red label, p]" (ZSMG).

Paratypes: 1 Å, same locality data as holotype, with additional label: "DNA / M. Balke / 3334 [green label, p]" (NMPC); 2 ♀♀, labelled: "Solomon Islands: C-Guadalcanal, / 0.5 km N Mbaole, 853 m, 2007, / 09°37.69S, 160°06.88E, K. Mailautoka [p]" (NMPC, ZSMG); 10 ÅÅ, 7 ♀♀, labelled: "SOLOMON IS.: / Guadalcanal Is. / Suta / 27.vi.1956 [p] // E.S.Brown / B.M.1957-201 [p]" (NHMUK, NMPC, ZSMG); 1 Å, labelled: "5327 [on side, hw] / SOLOMON IS. [red underlined] / Guadalcanal [p] / Suta / 27.vi. [hw] 195 [p] 6 [hw] / E.S.Brown [p] / Sangava / R [on reverse, hw] // E.S.BROWN coll / C.I.E. 1957-24 [hw] // E.S.Brown / B.M.1957-201 [p]" (NHMUK); 1 Å, labelled: "Å [p] // Type [round label with red frame, p] // SOLOMON IS: / GUADALCANAL / Tenaru R. headwaters. / 1820' 5.viii.53. / In hole in tree trunk. [hw] // Brit.Mus. / 1987-14 [p] // aC 2 [hw] // Copelatus / torosus Type! [hw] / J. Balfour-Browne det., 195 [p] 3 [hw]" (NHMUK). All paratypes with the respective printed red label.

Description of male holotype. *Habitus*: Elongate, oblong-oval, broadest shortly before mid-length of elytra; body distinctly convex in lateral view. Body outline continuous, without discontinuity between pronotum and elytra. Dorsal surface shiny (Fig. 4).

Colouration: Body colour pitchy brown; sides of pronotum (except for lateral margin) and appendages paler, ferruginous.

Head: Moderately broad, ca. $0.65 \times$ width of pronotum, trapezoidal. Anterior margin of clypeus indistinctly concave. Antenna with antennomeres long and slender. Reticulation consisting of moderately deeply impressed isodiametric meshes. Punctation double, consisting of coarse setigerous punctures, and very small punctures spread sparsely on surface; row of coarse punctures present around inner margin of eyes, few punctures present at frontal level of eyes, and several punctures anterolaterally to eyes in fronto-clypeal depressions.

Pronotum: Transverse (width/length ratio = 2.78), broadest between posterior angles, lateral margins moderately curved. Sides with lateral beading very thin and indistinct. Reticulation similar to that of head. Punctation similar to that of head; rows of coarse setigerous punctures present along anterior margin, laterally in longitudinal

depression close to sides, several punctures present also in basolateral depressions along basal margin. Disc of pronotum with shallow medial longitudinal scratch.

Elytra: Base of elytra as broad as pronotal base; lateral margins of elytra subparallel-sided in basal half, distinctly narrowing in apical half. Elytral striae absent. Reticulation similar to that of head and pronotum, meshes somewhat elongated longitudinally. Punctation consisting of coarse setigerous punctures and very fine sparse punctures. Coarse punctures arranged in three distinct longitudinal puncture lines: two discal and one lateral; another row of punctures present along lateral margin of elytra, and few coarse punctures present also in interspace between discal and lateral puncture lines.

Legs: Protibia modified, angled near base, distinctly broadened anteriorly, club shaped. Pro- and mesotarsomeres 1–3 distinctly broadened, with adhesive setae on their ventral side.

Ventral side: Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered laterally; reticulation almost effaced. Metaventrite with microsculpture consisting of polygonal meshes; lateral parts of metaventrite ("metasternal wings") tongue-shaped, slender. Metacoxal lines nearly complete, absent only very close to metaventrite. Metacoxal plates covered laterally with long, deep longitudinal strioles; reticulation consisting of extremely elongated, longitudinal polygonal meshes. Metacoxal processes rounded and incised at posterior margin. Abdominal ventrites I and II with longitudinal strioles; ventrites III and IV with oblique strioles laterally. Tuft of setae present antero-medially on ventrites III–V; ventrite VI with setigerous punctures laterally on either side. Abdominal reticulation consisting of elongate polygonal meshes, longitudinal on ventrites I and II, oblique on ventrite III and transverse on ventrites IV–VI. Punctation consisting of fine, sparsely distributed punctures.

Genitalia: Median lobe of aedeagus (Fig. 18) sickle-shaped, with evident dorsal and ventral sclerites; dorsal sclerite without surface sculpture and divided into two parts in apical half: left part shorter than right one, both slightly curved, with small crests, notches and truncate apexes (Fig. 18A, B); ventral sclerite divided into two parts apically: left part more strongly sclerotised, broader, shorter, with broadly pointed apex, right part longer, partly sclerotised (medially membranous), with elongate, thin apex in shape of very weak hook (Fig. 18C).

Lateral lobes (parameres) of narrow triangular form, with broader subdistal part due to curved setigerous dorsal margin; setae numerous, dense, and strong distally, and distinctly less numerous, weaker, and sparser basally (Fig. 18D).

Female. Identical to male in habitus. Protibia simple, not angled basally and only slightly broadened distally; pro- and mesotarsomeres not broadened, without adhesive setae.

Variability. All specimens of the type series agree well with the holotype. There is only slight variability in body colouration: some specimens have head with pale frontal part and dark vertex, and the sides of elytra are indistinctly paler than elytral disc. Few longitudinal strioles present in depression close to posterior angles of pronotum in one female. Small differences were detected also in the shape of the male median lobe: a notch on right part of dorsal sclerite can be almost absent in some specimens (cf. Figs 18B, 23).

Measurements. TL: 6.3-7.2 mm (mean value: 6.9 ± 0.2 mm); holotype: 6.9 mm. TL-h: 5.7-6.4 mm (mean value: 6.2 ± 0.2 mm); holotype: 6.3 mm. MW: 3.2-3.6 mm (mean value: 3.4 ± 0.1 mm); holotype: 3.4 mm.

Differential diagnosis. Based on the absence of elytral striae, the new species can be included into the *C. hydroporoides* species group (or *haemorrhoidalis*, in earlier studies). This species group contains currently 55 species distributed predominantly in Afrotropical and Neotropical regions (Nilsson and Hájek 2020). Only two species of this group were recently described from the Oriental (Maluku, Indonesia) and Australian regions (Papua New Guinea) by Hájek et al. (2010) and Megna et al. (2017), respectively.

Based on shape of the median lobe and paramere, *C. laevipennis* sp. nov. is without any doubt closely related to *C. variistriatus* sp. nov., which it resembles also in size, body shape, and colouration, and *C. urceolus* sp. nov. The type localities of *C. laevipennis* sp. nov. and *C. variistriatus* sp. nov. are only ca. 20 km apart, although they differ significantly in altitude. Due to great variability of elytral striation in *C. variistriatus* sp. nov. described below, we hesitated first to describe another species, which may represent only a non-striated population of one species. Finally, we have decided to describe both species, because of: 1) *C. laevipennis* sp. nov. has more parallel habitus than *C. variistriatus* sp. nov.; 2) there are no intermediate specimens, i.e., there are no specimens of *C. variistriatus* sp. nov. without elytral striae, and no specimens of *C. laevipennis* sp. nov. with traces of striae; 3) minor differences in the shape of the median lobe can be detected between both species, mainly in shape of the apexes of parts of the dorsal and ventral sclerites (cf. Figs 18, 22–25); 4) there is significant genetic divergence between both species: uncorrected genetic distances between the sequenced CO1 haplotypes of *C. laevipennis* sp. nov. and *C. variistriatus* sp. nov. are ca. 7% (pers. obs.).

Etymology. The species name is composed from Latin adjective *laevis* (*-is*, *-e*, = smooth) and noun *penna* (*-ae*, feminine, = wing), referring to smooth elytra without striae.

Distribution. The species is known only from medium altitude area (ca. 600–900 m) in north-central Guadalcanal.

Habitat. Largely unknown; according to label data, one specimen was collected in a water-filled hole in tree trunk.

Copelatus portior Guignot, 1956

Figures 5-6, 19

Copelatus portior Guignot, 1956: 53 (type locality: "New Hebrides [Vanuatu], Malekula Island").

Copelatus divisus Watts, 1978: 122 ("Seleo, Berlinhafen" [Papua New Guinea, Sandaun Province, Seleo Island); synonymy by Hendrich et al. (2019).

Material examined. *Guadalcanal*: 1 \Diamond , Honiara, M.V. light, 8.–12.ix.1953, J.D. Bradley leg.; 1 \Diamond , Honiara, Kukum, 1962, P.J.M. Greenslade leg.; 1 \Diamond , same data, but 19.v.1962; 1 \Diamond , same data, but 20.v.1963; 1 \Diamond , same data, but, 2.iii.1965; 1 \Diamond , 1



Figures 5, 6. Habitus of *Copelatus* 5 *C. portior* Guignot, 1956 (male, Guadalcanal; TL: 5.2 mm) 6 *C. portior* (striolate female, Papua; TL: 4.7 mm).

♀, same data, but 12.v.1966 (all NHMUK); 1 ♂, 1 ♀, Mt. Austine, Barana vill. env., 09°28.0'S, 159°58.4'E, 280 m, 23.xi.-8.xii.2013, J. Hájek leg. (NMPC, ZSMG).

Ontong Java: 1 ♂, 1 ♀, Keila, 31.i.1955, E.S. Brown leg. (NHMUK); 1 ♀, Leuaniua, 27.i.1955, E.S. Brown leg. (NHMUK).

Diagnosis. For complete description, see Hendrich et al. (2019). Medium sized (TL: 5.0–5.7 mm), oblong-oval species. Species variable in elytral colouration: elytra from almost uniformly dark brown to black with only base and lateral sides yellowish, to almost yellowish-orange coloured with dark stripes along elytral striae (Fig. 5). Elytron with well-impressed six discal striae and a submarginal stria. Female dimorphic; striolate form with dorsal surface almost black, matt and with coarse microreticulation and numerous strioles on elytra and pronotum (Fig. 6). Median lobe in lateral view sickle-shaped; broad and subparallel in basal two thirds, thin and regularly curved in apical third; a distinct hammer-like process present in two thirds on ventral side (Fig. 19). Parameres broad, "D"-shaped; apical lobes moderately long, club-shaped.

Distribution. The species is originally described from northern Papua New Guinea and Vanuatu. It is widely distributed in the Australasian Region: from Lesser Sunda Islands, through New Guinea, northern Australia, and Solomon Islands to Vanuatu. First record from the Solomon Islands.

Habitat. Two specimens from Barana were recently collected in a streamlet flowing through secondary forest and gardens near the village; both of them were found in calm water with decaying leaves on the bottom (Fig. 26).

Copelatus tulagicus Guignot, 1942

Figures 7, 8, 20

- *Copelatus apicalis* J. Balfour-Browne, 1939: 78 (type locality: "Solomon Islands: Tulagi; preoccupied by *Copelatus apicalis* Fairmaire, 1898: 465 [currently in genus *Madaglymbus* Shaverdo & Balke, 2008]).
- *Copelatus tulagicus* Guignot, 1942: 86 (as a replacement name for *Copelatus apicalis* J. Balfour-Browne, 1939: 78).

Type material. *Holotype*: \vec{O} , labelled: "Type [round label with red frame, p] // 1826 [hw] // SOLOMON IS. [p] / Tulagi / 3.viii.1934 / on leaf. [hw] / R.A.Lever [p] // Pres.by / Imp.Inst.Ent. / B.M.1936-90. [p] // Copelatus / apicalis, / \vec{O} Type sp. nov. / J.Balfour-Browne [hw]" (NHMUK).

Paratype: ♀, labelled: "Type [round label with red frame, p] // PAPUA: Kokoda [yellow underlined] / I,200ft.v.1933. / L.E.Cheesman. / B.M.1933-577. [p] // Copelatus / apicalis, / ♀ Type sp. nov. / J.Balfour-Browne [hw]" (NHMUK).

Additional material examined. *Guadalcanal*: $1 \Leftrightarrow$, Kukum, 28.iii.1958, E.S. Brown leg.; $1 \diamondsuit$, $1 \Leftrightarrow$, Kukum, 20.v.1963, P. Greenslade leg.; $1 \Leftrightarrow$, same data, but 29.v.1963; $3 \Leftrightarrow \Leftrightarrow$, same data, but 8.i.1965; $1 \Leftrightarrow$, same data, but 26.v.1962; $1 \diamondsuit$, Mt. Austen, xii.1965–i.1966, P. Greenslade leg. (all NHMUK); $1 \diamondsuit$, ca 4.5 km S of Barana vill., forest near "Japanese camp" at Moka river, 09°30.3'S, 159°58.9'E; 275 m, 5.–6. xii.2013, J. Hájek leg.; $1 \diamondsuit$, Mt. Austine, Barana vill. env., 09°28.0'S, 159°58.4'E, 280 m, 23.xi.-8.xii.2013, J. Hájek leg. (all NMPC). *Santa Isabel*: $1 \diamondsuit$, Ysabel, Gatere, 19.ii.1956, E.S. Brown leg. (NHMUK).

Diagnosis. Medium sized (TL: 5.8–6.8 mm), elongate, oblong-oval species. Elytra with transverse testaceous basal band, which does not reach either suture or lateral margin, and with relatively small apical testaceous spot (Figs 7, 8). Pronotum with short longitudinal strioles near posterior angles. Elytra with six well impressed discal striae and a submarginal stria: striae 1 and 5 beginning more posteriorly than other striae; submarginal stria long, beginning at elytral mid length. Female dimorphic; striolate form with dorsal surface matt and with coarse microreticulation and numerous strioles on pronotum and elytra, except for apex (Fig. 8). Median lobe hook-like shaped in lateral view, simple; broadened and with distinct pit in two thirds of its length, apically tapering and strongly curved dorsally (Fig. 20A–C). Parameres "D"-shaped; apical lobes moderately long (Fig. 20D).



Figures 7, 8. Habitus of *Copelatus* 7 *C. tulagicus* Guignot, 1942 (holotype; TL: 6.5 mm) 8 *C. tulagicus* (striolate female, Guadalcanal; TL: 6.1 mm).

Comments on classification. Based on the characteristic hook-like shape of the median lobe, *C. tulagicus* apparently belongs to a complex of species distributed in Sunda Islands and New Guinea, including *C. geniculatus* Sharp, 1882, *C. gentilis* Sharp, 1882, *C. lineatus* (Guérin-Méneville, 1838), *C. biroi* Guignot, 1956, and *C. subterraneus* Guéorguiev, 1978 (of the *C. irinus* species group) and several additional undescribed species; the most closely related species is probably *C. martinbaehri* Hendrich et al., 2019 described recently from southeastern PNG and northern Queensland.

Copelatus tulagicus was described based on a male from Solomons and female specimen from southeastern New Guinea (Kokoda). The conspecificity of the female with the male holotype is doubtful with the respect to recently described *C. martinbaehri* from Central Province (Papua New Guinea), which differs from *C. tulagicus* only in the shorter and straighter apical part of the male median lobe of the aedeagus.

Distribution. The species seems to be widely distributed across the New Guinea and the Solomon Islands.

Habitat. The specimen from Barana was recently collected together with *C. portior* in a streamlet flowing through secondary forest and gardens near the village (Fig. 26). The specimen from Moka River was collected in a small puddle near the river. Some specimens from Kukum were covered with moth scales and they were apparently collected at light.

Copelatus urceolus sp. nov.

http://zoobank.org/F1E81914-2D6C-4163-A5E9-6916D097F308 Figures 9, 21

Type locality. Solomon Islands, Guadalcanal, Vulavula River.

Type material. *Holotype:* ∂, labelled: "∂ [p] // Type [round label with red frame, p] // SOLOMON IS: / GUADALCANAL / 1720' 6.viii.53. [hw] // Small pool on top / of large boulder in Vulavula River. [hw] // Brit.Mus. / 1987-14 [p] // aD 2 [hw] // Copelatus / urceolus Type! [hw] / J. Balfour-Browne det., 195 [p] 3 [hw]" (NHMUK).

Description of male holotype. *Habitus*: Elongate, oblong-oval, broadest before mid-length of elytra; body distinctly convex in lateral view. Body outline continuous, without discontinuity between pronotum and elytra. Dorsal surface shiny (Fig. 9).

Colouration: Head, lateral parts of pronotum, appendages and prosternum orange-ferruginous; elytra, meso- and metaventrite, and abdomen pitchy brown; anterior margin and midpart of pronotum darkest, brown.

Head: Moderately broad, ca. $0.60 \times$ width of pronotum, trapezoidal. Anterior margin of clypeus indistinctly concave. Antenna with antennomeres long and slender. Reticulation consisting of moderately deeply impressed isodiametric meshes. Punctation double, consisting of coarse setigerous punctures, and very small punctures spread sparsely on surface; row of coarse punctures present around inner margin of eyes, few punctures present at frontal level of eyes, and several punctures anterolaterally to eyes in fronto-clypeal depressions.

Pronotum: Transverse (width/length ratio = 2.48), broadest between posterior angles, lateral margins moderately curved. Sides with lateral beading thin, but distinct except for anterior angles. Reticulation similar to that of head. Punctation similar to that of head; rows of coarse setigerous punctures present along anterior margin, laterally in longitudinal depression close to sides, several punctures present also in basolateral depressions along basal margin. Centre of disc with medial longitudinal smooth line.

Elytra: Base of elytra as broad as pronotal base; lateral margins of elytra curved, distinctly narrowing in apical half. Elytral striae absent. Reticulation similar to that of head and pronotum. Punctation consisting of coarse setigerous punctures and very fine sparse punctures. Coarse punctures arranged in three distinct longitudinal puncture lines: two discal and lateral; another row of punctures present along lateral margin of elytra, and few coarse punctures present also in interspace between discal and lateral puncture lines.

Legs: Protibia modified, angled near base, distinctly broadened anteriorly, club shaped. Pro- and mesotarsomeres 1–3 distinctly broadened, with adhesive setae on their ventral side.



Figures 9–12. Habitus of *Copelatus* 9 *C. urceolus* sp. nov. (holotype, TL: 5.1 mm) 10 *C. variistriatus* sp. nov. (holotype; TL: 6.6 mm) 11 *C. variistriatus* sp. nov. (male paratype with reduced striae; TL: 6.2 mm) 12 *C. variistriatus* sp. nov. (striolate female paratype; TL: 5.9 mm).

Ventral side: Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered; reticulation almost effaced. Metaventrite with microsculpture consisting of polygonal meshes; lateral parts of metaventrite ("metasternal wings") tongue-shaped, slender. Metacoxal lines nearly complete, absent only very close to metaventrite. Metacoxal plates covered laterally with long, deep longitudinal strioles; reticulation consisting of extremely elongated, longitudinal polygonal meshes. Metacoxal processes rounded and incised at posterior margin. Abdominal ventrites I–II with longitudinal strioles. Tuft of setae present antero-medially on ventrites III–V; ventrite VI with setigerous punctures laterally on either side. Abdominal reticulation consisting of elongate polygonal meshes, longitudinal on ventrites I and II, oblique on ventrite III, and transverse on ventrites IV–VI. Punctation consisting of fine, sparsely distributed punctures.

Genitalia: Median lobe of aedeagus (Fig. 21) sickle-shaped, with evident dorsal and ventral sclerites; dorsal sclerite without surface sculpture and divided into two parts in apical half: left part distinctly shorter than right one, parts slightly curved, with crests and broadly pointed apexes (Fig. 21A, B); ventral sclerite divided into two parts apically: left part sclerotised, broader, shorter, with broadly pointed apex, right part longer, partly sclerotised (apically membranous), asymmetrically concave, with long, thin, rounded apex (Fig. 21C).

Lateral lobes (parameres) of narrow triangular form, with broader subdistal part due to curved setigerous dorsal margin; setae numerous, dense, and strong distally, and distinctly less numerous, weaker, and sparser basally (Fig. 21D).

Female. Unknown.

Measurements. TL: 5.1 mm. TL-h: 4.5 mm. MW: 2.5 mm.

Differential diagnosis. Member of the *Copelatus hydroporoides* species group, see under *C. laevipennis* sp. nov. *Copelatus urceolus* sp. nov. is most likely related to *C. laevipennis* sp. nov. and *C. variistriatus* sp. nov. It differs from both mentioned species in smaller body length, different shape of median lobe of male genitalia (cf. Figs 19, 21, 22), and from the latter species also in absence of elytral striae.

Etymology. We adopted the manuscript name used by J. Balfour-Browne. Latin noun *urceolus* (*-i*, masculinum) means small pitcher or jug, referring probably to the habitat in which the type specimen was caught; the name is used in the nominative case, standing in apposition.

Distribution. The species is known only from the type locality in central Guadalcanal.

Copelatus variistriatus sp. nov.

http://zoobank.org/A5542699-AEEA-4B6F-A5F2-629054351795 Figures 10–12, 22, 24, 25

Type locality. Solomon Islands, Guadalcanal, 4.5 km S of Barana Village, Moka River near "Japanese camp", 09°30.3'S, 159°58.9'E.

Type material. *Holotype*: ♂, labelled: "Solomon Islands, GUADALCANAL / ca 4.5 km S of Barana vill., forest / nr. "Japanese camp" & Moka river / 09°30.3'S,

159°58.9'E; 275 m / Jiří Hájek leg., 5.–6.xii.2013 [printed] // HOLOTYPE 3° / *COPELATUS* / *variistriatus* sp. nov. / Hájek, Hendrich & Balke det. 2018 [red label, printed]" (NMPC). *Paratypes*: 9 3° , 5 9° , same data as holotype (NHMUK, NMPC); 9 3° , 11 9° , labelled: "Solomon Islands, GUADALCANAL / ca. 3.5 km SE of BARANA vill. / (drying up stream in shaded gorge) / 09°29.8'S, 159°59.5'E; 190 m / Jiří Hájek leg., 24.xi.-14.xii.2013 [printed]" (NHMW, NMPC, ZSMG). All paratypes with the respective printed red label.

Description of male holotype. *Habitus*: elongate oblong oval, broadest at midlength of elytra; body distinctly convex in lateral view. Body outline continuous, without discontinuity between pronotum and elytra. Dorsal surface shiny (Fig. 10).

Colouration: Body colour pitchy brown; head, sides of pronotum and appendages paler, ferruginous.

Head: Rather narrow, ca. 0.57 × width of pronotum, trapezoidal. Anterior margin of clypeus indistinctly concave. Antenna with antennomeres long and slender. Reticulation consisting of moderately deeply impressed isodiametric meshes. Punctation double, consisting of coarse setigerous punctures, and very small punctures spread sparsely on surface; row of coarse punctures present around inner margin of eyes, few punctures present at frontal level of eyes, and several punctures anterolaterally to eyes in fronto-clypeal depressions.

Pronotum: Transverse, broadest between posterior angles (width/length ratio = 2.91), lateral margins moderately curved. Sides with lateral beading very thin and indistinct. Reticulation similar to that of head. Punctation similar to that of head; rows of coarse setigerous punctures present along anterior margin, laterally in longitudinal depression close to sides, several punctures present also in basolateral depressions along basal margin. Few longitudinal strioles present in depressions close to posterior angles; disc of pronotum with shallow medial longitudinal scratch.

Elytra: Base of elytra as broad as pronotal base; lateral margins of elytra slightly diverging in basal half, distinctly narrowing in apical half. Eleven discal (but see variability) and a submarginal longitudinal striae present on each elytron: distance between stria 1 and suture twice bigger than distance between other discal striae; striae 1 and 2 absent at base; stria 10 present only as numerous strioles in basal third of elytra; even striae shortened apically. Submarginal stria rather short, present approximately in third fourth of elytral length. Few longitudinal strioles present in interspaces between suture, stria 1, and stria 2. Reticulation similar to that of head and pronotum, meshes somewhat elongated longitudinally. Punctation consisting of coarse setigerous punctures and very fine sparse punctures; coarse punctures present in row along lateral margin of elytra.

Legs: Protibia modified, angled near base, distinctly broadened anteriorly, club shaped. Pro- and mesotarsomeres 1–3 distinctly broadened, with adhesive setae on their ventral side.

Ventral side: Prosternum sinuate anteriorly, obtusely keeled medially. Prosternal process shortly lanceolate, in cross-section convex, apex obtuse; process distinctly bordered laterally; reticulation consisting of shallow, hardly perceptible polygonal meshes. Metaventrite with microsculpture consisting of polygonal meshes; lateral

parts of metaventrite ("metasternal wings") tongue-shaped, slender. Metacoxal lines nearly complete, absent only very close to metaventrite. Metacoxal plates covered with long, deep longitudinal strioles; reticulation consisting of extremely elongated, longitudinal polygonal meshes. Metacoxal processes rounded at posterior margin. Abdominal ventrites I–II with longitudinal strioles; ventrites III and IV with oblique strioles laterally. Tuft of setae present antero-medially on ventrites III–V; ventrite VI with setigerous punctures laterally on either side. Abdominal reticulation consisting of elongate polygonal meshes, longitudinal on ventrites I and II, oblique on ventrite III and transverse on ventrites IV–VI. Punctation consisting of fine, sparsely distributed punctures.

Genitalia: Median lobe of aedeagus (Fig. 22) sickle-shaped, with evident dorsal and ventral sclerites; dorsal sclerite without surface sculpture and divided into two parts in apical half: left part shorter than right one, both slightly curved, with small crests, notches and truncate apexes (Fig. 22A, B); ventral sclerite divided into two parts apically: left part more strongly sclerotised, broader, shorter, with broadly pointed apex, right part longer, partly sclerotised (medially membranous), with elongate, thin apex in shape of weak hook (Fig. 22C).

Lateral lobes (parameres) of narrow triangular form, with broader subdistal part due to curved setigerous dorsal margin; setae numerous, dense, and strong distally, and distinctly less numerous, weaker and sparser basally (Fig. 22D).

Female. Similar to male in habitus. Protibia simple, not angled basally and only slightly broadened distally; pro- and mesotarsomeres not broadened, without adhesive setae. Dimorphic; striolate form due to dense striolation matt; strioles present on whole surface of pronotum and elytra, thus elytral striation not recognisable: strioles longitudinal, usually very long, only rarely confluent (Fig. 12).

Variability. The specimens of the type series vary in dorsal body colouration: specimens from drying up stream are generally paler (ochreous) than specimens from Moka River. The highest variability is however in elytral striation: in some specimens short strioles present between suture and stria 1, suggesting the present of "true" stria 1, which is vanished in all studied specimens (thus, the first visible stria is actually stria 2); in addition, strioles presenting in basal third between striae 9 and 10 may confluent into distinct stria in some specimens. On the other hand, all even striae have tendency for reduction, they are often fragmented, persisting only as a few longitudinal strioles or missing completely; in the extreme, only four elytral striae (stria 1, 3, 5, 7) are well preserved on disc with striae 9 and 10 preserved as short strioles (Fig. 11). The submarginal stria is missing in ca. one third of all specimens. Generally, the striation is more complete in the specimens from Moka River than in specimens from drying up stream. Additional short strioles may occur irregularly between all discal striae in some specimens. Small differences were detected also in the shape of the male median lobe (cf. Figs 22B, 24, 25).

Measurements. TL: 5.7–6.7 mm (mean value: 6.4 ± 0.2 mm); holotype: 6.6 mm. TL-h: 5.2–6.1 mm (mean value: 5.7 ± 0.2 mm); holotype: 6.0 mm. MW: 2.9–3.4 mm (mean value: 3.2 ± 0.1 mm); holotype: 3.3 mm.

Differential diagnosis. Due to variable number of elytral striae, it is quite difficult to classify the new species within the traditional *Copelatus* species group (Sharp 1882; Guignot 1961; Guéorguiev 1968). We have tentatively included the species into the *C. trilobatus* species group, as the state of eleven dorsal striae and a submarginal stria is the most frequent condition in *C. variistriatus* sp. nov., and the other forms resulting from subsequent reduction of striae. However, the double distance between suture and stria 1 suggested possible presence of twelfth stria in the ground plan of the species, currently absent in all specimens studied.

The *C. trilobatus* species group includes up to now 24 species occurring in tropics of all continents (see under *C. bougainvillensis* sp. nov.). However, no species is similar to the new species. *Copelatus variistriatus* sp. nov. is without any doubts closely related to *C. laevipennis* sp. nov., from which it differs in slightly smaller body length, more oval habitus, presence of elytral striae, and minor differences in the shape of the median lobe (see also under *C. laevipennis* sp. nov.).

Etymology. The species name is composed from Latin adjectives *varius* (*-a*, *-um*, = diverse, variegated) and *striatus* (*-a*, *-um*, = with striae), referring to the variable number of elytral striae in the new species.

Distribution. The species is known so far only from two localities, ca. 1.5 km apart, along north coast of Guadalcanal.

Habitat. At the type locality, the species was collected in small side rock pools of a small forest river. At the other locality, the specimens were collected in puddles/pools with muddy bottom made by a temporary forest stream (Fig. 28). At both places it was collected together with *C. baranensis* sp. nov.

Copelatus sp. 1

Figure 13

Material examined. *Guadalcanal*: 1 ♀, 0.5 km N Mbaole, 09°37.69'S, 160°06.69'E, 2799 feet, 2007, K. Mailautoka leg. (ZSMG).

Diagnosis. Medium sized (TL: 6.3 mm), elongate, oblong-oval species. Head testaceous, with dark band posterior to eyes; pronotum brown blackish, with testaceous sides; elytra brown blackish, with broad transverse testaceous basal band and testaceous apical part. Pronotum with short longitudinal strioles laterally. Elytra with ten discal striae and a submarginal stria: striae 1, 3, 5, 7, 9, and 10 almost complete and well impressed; striae 2, 4, 6, and 8 present only as a series of short strioles between odd striae; submarginal stria short, split to several strioles on one side (Fig. 13).

Comments to classification. Based on presence of ten discal and a submarginal stria on elytra, the species can be included in the *C. erichsonii* species group. It could not be associated with any species currently known from the Solomon Islands. Without a male available for the study, we leave this taxon unidentified to species level.

Distribution. The species is known only from a single medium altitude locality in north-central Guadalcanal.



Figures 13, 14. Habitus of Copelatus 13 Copelatus sp. 1 14 Copelatus sp. 2.

Copelatus sp. 2

Figure 14

Material examined. *Guadalcanal*: 3 ♀ ♀, 0.5 km N Mbaole, 09°37.69'S, 160°06.69'E, 2799 feet, 2007, K. Mailautoka leg. (ZSMG).

Diagnosis. Medium sized (TL: 7.2–7.9 mm), elongate, oblong-oval species. Head testaceous, with dark band posterior to eyes; pronotum brownish, with broad testaceous sides; elytra brown blackish, with thin transverse testaceous basal band and somewhat paler brown apical part. Pronotum and elytra (except for apical fifth) densely covered with long longitudinal strioles. Elytral striation due to presence of striolae could not be observed; presence of at least five discal striae is perceptible in the non-striolate apical part of elytra (Fig. 14).

Comments to classification. The striolate female could not be associated with any current species group of *Copelatus*. They do not fit to any currently known species from the Solomon Islands. Without a male available for study, we leave this taxon unidentified to species level.

Distribution. The species is known only from a single medium altitude locality in north-central Guadalcanal.



Figure 15. Male genitalia of *Copelatus baranensis* sp. nov. (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figure 16. Male genitalia of *Copelatus bougainvillensis* sp. nov. (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figure 17. Male genitalia of *Copelatus kietensis* sp. nov. (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figure 18. Male genitalia of *Copelatus laevipennis* sp. nov. (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.


Figure 19. Male genitalia of *Copelatus portior* Guignot, 1956 (Australia) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figure 20. Male genitalia of *Copelatus tulagicus* Guignot, 1942 (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figure 21. Male genitalia of *Copelatus urceolus* sp. nov. (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figure 22. Male genitalia of *Copelatus variistriatus* sp. nov. (holotype) **A** median lobe in lateral view, right side **B** median lobe in lateral view, left side **C** median lobe in ventral view **D** left paramere in external view. Scale bar: 0.5 mm.



Figures 23–25. Variability of *Copelatus* male genitalia: median lobe in lateral view, left side 23 *Copelatus laevipennis* sp. nov. (paratype) 24, 25 *C. variistriatus* sp. nov. (paratypes). Scale bar: 0.5 mm.



Figures 26–28. Habitats of *Copelatus* in Guadalcanal **26** temporary forest stream near Barana village **27** shaded pool on the same stream **28** small temporary puddles in a gorge along the road from Barana to Lungga river.

Key to Copelatus species of Solomon Islands

The key is based mostly on male characters, since some species are similar in external morphology and in most cases, the male genitalia need to be studied for reliable species identification. The two unidentified species are not included in the key.

1	Elytron with six discal striae and a submarginal stria (6+1). Median lobe of ae-
	deagus simple, without division into dorsal and ventral scienites, sometimes with
_	Flytron with number of strige and degree of their development very variable
	among species and within one species $(0-11 + 0-1)$. Median lobe of aedeagus
	complex, with evident dorsal and ventral sclerites, which apically divided into
	two parts of different shape
2	Smaller (TL: 5.0-5.7 mm), broader species (Figs 5, 6). Median lobe of aedeagus
	with a median, hammer-like process on ventral side (Fig. 19) portion
_	Larger (TL: 5.8-6.8 mm), more elongate species (Figs 7, 8). Median lobe of ae-
	deagus simple, hook-like, without process (Fig. 20)tulagicus
3	Elytron with striae. Dorsal sclerite of median lobe of aedeagus with rugose surface
	sculpture distinctly visible in lateral view; apexes of two parts of dorsal and ventral
	sclerites without strong modification, elongate, more or less pressed together (Figs
	15–17)
_	Elytron with or without striae. Dorsal sclerite of median lobe of aedeagus without
	surface sculpture, smooth; apexes of two parts of dorsal and ventral scientes dif-
6	The result of the second seco
4	Larger species (11: 0.5 mm). Elytron black with large subapical testaceous spot;
	with 11 doisal and a submarginal stria (11g. 5). Male genitalia as in Fig. 17
_	Smaller species (TI : 5.2–6.2 mm) Flytron brownish black with testaceous trans-
	verse basal band, or almost uniformly ferruginous: with 11 dorsal striae and with
	or without submarginal stria (Figs 1, 2)
5	More parallel species: disc of elvtra brownish black (Fig. 1). Median lobe of aedeagus
-	and paramere broader, with more rounded apexes (Fig. 15) <i>baranensis</i> sp. nov.
_	More oval species; disc of elytra ferruginous, darker along striae (Fig. 2). Median
	lobe of aedeagus and paramere more elongate and slender apically (Fig. 16)
	bougainvillensis sp. nov.
6	Elytra without striae7
_	Each elytron with 11 dorsal and a submarginal stria. The striae can be reduced or
	additional strioles can be present (Figs 10–12). Male genitalia as in Fig. 22
	variistriatus sp. nov.
7	Smaller (TL: 5.1 mm), more oval species (Fig. 9). Male genitalia as in Fig. 21
	<i>urceolus</i> sp. nov.
_	Larger (1L: 6.3–/.2 mm), more parallel species (Fig. 4). Male genitalia as in Fig.
	18laevipennis sp. nov.

Discussion

With ten recorded species, our account of *Copelatus* from the Solomon Islands has to be considered very preliminary. The only island with extensive collecting efforts was Guadalcanal, from where we report eight species, four of which are described as new, two species are widespread in the Australian region, and two species known only from females remain unidentified. However, still only a small (north-central) area of that island was explored and covers only low and medium altitudes. Based on decades of our fieldwork experience in other parts of the world, we suggest that the diversity of Copelatus changes altitudinally with different microhabitats. That in turn means that the actual number of *Copelatus* species in Guadalcanal might be at least twice as high. Interestingly, the Copelatinae genus Exocelina Broun, 1886, highly diverse in Australia, New Guinea, and New Caledonia (and single species in Vanuatu and Hawaii), has not been reported from the Solomon Islands yet. Most species of *Exocelina* inhabit stream associated stagnant water habitats such as pools in intermittent streams, small waterfilled holes on rocks, and small areas stagnant water at the margin of streams and even the tiniest forest creeks. They can be found in wet gravel and leaves in otherwise dry creek beds. Their apparent absence in the Solomon Islands suggests that this habitat type could be filled by *Copelatus* species (see Toussaint et al. 2015).

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References

Balfour-Browne J (1939) On *Copelatus* Er. and *Leiopterus* Steph. (Col. Dytiscidae) with descriptions of new species. The Transactions of the Royal Entomological Society of London 88: 57–88. https://doi.org/10.1111/j.1365-2311.1939.tb00250.x

- Balke M, Gómez-Zurita J, Ribera I, Viloria A, Zillikens A, Steiner J, García M, Hendrich L, Vogler AP (2008) Ancient associations of aquatic beetles and tank bromeliads in the Neotropical forest canopy. PNAS 105: 6356–6361. https://doi.org/10.1073/ pnas.0710368105
- Balke M, Ribera I, Vogler AP (2004) MtDNA phylogeny and biogeography of Copelatinae, a highly diverse group of tropical diving beetles (Dytiscidae). Molecular Phylogenetics and Evolution 32: 866–880. https://doi.org/10.1016/j.ympev.2004.03.014
- Caetano DS, Bená D de C, Vanin SA (2013) Copelatus cessaima sp. nov. (Coleoptera: Dytiscidae: Copelatinae): first record of a troglomorphic diving beetle from Brazil. Zootaxa 3710: 226–232. https://doi.org/10.11646/zootaxa.3710.3.2
- Fairmaire L (1898) Matériaux pour la faune coléoptérique de la région malgache. 7^e note. Annales de la Société Entomologique de Belgique 42: 463–499.
- Erichson WF (1832) Genera Dyticeorum. Nietackianis, Berolini, [ii +] 48 pp. https://doi. org/10.5962/bhl.title.48804
- Guéorguiev VB (1968) Essai de classification des coléoptères Dytiscidae. I. Tribus Copelatini (Colymbetinae). Izvestija na Zoologitjeskija Institut s Musei Sofia 28: 5–45.
- Guignot F (1942) Seizième note sur les hydrocanthares. Bulletin Mensuel de la Société Linnéenne de Lyon 11: 86–88. https://doi.org/10.3406/linly.1942.9665
- Guignot F (1956) Dytiscides récoltés par le Dr. L. Biró en Nouvelle Guinée et dans l'Île de Java (Coleoptera). Annales Historico-Naturales Musei Nationalis Hungarici (Series Nova) 7: 51–60.
- Guignot F (1961) Revision des hydrocanthares d'Afrique (Coleoptera Dytiscoidea). 3. Annales du Musée Royal du Congo Belge, Série 8^{vo} (Sciences Zoologiques) 90: 659–995.
- Hájek J, Hendrich L, Balke M (2018) The *Copelatus doriae-masculinus* species complex in Greater Sunda, with description of three new species and a new synonymy (Coleoptera, Dytiscidae). Spixiana 41(1): 77–90.
- Hájek J, Hendrich L, Hawlitschek O, Balke M (2010) *Copelatus sibelaemontis* sp. nov. (Coleoptera: Dytiscidae) from the Moluccas with generic assignment based on morphology and DNA sequence data. Acta Entomologica Musei Nationalis Pragae 50: 437–443.
- Hendrich L, Shaverdo H, Hájek J, Balke M (2019) Taxonomic revision of Australian *Copelatus* Erichson, 1832 (Coleoptera, Dytiscidae, Copelatinae). ZooKeys 889: 81–152. https://doi. org/10.3897/zookeys.889.39090
- Manuel M, Deler-Hernández A, Megna YS, Hájek J (2018) Copelatus Erichson from the Dominican Republic, with the description of a new species, comments on elytral striation and faunistic notes on Antillean species (Coleoptera: Dytiscidae: Copelatinae). Zootaxa 4399: 371–385. https://doi.org/10.11646/zootaxa.4399.3.7
- Megna YS, Atthakor W, Manaono M, Hendrich L, Balke M (2017) A new species of *Copelatus* Erichson, 1832 (Coleoptera: Dytiscidae: Copelatinae) from the Adelbert Range of Papua New Guinea. Australian Entomologist 44: 49–55.
- Nilsson AN, Hájek J (2020) A World Catalogue of the Family Dytiscidae, or the diving beetles (Coleoptera, Adephaga). Version 1.I.2020. Distributed as a PDF file via Internet. http:// www.waterbeetles.eu [accessed 15 February 2020]

- Ranarilalatiana T, Bergsten J (2019) Discovery of specialist Copelatinae fauna on Madagascar: tropical forest floor depressions an overlooked terrestrial habitat for diving beetles (Coleoptera, Dytiscidae). ZooKeys 871: 89–118. https://doi.org/10.3897/zookeys.871.36337
- Sharp D (1882) On aquatic carnivorous Coleoptera or Dytiscidae. Scientific Transactions of the Royal Dublin Society (2)2: 179–1003. [pls 7–18] https://doi.org/10.5962/bhl.title.9530
- Toussaint EF, Hendrich L, Shaverdo H, Balke M (2015) Mosaic patterns of diversification dynamics following the colonization of Melanesian islands. Scientific Reports 5: e16016. https://doi.org/10.1038/srep16016
- Watts CHS (1978) A revision of the Australian Dytiscidae (Coleoptera). Australian Journal of Zoology, Supplementary Series 57: 1–166. https://doi.org/10.1071/AJZS057



A new species of Leptopelis (Anura, Arthroleptidae) from the south-eastern slope of the Ethiopian Highlands, with notes on the Leptopelis gramineus species complex and the revalidation of a previously synonymised species

Arthur Tiutenko¹, Oleksandr Zinenko²

l Friedrich-Alexander-Universität Erlangen-Nürnberg, Schlossplatz 4, 91054, Erlangen, Germany **2** V. N. Karazin National University, 4 Svobody sq., 61058, Kharkiv, Ukraine

Corresponding author: Arthur Tiutenko (arthur.tiutenko@fau.de)

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Abstract

A new ground-dwelling species of treefrog in the genus *Leptopelis* is described from the Harenna Forest in south-eastern Ethiopia. The description is based on morphology and acoustics and is supported by molecular data. The new species has a small body size, and the digital discs on fingers and toes are significantly more conspicuous than in other semi-fossorial members of the *L. gramineus* complex. It occupies forest habitats at lower altitudes and is separated ecologically and geographically from high-altitude species of the complex. One of them, a parapatric cryptic species from Bale and Arsi Mountains, is resurrected from synonymy of *L. gramineus* and given a new name, *L. montanus*. Genetic barcoding of specimens from both populations showed that they belong to two distinct lineages that had been revealed by recent phylogenetic research. To confirm the geographic separation of the studied populations, the collection area of *L. gramineus* types was verified through analysis of the diary and the final report of the 2nd expedition of V. Bottego, and through matching of the route described in it with modern maps. The type locality of *L. gramineus* sensu stricto is restricted to Gamo Gofa, Ethiopia. Following the results of recent phylogenetic studies, the range of *L. gramineus* is limited to west of the Great Rift Valley. An identification key to the named Ethiopian species of the genus is provided.

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Keywords

Bale Mountains, Ethiopia, Harenna Forest, *Leptopelis diffidens* sp. nov., *Leptopelis montanus* nom. nov., *Leptopelis rugosus, Pseudocassina ocellata, Pseudocassina rugosa*

Introduction

The genus *Leptopelis* Günther, 1859 is usually referred by the vernacular name 'Forest Treefrogs', or just 'Tree Frogs'. Indeed, the majority of its currently recognised ca. 50 members are arboreal or semi-arboreal frogs, found on tall grass, scrubs, and trees in various habitats across sub-Saharan Africa (Channing and Rödel 2019). A few members of the genus have been known to present an exception, being adapted to semifossorial way of life and occupying different habitats and ecological niches than the rest of the congeners. Two of these species, *Leptopelis bocagii* (Günther, 1865) and *L. gramineus* (Boulenger, 1898), have long been assumed to comprise several cryptic taxa (Largen 1977; Schiøtz 1999).

For L. gramineus, the evolutionary relationships between populations east and west of the Great Rift Valley (GRV) were outlined in the recent works dealing with phylogeography and evolution of amphibians in the Ethiopian Highlands (Mengistu et al. 2012; Freilich et al. 2016; Reyes-Velasco et al. 2018). The GRV separated L. gramineus into eastern and western lineages during the early Pliocene, a period that corresponds to major tectonic processes that shaped the geomorphology of the Rift. The complex and diverse relief and climate at both sides of the GRV were preconditions of further divergence in this group. The high levels of genetic differentiation between amphibian populations were revealed by earlier research on mitochondrial genes (Mengistu et al. 2012; Freilich et al. 2016); however, the appropriateness of separation of cryptic species in L. gramineus was doubted because little to no variation between populations was found when a small number of nuclear markers had been used. Reyes-Velasco et al. (2018) reassessed genome-wide levels of the divergence between species and populations in a more recent study involving both, mtDNA and ddRAD loci. Currently, at least five species are assumed to exist in the L. gramineus complex. The same phylogenetic study revealed genetic isolation of the forest population and high-altitude population of *L. gramineus* in the south of the Somali Plateau and confirmed the conclusions that we had drawn from observations we made in 2012-2019 and from material obtained during our fieldwork in the Harenna Forest, one of the last remaining natural forests in this part of Africa.

Material and methods

Material examined

The examined and compared material in museum collections comprises 105 specimens identified as *L. gramineus* and 86 specimens of six other *Leptopelis* species from the

Horn of Africa. The list of these specimens is provided in Suppl. material 6: Table S2. For collection names we use here the following abbreviations:

BMNH	Natural History Museum, London, UK;		
LIV	World Museum, National Museums Liverpool, UK;		
MSNG	G Museo Civico di Storia Naturale "Giacomo Doria", Genoa, Italy;		
PEM	Bayworld, Port Elizabeth, South Africa;		
ZMB	Naturhistorisches Museum, Berlin, Germany;		
ZNHM	Zoological Natural History Museum at Addis Ababa University, Addis		
	Ababa, Ethiopia;		
ZSM	Zoologische Staatssammlung, Munich, Germany.		

Morphological measurements and analysis

Morphometric measurements of 45 preserved specimens of three lineages of *L. gramineus* complex were performed with digital callipers with \pm 0.1 mm accuracy by the first author. For measured values see Suppl. material 5: Table S1.

The following abbreviations (mainly adopted from Watters et al. 2016) are used for measured parameters: SVL = snout-vent length (direct line distance from tip of snout to posterior margin of vent), HW = head width (at the widest point), HL = head length (from the posterior of the jaws to the tip of the snout), ED = eye diameter (horizontally from the anterior to posterior corner of the eye), **EN** = eye-naris distance (from the anterior corner of the eye to the posterior margin of the nostril), NS = narissnout distance (from the centre of a naris to the snout tip), SL = snout length (from the tip of the snout to the anterior corner of an eye), **IOD** = interorbital distance (the shortest distance between the orbits), UEW = upper eyelid width (greatest width of the upper eyelid margins), **IND** = internarial distance (shortest distance between the inner margins of the nostrils), TD = tympanum diameter (greatest horizontal width of the tympanum), **ETD** = eve-tympanum distance (from the anterior margin of the tympanum to the posterior corner of the eye), FLL = forearm length (from the flexed elbow to the base of the outer palmar tubercle), Fin1L = finger I length (from the proximal edge of the palmar tubercle to the tip of finger I), Fin2L = finger II length (from the proximal edge of the palmar tubercle to the tip of finger II), Fin2W = finger II terminal phalanx width (measured in the middle of the phalanx), Fin2DW = finger III disc width, Fin3L = finger III length (from the proximal edge of the palmar tubercle to the tip of finger III), Fin4L = finger IV length (from the proximal edge of the palmar tubercle to the tip of finger IV), **Fin4W** = finger IV terminal phalanx width (measured in the middle of the phalanx), Fin4DW = finger IV disc width (measured at the widest point of finger IV disc), TL = tibia length (distance from the outer surface of the flexed knee to the tibio-tarsal inflection), THL = thigh length (distance from the vent to the knee), TSL = tarsus length (from the tibio-tarsal articulation to the base of the inner metatarsal tubercle); **Toe1L** = toe I length (measured from the joint of basal phalanx), **Toe2L** = toe II length (measured from the joint of basal phalanx to the toe tip), **Toe3L** = toe III length (measured from the joint of basal phalanx to the toe tip), Toe4L = toe

IV length (measured from the joint of basal phalanx to the toe tip), **Toe4W** = toe IV terminal phalanx width (measured in the middle of the phalanx), **Toe4DW** = toe IV disc width (measured at the widest point of toe IV disc), **Toe5L** = toe V length (measured from the joint of basal phalanx to the toe tip), **IMT** = inner metatarsal tubercle length, **IMTW** = inner metatarsal tubercle width (at the widest point), **TailL** = tail length (measured from the vent to the tip of the tail).

Statistical analyses were performed with Statistica 8.0. To increase the statistical sample size, we pooled specimens of all ages and sexes in a single sample. To minimise the differences in measurements that are associated with the size of specimens, we divided these values by SVL. Characters with high proportion of missing values in the dataset were excluded. We performed principal component analysis first, to check if any strong signal exists in the study material. Subsequently, a multivariate canonical discriminant analysis was applied in order to assess the morphological distinctiveness and our ability to separate the species on a basis of external characters only. This analysis was done with specimens assigned to three groups according to their geographic origin within the clades determined in recent phylogenetic studies.

The metamorphosis stage of juvenile specimens and larvae we determined according to the table of Gosner (1960).

Toponymy and geo-referencing

Localities of collection vouchers and of historical records were geo-referenced with Garmin BaseCamp 4.8.8 and QGIS 3.6. Geographic coordinates at our own study sites were taken with Garmin GPSmap 66st. For geographic coordinates and elevation of the localities mentioned in the text see Suppl. material 7: Table S3. The names of settlements are spelled according to the database of National Geospatial-Intelligence Agency (geonames.nga.mil). For other toponyms that have no standardised names we used their spelling suggested us by local people (Oromo).

To ensure that the collection localities of our vouchers in the Harenna Forest do not coincide with the type locality and range of *L. gramineus* sensu stricto, we determined the collection area of *L. gramineus* types through analysis of the diary included in the final report of the 2^{nd} expedition of Vittorio Bottego, and through matching of the route described in it with modern maps.

The authors of phylogenetic studies of Ethiopian *Leptopelis* (Mengistu et al. 2012; Freilich et al. 2016; Reyes-Velasco et al. 2018) used a mix of toponyms, such as names of towns, of historical Abyssinian provinces, or of mountain ranges for their clades. Not all of the clades that they recognised coincide or overlap. Also, their number is different in each of these papers. Therefore, we cannot use any of the existing names here and have designed our own simple system of names that derived from historical Abyssinian toponyms 'Borana', 'Shewa', 'Sidamo', 'Arsi', and 'Bale'. In the list of Gen-Bank sequences (provided in Suppl. material 8: Table S4) we matched these names to the names that Mengistu et al. (2012), Freilich et al. (2016) used Reyes-Velasco et al. (2018) used in their publications. This "translation" aid should improve the understanding of the system of clades that we discuss here.

Molecular methods

We performed genetic barcoding of specimens from populations that we were studying in order to confirm their identity and to connect our results to the published work of other authors. Total genomic DNA was extracted with NeoPrep DNA kit (NeoGene, Kyiv, Ukraine) from muscle tissue samples obtained from *Leptopelis* specimens housed in the Zoological State Collection Munich (ZSM 63/2019, ZSM 80/2019, ZSM 83/2019) and from one non-collected specimen. A fragment of 16S gene was amplified with use of published primers 16Sar and 16Sbr and with reaction conditions as described in Bossuyt and Milinkovitch (2000) and Reyes-Velasco et al. (2018). For this process we used DreamTaq Green PCR Master Mix (2X) (ThermoFisher Scientific). The PCR products were purified according to the ExoSAP protocol and sent to a commercial sequencing company. The chromatograms were visually checked, verified and trimmed. The new sequences are deposited in NCBI GenBank under the following accession numbers: MN909551 (Gaysay Grasslands, Bale Mountains), MN909553 (Harenna Forest), MN909554 (Harenna Forest), MN909555 (Menz-Guassa).

We compared our sequences with already available GenBank records and assigned the populations of our interest to the lineages and haplogroups from previous studies (Mengistu et al. 2012; Freilich et al. 2016; Reyes-Velasco et al. 2018). All similar sequences were retrieved from NCBI GenBank by BLAST search and aligned with MUSCLE algorithm that is implemented in MEGA version X (Kumar et al. 2018). We created a phylogenetic tree with MrBayes 3.2.7a (Ronquist et al. 2012), using the GTR+G substitution model and 1.5 Mio. of generations MCMC, sampling every 100th tree and discarding 10% of trees as burn-in when summarising the results. The parameter convergence was checked using Tracer 1.7.1 (Rambaut et al. 2018). The final tree (see in Suppl. material 11) was visualised and annotated in MEGA version X. Since not all sequences from GenBank had the same length, we performed another analysis using restricted dataset of 50 sequences trimmed to the overlapped region of 435 nucleotides. A phylogenetic network was constructed using statistical parsimony in TCS (Clement et al. 2000). The list of GenBank sequences that we used in this study is provided in Suppl. material 8: Table S4.

Bioacoustics recordings

We recorded vocalisations of two *Leptopelis* species at 12 localities in the Harenna Forest (Gola, Hacho, Harawa, Haro Alati, Hordoba, Kaffa Guasaa, Manyate, Megano, Segoba, Sire, Woraba, Yagana) and of one parapatric species at one locality in Bale Mountains (Gaysay Grasslands). The taxonomic identity of these populations, hence of calling individuals, had been established through aforementioned genetic barcoding. The sound recordings were performed in May and June 2019 with a TASCAM DR-100MKII stereo recorder, processed with Adobe Audition CC 2019, analysed and visualised with Sonic Visualiser 4.0.1 (University of London). In the Harenna Forest the advertisement calls were recorded in the morning (9–11 a.m.), in the afternoon (2–4 p.m.) and after sunset (9–11 p.m.), at air temperatures of 19–26 °C and at no wind. At the high-altitude locality (Gaysay Grasslands) our activities in dark hours were not allowed for security reasons. We recorded there in the morning and in the afternoon at similar temperatures, however, at wind speed of ca. 30 km/h.

Results and discussion

Initially we became aware of a possibly new species of *Leptopelis* when we heard advertisement calls during our first trip and stays on glades in the Harenna Forest in March 2012: They differed from vocalisations that we had heard previously in a high-altitude population of *L. gramineus* in the Web Valley, Bale Mountains. We made the same observations during our next trips and recorded the calls in May–June 2019. Comparative examination of specimens from the Harenna Forest and from other populations distributed across Ethiopian Highlands increased our confidence that two species of burrowing *Leptopelis* live in parapatry north and south of the Harenna Escarpment and occupy different habitats. Genetic barcoding of individuals from both populations showed that they belong to two distinct lineages that had been revealed by recent phylogenetic research (Freilich et al. 2016; Reyes-Velasco et al. 2018).

Any further analyses and taxonomic decisions require clarity about the geographic range of *L. gramineus* sensu stricto. Since no species in this complex are sympatric, one of them with the range that includes the type locality can be considered as 'true' *L. gramineus*. Like many species of African herpetofauna described in the 19th and early 20th century, the type locality of *L. gramineus* was imprecisely given between two places separated from each other by several mountain ranges and by 180 km straight line. Fortunately, we know the exact route of Bottego's expedition and can approximately determine the position of collection sites along it, thus restrict the type locality to a more concrete geographic area.

Restriction of the type locality of L. gramineus

The often-used English vernacular name 'Badditu Forest Treefrog' seems to have been coined by Frank and Ramus (1995). It is misleading because the type specimens originate from the area "between Badditu and Dime", according to original description by Boulenger (1898), as well as to BMNH and to MSNG catalogues. Vannutelli and Citerni (1899: 606) who had accompanied Bottego in his expedition wrote in the final report clearly that the frogs, described by Boulenger as two new species, were obtained on the way from Badditu to Dime: "*I due nuovi graziosi Batraci, Megalixalus gramineus Blgr. e Hylambates Vannutellii Blgr., furono ambedue raccolti durante il percorso dai Badditù a Dimè.*"

Since no specimens tagged only with the locality 'Badditu' exist, it should be safe for us to assume that *L. gramineus* was not found by Bottego's expedition at the eastern shores of the lakes Abaya and Chamo, i.e., where the Badditu tribal territory is situated. This place cannot be found on modern maps because it is a historical tribe name that was used as toponym mainly by Italians. The members of this tribe called themselves



Figure 1. Fragment of a map from Vannutelli and Citerni 1899 showing the route that the 2nd expedition of Vittorio Bottego took in 1896 (dark red line). The part of the route where type specimens of *Leptopelis gramineus* were collected is marked with blue colour.

'Koyra' (Bryan and Tucker 1930; Cerulli 1956), and today this ethnic group is better known as 'Koora'. Their territory is restricted to an area north of the Burji tribal territory (Fig. 1), on a rugged mountain spur east of Lake Abaya (Cerulli 1956).

Bottego and his people came to Burji on 17 March 1896 (see Fig. 1) and from there went northward, along the eastern side of the GRV, through Badditu territory. They discovered the 'Lake Margherita' (now Lake Abaya) on 12 May having already left Badditu. They spent a month exploring this lake that is located in the Rift at elevation of 1175 m a.s.l. and left the area by the middle of June 1896. As for *L. gramineus*, we know that it does not occur at such low elevations, hence the collecting locality could not be there. Moreover, much of the area at the Lakes Abaya and Chamo is today in the popular Necisar National Park. This protected area is well studied, and extensive collections were deposited in a number of museums. *L. gramineus* have never been recorded there.

In the second half of June the Bottego expedition went further west, to explore the lower Omo River, and traversed mountain ranges of over 3000 m in altitude. Finally, they reached Dime – another tribal area around Mount Smith, today situated in northern part of Selamago district in Southern Nation, Nationalities and Peoples Region. After that they never went back to Badditu: In October 1896 the expedition returned to the north corner of Lake Rudolf (now Turkana) and went from there again north where Bottego was killed in a battle in Daga Roba on 17 March 1897.

In his revision of Ethiopian Leptopelis Largen (1977) gave an erroneous collection date of the type series, "5 July 1896", having mistaken May (the 5th month) for the 5th day of July. In his later publication (Largen 2001) he corrected this mistake and used roman numerals for both, May and July: "V-VII.1896". This agrees with the specimen tags and the catalogue information: Boulenger himself made an entry in the hand-written catalogue of the Natural History Museum that the two specimens he had received (and which are now treated as paralectotypes) had been collected "between" May and July 1896. This also corresponds to what we see on the original specimen tag that is still kept in the glass with the specimens, a handwritten note in Italian (probably by Vannutelli): "Dai Badditù a Dimè. Magg.-Lugh. 96. Sped. Bottego". We do not know for sure whether all specimens of the type series were collected at one single site. However, since not just one locality and date but a time span and a range between two places (Badditu and Dime) are provided, it appears more logical that there were several collecting sites. Obviously, we can exclude Mount Smith, because it is in Dime, and the specimens are known to have been collected on the way there. The Bottego expedition should have crossed approximately four mountain ranges in this area, including Gughe which is in vicinity of what is today Arba Minch town. There are a few specimens of *L. gramineus* from this area in collections, including nearby places Chencha, Dorse, Bonche Valley. The type specimens might have been collected there or on further mountain ranges which offer suitable climatic conditions and habitats (Coltorti et al. 2019). Unfortunately, when Capocaccia (1957) designated the lectotype of L. gramineus, she did not validate and restrict the type locality, as recommended by the ICZN, Recommendation 74E. Therefore, the whole area between Mt. Gughe and Mt. Smith, as well as Mt. Gughe itself can still be viewed as such. This Ethiopian province was called Gamu Gofa, and the tribal territory of Gofa was situated approximately half the way "from Badditu to Dime" (see Fig. 1). For further discussion in this paper we treat this area as type locality of *L. gramineus*: Ethiopia, Gamu Gofa, 6°29'N, 36°59'E.

With the lectotype series, BMNH 1947.2.10.19–20 (2 specimens) and MSNG 28564A–B (2 specimens), at least the following specimens housed in museum collections should be considered as topotypic: BMNH 1975.1618–9 (2 specimens), 9 km N of Chencha, 27.6.1975, leg. Yalden and Largen; ZNHM H.558/1–2 (2 specimens), Chencha, 1971, leg. Rupp; ZNHM H.678/1–3 (3 specimens), Chencha, 1972, leg. Rupp; ZNHM H.634/1–2 (2 specimens), 1 km SW of Chencha, 9.9.1972, leg. Clarke; BMNH 1975.1633–4 (2 specimens), 4 km N of Dorse, 28.4.1972, leg. Yalden and Largen; BMNH 1969.193, Bonche Valley, 1969, leg. Sandhurst Ethiopia Expedition.

Since *L. gramineus* has never been reported from the Badditu territory, the other vernacular name of this species (found, for instance, in Largen and Spawls 2010; Channing and Rödel 2019) appears to be more appropriate: 'Ethiopian Burrowing Treefrog'. The attribute 'burrowing' in the name reflects the established opinion that these frogs are fossorial (see Largen 1977; Schiøtz 1999; Largen 2001; Largen and Spawls 2010; Channing et al. 2012; Reyes-Velasco et al. 2018; Channing and Rödel 2019).

The confidence that our study sites do not lie in the distribution area of *L. gramineus* sensu stricto and available material allow a description of a new species.

Leptopelis diffidens sp. nov.

http://zoobank.org/E059D4EF-FFD4-40ED-B24F-A12AB7A998EF Figures 2–4

Type locality. Between rivers Welmel and Yadot, Harenna Forest, Bale Zone, Oromia Region, Ethiopia (6°35'N, 39°45'E).

Material. *Holotype*: ETHIOPIA • ♀; Segoba glade, Harenna Forest, Bale Zone, Oromia Region; 6°35'10.5"N, 39°44'30.7"E, 1770 m a.s.l.; 1 June 2019; A. Tiutenko leg.; "Found in a tussock near calling male on a flooded forest glade during rainy season"; ZSM 81/2019. Paratypes: Етнюры • 13; Woraba glade, Harenna Forest, Bale Zone, Oromia Region; 6°35'39.9"N, 39°45'15.2"E, 1800 m a.s.l.; 5 June 2019, A. Tiutenko leg.; "Found in wet grass near slow flowing temporary stream in rainy season"; GenBank: MN909553; ZSM 83/2019 • 1 juvenile; Woraba glade, Harenna Forest, Bale Zone, Oromia Region; 6°35'39.3"N, 39°45'14.5"E, 1800 m a.s.l.; 5 June 2019; A. Tiutenko leg.; "Found in marshy grass about 10 m from a slowly flowing stream on a flooded glade"; ZSM 82/2019 • 1[°]; Katcha, Bale Mts; 06°42'N, 39°44'E. 2400 m a.s.l.; 5 August 1986; Harenna Forest Expedition, M. J. Largen leg.; "Small stream near border between grassy clearing and dense Schefflera/Hagenia forest. Males calling from grass bordering stream"; LIV 1986.212.198 • 24 juveniles; Swamp near Shawe R., Bale Mts; 06°40'N, 39°44'E. 1980 m a.s.l.; 7 August 1986; Harenna Forest Expedition, M. J. Largen leg.; "Crawling up tall grasses surrounding swamp with open water and tall bushes, in Aningeria forest. Just metamorphosed juveniles, green and brown phases, latter with 3 longitudinal dark stripes, median confluent with interorbital bar"; LIV 1986.212.199-222.

Additional material. ETHIOPIA • 1 larva; Woraba glade, Harenna Forest, Bale Zone, Oromia Region; 6°35'39.0"N, 39°45'14.6"E, 1800 m a.s.l.; 5 June 2019; A. Tiutenko leg.; "temporary puddle"; ZSM 172/2019.

Diagnosis. Medium-sized (SVL of males ca. 24–29 mm, females ca. 35–40 mm) ground-dwelling and burrowing frog with robust body, relatively wide and short head, and short limbs. Terminal phalanges of toes and fingers expanded to small, but distinct discs. Only base phalanges of toes II–IV with broad web; on toe V the web extends along phalanges 1 and 2. The rest of toe phalanges with feeble fringe. Fingers are free of web or fringe. Light-brown or light olive-green from above; either no dorsal pattern, or three indistinct broad longitudinal bands (one vertebral and two dorsolateral) present that are slightly darker than the ground colour. Males with pectoral glands. Advertisement call: quiet, high-pitched singleton 'quack', repeated at intervals of ca. 20 seconds.

Description of holotype. ZSM 81/2019, adult female. SVL 35.9 mm. The head slightly wider than its length (HW/HL 0.77), ca. 1/3 of the SVL (HL/SVL 0.32). Body oval. Eyes rather large (ED/HL 0.39, ED/SL 1.15, UEW/HW 0.20), positioned laterally (IOD 4.2 mm) and directed slightly forwards. Pupil vertical. Snout strongly curved downwards, especially in front of the nares. Canthus rostralis rounded. Naris approximately at half the distance between eye and snout tip. Tympanum visible, very close to posterior border of the orbit (ETD ca. 1.0 mm), comparatively small: TD

1.4 mm, TD/ED 0.31. Hind limbs rather short TL+THL/SVL 0.76. Tibio-tarsal articulation reaches to the anterior of the tympanum. Outer metatarsal tubercle absent. Inner metatarsal tubercle very large (IMT/TSL 0.24), compressed, ca. half as broad as long (IMTW/IMT 0.57). Terminal phalanges of toes and fingers with small, but rather conspicuous discs, e.g., Fin2W/Fin2DW 0.35, Fin4W/Fin2DW 0.67, Toe4W/ Toe4DW 0.68. Fingers free of webbing. Toe I free of web. On toe II the web does not reach beyond phalanx 1. On toes III and IV it reaches to ca. 1/2 of the phalanx 2, and on toe V it even extends up to the joint of the phalanges 2 and 3. The web continues as a feeble fringe along the rest of the phalanges on all toes except toe I that is free of it. Dorsal skin finely granular, almost smooth, with scattered singleton tubercles. Feeble vomerine teeth form two small groups.

Colouration in life pale green-brown or grey-green from above. Feeble one vertebral and two dorsolateral bands, a little darker than the ground colour, bordered with small irregular tubercles. The dorsal colour transits to light green and blue at thighs and shoulders. Venter off white, feebly mottled with grey. Gular area without pattern. Black band along canthus rostralis on each side of the head extending over the nostril to the eye. Behind the eye it continues over the tympanum and above the shoulder to approximately the middle of the flank. This band is not outlined. Iris dark bronze.

Colouration in preservative: Dorsum grey. Venter pale, grey mottled. Dorsolateral pattern as in life.

Variation of paratypes. The paratype series comprises 27 specimens of various ages and sexes.

Paratype 1 (Fig. 2B): ZSM 83/2019, adult male. It has a much slenderer body than the holotype, whose body is almost round, and is much smaller than the female: SVL 21.8 mm versus 35.9 mm. The head is similarly large (HL/SVL 0.34), but even wider than in the female: HL/HW 0.88. Tympanum is of similar size: TD/ED 0.30. Pectoral glands visible. Digital discs are slightly larger: Fin2W/Fin2DW 0.51, Fin4W/ Fin4TW 0.72, Toe4W/Toe4DW 0.77. Skin on all parts of the body and head smooth. Colouration in life: Dorsal colouration is very similar to that of the holotype: Pale olive-green from above, with three indistinct longitudinal bands. Ventral colouration is different and pale, without pattern. Throat with some scattered dark spots. Sides of thighs with dark grey blotches and bands. Similar pattern also between flanks and venter; this pattern is absent in the holotype. Similar to holotype, a dark band extends from snout tip, over the eye and above tympanum; however, it does not continue at both sides of the body beyond the shoulder. Unlike in holotype, there are no dark blotches on flanks. Colouration in preservative like in life but dorsal colour is grey.

Paratype 2 (Fig. 2C): ZSM 82/2019, metamorph at Gosner stage 45. Very small individual, SVL 12.9 mm, with tail stub – 5.9 mm. Head is similarly large in comparison to body as in adult individuals: HL/SVL 0.34. Other proportions are similar: HL/HW 0.89, ED/HL 0.46, ED/SL 0.95, TD/ED 0.30, TL+THL/SVL 0.63, IMT/TSL 0.29. The inner metatarsal tubercle is flatter than in adults: IMTW/IMT 0.25. Digital discs are similarly conspicuous in this stage of the life cycle: Fin2W/Fin2DW



Figure 2. Types of *Leptopelis diffidens* sp. nov. (in dorsal and ventral view) **A** holotype, female, ZSM 81/2019 **B** paratype 1, male, ZSM 83/2019 **C** paratype 2, metamorph, ZSM 82/2019 **D** paratype 3, female, LIV 1986.212.198.

0.83, Toe4W/Toe4DW 0.75. In life, the colouration was very similar to that of the male Paratype 1.

Paratype 3 (Fig. 2D): LIV 1986.212.198, adult female with even larger SVL than in the holotype – 39.6 mm, but with almost identical morphology and very similar appearance: HL/SVL 0.32, HL/HW 0.79, ED/HL 0.36, ED/SL 0.91, TD/ED 0.39, TL+THL/SVL 0.69, IMT/TSL 0.27, IMTW/IMT 0.99, Fin2W/Fin2DW 0.51, Fin4W/Fin4TW 0.72, Toe4W/Toe4DW 0.72. It is an old specimen that had been probably fixated or even kept for some time in formalin prior to current storage in alcohol; therefore it is hardly possible to compare its colouration with more recently collected and immediately ethanol preserved specimens. In its current state the dorsum of this specimen appears bluish-grey; the venter is yellowish. No pattern is recognisable, except dark lateral band which is similar to that in the holotype. Probably the live colour of dorsum of the Paratype 3 has been more uniformly green.

Paratypes 4–27 (Fig. 4): LIV 1986.212.199–222, recently metamorphosed individuals with SVL 11.1–14.5 mm (see Suppl. material 5: Table S1 for morphometrics of each specimen). They closely resemble the male Paratype 1 in body shape but are smaller and lack pectoral glands. In comparison with Paratype 2, they lack tails, i.e., have been at a more advanced development stage (Gosner stage 46) at time of collecting, though they are of similar size or just slightly larger. Like all other specimens of the type series, they have more or less continuous dark lateral bands extending from the snout tip to the middle of the flanks or further. Eight specimens appear rather bluish, compared to the rest of the series whose dorsal colour is brown. This may indicate that they were green in life. Among brown specimens some have also bluish areas which may have been green in life as well. The ventral side is uniformly pale, without any spots or pattern.

Differential diagnosis. *Leptopelis diffidens* sp. nov. shares the following traits with the rest of the members of the genus *Leptopelis*: The terminal phalanx of finger kinked (out of alignment with the rest of phalanges); no fingers opposing each other; digital discs on fingers present; outer metatarsal tubercle absent; singleton subarticular tubercles; tympanum visible; pupil vertically elliptic; vomerine teeth arranged in two groups between choanae.

The digital discs both, on fingers and toes, are in the new species significantly more conspicuous than *L*. sp. 'Shewa' and in the parapartic high-altitude population in Bale and Arsi Mountains – *L. montanus* nom. nov. (Figs 5, 6). In the examined specimens of both high-altitude species the digital discs were virtually absent: On their fingers there are just feeble pads (Fin2W/Fin2DW 0.92, Fin4W/Fin4DW 0.86), and on toes the tips are even narrower than the phalanges (Toe4W/Toe4DW 1.8). The digital discs in *L. diffidens* sp. nov. are not significantly larger than in *L. gramineus* sensu stricto (Fig. 6). In two paralectotypes and five topotypic specimens that we measured the average ratio 'disc width to phalanx width' was 0.85 on finger II (Toe2W/Toe2DW) and 0.70 on toe IV (Toe4W/Toe4DW).

The examined males of both, *L.* sp. 'Shewa' and *L. montanus* nom. nov., are much larger than in the new species: SVL 27.2–40.6 mm (mean 35.6 mm). Females in these

two species are even larger, with SVL 47.5–56.6 mm (mean 51.5 mm). Generally, females of *L. diffidens* sp. nov. are sized like males of the montane species in this species complex (see comparison photographs in Suppl. material 12).

All examined males of *L. gramineus* sensu stricto (three specimens, including paralectotype BMNH 1947.2.10.20) were significantly larger than of *L. diffidens* sp. nov.: mean SVL 36.1 mm; the size difference between male and female in *L. gramineus* appears to be small. Two of the examined three females (including paralectotype BMNH 1947.2.10.19) had SVL 39.72 mm and 39.37 mm. The third female (BMNH 1975.1633) with SVL 24.39 mm may be immature.

A principal component analysis showed that the low-altitude geographic group from the Harenna Forest that we describe here as *L. diffidens* sp. nov. is separated from the rest of the groups along the first axis accumulating 44.99% of morphological variation. The other groups are partially separated along the second axis accumulating 10.85% of variation while the most divergent groups are *L. gramineus* sensu stricto and the highland populations from the east of the GRV (*L. montanus* nom. nov.). The specimens from 'Borana/Sidamo' population are placed between them and partially overlap with both. See this plot in Suppl. material 10.

A canonical discriminant analysis separated the three groups *L. diffidens* sp. nov., *L. montanus* nom. nov., and *L. gramineus* sensu stricto with high confidence (see Fig. 7): Wilks's Lambda approx. 0.00048, F(58, 26) = 20.111, p < .0000. Four measurements (HL, TL, Toe3L, Toe4L) had the largest contribution to the discriminant function. A discriminant analysis summary and standardised coefficients for canonical variables are provided in Suppl. material 1 and 3. In spite of moderate sample size and combining both sexes in a single multivariate analysis, we revealed strong differences in the body proportion between three lineages of the *L. gramineus* complex, manifested in head proportions, tibia, and longest toe lengths. As Lada et al. (2005) showed for European *Pelobates*, the fossorial way of life is a strong constraint for morphological variation and sexual dimorphism. Thus, the morphological divergence that we observed in the studied species of *L. gramineus* complex may be related to different extent of fossoriality.

Compared to all other frogs currently assigned to *L. gramineus*, the new species appears to have smoother skin on dorsum, with just some small singleton warts. *L. diffidens* sp. nov. differs from other members of the complex also in overall dorsal colouration: It is rather grey-green or pale beige while *L. gramineus* and other high-altitude species of this complex have more bright green or yellow-brown colouration. Furthermore, the new species has no such dorsal pattern of scattered blotches and ocelli as in the parapatric *L. montanus* nom. nov. The latter, in turn, usually lacks continuous black lateral bands that seems to be typical for *L. diffidens* sp. nov. (Fig. 3). Canthus rostralis in *L. diffidens* sp. nov. is not outlined light yellow or light green as in other members of this complex.

From non-sympatric, but also ground-dwelling, *L. bocagii* the new species differs by less robust body form, smaller head, rounded canthus rostralis (versus strongly angled in *L. bocagii*). The digital discs in *L. bocagii* are similarly expanded except on toes IV and V where they seem to be larger. The inner metatarsal tubercle is similarly large



Figure 3. Adult females of three species of the *Leptopelis gramineus* complex in life, shown to the same scale **A** *L. diffidens* sp. nov., Harenna Forest (holotype, ZSM 81/2019) **B** *Leptopelis montanus* nom. nov., Gaysay Grasslands, Bale Mountains (not collected) **C** *L.* sp. 'Shewa', Menz-Guassa (not collected).



Figure 4. Paratypes 4–28, immature individuals, LIV 1986.212.199–222 (in dorsal and ventral views).



Figure 5. Comparison of digital discs (on right hand and foot) and of pedal webbing in A *Leptopelis diffidens* sp. nov., and B *L. montanus* nom. nov.

and compressed. Unlike in *L. bocagii* that has strong vomerine teeth, they are feeble in *L. diffidens* sp. nov. and additionally, the colouration of *L. bocagii* is very different, see Largen (1977) for a detailed description and illustration.

Leptopelis diffidens sp. nov. is generally ground-dwelling and does not occur on trees or scrubs, although males and juveniles may occasionally climb grass. From all scansorial members of the genus distributed at the Horn of Africa, including sympatric L. ragazzii (Boulenger, 1896), it differs by much more robust body (particularly in females), comparatively small head, shorter limbs, reduced digital discs. Moreover, L. ragazzii has a flatter head with less curved snout and comparatively larger eyes. This applies also to non-sympatric L. vannutellii (Boulenger, 1898), which does not differ morphologically from L. ragazzii. Both, L. ragazzii and L. vannutellii, have small inner metatarsal tubercle and well-developed webbing on feet, extending on toes III and IV well beyond the joint of the phalanges 1 and 2. Similarly to L. ragazzii that has two clearly different colour phases, bright green and brown, occurring together. Leptopelis diffidens sp. nov. exhibits such dichromatism as well, although not that distinct: unlike in L. ragazzii, the brown phase in the new species is not purely brown, but rather olive-green or grey-brown. No plain green phase was ever recorded, but grey-green or pale green individuals occur in populations of L. vannutellii. Overall, the colour of L. vannutellii is not like that of L. diffidens sp. nov.

The non-sympatric species *L. susanae* and *L. yaldeni* Largen, 1977 have much broader digital discs, small inner metatarsal tubercles, and more extensive webbing



Figure 6. Pedal webbing of five members of the *Leptopelis gramineus* complex A *L. diffidens* sp. nov., LIV 1986.212.198 B *L. gramineus*, BMNH 1947.2.10.19 (paralectotype) C *L. gramineus*, BMNH 1975.1619
D *L.* sp. 'Borana/Sidamo', PEM 3821 E *L. montanus* nom. nov., BMNH 1975.1621 F *L.* sp. 'Shewa', BMNH 1969.969.

on toes III and IV than the new species. Both are scansorial forms with more slender bodies. *Leptopelis yaldeni* has two colour phases which are clearly distinct, green and brown, like in *L. ragazzii*. Similar to *L. diffidens* sp. nov., *L. susanae* does not show such clear dichromatism, but its general colouration clearly differs very much from the new species (compare photographs and description in Largen 1977).

Another species, *L. concolor* Ahl, 1929, recorded from the Juba river basin, southeast of the distribution area of *L. diffidens* sp. nov., is a scansorial form with a similar body size that occupies coastal savanna habitats. Similar to the new species, it has reduced pedal webbing but larger discs, particularly on toes III, IV, and V. Like other



Figure 7. Grouping of three species of *Leptopelis gramineus* complex on a plane of two canonical roots. Discriminant analysis summary and standardised coefficients for canonical variables are provided in Suppl. material 1 and 3.

arboreal *Leptopelis*, it has a slender body and a large head. The colouration of *L. concolor* is rather constant: light brown from above, with dark interorbital bar and an indistinct reversed 'Y' on dorsum.

Advertisement call. Males usually call from tussocks surrounded by water, but also from tall and dense grass at margins of pools and puddles, and occasionally from shrubs surrounding the glade. The animal sits near the roots of the grass, but not on grass and not in water. When approached, it stops calling and becomes silent for many minutes. The calls are rather weak and may be difficult to perceive in places with background chorus of other frogs, particularly of *Ptychadena*, or with loudly shouting birds. During a rainy season, in May–June, we did not notice any significant difference in the intensity of vocalisations between day and night. However, in the same season we observed more calling individuals and more frequent calls in the parapatric population of *L. montanus* nom. nov. in the Bale Mountains.

Like other Ethiopian members of this species complex (Largen 1977; Schiøtz 1999), the advertisement call of *L. diffidens* sp. nov. is a 'quack'. It consists of a single note that contains eight pulses with interval of ca. 20 ms. The tone frequency is 990–2700 Hz (Fig. 8A). It is higher than in the call of the high-altitude member of the *L. gramineus* complex from the Bale Mountains that also consists of a single note, however, of five pulses and with a frequency of 344–3270 Hz (Fig. 8B). According to our observations, *L. diffidens* sp. nov. repeated their 'quack' with equal intervals of ca. 20 seconds. Another male may use the pause for his call. We did not observe two or more males



Figure 8. A comparison of singleton advertisement calls of *Leptopelis diffidens* sp. nov. and *L. montanus* nom. nov. **B** oscillograms (top) and spectrograms (bottom) of advertisement calls of *L. diffidens* sp. nov., *L. montanus* nom. nov.), and *L. ragazzii* within 40 seconds.

calling synchronously. Largen (1977) mentioned a 'scream' that sometimes precedes the 'quack' in *L. gramineus* from high-altitude population (*L. montanus* nom. nov.). We did not observe such vocalisations in the new species at any localities we visited.

The vocalisation of *L. ragazzii* partially resembles that of *L. diffidens* sp. nov. in containing a 'quack' sound of similar pitch and frequency. However, it is normally preceded by a screech, lasting for ten or more seconds, that consists of 4 or 5 notes with increasing pitch and frequency (Fig. 8C). *L. diffidens* sp. nov. does not produce such sounds.

Genetics. The 16S mitochondrial gene sequence (GenBank no. MN909554) of the specimen ZSM 83/2019 (Paratype 1) matches to 99.8% the corresponding sequences obtained by other workers from tissue samples of frogs that had been collected in the Harenna Forest at Katcha and in vicinity of Rira and identified as *L. gramineus*. Freilich et al. (2016) applied phylogenetic reconstructions based on the COI gene and found four highly divergent mitochondrial lineages in *L. gramineus* with average distance of 6.4–8.0% between them. They restricted three of these clades to the east of the GRV and called 'Arsi', 'Kibre Mengist' and 'Kasha'. These correspond to what we call here *L. montanus* nom. nov., *L.* sp. 'Borana/Sidamo', and *L. diffidens* sp. nov. (see Suppl. material 8: Table S4).

As Reyes-Velasco et al. (2018) showed with their genetic study using multiple RAD loci, the population from the Harenna Forest and the related population from Bore and Kibre Mengist area (L. sp. 'Borana/Sidamo') represent together a sister clade of L. susanae. This lineage diverged from the high-altitude species L. montanus nom. nov. around 4 my ago, during the Pliocene. The split between eastern and western lineages of the L. gramineus complex took place ca. 6 my ago. Therefore, the new species from the Harenna Forest is more distantly related to species from the west of the GRV, hence also to L. gramineus sensu stricto.

In order to align our material to the already defined groups we have constructed a statistical parsimony network of 16S haplotypes (showed in Fig. 9). Both, the paratype of *L. diffidens* sp. nov. (GenBank no. MN909554) and the sequenced specimen from the Gaysay Grasslands (GenBank no. MN909551) clustered respectively, with the representatives of the geographic groups of the low-altitude Harenna Forest (i.e., clades 'Harenna' sensu Reyes-Velasco et al. 2018 and 'Kasha' sensu Freilich et al. 2016) and of the high-altitude population that Freilich et al. (2016) called 'Arsi' and Reyes-Velasco et al. (2018) called 'Bale Mountains'.

Etymology. The Latin adjective *diffidens* (used here in nominative singular) means diffident, anxious, shy, mistrustful. This name refers to the behaviour of this frog that appears even more cryptic and defensive than *L. gramineus* from other populations. The vocalisation of a male is so quiet that the animal is very difficult to locate and to spot. When approached, at a distance of only 5 m, it stops calling and remains silent until the disturber has gone away or has not moved for at least 10 minutes. As a vernacular name for this species, we propose 'Harenna Burrowing Treefrog'.

Distribution and habitat. According to our current knowledge, the new species is endemic to the Harenna Forest where it occurs at elevations of 1400–2300 m a.s.l. We observed these frogs and recorded vocalisations at the following localities, which are glades in a tall forest: Gola, Hacho, Harawa, Haro Alati, Hordoba, Kaffa Guasaa,



Figure 9. Network of haplotypes and a map showing the distribution of the *Leptopelis gramineus* species complex members: *L. gramineus* (filled circle), *L. sp.* 'Shewa' (empty circle), *L. montanus* nom. nov. (empty triangle), *L. diffidens* sp. nov. (filled square), *L. sp.* 'Borana/Sidamo' (empty diamond). Type localities of the described species are marked with a cross. The colours on the haplotypes chart correspond to various groups recognised by Mengistu et al. (2012), Freilich et al. (2016) and Reyes-Velasco et al. (2018). The symbols in the legend correspond to populations shown on the map.

Manyate, Megano, Segoba, Sire, Woraba, and Yagana (Fig. 9). This species was also collected many times from Katcha and nearby localities.

Leptopelis diffidens sp. nov. seems to be confined to glades in the forest that are temporarily flooded with rain water during rainy season. These frogs do not live in areas of dense high trees, where they are replaced by *L. ragazzii*, typically found along streams. These two species never occur syntopically. We also did not encounter *L. diffidens* sp. nov. in man-made open areas in the forest, such as clearings, plantations, or settlements. However, we found two males of *L. ragazzii* (brown and green phase) on Aboye, a large clearing regularly used for barley plantation. Moreover, unlike *L. ragazzii* and the highland members of the *L. gramineus* complex whose larvae live in fastflowing water, the new species uses lentic or slow-flowing waterbodies for breeding and does not occur at fast flowing streams (Fig. 9). We assume therefore that this frog requires a certain flora composition and environmental, particularly hydrological, conditions that are available only in natural glades.

Leptopelis diffidens sp. nov. shares breeding sites with *Phrynobatrachus inexpectatus*, *Ptychadena* sp., and *Xenopus clivii*. We observed this even in the middle of Manyate, a large village in the south of the Harenna Forest, because it has been built around a natural glade that retained its flora and hydrological characteristics.

An altitudinal and ecological barrier exists between *L. diffidens* sp. nov. and the not-yet-named montane grassland-dwelling *L. montanus* nom. nov.: *L. diffidens* sp. nov. does not ascend the Harenna Escarpment and is absent already in the sub-alpine heathlands, i.e., above 2500 m a.s.l. On the other hand, *L. montanus* nom. nov. that is distributed in the high-altitude areas, including the Sanetti Plateau, was not recorded in the areas directly adjacent to the Harenna Escarpment that have elevations around 4000 m a.s.l.; hence, no contact zone seems to exist here (Fig. 9). *Leptopelis diffidens* sp. nov. may not range west beyond the Ladamo mountain chain because suitable glades are absent there. This ridge also seems to isolate *L. diffidens* sp. nov. from populations of *L.* sp. 'Borana/Sidamo', a not-yet-named sister clade which was reported from localities further west, in Borana and in northern Sidamo (Reyes-Velasco et al. 2018). In the east, the range of *L. diffidens* sp. nov. reaches into Berbere area of the Harenna Forest where the forest gradually transits to bushland and savanna. The southern range boundary seems to be at Manyate and Haro Alati glade. South of this line glades disappear and the forest merges with Acacia bushland.

Sexual characters. Males can be distinguished from females by a much smaller body size, less robust body, larger head, and the presence of pectoral glands.

Natural history. *Leptopelis diffidens* sp. nov. is a ground-dwelling and semi-fossorial frog that spends most of its life hidden. In the dry season it is nocturnal, but in the rainy season we did not notice a significant difference between diurnal and nocturnal activity patterns at least in males.

Since we found a gravid female of *L. diffidens* sp. nov. and individuals at the last stages of metamorphosis on the same day and at the same locality, we assume that this species breeds more than once a year. Its breeding seasons appear to be out of sync with that of the parapatric high-altitude population of *L. montanus* nom. nov. in the Bale

Mountains. We found metamorphs of *L. diffidens* sp. nov. (ZSM 82/2019 and ZSM 172/2019) in June, at the end of the short rainy season. A few days earlier we had visited a population of *L. montanus* nom. nov. in the Gaysay Grasslands and found neither metamorphs nor larvae. The rainy season there had just begun. In the Web Valley, Bale Mountains, we observed large tadpoles of *L. montanus* nom. nov. in the middle of February, at the end of a dry season. However, just a week later at Katcha in the Harenna Forest we found all puddles dried, and no frogs were around. During another trip, we found tadpoles in advanced development stages and metamorphs of *L. montanus* nom. nov. in vicinity of Dinsho village, Bale Mountains, in early November but saw neither adults nor larvae of *L. diffidens* sp. nov. after that in the Harenna Forest where the rainy season was approaching its end.

Larva. The tadpole of *L. gramineus* has been described by Largen (1977) based on specimens from high-altitude populations and compared with the tadpole of sympatric *L. ragazzii* by Tiutenko and Zinenko (2019). Considering the morphological similarity and close phylogenetic relationship of *L. diffidens* sp. nov. we expect the tadpole of the new species to be similar to the tadpoles in the rest of this species complex. Except for one specimen ZSM 172/2019 at Gosner stage 43, no larvae of *L. diffidens* sp. nov. have been collected so far. This specimen was at time of collection shortly before leaving water and resembles an adult *L. diffidens* sp. nov. closely enough to permit positive identification. It has completely developed forelimbs. Instead of larval mouthparts it has an almost completely developed oral cavity and even a tongue. However, the development of the mouth opening is incomplete: it is only 2.4 mm wide, oval, and directed forward. Additionally, the tympanum is not yet present. All measurements are a little larger than those of another specimen (ZSM 82/2019) that we found at the same locality in a more advanced metamorphotic stage (see Suppl. material 5: Table S1).

Status of other cryptic species in *Leptopelis gramineus* **complex.** Largen (1977) pointed out in his review of the Ethiopian *Leptopelis* that there is a "*great variation of size which has been demonstrated between, but not within, populations*" of *L. gramineus.* Since his so-called 'populations' are now treated as cryptic species, the pattern of these size differences has become clearer. The high-altitude species are generally considerably larger than the low-altitude ones. The 12 small male specimens from Wando (BMNH 1973.2164-2176) that Largen mentions are probably of the species that we call here *L.* sp. 'Borana/Sidamo' which is closely related to *L. diffidens* sp. nov. that is characterised by the same 'dwarfism'. The largest (BMNH 1975.1638) of the 27 males examined of this species measured 29.6 mm. The largest of four females examined measured 40.0 mm. The mean SVL values are 25.4 (males) and 38.6 mm (females).

The population at the high-altitude lake Wonchi that Largen also mentions as an example of small-sized *L. gramineus* requires an additional study. Unfortunately, we did not find the specimens that Largen refers to (AAU H.548/1–2 and AAU H.718/1–4) during our visit in the ZNHM. Geographically this population should be within the range of *L.* sp. 'Shewa', and the locality is situated at high elevation of almost 3500 m a.s.l. This was also confirmed by DNA barcoding performed by Mengistu et al. (2012). Whether small body size is typical for this population, is yet to be determined.



Figure 10. A, B Habitats of *Leptopelis diffidens* sp. nov. in the Harenna Forest – Segoba glade and Manyate village **C** habitat of *L. montanus* nom. nov. in the Web Valley, Bale Mountains **D** habitat of *L.* sp. 'Shewa' in Menz-Guassa.

For resolving the taxonomic status of the western populations of L. gramineus, new material and DNA samples from the type locality or from nearby highlands in Ethiopian administrative zones Gamo Gofa, Keffa, Wolayita, Kenta, and Dawuro are required together with further studies of morphological differences, bioacoustics, ecology, and contact zones. Mengistu et al. (2012) mentioned tissue samples of specimens from Gamo Gofa, but did not report any further results of their analysis. Other workers (Freilich et al. 2016; Reyes-Velasco et al. 2018) did not include topotypic material in their published results. Reyes-Velasco et al. (2018) had obtained nuclear DNA sequences of vouchers from Dorse and Chencha (Gamo Gofa), and from Bonga (Keffa), places at and close to the type locality, but apparently did not use them in their analysis for some reason. Jimma is the geographically closest locality from which DNA sequences were analysed so far. Reyes-Velasco et al. (2018) found that this population is rather different from L. gramineus further north, around Addis Ababa, Wonchi, and at other localities in Shewa and Gojam (L. sp. 'Shewa'). We compared specimens of L. gramineus sensu stricto (paralectotypes and topotypic specimens housed in BMNH) with specimens from the north and found considerable difference between these populations in digital discs size, skin surface structure, body size and colouration. Therefore, it appears likely that the 'northern' population (L. sp. 'Shewa') represents a different species. Ahl (1924: 9) described a new species Pseudocassina rugosa, with type locality in Meta. In his later work he assigned it to the genus Leptopelis (Ahl 1931: 222) without

any explanation. Largen synonymised *L. rugosus* with *L. gramineus* in the course of a revision of Ethiopian *Leptopelis* (Largen 1977). If comparison of genetic, acoustic, and morphological data with topotypic material would confirm a sufficient level of divergence, reinstatement of *Leptopelis rugosus* (Ahl, 1924) may be considered. Also, it is not clear how far this putative species ranges southwards, so there is no confidence that it does reach the type territory of *L. gramineus*.

In the same paper Ahl (1924) described Pseudocassina ocellata, another species that Largen (1977) synonymised with L. gramineus. Its type locality is Dida Plateau ('Hochebene Didda') which he first erroneously placed in Somalia, as he did with some other specimens collected by Neumann and Erlanger (see discussion in Largen 1975). This plateau is in fact situated in the Arsi Mountains, and the Leptopelis population there is certainly conspecific with frogs from other localities in Arsi and Bale. According to the published genetic data, this clade is reproductively isolated from other lineages of L. gramineus complex to the west from the GRV and could have limited exchange of genetic material with Kofele lineages sensu Reyes-Velasco et al. (2018). Therefore, we resurrect this species from synonymy and raise L. ocellatus (Ahl, 1924) to the status of a valid species in genus *Leptopelis*. As the vernacular English name we propose 'Ocellated Burrowing Treefrog' because it describes the typical colouration of this animal and matches the original description by Ahl (1924). However, the Latin name ocellatus is not available in this genus because a valid species from West Africa was described earlier and now bears the same name: L. ocellatus (Mocquard, 1902). In accordance with Article 50 and Paragraph 3 of Article 60 of ICZN, we establish a substitute name Leptopelis montanus nom. nov. for Leptopelis ocellatus (Ahl, 1924) to resolve its homonymy with Leptopelis ocellatus (Mocquard, 1902).

Etymology. The new specific name *montanus* (living in mountains, montane) refers to the fact that this species ranges into afromontane areas up to elevation of almost 4000 m a.s.l., thus being a *Leptopelis* with probably the highest altitudinal distribution. The name is an adjective in nominative singular.

Key to the Ethiopian species of the genus Leptopelis

This key covers only species of the genus that are currently known from Ethiopia and were formally described. Species candidates are not included due to lack of knowledge about their morphological traits.

1	Metatarsal tubercle small, length not exceeding 6% of SVL	2
_	Metatarsal tubercle large, length ca. 8% of SVL	5
2	Tibia long, ca. 50% of SVL or longer	3
_	Tibia moderate, shorter than 50% of SVL	
3	Blue-green colouration at axilla and groin	vannutellii
_	No blue-green colouration at axilla and groin	ragazzii
4	Dorsal pattern (dark triangle) not confluent with interorbital bar	yaldeni
_	Dorsal pattern (mid-dorsal stripe) confluent with interorbital bar	susanae

5 Canthus rostralis st	trongly angled	bocagii
 Canthus rostralis re 	ounded	6
6 Digital discs distine	ct, at least 30% wider than terr	ninal phalanx7
 Digital discs indistir 	nct, not wider than terminal pha	lanx <i>montanus</i> nom. nov.
7 Dorsal skin smooth	h, with small singleton warts	<i>diffidens</i> sp. nov.
– Dorsal skin rough .	~	gramineus
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References

- Ahl E (1924) Über eine Froschsammlung aus Nordost-Afrika und Arabien. Mitteilungen aus dem Zoologischen Museum in Berlin 11: 1–12. https://doi.org/10.1002/mmnz.4830110102
- Ahl E (1931) Amphibia, Anura III, Polypedatidae. Das Tierreich 55: [xvi +] 477 pp. https:// doi.org/10.1515/9783111625430
- Bossuyt F, Milinkovitch MC (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. Proceedings of the National Academy of Sciences of the United States of America 97: 6585–6590. https://doi. org/10.1073/pnas.97.12.6585
- Boulenger GA (1898) Concluding report on the late Capt. Bottegos collection of reptiles and batrachians from Somaliland and British East Africa. Annali del Museo Civico di Storia Naturale di Genova, Serie 2, 18: 715–722.
- Bryan MA, Tucker AN (1930) Practical Orthography of African Languages. Linguistic Surveys of Africa. International African Institute. Oxford University Press, London, 24 pp.
- Capocaccia L (1957) Catalogo dei tipi di anfibi del Museo Civico di Storia Naturale di Genova. Annali del Museo Civico di Storia Naturale di Genova, Serie 3, 69: 208–222.
- Cerulli E (1956) Peoples of South-West Ethiopia and its Borderland. North Eastern Africa, part III. Ethnographic Survey of Africa. International African Institute, London, 194 pp.
- Channing A, Rödel M-O (2019) Field Guide to the Frogs and other Amphibians of Africa. Struik Nature, Cape Town, 407 pp.
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x
- Coltorti M, Peruccini P, Arthur KJW, Arthur J, Curtis M (2019) Geomorphology, soils and palaeosols of the Chencha area (Gamo Gofa, south western Ethiopian Highlands). Journal of African Earth Sciences 151: 225–240. https://doi.org/10.1016/j.jafrearsci.2018.12.018
- Frank N, Ramus E (1995) Complete Guide to Scientific and Common Names of Amphibians and Reptiles of the World. N. G. Publishing Inc., Pottsville, 377 pp.
- Freilich X, Anadon JD, Bukala J, Calderon O, Chakraborty R, Boissinot S (2016) Comparative Phylogeography of Ethiopian anurans: Impact of the Great Rift Valley and Pleistocene climate change. BMC Evolutionary Biology 16(206): 1–19. https://doi.org/10.1186/ s12862-016-0774-1
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190.
- Günther ACLG (1859 ["1858"]) Catalogue of the Batrachia Salientia in the Collection of the British Museum. Taylor and Francis, London, [xvi +] 160 pp. [pls 1–12]
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547– 1549. https://doi.org/10.1093/molbev/msy096
- Lada GA, Borkin LJ, Litvinchuk SN (2005) Morphological variation in two cryptic forms of the common spadefoot toad (*Pelobates fuscus*) from eastern Europe. In: Ananjeva N, Tsinenko O (Eds) Herpetologia Petropolitana. Proceedings of the 12th Ordinary General Meeting of the Societas Europea Herpetologica. 12–16 August 2003. St. Petersburg, 53–56.
- Largen MJ (1975) The status of the genus Kassina (Amphibia Anura Hyperoliidae) in Ethiopia. Monitore Zoologico Italiano. Nuova Seria, Supplemento. Firenze 6: 1–28. https://doi.org /10.1080/03749444.1975.10736806
- Largen MJ (1977) The status of the genus *Leptopelis* (Amphibia Anura Hyperoliidae) in Ethiopia, including descriptions of two new species. Monitore Zoologico Italiano. Nuova Serie, Supplemento. Firenze 9: 85–136. https://doi.org/10.1080/03749444.1977.10736845
- Largen MJ (2001) Catalogue of the amphibians of Ethiopia, including a key for their identification. Tropical Zoology 14(2): 307–402. https://doi.org/10.1080/03946975.2001.10531159
- Largen MJ, Spawls S (2010) Amphibians and Reptiles of Ethiopia and Eritrea. Edition Chimaira, Frankfurt am Main, 693 pp.
- Laurent RF (1972) Review: "The morphology, systematics, and evolution of the Old World treefrogs (Rhacophoridae and Hyperoliidae), by S. S. Liem (1970)". Copeia 1972: 198–201. https://doi.org/10.2307/1442811
- Mengistu AA, Nagel P, Getahun A, Saber SA, Menegon M, Gower D, Sá R de, Loader S (2012) Phylogeography of *Leptopelis* (Arthroleptidae, Amphibia) in the Ethiopian Highlands: Molecular and Morphological Investigation. In: Mengistu AA (Ed.) Amphibian Diversity, Distribution and Conservation in the Ethiopian Highlands. Morphological, Molecular and Biogeographic Investigation on *Leptopelis* and *Ptychadena* (Anura). PhD Thesis. University Basel, 35–73.

- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi. org/10.1093/sysbio/syy032
- Reyes-Velasco J, Manthey JD, Freilich X, Boissinot S (2018) Diversification of African tree frogs (genus *Leptopelis*) in the highlands of Ethiopia. Molecular Ecology 27: 2256–2270. https://doi.org/10.1111/mec.14573
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029
- Schiøtz A (1999) Treefrogs of Africa. Edition Chimaira, Frankfurt am Main, 350 pp.
- Tiutenko A, Zinenko O (2019) Tadpole of *Leptopelis ragazzii* (Boulenger, 1896), Shoa Forest Tree Frog (Anura, Arthroleptidae). Herpetozoa 32: 10–15. https://doi.org/10.3897/ herpetozoa.32.e35742
- Vannutelli L, Citerni C (1899) L'Omo; viaggio d'esplorazione nell'Africa Orientale. Heopli, Milano, 704 pp.
- Watters JL, Cummings ST, Flanagan RL, Siler CD (2016). Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. Zootaxa 4072: 477–495. https://doi.org/10.11646/zootaxa.4072.4.6

Discriminant function analysis summary

Authors: Oleksandr Zinenko

Data type: statistics

Explanation note: Supplementary data of multivariate canonical discriminant analysis. Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Factor Structure Matrix

Authors: Oleksandr Zinenko Data type: statistics Explanation note: Supplementary data of multivariate canonical discriminant analysis. Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1023.53404.suppl2

Supplementary material 3

Standardized Coefficients

Authors: Oleksandr Zinenko

Data type: statistics

Explanation note: Supplimentary data of multivariate canonical discriminant analysis.

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Supplementary material 4

Mean plots of morphometric characters grouped by species

Authors: Oleksandr Zinenko

Data type: statistics

Explanation note: Supplimentary data of multivariate canonical discriminant analysis. Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Table S1. Morphometric measurements of specimens from three populations of *Leptopelis gramineus* complex

Authors: Arthur Tiutenko

Data type: morphological

- Explanation note: The table contains morphometric data from 45 specimens found in collections., including the holotype and the paratypes of *L. diffidens* sp. nov. For meaning of abbreviations see Material and methods in the main article text.
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Link: https://doi.org/10.3897/zookeys.1023.53404.suppl5

Supplementary material 6

Table S2. Examined material

Authors: Arthur Tiutenko

Data type: specimen list

- Explanation note: List of examined 105 specimens of Leptopelis gramineus complex and 86 specimens of six other Leptopelis species occuring sympatrically or in nearby geographic areas.
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Table S3. Gazetteer

Authors: Arthur Tiutenko

Data type: geographic

- Explanation note: List of geographic locations mentioned in the text of the article with their geographic coordinates and elevation.
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Supplementary material 8

Table S4. GenBank sequences

Authors: Arthur Tiutenko

Data type: phylogenetic

- Explanation note: This table contains accession numbers of all mtDNA (16S) sequences used for this study that are published in GenBank along with names of clades (given by the authors) that these sequences belong to.
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Link: https://doi.org/10.3897/zookeys.1023.53404.suppl8

Supplementary material 9

Data table of geographic locations

Authors: Arthur Tiutenko

Data type: geographic

Explanation note: Gazetteer in a software readable format.

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Principal component analysis plot

Authors: Oleksandr Zinenko

Data type: statistics

Explanation note: Projection of the cases on the factor-plane (1×2) .

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Link: https://doi.org/10.3897/zookeys.1023.53404.suppl10

Supplementary material II

Phylogenetic tree

Authors: Oleksandr Zinenko

Data type: phylogenetic

Explanation note: Phylogenetic tree built from 16S mtDNA sequences.

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Link: https://doi.org/10.3897/zookeys.1023.53404.suppl11

Supplementary material 12

Comparison of mature female *Leptopelis diffidens* sp. nov. and mature male *L. montanus* nom. nov.

Authors: Arthur Tiutenko

Data type: multimedia

- Explanation note: Adult females of the new speices have a similar size as adult males of the parapatric species from Bale Mountains. This plate allows visual comparison of two specimens.
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RESEARCH ARTICLE



Two new species of the genus Deltomerodes Deuve, 1992 (Coleoptera, Carabidae, Patrobini) from Xizang, China

Weifeng Yan¹, Hongliang Shi¹, Hongbin Liang², Juan Shi¹

I College of Forestry, Beijing Forestry University, Beijing 100083, China **2** Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

Corresponding author: Juan Shi (shi_juan@263.net)

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Abstract

Two new species of the carabid genus *Deltomerodes* are described from Xizang (China): *D. ovicollis* **sp. nov.** (type locality: Doxong La, Mêdog) and *D. conaensis* **sp. nov.** (type locality: Rama La, Cona). The new species are amply described, illustrated, and their distributions are mapped. An updated key to all eight known Chinese *Deltomerodes* species is provided.

Keywords

Alpine insect, key, morphology, Tibetan Plateau

Introduction

Deltomerodes Deuve, 1992 (Patrobini, Deltomerodina), the sole genus of the subtribe Deltomerodina Zamotajlov, 2002, is a small genus, previously containing thirteen species (Map 1), known from China, Nepal and India (Zamotajlov 2017). This subtribe was erected for the most primitive monophyletic group of the tribe Patrobini Kirby, 1837, the sister group to the other three subtribes based on the results of a phylogenetic analysis (Zamotajlov 2002). In China, six species of *Deltomerodes* have been recorded so far in Yunnan, Sichuan and Xizang (Deuve 1992; Zamotajlov 1999). They can be readily differentiated from other Patrobini from China by having all tarsi pubescent

dorsally. The other seven *Deltomerodes* species were recorded in Nepal and North India (Schmidt 1994, 1995, 1996, 1998; Schmidt and Hartmann1998; Zamotajlov 1999).

During sorting of the results of a recent expedition in Xizang, two series of specimens from Mêdog and Cona counties were readily recognized as two new species of the genus *Deltomerodes*. Therefore, the main purpose of this paper is to describe these two new species and provide an updated key to all known Chinese *Deltomerodes* species.

Materials and methods

The specimens examined, including all types of the new species, are deposited in the collections of the Institute of Zoology, Chinese Academy of Science, Beijing, China (**IZAS**). The specimens were collected under stones or by pitfall traps and kept in zip-lock plastic bags with tissue paper soaked in 75% ethanol. The male genitalia were treated with a 10% KOH solution at room temperature for 12 h to show features of the endophallus. The female genitalia were treated with a 10% KOH solution at room temperature for 12 h to show features of the endophallus. The female genitalia were treated with a 10% KOH solution at room temperature for 12 h, then stained in chlorazol black for 10 s and rinsed with 95% ethanol for 10 s. The male endophallus and the female genitalia were captured in a solution of glycerine. Images of the habitus and characters were captured with a Nikon D7500 camera attached to a Nikon SMZ18 microscope, then stacked with the software Helicon Focus 6.7.1 (Shi et al. 2013). Terminology for female genitalia follows Deuve (1993).

Abbreviations of measurements used in the paper are as follows: L, overall length from apex of mandibles to apex of elytra; HW, width of head, as greatest transverse distance of head; PL, length of pronotum, as linear distance from anterior to basal margin, measured along midline; PW, width of pronotum, as greatest transverse distance of pronotum; EL, length of elytra, as linear distance from anterior end of elytral lateral groove to apex of elytra, measured along elytral suture; EW, width of elytra, as greatest transverse distance of closed elytra. Abbreviations used for parts of the thorax are as follows: msem, mesepimeron; mset, mesepisternum; msst, mesosternum; mtet, metepisternum; mtst, metasternum. Abbreviations used for female genitalia are as follows: co, common oviduct; bc, bursa copulatrix; bcs, annular sclerite of bursa copulatrix; ep IX, epipleurite IX; gc I, gonocoxite I; gc II, gonocoxite II; sg, spermathecal gland; sd, spermathecal duct; sp, spermatheca.

Taxonomy

Deltomerodes Deuve, 1992

Type species. Deltomerodes memorabilis Deuve, 1992.

Diagnosis. The genus can be identified by the following combination of character states: Slender, medium-sized Partrobini, body elongate, length 8.5–12.0 mm; dorsal side black to reddish-brown. Head large with small eyes; first antennomere unisetose or with one seta distinctly longer than the others; two to four pairs of setae between eyes



Map 1. Distribution map for *Deltomerodes* species. Distributions are listed in the checklist below (magenta for new species, red for recorded species).

and neck constriction; mandibles normal, right mandible with a tooth beneath basal inner margin; terminal maxillary palpomere fusiform, broadest in middle; submentum with two pairs of setae in males, one to four pairs (usually two) in females. Pronotum ovate, longitudinal, cordate to subquadrate; lateral margins usually with one pair of setae before middle (two pairs in *D. memorabilis*); posterior angles usually with one pair of setae (two or three pairs in *D. schmidti*). Elytra oblong-ovate, shoulders rather narrow, oblique to slightly angulate; discal setiferous pores present on the third interval, usually also on the fifth and/or seventh intervals; striae more or less smoothed laterally. Thorax with suture between mesepisternum and mesosternum joining anterior margin of mesepimeron. All tarsomeres pubescent dorsally; fifth meso- and metatarsomeres with two to five pairs of setae ventrally. Apical lamella of aedeagus narrow, more or less bent dorsally in lateral view and twisted rightwards in dorsal view; endophallus with two groups of copulatory pieces: the proximal one near middle, usually loop-shaped (straight in *D. conaensis* sp. nov.); one or two spiniform distal pieces near distal margin of apical orifice. Parameres with short apical projections, left one somewhat obtuse or truncated at apex. Female gonopods dimerous, gonocoxite I glabrous; gonocoxite II thin, with one or two small subapical setae in one fovea; reproductive tract with a wellmarked annular sclerotized ring on bursa copulatrix.

Comparision. *Deltomerodes* is rather similar to *Deltomerus* and *Platidiolus*, and these are the only three genera with tarsi pubescent dorsally in Patrobini. *Deltomerus* also shares some other characters with *Deltomerodes*, including: body slender; fifth and seventh intervals of elytra usually with additional discal setiferous pores; fifth meso- and metatarsomeres with several setae ventrally. But *Deltomerus* is different in many characters: (1) first antennomere plurisetose, with several long setae; (2) submentum with more than two pairs

of setae in males; (3) pronotum lateral margins plurisetose before middle (in *Deltomerodes*, only *D. memorabilis* with two pairs of mid-lateral setae); (4) all elytral striae distinct, subequally developed; (5) basal loop-shaped sclerite of endophallus absent (in *Deltomerodes*, only *D. conaensis* sp. nov. without basal loop-shaped sclerite). *Platidiolus* is differentiated from *Deltomerodes* by: (1) smaller size, body length less than 6 mm; (2) apical maxillary palpomere broadest at base, attenuate towards apex; (3) submentum with more than two pairs of setae in males; (4) elytra with discal setiferous pores only on the third interval.

Checklist of Deltomerodes species

- 1 D. murzini Zamotajlov, 1999: 238: CHINA (Yunnan: Yulongxueshan)
- 2 D. memorabilis Deuve, 1992: 82: CHINA (Yunnan: Habaxueshan)
- 3 D. kryzhanovskii Zamotajlov, 1999: 244: CHINA (Sichuan: Zhuotala)
- 4 D. miroshnikovi Zamotajlov, 1999: 242: CHINA (Sichuan: Cholashan)
- 5 *D. ovicollis* sp. nov.: CHINA (Xizang: Doxong La)
- 6 D. zolotichini zolotichini Zamotajlov, 1999: 250: CHINA (Xizang: Serkyimla)
- 6' D. zolotichini similis Zamotajlov, 1999: 251: CHINA (Xizang: Serkyimla)
- 7 D. conaensis sp. nov.: CHINA (Xizang: Rama La)
- 8 D. wrasei Zamotajlov, 1999: 240: CHINA (Xizang: Gangdisêshan)
- 9 D. stenomus (Andrewes, 1936: 61): INDIA (Sikkim: Jalep)
- 10 D. schawalleri schawalleri Schmidt, 1998: 6: NEPAL (Province No. 1: Tangjala)
- 10' D. schawalleri miniangularis Schmidt, 1998: 8: NEPAL (Province No. 1: Meropapala)
- 11 D. sciakyi Schmidt, 1996: 145: NEPAL (Province No. 1: Dingboche)
- 12 D. schmidti Zamotajlov, 1999: 248: NEPAL (Province No. 1: Mt. Everest)
- 13 D. chulii Schmidt, 1995: 19: NEPAL (Gandaki: Manaslu Himal)
- 14 D. nepalensis nepalensis Schmidt, 1994: 132: NEPAL (Gandaki: Muktinath Himal)
- 14' D. nepalensis gracilis Schmidt, 1995: 20: NEPAL (Gandaki: Pisang Himal)
- 15 D. grilli Schmidt & Hartmann, 1998: 33: NEPAL (Karnali: Sisne Himal)

Key to Chinese species of Deltomerodes

- - (Fig. 17); body less slender, humeri distinct, rounded to slightly angulate...**3**

angles, posterior margin strongly protruded medially; seventh elytral interval with four to six setiferous pores; Xizang (Doxong La)..... Elytral fifth interval without setiferous pores; apical lamella of male genitalia 3 broader, gradually acuminate at apex (Zamotajlov 1999: 243, figs 29-40); Xizang4 Elytral fifth interval with one to seven setiferous pores; apical lamella of male genitalia narrower, abruptly acuminate at apex (male of D. kryzhanovskii not known); Sichuan, Yunnan.....7 Pronotum cordate, strongly narrowed to base, its sides with distinct sinuation 4 before posterior angles; Xizang (Serkyimla)......5 Pronotum subcordate or subquadrate, less narrowed to base, its sides with Aedeagus larger, apex more markedly curved dorsally, apical copulatory piece 5 longer, apex of parameres broader (Zamotajlov 1999: 245, figs 41-48)..... Aedeagus smaller, apex less markedly curved dorsally, apical copulatory piece 6 Elytral scutellar stria less developed; sides of pronotum slightly sinuate before posterior angles, which are subrectangular, slightly protruding laterally; endophallus with two distal spiniform pieces, basal loop-shaped sclerite of endophallus absent; Xizang (Rama La) D. conaensis sp. nov. Elytral scutellar stria developed; sides of pronotum not sinuate before posterior angles, which are obtuse, not protruding laterally; endophallus with one distal spiniform piece, basal loop-shaped sclerite of endophallus present; Xizang (Gangdisêshan) D. wrasei 7 Head with three pairs of setae between eyes and neck constriction; both elytral scutellar pore and stria absent; female with four setae on each side of submentum; pronotum about as wide as head (PW/HW = 1.03-1.08); Yunnan Head with two pairs of setae between eyes and neck constriction; both elytral scutellar pore and stria present; female with two setae on each side of submentum; pronotum slightly wider than head (PW/HW = 1.10-1.11); 8 Pronotum anterior angles distinct, lateral margins with more or less distinct sinuation before hind angles; shoulders rounded; elytral striae shallow, with indistinct and irregular punctures, fifth interval with two to four setiferous pores, umbilicate series composed of ten to fourteen pores; Sichuan (Cholas-Pronotum anterior angles indistinct, lateral margins without sinuation before hind angles; shoulders angulate; elytral striae deep, with large and regular punctures, fifth interval with one or two setiferous pores, umbilicate series composed of nine or ten pores; Sichuan (Zhuotala) D. kryzhanovskii

Deltomerodes ovicollis sp. nov.

http://zoobank.org/6ACFCC70-BE8C-415C-B279-B86E37B771BD Figs 1–14

Type locality. China, Xizang, Mêdog County, Doxong La (= Duoxiong La, 29.4890°N, 94.9527°E, 4210 m).

Type material. *Holotype*: male (IZAS), "Xizang, Mêdog County, Doxong La pass, 29.4860°N, 94.9527°E, 4210m, 2011 VII. 22, Yang XD lgt."; "HOLOTYPE⁽⁾ *Deltomerodes ovicollis* sp. nov., des. YAN & SHI, 2021" [red label]. *Paratypes*: 1 female (IZAS), same data as holotype but labeled as paratype; 4 females (IZAS), "China, Tibet, Lage to Pai Township. foot path. pitfall trap. 29.4897°N, 94.9533°E"; "4090 m. 2015. 8.8-10. D3. Liang H. B. coll. IOZCAS".

Diagnosis. The new species is unique in the genus *Deltomerodes* in having the following two features: (1) pronotum ovate with posterior margin strongly protruded medially; (2) first antennomere with two or three short accessory setae. In addition, the new species can be diagnosed from congeners by the combination of following characters: head with three pairs of setae between eyes and neck constriction; pronotum lateral margins with one pair of setae before middle, not sinuate before posterior angles; elytral discal pores present on third, fifth and seventh intervals; fifth meso- and metatarsomeres with two pairs of setae ventrally; armature of endophallus consisting of a large loop-shaped proximal copulatory piece and a long spiniform distal piece.

Description. Habitus as in Figure 1. Medium-sized for a Patrobini species (L = 9.3-10.7 mm; EW = 3.3-3.5 mm).

General appearance: Dorsal side dark brown to reddish-brown, shiny, without metallic luster; head, pronotum and elytra brown to reddish-brown; mandibles, antennae and legs a little paler, palpi reddish-brown; ventral side largely dark brown to reddish-brown. Dorsal surface of forebody smooth except for basal foveae of pronotum slightly punctate. Microsculpture invisible on head and pronotum, isodiametric on the entire elytra.

Head: Broad and ovate; surface smooth, without distinct punctures. Mandibles not elongated, right mandible with a weakly protruding tooth beneath the basal inner margin; terminal maxillary palpomere broadest in the middle, penultimate and antepenultimate palpomeres with an apical ring of setae; ligula with two adjacent apical setae. Antennae pubescent beginning with third antennomere; first antennomere plurisetose, with two or three short setae and one primary seta distinctly longer than the others; second antennomere glabrous except for apical ring of four to five setae (Fig. 4). Eyes small, very slightly convex; temporae tumid, much longer than eye diameter; neck constriction rather deep. Frontal furrows short, shallow but distinct, extended to level of middle of eyes, slightly divergent posteriorly, finely rugose anteriorly. Head with three setae between eyes and neck constriction on each side: one supraorbital seta near middle level of eyes (in one female with one additional seta on one side), two setae between posterior margins of eyes and neck constriction, a little distant from the neck constriction.



Figure 1. *Deltomerodes ovicollis* sp. nov. (holotype). Habitus and labels (the white dots represent the setiferous pores on the left side of the specimen). Scale bar: 1.0 mm.

tion. Mentum with two deep longitudinal foveae basally, tooth narrow and bifid, apical notch shallow; submentum with two setae on each side in both sexes (Fig. 2).

Pronotum: Longitudinal ovate, rather narrow, PW/PL = 0.95–1.05, slightly wider than head, PW/HW = 1.12–1.25, widest near anterior third, fairly convex, moderately constricted posteriorly. Anterior margin near straight; anterior angles indistinct, not protruding forward; lateral margins broadly rounded in front, without sinuation before posterior angles; posterior margin strongly protruded, forming a wide median peduncle and short incisions near posterior angles (Fig. 3); posterior angles obtuse, their apices slightly pointed outwards. Anterior transverse impression shallow and smooth; basal foveae shallow, finely punctate and wrinkled; disc smooth, median line shallow but distinct, obliterated at both extremities; lateral grooves extremely narrow, not



Figures 2–6. *Deltomerodes ovicollis* sp. nov. (holotype) 2 labium 3 pronotum 4 first two segments of antenna 5 lateral side of the fifth metatarsomere 6 ventral side of thorax. Scale bars: 0.5 mm (a for 2, 3, b for 4–6).

punctate. Lateral margins each with one seta near anterior third, another seta situated a little before posterior angle.

Elytra: Oblong-ovate, convex; EL/EW = 1.62–1.74, EW/PW = 1.70–1.83, EL/PL = 2.75–2.95, widest near posterior third, constricted both anteriorly and posteriorly; humeri narrow and oblique, humeral tooth hardly visible; lateral groove narrow. Intervals slightly convex; inner striae well incised, coarsely punctate basally, lateral striae shallower and finer punctate; scutellar stria inconspicuous, only with one or two punctures and a superficial impression, scutellar pore present at base of second interval or second stria; third interval with five to seven setiferous pores, adjoining the third stria or placed on the third interval, the first one near base; fifth interval with three to seven setiferous pores, all in basal half of elytra; seventh interval with four to six setiferous pores in basal half; umbilicate series composed of ten to thirteen pores, basal ones not aggregated.

Ventral side: Largely smooth, lateral areas of mesepisternum very finely wrinkled; mesepimeron narrow, slightly widened laterally, suture separating mesepisternum and



Figures 7–10. *Deltomerodes ovicollis* sp. nov. (holotype) **7** left paramere of aedeagus (dotted line shows damaged apex) **8** right paramere of aedeagus **9** median lobe of aedeagus, left lateral view (**9A**), dorsal view (**9B**) **10** endophallus of aedeagus, left lateral view (**10A**), dorsal view (**10B**). Scale bar: 0.5 mm.

mesosternum joins anterior margin of mesepimeron (Fig. 6); metepisternum long and narrow, not punctate. Lateral areas of abdominal sternites finely rugose, sternites IV to VI each with one pair of setae near middle; sternite VII with one seta on each side in males and two in females. Female sternite VIII with anterolateral apophyses long, longitudinal depigmentation distinct, posterointernal depigmentation present (Fig. 13). Female tergite VIII with anterolateral apophyses quite short; longitudinal carinae absent, transverse ones short and laterally placed, median sclerotization absent, basal depigmentation present, apical depigmentation absent (Fig. 12).

Legs: Males with the basal two protarsomeres slightly expanded, second protarsomere distinctly wider than third, which is near triangular; fourth protarsomere slightly bilobed; metatrochanter normal, apex not protruding; all tarsomeres pubescent dorsally; fifth meso- and metatarsomeres with two pairs of setae ventrally (Fig. 5).

Male genitalia: Median lobe of aedeagus strongly bent at base, gutter-shaped and opened dorsally; in lateral view apical lamella slightly curved dorsally; in dorsal view



Figures 11–14. *Deltomerodes ovicollis* sp. nov. (paratype female) **11** female reproductive tract **12** female tergite VIII **13** female sternite VIII **14** gonocoxa, right lateral view (**14A**), dorsal view (**14B**). Scale bars: 0.5 mm (a for **11–13**, b for **14**).

apical lamella gradually attenuated towards apex and fairly twisted rightwards, apex round-truncate, forming a faint hook to the right (Fig. 9). Armature of endophallus consisting of a large loop-shaped proximal copulatory piece and a long spiniform distal piece (Fig. 10). Left paramere larger than right one, both short and gradually contracted towards apex but left one more truncated at apex, both with one long apical seta and one or two short subapical setae (Figs 7, 8).

Female genitalia: Gonocoxite I glabrous; gonocoxite II narrow and slightly curved, blunt-rounded at apex, with two small setae in one fovea at subapical inner margin (Fig. 14). Reproductive tract with well-marked annular sclerotized ring about 0.37 mm in diameter on bursa copulatrix, spermatheca small and tubular, spermathe-



Map 2. Distribution map for *Deltomerodes ovicollis* sp. nov. and adjacent taxa: *D. ovicollis* sp. nov. (red); *D. zolotichini zolotichini* (blue); *D. zolotichini similis* (magenta).

cal duct well developed, spermathecal gland inserted near apex of spermathecal duct, much longer than spermathecal duct (Fig. 11).

Distribution. This species is known only from the type locality of Doxong La in Mêdog County, Xizang (Map 2).

Etymology. The scientific name of the new species comes from Latin and refers to its very special ovate pronotum.

Remarks. Zamotajlov (1999) recognized five species groups in the genus Deltomerodes, among which three were from China. Most of the Chinese species (four of six) were assigned to the *murzini*-group, whereas *D. zolotichini* and *D. memorabilis* are in their own species groups. According to Zamotajlov (1999), the new species can be keyed to the *murzini*-group for some characters: (1) head with less than four pairs of setae between eyes and neck constriction; (2) pronotum not cordate, without distinct sinuation before posterior angles, sides with one seta in anterior third. However, it is quite different from all other members of this species group not only for its very special pronotum shape (ovate with posterior margin strongly protruded) but also for other important features: (1) fifth meso- and metatarsomeres with two pairs of setae ventrally vs. with three or four pairs of setae in other species of this group; (2) first antennomere with two or three short accessory setae vs. without short setae; (3) seventh elytral interval with four or more discal pores vs. with one or two setiferous pores. Additionally, the new species is more similar to D. memorabilis mainly in aspects of the humeral shape and in the chaetotaxy of the elytral intervals and fifth tarsomeres, though the differences between the two species are rather distinct following the key. Thus, the new species may be either assigned to the *memorabilis*-group or assigned to its own group.

Deltomerodes conaensis sp. nov.

http://zoobank.org/52BCBE25-A05D-4FC4-A151-C81D2380673A Figs 15–28

Type locality. China, Xizang: Cona County, Rama La pass (28.3037°N, 91.8834°E, 5019 m).

Type material. *Holotype*: male (IZAS), "Xizang, Lhoka pref., Cona county, Quchomo Xiang, Rama La pass, alpine meadow, 28.3037°N, 91.8834°E, 5019m"; " under stone, 2019. VII. 24, Shi HL, Yan WF & Zhu PZ lgt., Exp. BJFU 2019"; "HOL-OTYPE *Deltomerodes conaensis* sp. nov., des. YAN & SHI, 2021" [red label]. *Paratypes*: 9 males and 7 females (IZAS), same data as holotype but labeled as paratypes.

Diagnosis. The new species can be distinguished within the genus by the combination of: head generally with two pairs of setae between eyes and neck constriction; pronotum subcordate, lateral margins slightly sinuate before posterior angles, which are slightly pointed outwards; humeri distinct, rounded; scutellar pore present; elytral discal pores only present on third and seventh intervals; fifth meso- and metatarsomeres with three to four pairs of setae ventrally; endophallus consisting of a straight and weakly sclerotized proximal copulatory piece and two distal spiniform pieces.

Description. Habitus as in Figure 15. Medium-sized for a Patrobini species (L = 9.7–11.5 mm; EW = 3.2–3.7 mm).

General appearance: Dorsal side dark brown to black, shiny, without metallic luster; head, mandibles, pronotum and elytra brown; antennae and legs a little paler, palpi reddish-brown; ventral side largely dark brown, abdominal sternum brown. Dorsal side smooth except for basal foveae of pronotum slightly punctate. Microsculpture invisible on head and pronotum, isodiametric on elytra, distinct in apical third, hardly visible near base.

Head: Broad and ovate; surface smooth, without coarse punctures. Mandibles not elongated, right mandible with a weakly protruding tooth beneath basal inner margin; terminal maxillary palpomere broadest in middle; ligula with two adjacent apical setae. Antennae pubescent beginning with third antennomere; first antennomere with only one long seta; second antennomere glabrous except for apical ring of setae (Fig. 16). Eyes small, very slightly convex; temporae tumid, much longer than eye diameter; neck constriction shallower than in *D. ovicollis* sp. nov. Frontal furrows short and rather shallow, extended to level of posterior edge of eyes, slightly divergent posteriorly; with two setae between eyes and neck constriction on each side (two specimens with three or four setae on one side), including one supraorbital seta near middle level of eyes, and one seta between posterior margins of eyes and neck constriction, not adjoining to neck constriction. Mentum with two deep longitudinal foveae basally, tooth narrow and bifid, apical notch shallow; submentum with two setae on each side in both sexes (Fig. 18).

Pronotum: Longitudinal-subcordate, rather narrow, PW/PL = 1.00–1.15, slightly wider than head, PW/HW = 1.11–1.21, widest near anterior third, fairly convex, moderately constricted posteriorly. Anterior margin near straight; anterior angles rounded, somewhat distinct, not protruding anteriorly; lateral margins broadly rounded in



Figure 15. *Deltomerodes conaensis* sp. nov. (holotype). Habitus and labels (the white dots represent the setiferous pores on the left side of the specimen). Scale bar: 1.0 mm.

front, with slight sinuation before posterior angles; posterior margin nearly straight to slightly rounded; posterior angles obtuse to rectangular, apex sharp, slightly pointed outwards. Anterior transverse impression shallow and smooth; basal foveae shallow, finely punctate and wrinkled; disc smooth, median line shallow, obliterated at both extremities; lateral grooves narrow, not punctate. Lateral margins each with one seta before middle (two specimens with two setae on one side or both sides), another seta situated a little before posterior angle (Fig. 19).

Elytra: Oblong-ovate, convex; EL/EW = 1.69–1.74, EW/PW = 1.64–1.74, EL/PL = 2.80–2.84, widest near posterior third; humeri distinct, rounded, with small humeral tooth. Intervals slightly convex; inner striae well incised, finely punctate basally, lateral striae shallower; scutellar stria inconspicuous, only with one or two punctures



Figures 16–19. *Deltomerodes conaensis* sp. nov. (holotype) 16 first two segments of antenna 17 lateral side of fifth metatarsomere 18 labium 19 pronotum. Scale bars: 0.5 mm (a for 16, 17, b for 18, 19).

and a superficial impression, scutellar pore present at base of second interval; third interval with three to four setiferous pores, all adjoining the third stria, the first one near basal sixth; seventh interval with one or two setiferous pores near basal sixth; umbilicate series composed of nine to twelve pores, basal ones not aggregated.

Ventral side: Prosternum smooth, propleuron densely punctate; mesosternum and mesopleuron wrinkled, mesopleuron with sporadic coarse punctures; mesepimeron narrow, slightly widened laterally, suture separating mesepisternum and mesosternum joins anterior margin of mesepimeron; metepisternum rather long and narrow, not punctate. Lateral areas of abdominal sternites finely rugose, sternites IV to VI each with one pair of setae near middle; sternite VII with one seta on each side in males and two in females. Female sternite VIII with anterolateral apophyses long, with both longitudinal and transverse depigmentation distinct, posterointernal depigmentation present (Fig. 26). Female tergite VIII with anterolateral apophyses quite short; longitudinal carinae absent, transverse ones short and laterally placed, median sclerotization absent, basal depigmentation indistinct, apical depigmentation absent (Fig. 25).

Legs: Males with basal two protarsomeres slightly expanded, second protarsomere distinctly wider than third, which is near triangular; fourth protarsomere



Figures 20–23. *Deltomerodes conaensis* sp. nov. (holotype) 20 left paramere of aedeagus 21 right paramere of aedeagus 22 median lobe of aedeagus, left lateral view (22A), dorsal view (22B) 23 endophallus of aedeagus, left lateral view (23A), dorsal view (23B). Scale bar: 0.5 mm.

slightly bilobed; metatrochanter normal, apex not protruding; all tarsomeres pubescent dorsally; fifth meso- and metatarsomeres with three or four pairs of setae ventrally (Fig. 17).

Male genitalia: Median lobe of aedeagus strongly bent at base, gutter-shaped and opened dorsally; in lateral view apical lamella moderately curved dorsally; in dorsal view apical lamella gradually attenuated towards apex and faintly twisted rightwards, apex rounded, forming a prominent hook to the right (Fig. 22). Armature of endophal-lus consisting of a straight and weakly sclerotized proximal copulatory piece and two spiniform distal pieces, the dorsal one large and stout, the left one much narrower (Fig. 23). Left paramere larger than right one, both short and gradually contracted towards apex but the left one more truncated at apex, both with two long apical setae and one or two short subapical setae (Figs 20, 21).

Female genitalia: Gonocoxite I glabrous; gonocoxite II narrow and slightly curved, blunt-rounded at apex, with two small setae in one fovea at subapical inner margin (Fig. 28). Reproductive tract with well-marked annular sclerotized ring about



Figures 24–28. *Deltomerodes conaensis* sp. nov. (paratype female) 24 female reproductive tract 25 female tergite VIII 26 female sternite VIII 27 spermatheca 28 gonocoxa, right lateral view (28A), dorsal view (28B). Scale bars: 0.5 mm (a for 24–26, b for 27, 28).

0.35 mm in diameter on bursa copulatrix; spermatheca tubular, very small, without spermathecal duct and spermathecal gland (Figs 24, 27).

Distribution. This species is known only from the type locality of Rama La in Cona County, Xizang (Map 3).



Map 3. Distribution map for *Deltomerodes conaensis* sp. nov. (red).

Etymology. The scientific name of the new species refers to its type locality in Cona County.

Remarks. According to the key provided by Zamotajlov (1999), the new species belongs to the *murzini*-group for some characters: (1) head with less than four pairs of setae between eyes and neck constriction; (2) pronotum not cordate, without distinct sinuation before posterior angles, sides with one seta in anterior third; (3) fifth mesoand metatarsomeres with three or four pairs of setae ventrally. Within this species group, the new species seems to be closest to *D. wrasei* for their adjacent distribution and morphological similarities in chaetotaxy, pronotum shape and in the apical lamella of the aedeagus. However, the armature of the endophallus in *D. conaensis* sp. nov. has a straight and weakly sclerotized proximal copulatory piece. The new species is different from all other known species of the genus, in which the proximal copulatory piece is well-sclerotized and loop-like.

Discussion

The genus *Deltomerodes* is well defined by its particular appearance and morphological characters, while relationships among its members remain unclear. Therefore, more materials are needed for a more extensive phylogenetic analysis, especially in consideration of some undetermined species with only females available and an insufficient number of specimens from the potential distribution area of the genus, including Southern Xizang and Bhutan.

The specimens of *D. conaensis* sp. nov. were found under stones along a dry creek bed formed by seasonal snow melting in the alpine meadow macrohabitat at about 5000 m altitude. The new species of *Deltomerodes* co-occur with *Nebria superna* Andrewes, a supra-high-altitude species widely distributed in the Tibetan Plateau. It is

noticeable that all specimens of *D. conaensis* sp. nov. were found only in a small (about twenty square meters) area slightly elevated above the creek bed slightly protruding from the creek bed, while *Nebria superna* was found extensively all along the creek bed. This suggests that species of *Deltomerodes* are more stenoecic and occur only in some rather humid microhabitats at high altitudes.

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References

- Andrewes HE (1936) Papers on Oriental Carabidae XXX. Annals and Magazine of Natural History (10) 18: 54–65. https://doi.org/10.1080/00222933608655174
- Deuve T (1992) *Deltomerodes memorabilis* n. gen., n. sp., carabique Patrobinae des hautes montagnes du Yunnan. Revue Française d'Entomologie (N.S.) 14: e82.
- Deuve T (1993) L'abdomen et les genitalia des femelles de Coléoptères Adephaga. Mémoires du Muséum d'Histoire Naturelle. Zoologie 155: e184.
- Kirby W (1837) Part the fourth and last. The insects. In: Richardson J (Ed.) Fauna Boreali-Americana; or the zoology of the northern parts of British America: containing descriptions of the objects of natural history collected on the late Northern Land Expeditions, under command of captain Sir John Franklin, R.N. Norwich: J. Fletcher, [xxxix +] 329 [+ 1] pp.[, 8 pls].
- Schmidt J (1994) Beschreibungen neuer Arten der Carabidae aus Nepal (1): Gattungen *Trechus, Deltomerodes, Pterostichus* (Insecta: Coleoptera). Reichenbachia 30(21): 129–135.
- Schmidt J (1995) Beschreibungen neuer Arten der Carabidae aus Nepal (2): Gattungen Deltomerodes, Pterostichus, Xestagonum (Insecta: Coleoptera). Reichenbachia 31(5): 19–25.
- Schmidt J (1996) Beschreibungen neuer Arten der Carabidae aus Nepal (3): Gattungen Broscus, Deltomerodes, Xestagonum (Insecta: Coleoptera). Reichenbachia 31(26): 143–154.
- Schmidt J (1998) Beschreibungen neuer Arten der Carabidae aus Nepal (4): Gattungen Broscus, Deltomerodes, Xestagonum (Insecta: Coleoptera). Entomologische Abhandlungen und Berichte aus dem Staatlich Museum für Tierkunde in Dresden 58(1): 5–28.

- Schmidt J, Hartmann M (1998) Deltomerodes grilli sp. n., der erste Nachweis einer alpinen Art der Tribus Patrobini aus West-Nepal (Coleoptera, Carabidae). Mitteilungen der Münchner Entomologischen Gesellschaft 88: 33–36.
- Shi HL, Zhou HZ, Liang HB (2013) Taxonomic synopsis of the subtribe Physoderina (Coleoptera, Carabidae, Lebiini), with species revisions of eight genera. ZooKeys 284: 1–129. https://doi.org/10.3897/zookeys.284.3983
- Zamotajlov AS (1999) Redefinition of the genus *Deltomerodes* Deuve, 1992, with the description of new species (Coleoptera Carabidae Patrobinae). In: Zamotajlov A, Sciaky R (Eds) Advances in Carabidology. Papers Dedicated to the Memory of Prof. Dr. Oleg L. Kryzhanovskij. Krasnodar: MUISO Publishers, 473 pp. [Pp. 229–258.]
- Zamotajlov AS (2002) Inferring phylogenetic system of the carabid subfamily Patrobinae (Coleoptera, Carabidae). St. Petersburg: Zoological Institute of Russian Academy of Sciences, 145 pp. [Meetings in memory of N.A. Cholodkovsky; Iss. 55] [In Russian]
- Zamotajlov AS (2017) Tribe Patrobini Kirby, 1837. Catalogue of Palaearctic Coleoptera, Volume 1: Archostemata – Myxophaga – Adephaga. 1477 pp. [p. 11, 456–465.]

RESEARCH ARTICLE



DNA barcoding and morphology reveal a new cryptic species of *Nagiella* (Lepidoptera, Crambidae, Spilomelinae) from Japan

Yuki Matsui¹, Hideshi Naka², Utsugi Jinbo³

1 The United Graduated School of Agricultural Sciences, Tottori University, Tottori, Japan **2** Faculty of Agriculture, Tottori University, Tottori, Japan **3** National Museum of Nature and Science, Tsukuba, Ibaraki, Japan

Corresponding author: Yuki Matsui (mothya22@gmail.com)

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Abstract

Nagiella tristalis Matsui & Naka, **sp. nov.** is described from Japan, based on DNA barcoding and morphological evidence. The two species previously known from Japan, *N. quadrimaculalis* and *N. inferior*, are diagnosed. Photographs of adults, including male and female genitalia of the three species, are provided.

Keywords

DNA barcodes, genitalia, Patania, Pleuroptya, Rubus buergeri

Introduction

Nagiella Munroe, 1976 was established as a replacement name for *Nagia* Walker, 1866 (type species: *Nagia desmialis* Walker, 1866), which is a junior homonym of *Nagia* Walker, 1858 (Lepidoptera, Noctuidae). Munroe (1976) included *Scopula quadrimaculalis* Kollar & Redtenbacher, 1844 and *Sylepta inferior* Hampson, 1899 in this genus, and he described *N. hortulatoides* Munroe, 1976. Munroe also treated the type species, *N. desmialis*, as a synonym of *N. quadrimaculalis*. Ullah et al. (2017) studied this genus in China and described *N. occultalis* Ullah & Yang, 2017, which is a cryptic species of the *N. quadrimaculalis* species complex. Lu and Du (2020) described another species,

N. bispina Lu & Du, 2020 from China. So far, this genus comprises five species, i.e., *N. quadrimaculalis*, *N. inferior*, *N. hortulatoides*, *N. occultalis*, and *N. bispina*. These species are often confused due to their similar appearance; in these species, the ground color is uniformly greyish and each wing has a conspicuous white spot on each wing, except for *N. hortulatoides*.

Two species, *N. quadrimaculalis* and *N. inferior*, have been recorded in Japan under the genera *Sylepta* Hübner, 1823 (Shibuya 1928, 1929), *Nagia* (Mutuura 1957), and *Pleuroptya* Meyrick, 1890 (Inoue 1982; Sasaki and Yamanaka 2013). In this paper, we describe and illustrate *N. tristalis* sp. nov., a cryptic species of the *N. quadrimaculalis* species complex, from Tottori Prefecture, Japan. We also provide a phylogenetic hypothesis of relationships based on the mitochondrial COI region for the three Japanese *Nagiella* species and *N. occultalis*.

Material and methods

Sampling insect specimens

Most specimens of *N. tristalis* were obtained by collecting the larvae in rolled leaves of *Rubus buergeri* Miq. (Rosaceae) during the winter and then rearing them by the method as described below. We also collected the adults of *Nagiella* species and *Patania ruralis* (Scopoli) (to use as the outgroup in the phylogenetic analysis) from various localities of Japan, by light-trap and daytime search. In addition, several specimens of *Nagiella* species were obtained by rearing eggs with the method described below.

Female moths were placed in plastic cups (Clean Cup 129 Pi 860B, with lid Clean Cup 129 Pi FSL [Risupack, Gifu, Japan]; diameter 129 mm, height 130 mm) with fresh leaves of *Rubus buergeri* or *R. trifidus* Thunb. for egg laying. The hatched larvae were reared using fresh leaves of *R. buergeri* or *R. trifidus* under a 14L:10D photoperiod at 25 ± 2 °C and 50–60% relative humidity until pupation, and the resulting pupae were kept in the same conditions until the emergence of adults.

The holotype of the new species is deposited in the National Museum of Nature and Science (NSMT; Tsukuba, Ibaraki, Japan), and the paratypes are stored in the authors' private collections.

Genitalia preparation

Before examining the male and female genitalia, the abdomen was detached from the specimen and soaked in a 10% potassium hydroxide (KOH) solution. The soaked abdomen was kept at room temperature overnight and then incubated at 60 °C for 3–6 h. After incubation, the abdomen was transferred into a glass dish with 70% ethanol, and the genitalia were detached from the abdomen under a stereomicroscope (LW-820T; Wraymer Inc., Osaka, Japan) using scissors and tweezers. The genitalia were stained with merbromin in 70% ethanol and mounted on a glass

slides in Euparal. The photographs of the whole genitalia were captured with a stereomicroscope (SZX10; Olympus Corp., Tokyo, Japan) and a digital camera (DP25; Olympus Corp., Tokyo, Japan). The magnified views of genital structures were captured by an upright microscope (BX53; Olympus Corp., Tokyo, Japan) with a digital camera (DP21; Olympus Corp., Tokyo, Japan). Genital structures were measured on the screen by Fiji (Schindelin et al. 2012), based on the photographs and whole genitalia lengths measured by a ruler. As references for the terminology, we followed Kristensen (2003) and Kirti and Gill (2007) for the genitalia and Ullah et al. (2017) for the wing maculation.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from the middle legs of the moths using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The legs were crushed using BioMasher II (FUJI-FILM Wako Pure Chemical Co., Osaka, Japan) and incubated with Proteinase K (Ta-kara Bio Corp., Shiga, Japan) for 3–7 d at 60 °C to elute DNA. Subsequent procedures followed the manufacturer's protocol of the DNeasy Tissue Kit.

The mitochondrial COI gene was amplified using the primers TY-J-1460-Spilo (forward: TACAATTTATCGCTTAATACTCAGCC) and TL2-N-3014-Spilo (reverse: TCCATTACATATAATCTGCCATATTA). These primers were based on TY-J-1460 and TL2-N-3014 (Simon et al. 1994) and were modified for Spilomelinae species based on the whole mitochondrial sequences of *Cnaphalocrocis medinalis* (Guenée, 1854) (accession number: JN246082) and *Haritalodes derogata* (Fabricius, 1775) (accession number: KR233479) from GenBank (https://www.ncbi. nlm.nih.gov/genbank/). The composition of the PCR reaction solution was as follows; 12.5 μ l Q5 High-Fidelity DNA Polymerase (New England Biolabs Japan, Tokyo, Japan); 125 nmol of forward and reverse primers; 1 μ l DNA extract; and sterilized water was added up to 25 μ l in total volume. The PCR amplification was performed in the following programs; initial denaturation phase at 94 °C for 60 s; 40 cycles at 94 °C for 30 s, 54 °C for 60 s, 72 °C 90 s; and final extension phase at 72 °C for 10 min.

The PCR products were checked by electrophoresis on a 1% agarose gel and were purified using NucleoSpin Gel and PCR Clean-up (Takara Bio Corp., Shiga, Japan). Sequencing was conducted at Premix2 analysis service (Fasmac Co., Ltd, Kanagawa, Japan) using the primers LCO1490 (forward: GGTCAACAAATCATAAAGATATTGG) and HCO2198 (reverse: TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994). The sequences obtained in this study were deposited into DDBJ (https://www. ddbj.nig.ac.jp/). The accession numbers of these sequences are listed in Table 1.

Phylogenetic analysis and BOLD Barcode Index Number clustering

To construct the phylogenetic tree, we downloaded the sequences of *N. inferior*, *N. quadrimaculalis*, and *N. occultalis* (two sequences, respectively) from GenBank.

Patania ruralis was included as the outgroup because *Patania* (= *Pleuroptya*) is considered to be closely related to *Nagiella* based on male and female genitalia (e.g., Munroe 1976; Inoue 1982), but wing maculation, host plants, and the results of the phylogenetic analysis of Lu and Du (2020) suggest they are clearly different (see also Discussion for the differences between *Patania* and *Nagiella*). The sequences were aligned using MEGA 7.0 (Kumar et al. 2016). A neighbor-joining (NJ) tree was constructed using MEGA 7.0 based on Kimura 2-parameter model (Kimura 1980), and the bootstrap values were calculated with 1,000 replicates. Detection of variation sites and the number of intra/interspecific substitutions were calculated using MEGA 7.0.

DNA barcoding employs DNA sequences in a short and standardized gene region to facilitate species identification. BOLD (http://www.boldsystems.org/) is an international repository of DNA barcodes (Ratnasingham and Hebert 2007). The sequences in BOLD are clustered depending on their divergences and each cluster is given a unique Barcode Index Number (BIN) (Ratnasingham and Hebert 2013), an identifier for DNA barcode-based cluster corresponding to species. We searched the BOLD database for BINs that matched sequences obtained in this study.

Results

DNA sequence analysis

We successfully obtained 626 bp sequences of the COI barcode region of the seven specimens of *Nagiella* treated. Variation was detected at 58 sites (9.3%) in these 13 sequences. The number of intraspecific substitutions ranged from 0 to 3 (0–0.5%) while the number of interspecific substitutions ranged from 18 to 41 (2.9–6.5%) (Table 2).

Species	Location	DDBJ accession no.	
Nagiella inferior	Japan: Yamaguchi, Akiyoshidai	LC527425	
N. inferior	Japan: Tottori, Wakasa, Hyonosen	LC527427	
N. inferior	Japan: Shimane, Iinan, Kusandao	LC527428	
N. quadrimaculalis	Japan: Tottori, Daisen	LC527424	
N. tristalis	Japan: Tottori, Tottori, Sourokubara	LC527426	
N. tristalis	Japan: Tottori, Tottori, Uemachi	LC527429	
N. tristalis	Japan: Tottori, Tottori, Sourokubara	LC527430	
Patania ruralis	Japan: Tottori, Tottori, Hashimoto	LC527431	

Table 1. Genetic sample information for the material included in this study with accession numbers.

Table 2. Mean number of intra (in bold) / interspecific substitutions in mitochondrial COI (626 bp) among four *Nagiella* species.

Species	Nagiella tristalis	N. inferior	N. occultalis	N. quadrimaculalis
Nagiella tristalis $(n = 3)$	0.7			
N. inferior $(n = 5)$	35.5	1.8		
N. occultalis $(n = 2)$	36.7	28.8	0	
N. quadrimaculalis (n = 3)	40.3	36.5	18.3	0.7



Figure 1. Neighbour-joining (NJ) tree based on mitochondrial COI (626 bp) for 13 sequences of four *Nagiella* species. Nucleotide substitution model based on Kimura 2-parameter model (Kimura, 1980). Bootstrap value was calculated with 1,000 replicates (values <50 are not shown).

In the BOLD database, the sequence of *N. quadrimaculalis* obtained in this study corresponds to BOLD:AAD8178, and that of *N. inferior* corresponds to BOLD:AAE4571, while that of *N. tristalis* did not corresponded to any BIN.

The NJ tree (Fig. 1) shows the four monophyletic clades that correspond to morphologically different *Nagiella* species with strong supports (bootstrap value of 100 for each species). The NJ tree also supports the close relationship between *N. tristalis* and *N. inferior* with moderate support (bootstrap value 77), and indicates *N. occultalis* as the sister species of *N. quadrimaculalis* with high support (bootstrap value 90).

Taxonomy

Key to Japanese Nagiella species

 2 Ground color of both wings lighter, postmedial line distinct especially in the hindwing; large, comma-shaped white spots at end of discal cell in each wing, usually larger; subdiscal white spot of forewing usually quadrilateral, distinct; base of discal cell of hindwing white; valva of male genitalia dorsally straight margined subapically; anterior apophysis of female genitalia slightly incurved to dorsally, expansion of the base sharply triangular; signum of female genita-Ground color of both wings darker, postmedial line obscure; large, commashaped, white spots at end of discal cell in each wing, usually smaller, especially in the hindwing; subdiscal white spot of forewing rounded, small and blurry; base of discal cell of hindwing concolorous with ground color; dorsal margin of valva of male genitalia slightly incurved subapically; anterior apophysis of female genitalia straight and narrow, expansion of the base broadly triangular; signum of female genitalia nearly elliptic, larger (diameter

Nagiella tristalis Matsui & Naka, sp. nov.

http://zoobank.org/12764D16E-5465-4859-ADD8-9C1C0C4D06A7 Figures 2A, B, 3A–C, 6A–C

Type material. *Holotype.* (), Japan: Sourokubara, Tottori City, Tottori Pref., 35.46°N, 134.11°E, 110 m, 7 Nov. 2019 (F1 emerged), Y. Matsui leg., preserved in National Museum of Nature and Science, NSMT-I-L-75637. *Paratypes.* 2933, Same locality as holotype, 5 Mar. 2018, 6 Apr. 2018, 4 May 2018, 13 Sep. 2018 (emerged), H. Naka, and Y. Matsui leg.; 1936, Setagura, Tottori City, Tottori Pref., 35.47°N, 134.12°E, 45 m, 7-22 Mar. 2019 (emerged), 23 Sep. 2019 (F1 emerged), H. Naka leg.; 2^Q, Uemachi, Tottori City, Tottori Pref., 35.50°N, 134.24°E, 40 m, 5 and 10 Feb. 2019 (emerged), Y. Matsui leg.; 1^Q, Mt Honjin-yama, Tottori City, Tottori Pref., 35.51°N, 134.26°E, 110 m, 24 Jun. 2012, Y. Matsui leg.; 1Å, Tokumaru, Yazu Town, Tottori Pref., 35.37°N, 134.34°E, 145 m, 20 Aug. 2014, H. Naka leg. Other specimens. 19, Mt Takao, Tokyo To, 19 Jul. 1960, T. Ebato leg. (NSMT-I-L-75536); 13, Nashimoto, Shizuoka Pref., 23 May 1953, T. Ebato leg. (NSMT-I-L-75538); 13, ditto, 5 Jun. 1959, T. Ebato leg. (NSMT-I-L-75537); 1Å, ditto, 10 Jun. 1961, T. Ebato leg. (NSMT-I-L-75539); 2Å, ditto, 24 Aug. 1966, T. Ebato leg. (NSMT-I-L-75534, 75535); 1^Q, Kuragari-Valley, Nukata Town, Aichi Pref., 26 Jun. 1993, A. Sasaki leg. (NSMT-I-L-75593); 1⁽²⁾, Sugano, Tokuyama City, Yamaguchi Pref., 27 Jun. 1993, T. Ikenoue leg. (NSMT-I-L-75594); 12, Shimomyo, Aira Town, Kagoshima Pref., 28 May 1992, Y. Yanagita leg. (NSMT-I-L-75596); 1^Q, Kamitsuru, Izumi City, Kagoshima Pref., 14 Jul. 1992, Y. Yanagita leg. (NSMT-I-L-75595); 1&, Mt Ishizukadake, I. Yakushima, Kagoshima Pref., 5 Aug. 1958, B.T. leg. (NSMT-I-L-75607); 13, Nagata, I. Yakushima, Kagoshima Pref., 3 Oct. 2006, M. Owada and T. Fukuda leg. (NSMT-I-L-75606); 1^Q, Chuo-rindo, Uken, I. Amamiohshima, Kagoshima Pref., 13 Oct. 1988, M. Owada leg. (NSMT-I-L-75541); 3Å, ditto, 22 Apr. 2009,



Figure 2. *Nagiella* adults, dorsal view A *N. tristalis* sp. nov. male B *N. tristalis* sp. nov. female C *N. inferior* male D *N. inferior* female E *N. quadrimaculalis* male F *N. quadrimaculalis* female. Scale bars: 5 mm.

M. Owada leg. (NSMT-I-L-75609 to 75611); 1♀, Kinsakubaru, Naze, I. Amamiohshima, Kagoshima Pref., 11 Oct. 1988, M. Owada leg. (NSMT-I-L-75542); 1♂, Mt Yuwan-dake, I. Amamiohshima, Kagoshima Pref., 12 Oct. 1988, M. Owada leg. (NSMT-I-L-75543); 1♀, Naze, I. Amamiohshima, Kagoshima Pref., 25 Jun. 1968, Y. Kishida leg. (NSMT-I-L-75540); 1♂, Shinokawa, Setouchi, I. Amamiohshima, Kagoshima Pref., 21 Apr. 2009, M. Owada leg. (NSMT-I-L-75608); 1♂, Mikyo, I. Tokunoshima, Kagoshima Pref., 31 Oct. 1992, M. Owada leg. (NSMT-I-L-75544);

1∂, Gogayama, I. Okinawajima, Okinawa Pref., 30 Mar. 1974, T. Naito leg. (NSMT-I-L-75612); 1♀, Seifuautaki, Chinen-son, I. Okinawajima, Okinawa Pref., 16 Aug. 1980, R. Sato leg. (NSMT-I-L-75613); 1∂, same data as for preceding (NSMT-I-L-75614); 1♀, Ôkuni-bashi, Kunigami-son, I. Okinawajima, Okinawa Pref., 21 Apr. 2001, A. Sasaki leg. (NSMT-I-L-75615).

Etymology. The specific epithet refers to the darker wing color in comparison to that of *N. inferior*, and the habitat of this species is a shaded place.

Diagnosis. This new species is similar to *N. inferior* and *N. quadrimaculalis*, also distributed in Japan, but it can be distinguished by the following characters: length of forewing 12.0–13.0 mm; vertex with erect, dull-orange scales; subdiscal white spot of forewing rounded, small, and blurry; base of discal cell of hindwing identical to ground color; dorsal margin of valva of male genitalia slightly incurved subapically; anterior apophysis of female genitalia straight and narrow; signum of female genitalia nearly elliptical, larger than in *N. inferior* (diameter 0.09–0.14 mm). This species is also similar to *N. occultalis* and *N. bispina* distributed in China, but *N. occultalis* has the following differences: subdiscal white spot of forewing narrowed or elongated, tuba analis of male genitalia sclerotized, gnathos of male genitalia elongated and narrow at the base; *N. bispina* exhibits the following differences: gnathos of male genitalia absent, phallus of male genitalia with a hook-shaped cornutus, corpus bursae of female genitalia with two thorn-like signa. From *N. hortulatoides*, the new species can be easily distinguished by the wing maculation.

Description (Fig. 2A, B). *Head:* frons brownish grey, smooth. Vertex with erect, dull-orange scales. Labial palpus upturned, dorso-laterally dark brown, ventro-mesally pale white. Antenna dark brown; flagellum filiform with golden cilia ca 1/4 the diameter of flagellum in male.

Thorax and abdomen: dorsally brownish grey; patagium and tegula with ochreous brown. Ventrally milky white.

Wings: length of forewing 12.0–13.0 mm. Ground color of both wings brownish grey, with a large comma-shaped white spot at end of discal cell (the bases of R_5 to M_3), that of the hindwing somewhat small; cilia concolorous with ground color; postmedial line obscure. Subdiscal white spot of forewing rounded, small and blurry. Base of discal cell of hindwing concolorous with ground color.

Male genitalia (Fig. 3A–C): uncus short, subtrapezoid, blunt on posterior margin. Gnathos nearly triangular, short and small, apex blunt. Tuba analis elongate, not sclerotized, length ca 0.6 times that of valva. Transtilla subtriangular, completely connected medially. Saccus short, anterior margin rounded. Valva somewhat narrow, length ca 3.3 times that of width, dorsal margin slightly incurved subapically; costa more or less inflated with several setae at apex; clasper large, down curved, the apex blunt. Phallus cylindrical, without cornutus.

Female genitalia (Fig. 6A–C): ovipositor lobe oblong, setose. Anterior apophysis ca 2 times length of posterior apophysis, narrow, almost straight, with triangular expansion near base. Antrum trapezoid, sclerotized at collar. Ductus bursae almost equal in length to corpus bursae, membranous with wrinkles, slightly narrowed at posterior



Figure 3. Male genitalia of *Nagiella tristalis* sp. nov. **A** whole genitalia **B** gnathos, magnified view **C** apex of valva, magnified view.

end. Corpus bursae pear-shaped, membranous; signum nearly elliptical, sparsely covered with denticles.

Biology. In Honshu, Japan, adults are found in May to September, and they are considered bivoltine. They appear to be hardly attracted to light. Larvae feed on *Rubus buergeri* and the middle instar larvae overwinter in its leaves.

Distribution. Japan: Honshu (Tokyo, Shizuoka, Aichi, Tottori, Yamaguchi), Kyushu (Kagoshima), Ryukyu Islands (Yakushima, Amamioshima, Tokunoshima, Okinawajima).

Remarks. The shapes of the uncus and gnathos show intraspecific variations, i.e., in several specimens, the posterior margin of the uncus is slightly notched medially, and the projection of the gnathos is smaller than that shown on Figure 3B. Also, in *N. inferior*, the shape of the gnathos varies similarly as in *N. tristalis*. Therefore, these characters are unsuitable for diagnosis of *N. tristalis* and *N. inferior*. Similarly, Ullah et al. (2017) proposed the shape of the claspers as a diagnostic character for distinguishing between *N. occultalis* and *N. quadrimaculalis*, but we could not find any clear difference among *N. tristalis*, *N. inferior*, and *N. quadrimaculalis*, because of the overlap in intraspecific variations.

Nagiella inferior (Hampson, 1899)

Figures 2C, D, 4A–C, 7A–C

Botys quadrimaculalis Motschulsky, 1861: 1: 37 (preoccupied).

Pleuroptya quadrimaculalis: Bae, 2001: 122–124, pl. 5 fig. 172; Park et al. 2007: 177, 239 fig. 94; Bae et al. 2008: 148–149, fig. 167.

Sylepta inferior Hampson, 1899: 724; Shibuya 1928: 228; Shibuya 1929: 189.

Nagia inferior: Mutuura, 1957: 122, pl. 21 fig. 636.

Nagiella inferior: Munroe, 1976: 878; Lu and Du 2020: 149, fig. 5, figs 5, 10, 13.

Pleuroptya inferior: Inoue, 1982: 1: 343; 2: 234, 454, pl. 40, fig. 16; Li et al. 2012:

625–626, pl. 18, fig. 416; Sasaki and Yamanaka 2013: 81, 451.

Material examined. Japan: 13, Tohro, Hokkaido, 3 Jul. 1962, T. Ebato leg., (NS-MT-I-L-75498); 1 , Riv. Rusagawa, Shiretoko, Hokkaido, 26 Jul. 1962, K. Tsuchiya leg. (NSMT-I-L-75523); 1Å, Sapporo, Hokkaido, 6 Jul. 1933, collector unknown (NSMT-I-L-75529); 1∂, Shumarinai, Hokkaido, 20 Jul. 1998, Y. Kishida leg. (NS-MT-I-L-75569); 1⁽²⁾, Shibecha, Hokkaido, 8 Jul. 1958, K. Jinbo leg. (NSMT-I-L-75574); 2Å, Akan, Hokkaido, 13 Jul. 1958, K. Jinbo leg. (NSMT-I-L-75575, 75576); 13, Toubai, Nemuro City, Hokkaido, 5 Aug. 2013, U. Jinbo leg. (NSMT-I-L-37577); 13, Sannai-Ishizawa, Honjoh City, Akita Pref., 29 Jun. 1975, A. Sasaki leg. (NSMT-I-L-75548); 1Å, ditto, 29 Jun. 1978, A. Sasaki leg. (NSMT-I-L-75545); 2∂, ditto, 15 Jun. 1979, A. Sasaki leg. (NSMT-I-L-75546, 75547); 1♀, Shinzan Park, Honjoh City, Akita Pref., 11 Jul. 1977, A. Sasaki leg. (NSMT-I-L-75549); 1&, Uwanodai, Kawabe Town, Akita Pref., 5 Jul. 1977, A. Sasaki leg. (NSMT-I-L-75555); 1^Q, ditto, 12 Jul. 1977, A. Sasaki leg. (NSMT-I-L-75551); 1^Q, ditto, 26 Jun. 1978, A. Sasaki leg. (NSMT-I-L-75552); 1♀, ditto, 19 Jul. 1979, A. Sasaki leg.; 1♂, ditto, 19 Jul. 1979, A. Sasaki leg. (NSMT-I-L-75556); 13, ditto, 11 Jun. 1980, A. Sasaki leg. (NSMT-I-L-75550); 13, ditto, Akita Pref., 2 Jul. 1980, A. Sasaki leg. (NSMT-I-L-75553); 1^Q, Kamibiguchi, Gojohme Town, Akita Pref., 26 Jul. 1979, A. Sasaki leg. (NSMT-I-L-75557); 13, Tazawako Height, Tazawako Town, Akita Pref., 20 Aug. 1979, A. Sasaki leg. (NSMT-I-L-75558); 1⁽²⁾, Chikogi-zaki, Hachimori Town, Akita Pref., 13 Jul. 1979, A. Sasaki leg. (NSMT-I-L-75559); 12, Niida, Akita City, Akita Pref., 16 Jun. 1978, A. Sasaki leg. (NSMT-I-L-75560); 1Å, Asahimata, Akita City, Akita Pref., 16 Jul. 1980, A. Sasaki leg. (NSMT-I-L-75561); 13, Nibetsu, Akita City, Akita Pref., 3 Jul. 1980, A. Sasaki leg. (NSMT-I-L-75562); 13, ditto, 23 Jun. 1987, A. Sasaki leg. (NSMT-I-L-75563); 13, Mt Takao, Yuma Town, Akita Pref., 8 Jul. 1985, A. Sasaki leg. (NSMT-I-L-75564); 1⁽²⁾, Toshi, Nikaho Town, Akita Pref., 18 Jul. 1995, A. Sasaki leg. (NSMT-I-L-75565); 1⁽²⁾, ditto, 19 Aug. 1984, A. Sasaki leg. (NSMT-I-L-75566); 1⁽²⁾, Matsusaka, Ōno-dai, Moriyoshi Town, Akita Pref., 13 Jul. 1996, A. Sasaki leg. (NSMT-I-L-75567); 12, Aburato, Tsuruoka City, Yamagata Pref., 11 Jul. 1990, A. Sasaki leg. (NSMT-I-L-75568); 13, Futamata-Spa, Fukushima Pref., 6 Aug. 1967, T. Ebato leg. (NSMT-I-L-75497); 1⁽²⁾, Shiozawa-Spa, Fukushima Pref., 5 Aug. 1967, T. Ebato leg. (NSMT-I-L-75501); 13, ditto, 6 Aug. 1967, T. Ebato leg. (NSMT-I-L-75519); 1⁽²⁾, ditto, 29 Jun. 1968, T. Ebato leg. (NSMT-I-L-75518); 2⁽²⁾, Hanashiki-Spa, Gunma Pref., 22 Jun. 1963, T. Ebato leg. (NSMT-I-L-75459, 75460); 1 \bigcirc , same data as for preceding, (NSMT-I-L-75487); 1 \bigcirc , ditto, 8 Jul. 1961, T. Ebato leg. (NSMT-I-L-75492); 3⁽²⁾, Uenohara, Gunma Pref., 8 Jul.


Figure 4. Male genitalia of *Nagiella inferior* **A** whole genitalia **B** gnathos, magnified view **C** apex of valva, magnified view.

1961, T. Ebato leg. (NSMT-I-L-75488 to 75490); 1♂, Kawaburu-Spa, Gunma Pref., 1 Jul. 1967, T. Ebato leg. (NSMT-I-L-75491); 1♂, Kitakaruizawa, Gunma Pref., 12 Jul. 1970, T. Okada leg. (NSMT-I-L-75528); 1Å, Minakami, Gunma Pref., 22 Jul. 1931, collector unknown (NSMT-I-L-75530); 1° , same data as for preceding (NS-MT-I-L-75531); 13, Mt Mitsumine, Saitama Pref., 15 Jul. 1961, T. Ebato leg. (NS-MT-I-L-75461); 19, Shikanoyu, Titibu, Saitama Pref., 26 Jul. 1933, collector unknown (NSMT-I-L-75532); 13, Bushi, Iruma City, Saitama Pref., 10 Sep. 1979, H. Inoue leg. (NSMT-I-L-75635); 1⁽²⁾, Kameyama, Chiba Pref., 12 Aug. 1963, T. Ebato leg. (NSMT-I-L-75517); 1⁽²⁾, Kiyose, Tokyo To, 10 Jun. 1956, T. Ebato leg. (NSMT-I-L-75502); 2♀, ditto, 18 Aug. 1958, T. Ebato leg. (NSMT-I-L-75503, 75504); 1♀, ditto, 15 Aug. 1959, T. Ebato leg. (NSMT-I-L-75505); 1^Q, ditto, 2 Jul. 1959, T. Ebato leg. (NSMT-I-L-75506); 13, ditto, 12 Jun. 1958, T. Ebato leg. (NSMT-I-L-75507); 1^Q, ditto, 31 Aug. 1958, T. Ebato leg. (NSMT-I-L-75508); 1^Q, ditto, 18 Aug. 1959, T. Ebato leg. (NSMT-I-L-75509); 12, ditto, 2 Jul. 1957, T. Ebato leg. (NSMT-I-L-75511); 1∂, ditto, 4 Jun. 1958, T. Ebato leg. (NSMT-I-L-75512); 1♀, Ohizumi, Tokyo To, 18 Aug. 1966, T. Ebato leg. (NSMT-I-L-75513); 2Å, Mt Takao, Tokyo To, 27 Jun. 1959, T. Ebato leg. (NSMT-I-L-75514, 75515); 1Å, ditto, 7 Jun. 1961, T. Maenami leg. (NSMT-I-L-75527); 1Å, ditto, 19 Jun. 1996, U. Jinbo leg. (NSMT-I-L-33621); 1,7, Mt Mihara, Tokyo To, 31 May 1962, R. Aoki leg. (NSMT-I-L-75521); 12, Mt Mitake, Tokyo To, 16 Jul. 1960, T. Maenami leg. (NSMT-I-L-75526); 1Å, ditto, 20 Aug. 1959, T. Ebato leg. (NSMT-I-L-75510); 1¢, ditto, 25 Jul. 1998, U. Jinbo leg. (NSMT-I-L-36058); 13, Institute of Nature Study, Minatoku, Tokyo To, 6 Jun. 2017, U. Jinbo leg. (NSMT-I-L-55639); 1⁽²⁾, Hodokubo, Hino City, Tokyo To, 3 Jun. 1990, U. Jinbo leg. (NSMT-I-L-75626); 12, ditto, 16 Jun. 1990, U. Jinbo leg. (NSMT-I-L-75627); 13, ditto, 3 Aug. 1991, U. Jinbo leg. (NS-MT-I-L-75628); 1♀, ditto, 7 Sep. 1991, U. Jinbo leg. (NSMT-I-L-75629); 1♀, ditto, 23 Aug. 1992, U. Jinbo leg. (NSMT-I-L-75630); 13, Yokozawairi, Itsukaichi Town, Tokyo To, 18 Jun. 1994, U. Jinbo leg. (NSMT-I-L-75631); 1Å, ditto, Tokyo To, 27 Aug. 1994, U. Jinbo leg. (NSMT-I-L-75632); 12, Imperial Palace, Chiyoda-ku, Tokyo To, 26 May 2009, Y. Arita et al. leg. (NSMT-I-L-22523); 1Å, ditto, 7 Sep. 2010, [Malaise trap] (NSMT-I-L-22525); 1Å, ditto, 9 Aug. 2011, [Malaise trap] (NSMT-I-L-28051); 1^Q, ditto, 4 Sep. 2012, Y. Arita, H. Nakajima, M. Owada, Y. Kishida and U. Jinbo leg. (NSMT-I-L-31522); 13, Noborito, Tokyo To, 27 May 1932, collector unknown (NSMT-I-L-75533); 1Å, Nishitanzawa, Kanagawa Pref., 18 Jun. 1966, Y. Kishida leg. (NSMT-I-L-75525); 1♀, Mt Myôjô-san, Itoigawa City, Niigata Pref., 7 Aug. 1999, A. Sasaki leg. (NSMT-I-L-75570); 13, Teradomari, Niigata Pref., 9 Aug. 2005, R. Sato leg. (NSMT-I-L-75571); 1Å, ditto, 7 Jun. 2005, T. Naito leg. (NSMT-I-L-75572); 1Å, Yuzurihara, Yamanashi Pref., 23 Jun. 1945, T. Ebato leg. (NSMT-I-L-75499); 13, Kiyosato, Yamanashi Pref., 22 Jul. 1967, T. Ebato leg. (NSMT-I-L-75500); 13, Karuizawa, Nagano Pref., 21 Jul. 1958, T. Ebato leg. (NSMT-I-L-75493); 1Å, Miyota, Nagano Pref., 17 Aug. 1965, T. Ebato leg. (NSMT-I-L-75494); 1∂, Tobira-Spa, Nagano Pref., 14 Jul. 1957, T. Ebato leg. (NSMT-I-L-75495); 1♀, Kumanotaira, Nagano Pref., 7 Jul. 1962, T. Ebato leg. (NSMT-I-L-75496); 1Å, ditto, 25 Jun. 1944, H. Inoue leg. (NSMT-I-L-75520); 13, Nashimoto, Shizuoka Pref., 10 Jun. 1961, T. Ebato leg. (NSMT-I-L-75516); 2Å, Asagiri Plateau, Shizuoka Pref., 35.42°N, 138.59°E, 910 m, 27 Aug. 2019, Y. Matsui leg.; 1∂, Gujo-Rokunori, Gifu Pref., 5 Jul. 1966, S. Sawatani leg. (NSMT-I-L-75573); 13, Mt Hyôno-sen, Wakasa Town, Tottori Pref., 35.35°N, 134.49°E, 860 m, 6 Nov. 2017 (F, emerged), Y. Matsui leg.; 1Å, ditto, 21 Jun. 2020, Y. Matsui leg.; 1Å, Hirodomeno, Wakasa Town, Tottori Pref., 35.41°N, 134.45°E, 800 m, 4 Sep. 2015, Y. Matsui leg.; 4♀6♂, Tokumaru, Yazu Town, Tottori Pref., 35.37°N, 134.34°E, 145 m, 9-11 Aug. 2013, 10-20 Oct. 2014 (F, emerged), H. Naka leg.; 2, Wakabadai-kita, Tottori City, Tottori Pref., 35.45°N, 134.26°E, 40 m, 6 Jun. 2012, 22 Aug. 2014, Y. Matsui leg.; 1∂, Kôchi, Shikano Town, Tottori City, Tottori Pref., 35.40°N, 134.00°E, 495 m, 12 Jun. 2020, Y. Matsui leg.; 3Å, Mt Daisen, Kôfu Town, Tottori Pref., 35.35°N, 133.55°E, 910 m, 30 Sep.–2 Oct. 2017 (F, emerged), Y. Matsui leg.; 2♂, Mt Senjô-san, Kotoura Town, Tottori City, 35.43°N, 133.60°E, 385 m, 15 Jul. 2019, Y. Matsui leg.; 1∂, Ichibata, Izumo City, Shimane Pref., 27 May 1967, T. Maenami leg. (NSMT-I-L-75522); 19, same data as for preceding (NSMT-I-L-75524); 1913, Kusandao, Înan Town, Shimane Pref., 35.06°N, 132.83°E, 915 m, Oct. 2017 (F, emerged), Y. Matsui leg.; 1, Sugano, Tokuyama City, Yamaguchi Pref., 3 Jun. 1994, T. Ikenoue leg. (NSMT-I-L-75577); 1^Q, Yunoki, Tokuji Town, Yamaguchi Pref., 15 Jul. 1995, T. Ikenoue leg. (NSMT-I-L-75578); 2♀, Mt Tokusagamine, Yamaguchi Pref., 3 Aug. 1996, T. Ikenoue leg. (NSMT-I-L-75579, 75580); 13, Jakuchikyô, Yamaguchi Pref., 27 Jul. 1995, T. Ikenoue leg. (NSMT-I-L-75581); 1♂, Akiyoshi-dai, Mine City, Yamaguchi, 34.24°N, 131.31°E, 240 m, 16 Sep. 2018, Y. Matsui leg.; 1^Q, Kamitsuru, Izumi City, Kagoshima Pref., 14 Jul. 1992, Y. Yanagita leg. (NSMT-I-L-75582); 1♀, Shin-Wase-Tunnel, I. Amamiohshima, Kagoshima Pref., 27 Mar. 2009, M. Owada and M. Kimura leg. (NSMT-I-L-75617); 1♂, Yona, I. Okinawajima, Okinawa Pref., 1 Apr. 1964, T. Nagano leg. (NSMT-I-L-75618); 1♂, Seifuautaki, Chinen-son, I. Okinawajima, Okinawa Pref., 8 Aug. 1980, R. Sato leg. (NSMT-I-L-75619); 2♂, Mt Terukubi-yama, Kunigami-son, I. Okinawajima, Okinawa Pref., 10 Aug. 1980, R. Sato leg. (NSMT-I-L-75620, 75621); 1♂, Haneji, Nago City, I. Okinawajima, Okinawa Pref., 17 Aug. 2001, M. Kimura leg. (NSMT-I-L-75622); 1♂, ditto, 9 Aug. 2002, M. Kimura leg. (NSMT-I-L-75623); 1♂, ditto, 12 Aug. 2002, M. Kimura leg. (NSMT-I-L-75624); 1♂, Takeda-Rindo, I. Ishigakijima, Okinawa Pref., 5 Jun. 2007, M. Kimura leg. (NSMT-I-L-75625).

Diagnosis. Adult (Fig. 2C, D). Forewing length 9.0-12.5 mm. This species is similar to *N. tristalis* and *N. quadrimaculalis*, but can be distinguished by the following characters: forewing shorter; vertex scales lighter than in N. tristalis and N. quadrimaculalis; subdiscal white spot of forewing usually quadrilateral and distinct; base of discal cell of hindwing broadly white; gnathos of male genitalia nearly triangular, short and small, apex rounded (Fig. 4B); valva of male genitalia dorsally straight margined subapically (Fig. 4C); anterior apophysis of female genitalia slightly curved to dorsally, expansion of the base is sharply triangular (Fig. 7B); signum of female genitalia circular, smaller than in N. tristalis (diameter 0.05–0.06 mm) (Fig. 7C). This species is also similar to N. occultalis, but N. occultalis has the following differences: forewing length 15–16 mm; subdiscal white spot of forewing narrowed or elongated; base of discal cell of hindwing concolorous with ground color; tuba analis of male genitalia sclerotized; gnathos of male genitalia elongated and narrow at the base. Lu and Du (2020) also mentioned genital differences between these species, and as far as we can see from the specimen image by Lu and Du (2020), N. bispina is externally distinguishable from this species by base of discal cell of the hindwing being concolorous with the ground color.

Distribution. Japan, mainland China, Taiwan, Korea, Russia (southeast), India. **Host plant.** *Rubus buergeri* Miq., *R. trifidus* Thunb. (laboratory reared).

Remarks. Our identification of this species is based on characters of external morphology (Motschulsky 1861; Inoue 1982; Li et al. 2012; Sasaki and Yamanaka 2013; Lu and Du 2020) and male genitalia (Li et al. 2012; Lu and Du 2020). The species status was confirmed by DNA barcodes.

Nagiella quadrimaculalis (Kollar & Redtenbacher, 1844)

Figures 2E, F, 5A-C, 8A-C

Scopula quadrimaculalis Kollar and Redtenbacher 1844: IV: 492. Coptobasis quadrimaculalis: Lederer, 1863: 429–430; pl. 16 fig. 12. Nagia desmialisWalker, 1866: 1320. Sylepta quadrimaculalis: Shibuya, 1928: 229; pl. 8 fig. 14; Shibuya 1929: 189. Nagia quadrimaculalis: Mutuura, 1957: 122, pl. 21 fig. 635.

- *Pleuroptya quadrimaculalis*: Inoue, 1982: 1: 343; 2: 234, 454, pl. 40 fig. 17; Li et al. 2012: 624–625, pl. 18 fig. 415; Yamanaka 1995: 187, pl. 125 fig. 21; Sasaki and Yamanaka 2013: 81, 451.
- *Nagiella quadrimaculalis*: Munroe, 1976: 878; Ullah et al. 2017: 70–72, figs. 2B, 4B, table 3; Lu and Du 2020: 149, fig. 6, figs. 11, 14.

Material examined. Japan: 13, Marumori, Onikôbe, Narugo, Miyagi Pref., 30 Jul. 1997, M. Tanaka leg. (NSMT-I-L-75588); 13, Kirei-pass, Sumison, Miyazaki Pref., 9 Jul. 1992, Y. Yanagita leg. (NSMT-I-L-75592); 12, Hiromorigawa, Akita Pref., 14 Aug. 1988, A. Sasaki leg. (NSMT-I-L-75583); 1^Q, Garo-Kyo, Fujisato Town, Akita Pref., 28 Jul. 2002, A. Sasaki leg. (NSMT-I-L-75584); 1^Q, Yoroibata-Dam, Tazawako Town, Akita Pref., 26 Aug. 1989, A. Sasaki leg. (NSMT-I-L-75585); 20, Tose, Tamagawa, Tazawako Town, Akita Pref., 21 Aug. 1993, A. Sasaki leg. (NSMT-I-L-75586, 75587); 1Å, Futamata-Spa, Fukushima Pref., 6 Aug. 1967, T. Ebato leg. (NSMT-I-L-75462); 1^Q, Houshi, Gunma Pref., 19 Jul. 1957, T. Ebato leg. (NSMT-I-L-75466); (NSMT-I-L-75472), 13, Kawaburu-Spa, Gunma Pref., 1 Jul. 1967, T. Ebato leg.; 13, Mt Takao, Tokyo To, 23 Jun. 1951, T. Haruta leg. (NSMT-I-L-75479); 12, ditto, 10 Jul. 1960, T. Ebato leg. (NSMT-I-L-75463); 13, ditto, 26 Jun. 1959, T. Ebato leg. (NSMT-I-L-75464); 12, ditto, 27 Jun. 1959, T. Ebato leg. (NSMT-I-L-75474); 1⁽²⁾, Nippara, Tokyo To, 8 Aug. 1961, T. Ebato leg. (NSMT-I-L-75465); 1 \bigcirc , same data as for preceding (NSMT-I-L-75477); 2 $\stackrel{\circ}{\xrightarrow{}}$, ditto, 2 Sep. 1961, T. Ebato leg. (NSMT-I-L-75475, 75476); 1^Q, Mt Mitake, Tokyo To, 27 Aug. 1960, T. Maenami leg. (NSMT-I-L-75486); 1Å, ditto, 20 Jun. 1996, U. Jinbo leg. (NSMT-I-L-36059); 1♀, Ohnita, Ohme City, Tokyo To, 18 Aug. 1996, U. Jinbo leg. (NSMT-I-L-75634); 1⁽²⁾, Yuzurihara, Yamanashi Pref., 1 Sep. 1945, T. Ebato leg. (NSMT-I-L-75471); 1∂, ditto, 2 Sep. 1945, T. Ebato leg. (NSMT-I-L-75469); 1∂, ditto, 5 Sep. 1945, T. Ebato leg. (NSMT-I-L-75470); 13, ditto, 23 Sep. 1954, T. Ebato leg. (NSMT-I-L-75468); 13, Sagashio-Spa, Yamanashi Pref., 9 Aug. 1969, T. Ebato leg. (NSMT-I-L-75473); 2Å, Ashiyasu, Yamanashi Pref., 16 Jul. 1977, T. Ebato leg. (NSMT-I-L-75480, 75482); 13, ditto, 6 Aug. 1977, T. Ebato leg. (NSMT-I-L-75483); 1^Q, ditto, 19 Jul. 1980, T. Ebato leg. (NSMT-I-L-75478); 1^A, Nishiyama-Spa, Yamanashi Pref., 17 Aug. 1981, T. Ebato leg. (NSMT-I-L-75481); 12, Hirayu, Gifu Pref., 7 Aug. 1953, T. Haruta leg. (NSMT-I-L-75467); 13, Gujo-Rokunori, Gifu Pref., 1 Jul. 1966, S. Sawatani leg. (NSMT-I-L-75589); 13, Osugi-dani, Wakayama Pref., 4 Aug. 1976, S. Nakatani leg. (NSMT-I-L-75484); 1♂, Shimakawa-Osugi-dani, Wakayama Pref., 5 Jul. 1975, S. Nakatani leg. (NSMT-I-L-75485); 13, Tatsumi-tôge, Tottori City, Tottori Pref., 35.32°N, 134.01°E, 670 m, 2 Jul. 2019, Y. Matsui leg.; 2♀3♂, Mt Daisen, Kôfu Town, Tottori Pref., 35.35°N, 133.55°E, 910 m, 30 Sep.-29 Oct. 2017, and 18 Apr. 2018 (F, emerged), Y. Matsui leg.; 1∂, Suemochi, Shikano Town, Tottori City, Tottori Pref., 35.45°N, 134.09°E, 220 m, 1 Jun. 2020, Y. Matsui leg.; 1⁽²⁾, Kôchi, Shikano Town, Tottori City, Tottori Pref., 35.40°N, 134.00°E, 495 m, 12 Jun. 2020, Y. Matsui leg.; 2♀1♂, ditto, 12–16 Aug. 2020 (F, emerged), Y. Matsui leg.; 1⁽²⁾, Sourokubara, Tottori City, Tottori Pref., 35.46°N,



Figure 5. Male genitalia of *Nagiella quadrimaculalis* **A** whole genitalia **B** gnathos, magnified view **C** apex of valva, magnified view.



Figure 6. Female genitalia of *Nagiella tristalis* sp. nov. **A** whole genitalia **B** anterior apophysis, magnified view **C** signum, magnified view.

134.11°E, 110 m, 19 Jul. 2020 (larvae: collected from *Rubus buergeri*), 20 Aug. 2020 (emerged), Y. Matsui leg.; 1♀1♂, Toyofusa, Daisen Town, Tottori Pref., 35.41°N, 133.55°E, 680 m, 2 Sep. 2020 (larvae: collected from *R. palmatus*), 19–20 Oct. 2020



Figure 7. Female genitalia of *Nagiella inferior* **A** whole genitalia **B** anterior apophysis, magnified view **C** signum, magnified view.

(emerged) Y. Matsui leg.; 1° , Yakawa, Okuizumo Town, Shimane Pref., 35.10° N, 133.13° E, 680 m, 6 Sep. 2013, Y. Matsui leg.; 2° , Omogokei, Kumakôgen Town, Ehime Pref., 33.72° N, 133.10° E, 700 m, 8 Jun. 2019, Y. Matsui leg.; 1° , Shimomyo, Aira-Cho, Kagoshima Pref., 28 May 1992, Y. Yanagita leg. (NSMT-I-L-75590); 1° , Tobi, Miyanojo-cho, Kagoshima Pref., 26 May 1992, Y. Yanagita leg. (NSMT-I-L-75591); 1° , Mt Ishizukadake, I. Yakushima, Kagoshima Pref., 5-6 Aug. 1958, B.T. leg. (NSMT-I-L-75599); 1° , same data as for preceding (NSMT-I-L-75600); 4° , ditto, 17 Jul. 1970, K. Tobi leg. (NSMT-I-L-75601 to 75604); 1° , same data as for preceding (NSMT-I-L-75605).

Diagnosis. Adult (Fig. 2E, F). Forewing length 15.5–18 mm. This species is similar to *N. tristalis* and *N. inferior*, but can be distinguished by the following characters: forewing longer; vertex scales brown, darker than in *N. inferior*; cilia cream white at Cu, to $A_{1,2}$ for forewing, Cu, to CuP for hindwing; subdiscal white spot of fore-



Figure 8. Female genitalia of *Nagiella quadrimaculalis* **A** whole genitalia **B** anterior apophysis, magnified view **C** signum, magnified view.

wing quadrilateral or rounded, rather distinct; base of discal cell of hindwing partially white; gnathos of male genitalia slender and elongated (Fig. 5B); valva of male genitalia broader than in *N. tristalis* and *N. inferior*, with straight dorsal margin subapically (Fig. 5C); anterior apophysis of female genitalia slightly curved, expanded near bases, but not triangular (Fig. 8B); signum of female corpus brusae larger than that of *N. tristalis* and *N. inferior* (diameter 0.16–0.19 mm), with sharp projections at both edges of posterior margin (Fig. 8C). Ullah et al. (2017) provided diagnostic characters to distinguish this species from *N. occultalis*. Lu and Du (2020) mentioned genital differences between these species, as far as we can see, the specimen image of *N. bispina* by Lu and Du (2020) is externally distinguishable from this species by the cilia of each wing being concolorous with the ground color.

Distribution. Japan, mainland China, Taiwan, Korea, Russia (southeast), Southeast Asia, Nepal, India.

Host plant. Rubus buergeri Miq., R. palmatus Thunb. (in the field), R. buergeri, R. trifidus Thunb. (laboratory reared).

Remarks. *Nagia incomitata* Swinhoe, 1894 has long been considered a synonym of *N. quadrimaculalis*, but based on the investigation of the type specimen, Lu and Du (2020) considered it likely to belong to *Nosophora* Lederer, 1863. We also follow this taxonomic treatment.

Our identification of this species in this study was based on external morphology (Kollar and Redtenbacher 1844; Inoue 1982; Li et al. 2012; Sasaki and Yamanaka 2013; Ullah et al. 2017; Lu and Du 2020) and male genitalia (Li et al. 2012; Ullah et al. 2017; Lu and Du 2020). The species status was confirmed by DNA barcodes.

Discussion

Recently, the integration of DNA barcoding and morphological approaches has accelerated various stages of taxonomic studies, such as species identification and description, re-investigation of taxa, as well as detecting cryptic species, also in Spilomelinae (Sutrisno 2005; Haines and Rubinoff 2012; Mally et al. 2016; Ullah et al. 2017; Ullah et al. 2017; Mally et al. 2019; Yang et al. 2019; Lu and Du 2020). In the genus *Nagiella*, these approaches led to the discovery of *N. occultalis* and *N. bispina* from China (Ullah et al. 2017; Lu and Du 2020), as well as *N. tristalis* from Japan (this study). Combined morphological and molecular biological studies might lead to the discovery of additional new species of this genus also in other regions.

The NJ tree shows *N. inferior* + *N. tristalis* and *N. quadrimaculalis* + *N. occultalis* to be sister groups (Fig. 1). The morphological evidence also supports these relationships, i.e., the gnathos of the male genitalia is short and triangular in *N. inferior* and *N. tristalis* (Figs 3B, 4B), while that of *N. quadrimaculalis* and *N. occultalis* is elongated (Fig. 5B); the signum of the female corpus bursae lacks projections in *N. inferior* and *N. tristalis* (Figs 6C, 7C), while that of *N. quadrimaculalis* has projections (Fig. 8C), although that of *N. occultalis* is unknown.

As the species of the genus *Nagiella* are very similar in appearance to each other (except for *N. hortulatoides*), DNA barcoding (see Material and methods) may provide very useful information for the identification of species in this genus. However, the species information for this genus in BOLD probably contains some misidentifications. For example, BOLD:AAD8178 cluster contains a record named "*Pleuroptya inferior*". This cluster can be identified as *N. quadrimaculalis* based on the results of this study and the specimen images in the deposited data. On the other hand, the sequences of *N. inferior* in this study corresponded to the BOLD:AAD8178 is probably a misidentification. The user must judge whether the information in the database is based on correct identifications or not.

Host plant records of *Rubus buergeri* and *R. sieboldii* Blume for *N. inferior*, and *R. buergeri* for *N. quadrimaculalis* were known from Japan (Sasaki and Yamanaka 2013). However, in these published host plant records the *Nagiella* species may have

been confused. In our laboratory, the three *Nagiella* species (including *N. tristalis*, reared from eggs) fed on *R. buergeri* and *R. trifidus*. In the field, we found larvae of *N. quadrimaculalis* feeding on *R. buergeri* and *R. palmatus*, and *N. tristalis* feeding on *R. buergeri*. In Tottori Prefecture, Japan, where the distribution of *N. tristalis* and *N. inferior* overlap, we could not find larvae of the latter species in the field, although where we found the larvae of the former species on *R. buergeri* in winter. This suggests that either 1) *R. buergeri* is not the native host plant of *N. inferior*, or that 2) *N. inferior* has a different overwintering strategy than *N. tristalis*. Contrary to our results, Fan and Piao (2013) recorded *Rhus chinensis* Mill. (Anacardiaceae) as a host plant for *N. quadrimaculalis*. The native host plants of the genus *Nagiella* needs further investigation.

The species of Nagiella have been placed in various genera, namely Coptobasis Lederer, Pleuroptya Meyrick, and Sylepta Hübner (e.g., Lederer 1863; Hampson 1899; Shibuya 1928; Inoue 1982; Irungbam et al. 2016). Munroe (1976) separated Nagiella from Pleuroptya and its related genera by the following characters: uncus short and wide, valva with a large oblique clasper, saccus relatively small and simple, and type of wing maculation (consisting of greyish ground color and a conspicuous white spot on each wing). Inoue (1982) placed N. inferior and N. quadrimaculalis in *Pleuroptya*, but no evidence for this treatment was provided. Inoue's opinion was followed by many authors (e.g., Li et al. 2012; Sasaki and Yamanaka 2013; Irungbam et al. 2016). Kirti and Gill (2007) treated Pleuroptya as a synonym for Patania Moore, because the male genitalia of their respective type species share congeneric characters as follows: valva leaf-like, uncus with a truncate posterior margin, gnathos absent, tuba analis elongate, and cornutus present in phallus. Ullah et al. (2017) regarded Nagiella as a valid genus based on the following characters: gnathos present, valva broader than that of Patania with stout subapical setae, and phallus without cornutus. However, several characters such as the presence or absence of a gnathos and the presence or absence of a cornutus in the phallus are shared with some *Patania* species. For example, P. clava Xu & Du, 2016 possesses a developed, finger-like gnathos (Xu and Du 2016), P. balteata (Fabricius, 1798) has an elongated gnathos (Leraut 2005; Slamka 2013), P. accipitralis (Walker, 1866) is missing cornuti in the phallus (Leraut 2005), and P. obfuscalis (Yamanaka, 1998) possesses a bunch of setae medially on the costa of the valva (Yamanaka 1998). In addition, the male genitalia of N. bispina described by Lu and Du (2020) lack the gnathos and possess a hook-shaped cornutus. Therefore, we tentatively regard *Nagiella* as a valid genus based on the following available characters: large oblique clasper, wing maculation, and host plants. Although in N. hortulatoides the wing maculation is different, this taxon is obviously included in Nagiella based on characters of the male genitalia as shown by the phylogenetic results of Lu and Du (2020). Although the separation of Nagiella has been accepted by many authors, such as Kirti and Sodhi (2001), Rose (2002), Mally et al. (2019), and Nuss et al. (2003–2020), further comprehensive genitalic studies and also molecular phylogenetic analyses are indispensable to reveal details of the taxonomic status of Patania, Pleuroptya, and Nagiella.

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References

- Bae YS (2001) Economic insects of Korea 9: Pyraloidea: Pyraustinae & Pyralinae (Lepidoptera). Insecta Koreana Suppl. 16: 1–251.
- Bae YS, Byun BK, Paek MK (2008) Pyralid Moths of Korea (Lepidoptera, Pyraloidea). Korea National Arboretum, Seoul, 148–149.
- Fan LH, Piao MH (2013) Larval descriptions for four species in the genus *Pleuroptya* (Lepidoptera: Crambidae: Spilomelinae) from China, with notes on biology. Entomotaxonomia 35: 45–52.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
- Haines PW, Rubinoff D (2012) Molecular phylogenetic of the moth genus Omiodes Guenée (Crambidae: Spilomelinae), and the origins of the Hawaiian lineage. Molecular Phylogenetics and Evolution 65: 305–316. https://doi.org/10.1016/j.ympev.2012.06.021
- Hampson GF (1899) A revision of the moths of the subfamily Pyraustinae and family Pyralidae. Proceedings of the Zoological Society of London 1898: 712–725.
- Inoue H (1982) Pyralidae. In: Inoue H, Sugi S, Kuroko H, Moriuti S, Kawabe A (Eds) Moths of Japan (Vols 1, 2), Kodansha, Tokyo, 1: 307–404; 2: 223–254; 36–48; 228: 296–314.
- Irungbam JS, Chib MS, Wangdi K (2016) Taxonomic review of the superfamily Pyraloidea in Bhutan (Lepidoptera). Journal of Asia-Pacific Biodiversity 9: 355–382. https://doi. org/10.1016/j.japb.2016.06.004
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581
- Kirti JS, Gill NS (2007) Revival of genus *Patania* Moore and reporting of a new species *menoni* (Pyraustinae: Pyralidae: Lepidoptera). Journal of Entomological Research 31: 265–275.
- Kirti JS, Sodhi JS (2001) A systematic list of Pyraustinae of northeastern India (Pyralidae: Lepidoptera). Zoos' Print Journal 16: 607–614. https://doi.org/10.11609/JoTT. ZPJ.16.10.607-14

- Kollar V, Redtenbacher L (1844) Aufzählung und Beschreibung der von Freiherrn Carl v. Hügel auf seiner Reise durch Kaschmir und das Himaleyagebirge gesammelten Insecten. In: Hügel C von (Ed.) Kaschmir und das Reich der Siek. Band 4. Hallbergersche Verlagshandlung, Stuttgart, 395–564.
- Kristensen NP (2003) Skeleton and muscles: adults. Handbook of Zoology 4: 39–132. https:// doi.org/10.1515/9783110893724.39
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https:// doi.org/10.1093/molbev/msw054
- Lederer J (1863) Beitrag zur Kenntnis der Pyralidinen. Wiener Entomologische Monatschrift 7: 429–430.
- Leraut P (2005) Contribution à l'étude des genres *Pyralis* Linnaeus, *Pleuroptya* Meyrick et *Haritalodes* Warren (Lepidoptera, Pyraloidea). Revue Française d'Entomologie 27: 77–94.
- Li H et al. (2012) Microlepidoptera of Qinling Mountains. Science Press, Beijing, 624–626.
- Lu XQ, Wan JP, Du XC (2019) Three new species of *Herpetogramma* Lederer (Lepidoptera, Crambidae) from China. ZooKeys 865: 67–85. https://doi.org/10.3897/zookeys.865.35111
- Lu XQ, Du XC (2020) Revision of *Nagiella* Munroe (Lepidoptera, Crambidae), with the description of a new species from China. ZooKeys 964: 143–159. https://doi.org/10.3897/ zookeys.964.55703
- Mally R, Segerer AH, Nuss M (2016) Udea ruckdescheli sp. n. from Crete and its phylogenetic relationships (Pyraloidea, Crambidae, Spilomelinae). Nota Lepidopterologica 39: 123–135. https://doi.org/10.3897/nl.39.9090
- Mally R, Hayden JE, Neinhuis C, Jordal BH, Nuss M (2019) The phylogenetic systematics of Spilomelinae and Pyraustinae (Lepidoptera: Pyraloidea: Crambidae) inferred from DNA and morphology. Arthropod systematics and Phylogeny 77: 141–204. https://doi. org/10.26049/ASP77-1-2019-07
- Motschulsky VI (1861) Insectes du Japon. Etudes entomologiques 9: 4–39.
- Munroe EG (1976) New genera and species of Pyraustinae (Lepidoptera: Pyralidae), mainly from the collection of the British Museum (Natural History). The Canadian Entomologist 108: 873–884. https://doi.org/10.4039/Ent108873-8
- Mutuura A (1957) Pyralidae. In: Esaki T (Ed.) Icones Heterocerorum Japonicorum in coloribus naturalibus. Hoikusha Publishing, Osaka, 318 pp. [pp. 93–147] [in Japanese]
- Nuss M, Landry B, Mally R, Vegliante F, Tränkner A, Bauer F, Hayden J, Segerer A, Schouten R, Li H, Trofimova T, Solis MA, De Prins J, Speidel W (2003–2020) Global Information System on Pyraloidea. http://www.pyraloidea.org/ [accessed 2 May 2020]
- Park KJ, Bae YS, Cuong NN, Nha PV, Vuong PT (2007) Moths of North Vietnam. Center for Insect Systematics, Junghaeng-Sa, 342 pp.
- Ratnasingham S, Hebert PD (2007) BOLD: the Barcode of Life Data System (http://www. barcodinglife.org). Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x
- Ratnasingham S, Hebert PD (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PLoS ONE 8: e66213. https://doi.org/10.1371/journal. pone.0066213

- Rose HS (2002) An inventory of the moth fauna (Lepidoptera) of Jatinga, Assam, India. Zoos' Print Journal 17: 707–721. https://doi.org/10.11609/JoTT.ZPJ.17.2.707-21
- Sasaki A, Yamanaka H (2013) Spilomelini. In: Nasu Y, Hirowatari T, Kishida Y (Eds) The Standard of Moths in Japan IV. Gakken Education Publishing, Tokyo, 74–84. [433–478.]
- Schindelin J, Arganda-Carreras I, Frise E, Kayni V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nature Methods 9: 676–682. https://doi.org/10.1038/nmeth.2019
- Shibuya J (1928) The systematic study on the Formosan Pyralidae. Journal of the Faculty of Agriculture, Hokkaido Imperial University 22: 228–229.
- Shibuya J (1929) On the known and unrecorded species of the Japanese Pyraustinae (Lepid.). Journal of the Faculty of Agriculture, Hokkaido Imperial University 25: 151–242.
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651– 701. https://doi.org/10.1093/aesa/87.6.651
- Slamka F (2013) Pyraloidea of Europe: Identification, Distribution, Habitat, Biology. Pyraustinae & Spilomelinae (Vol. 3). František Slamka, 357 pp.
- Sutrisno H (2005) Molecular phylogeny of *Agrioglypta* Meyrick and *Talanga* Moore (Lepidoptera: Crambidae; Spilomelinae) inferred from nuclear EF-1α Gene, Hayati Journal of Biosciences 12: 45–49. https://doi.org/10.1016/S1978-3019(16)30323-0
- Swinhoe C (1894) New Pyrales from the Khasia Hills. Annals and Magazine of Natural History, including Zoology, Botany and Geology, London (Series 6) 14: 197–210. https://doi.org/10.1080/00222939408677791
- Ullah M, Yang Z, Qiao P, Zhang Y (2017) A new cryptic species of *Nagiella* Munroe from China revealed by DNA barcodes and morphological evidence (Lepidoptera, Crambidae, Spilomelinae). ZooKeys 679: 65–76. https://doi.org/10.3897/zookeys.679.11960
- Ullah M, Dong Y, Qiao P, Zhang Y, Yang Z (2018) Delineating closely related species of *Tyloste-ga* Meyrick (Lepidoptera: Crambidae: Spilomelinae) from mainland China using DNA barcodes. Mitochondrial DNA Part A 29: 1121–1127. https://doi.org/10.1080/2470139 4.2017.1419213
- Walker F (1866) Supplement 4. List of the Specimens of Lepidopterous Insects in the Collection of the British Museum London 34: 1121–1533.
- Xu D, Du XC (2016) A new species of *Patania* from the Hainan Island, China (Lepidoptera, Crambidae). ZooKeys 614: 129–135. https://doi.org/10.3897/zookeys.614.8810
- Yamanaka H (1995) Pyralidae of Nepal (I). Tinea 14: 182–193.
- Yamanaka H (1998) Pyralidae of Nepal (II). Tinea 15 (Supplement I): 99-115.
- Yang Z, Ullah M, Landry JF, Miller SE, Rosati ME, Zhang Y (2019) Reassessment of the moth genus *Bacotoma*, with a new species from Hainan Island (Lepidoptera: Crambidae: Spilomelinae). Insect Systematics & Evolution 51: 384–407. https://doi. org/10.1163/1876312X-00002205