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A review of Gryllidae (Grylloidea) with the description of one new species and four new distribution records from the Sindh Province, Pakistan

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Abstract

Seventeen species of the family Gryllidae were reviewed and a *Modicogryllus sindhensis* is described herein as new. Four species, namely *Acheta hispanicus* Rambur, 1838, *Gryllus septentrionalis* F. Walker, 1869, *Callogryllus saeedi* Saeed, 2000, and *Miogryllus itaquiensis* Orsini & Zefa, 2017 are recorded as new country and state records. Differences between similar species and a taxonomic key to the species of Sindh are provided.

Keywords

Acheta, *Callogryllus*, *Miogryllus*, *Modicogryllus*, new distribution record, review, taxonomic key

Introduction

Crickets are representative of superfamily Grylloidea with six (four families: Myrmecophilidae, Gryllotalpidae, Mogoplistidae and Gryllidae) Baissogryllidae Gorochov, 1985, Gryllidae Laicharting, 1781, Mogoplistidae Costa, 1855, Phalangopsidae Blanchard, 1845, Protogryllidae Zeuner, 1937 and Trigonidiidae Saussure, 1874 (Cigliano

2021). The group dates back from the Triassic Period and today includes 3,700 for all species of orthopterans known living and 43 extinct species, 22 extant and 27 extinct subfamilies, and 528 extant and 27 extinct genera (Resh and Carde 2009). The Orthoptera Species File is a taxonomic database of the world's Orthoptera including grasshoppers, katydids, crickets, and related insects, both living and fossil. It has full taxonomic and synonymic information for more than 29,060 valid species and includes 47,500 scientific names and 106,200 specimen records.

Crickets live in virtually all terrestrial habitats from treetops to a meter or more beneath the ground. Field crickets live in oligotrophic, dry, barren habitats. Crickets are abundantly found at night but conceal themselves in thick vegetation, leaf litter, and under stones and rocks. Crickets are drab, or brightly and intensely coloured. Crickets have an incomplete metamorphosis with three life stages viz., egg, nymph, and adults. Females insert their eggs in soil and lay their egg on plants (Alexander 1962).

The classification of the Gryllidae has been established by Henri de Saussure in a remarkable monograph published in Geneva in the years 1877 and 1878. In this thorough work, the author points out the most important morphological characters and establishes the larger divisions of the group. Although a great number of species have been described since the publication of Saussure's work, it remains the basis of the modern classification of the Grylloidea. The Gryllidae are abundant throughout Sindh, the most cultivated region of Pakistan that are damaged by mole crickets, ground crickets, field crickets, house crickets, etc. The Gryllidae live in different types of habitats such as moist soil, herbs, shrubs, grasses, and vegetation. The fauna of Gryllidae from Sindh is insufficiently known. It was therefore felt necessary to revise the family from this region. Descriptions, taxonomic keys, and illustrations for all 17 known species are provided; bionomics and ecological accounts are also briefly discussed. In this manuscript we offer one new species and four new records from Pakistan, which aid in filling the gaps in our knowledge of the Gryllidae of Pakistan and bring information up to date.

Materials and methods

All specimens were collected from different agricultural crops in various districts of Sindh. Material was brought to Entomology and Bio-control Research Laboratory (**EBCRL**), Department of Zoology, University of Sindh, Jamshoro. Methodology for euthanasia was adapted from Vickery and Kevan (1983) and Riffat and Wagan (2015) with slight modifications: specimens were killed by using potassium cyanide or chloroform in standard entomological killing bottles for 5–10 minutes. Samples were not left longer because their colours could change.

Pinning of samples was done quickly after killing. An insect pin was inserted on the pronotum posterior to transverse sulcus, slightly to the right of the median carina. The head was directed slightly downwards on the stretching board. The left wings were set with the long axis of the body nearly at a right angle to the pin. The posterior legs were bent beneath the body to minimize the possibility of breakage and to occupy a smaller area. The abdomen was dropped below the wings and not obscured by the hind legs.

Fully dried specimens were preserved in insect cabinets with labels providing collection date, habitat, locality, and collector's name. Naphthalene balls ($C_{10}H_8$) were placed in boxes to prevent the attack of ants and other insects. Specimens were identified through the bibliographies given by Riffat and Wagan (2015), and Orthoptera Species File (**OSF**) (Cigliano et al. 2020) was consulted.

Photographs of the various species were prepared. Line drawings were made with a camera lucida fitted on a microscope (Ernst Leitz Wetzlar Germany 545187) and these were improved with the help of the softwares Adobe illustrator CC-2015 and Adobe Photoshop CS.

Measurements of various body parts were calculated in millimetres (mm) using the microscope (Oculas), 10 × 10 graph, compass, divider, and ruler. Abbreviations used in the text are as follows.

LH Length of head;
LF Length of femur;
LP Length of pronotum;
LT Length of tegmen;
LT Length of tibia;
LT length of tarsus;
TBL total body length;
TN Tag Number;
SEMJ Sindh Entomological Museum Jamshoro.

Species distributions were mapped using latitude and longitude information for available sites of species. The material (TN: 802 SEM) has been deposited in Sindh Entomological Museum Jamshoro (**SEMJ**), Department of Zoology, University of Sindh, Jamshoro. Pakistan.

Taxonomic account

Family Gryllidae
Subfamily Gryllinae
Tribe Gryllini
Genus *Acheta* Linnaeus, 1758

***Acheta domesticus* (Linnaeus, 1758)**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov**. • 2♂, 8♀; Riffat, Surriya; 28 Aug. 2019; Mithi 24.7436°N, 69.8061°E, 11♂, 17♀; Riffat, Surriya; 30 Aug. 2019; Naushahro feroze

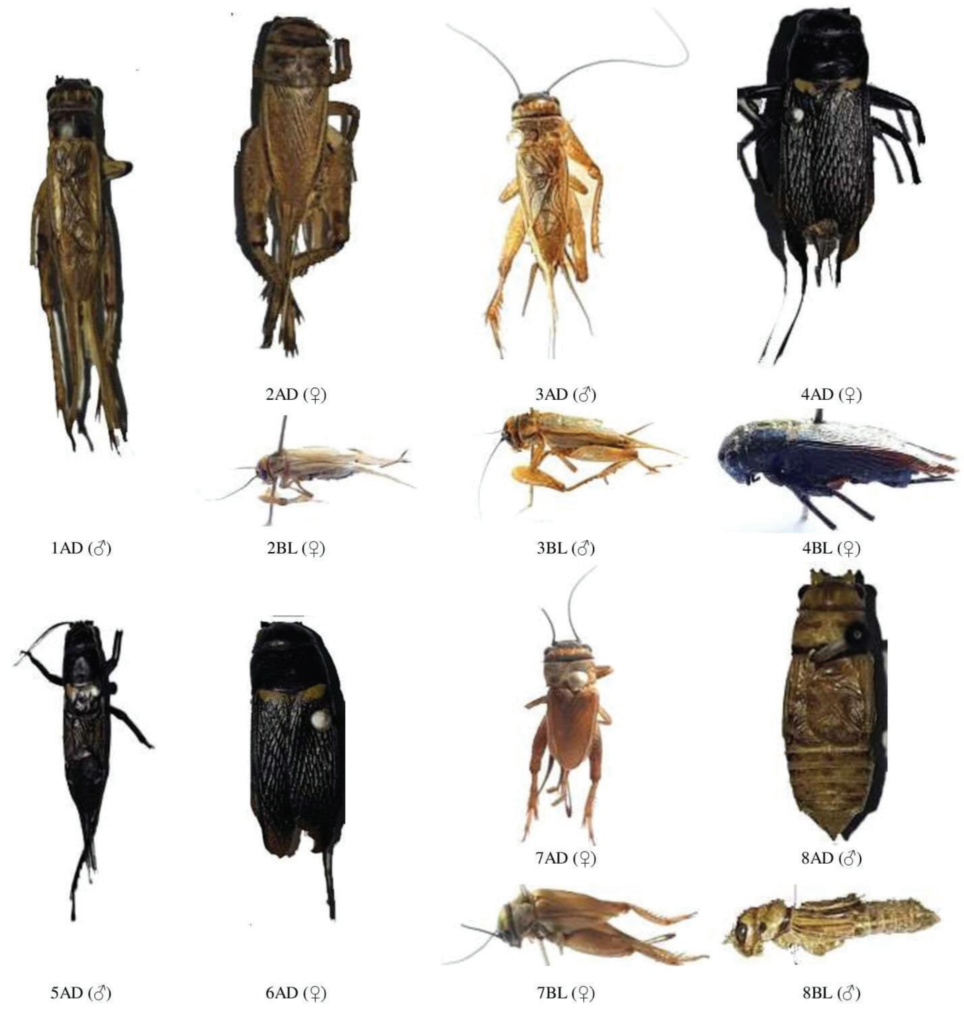

Figure 1. A male and female dorsal and lateral view of Gryllidae species. Subfamily Gryllinae: 1, 2 *Acheta domesticus* ♂♀, 3 *A. hispanicus* ♂, 4, 5 *Gryllus (Gryllus) bimaculatus* ♀♂, 6 *G. (Gryllus) campestris* ♀, 7 *G. septentrionalis* ♀, 8 *Gryllodes sigillatus* ♂ **B** male and female dorsal and lateral view of Gryllidae species. Subfamily Gryllinae: 9 *Gryllodes supplicans* ♀, 10 *Teleogryllus (Brachyteleogryllus) occipitalis* ♀, 11,12 *T. (Brachyteleogryllus) commodus* ♂♀, 13 *Modicogryllus sindhensis* sp. nov. ♀, 14 *Svercus palmetorum* ♀ **C** male and female dorsal and lateral view of Gryllidae species. Subfamily Gryllinae: 15 *Miogryllus itaquiensis* ♀, 16 *Callogryllus saeedi* ♀, 17 *C. ovilongus* ♀, 18 *C. bilineatus* ♀, 19 *Lepidogryllus siamensis* ♀, Subfamily Oecanthinae: 20 *Oecanthus fultoni* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

26.8463°N, 68.1253°E, 3♀; Surriya, Riffat; 3 Sep. 2019; Chachro 25.1156°N, 70.2557°E, 5♂, 11♀; Riffat, Surriya; 11 Sep. 2019; Umerkot 25.3549°N, 69.7376°E, 5♂, 16♀; Surriya, Riffat; 12 Sep. 2019; Nara 34.6851°N, 135.8048°E, 12♂, 24♀; Surriya, Riffat; 17 Sep. 2019; Nagarparkar 24.3572°N, 70.7555°E, 1♂, 4♀; 14 Aug. 2019; Tharparkar

Table 1. Distribution of Gryllidae species in different areas of Sindh, with numbers collected at each locality.

Species	Mithi	Naushahro feroze	Chachro	Umerkot	Nara	Nagarkarkar	Tharparkar	Sanghar	Islamkot
Acheta domesticus	10	28	03	16	21	36	05	11	09
Acheta hispanicus	01	—	—	—	—	—	—	—	—
Gryllus (Gryllus) bimaculatus	09	02	07	12	02	22	17	04	15
G(Gryllus) campestris	—	—	08	33	03	19	23	—	11
Gryllus septentrionalis	—	—	—	01	—	—	—	—	—
Gryllodes sigillatus	02	09	18	24	—	13	05	—	—
Gryllodes supplicans	—	—	—	01	02	—	—	—	—
Callogryllus saeedi	—	—	—	—	—	—	—	05	—
Callogryllus ovilongus	—	—	—	—	—	04	—	—	—
Callogryllus bilineatus	—	—	—	—	—	—	—	—	02
Modicogryllus sindhensis	—	—	—	01	—	—	—	—	—
Teleogryllus (Brachyteleogryllus) occipitalis	01	—	—	—	—	—	—	—	—
T.(Brachyteleogryllus) commodus	—	—	—	—	—	02	—	—	—
Lepidogryllus siamensis	—	—	—	01	—	—	—	—	—
Svercus palmetorum	—	—	—	—	—	—	02	—	—
Miogryllus itaquiensis	—	—	01	—	—	—	—	—	—
Oecanthus fultoni	—	—	—	01	—	—	—	—	—

24.8777°N, 70.2408°E, 2♂, 9♀; Riffat, Surriya; 16 Aug. 2019; Sanghar 26.0436°N, 68.9480°E, 1♂, 8♀; Riffat, Surriya; 17 Aug. 2019; Islamkot 24.7014°N, 70.1783°E.

Description. Medium size, pubescent and deep. General colouration light fulvous or testaceous (Fig. 1A). Head brown with two variables extending testaceous bands (Fig. 2A, B). Pronotum adorned with two large brown bands (Fig. 4A, B). Elytra extending to the apex of abdomen. Wings usually larger than the elytra (Fig.8A, B). Legs yellowish with a few brown spots. Posterior tibia armed with eleven spines on the basal side (Fig. 6A, B. Ovipositor large and acute.

Male: LH 2.25 ± 0.15 (mm), LP 3.5 ±1.4 (mm), LT 4.5 ± 1.73 (mm), LF 11.0 ± 2.08 (mm), LT 6.01 ± 1.0 (mm), LT 4.9 (mm), TBL 15.33 ± 4.2 (mm) **Female:** LH 3.26 ± 2.8 (mm), LP 3.83 ± 1.50 (mm), LT 4.7 ± 1.23 (mm), LF 14.0 ± 4.11 (mm), LT 7.33 ± 2.06 (mm), LO 10.66 ± 2.94 (mm), TBL 16 ± 3.05 (mm).

Ecology. *Acheta domesticus* is broadly distributed in the field. They complete their life cycle within 60–70 days. Agricultural crops affected by this species are *Tritium aestivum* (wheat), *Oryza sativa* (rice), *Sacharum officinarium* (sugarcane), and *Dactyloctenium aegyptium* (common lawn grasses).

Global distribution. Czech Republic, Greece, Peloponnese, Patras, Yugoslavia, Serbia, USA, India, Pakistan (Cigliano et al. 2020).

Remarks. *Acheta domesticus* is generally recognised as the house cricket, cosmopolitan in nature. The presence of this species was reported by Chopard (1969) from Himalayas, Srinagar, and Kashmir, at 6000 ft a.s.l. Previously, Ghouri (1961) stated that *A. domesticus* and other species were severe pests of many crops in Pakistan, and Malik (2012) also stated it from human habitation. At present we have recorded this species from Chachro (25.1156°N, 70.2557°E). We have collected large numbers of specimens from agricultural fields and confirm that it is a pest of various crops.

Figure 2. Male and female head dorsal view of Gryllidae species. Subfamily Gryllinae: 1, 2 *Acheta domesticus* ♂♀, 3 *A. hispanicus* ♂, 4, 5 *Gryllus (Gryllus) bimaculatus* ♂♀, 6 *G. (Gryllus) campestris* ♀, 7 *G. septentrionalis* ♀, 8-*Gryllodes sigillatus* ♂, 9 *Gryllodes supplicans* ♀, 10 *T. (Brachyteleogryllus) commodus* ♂. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

Acheta hispanicus Rambur, 1838

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♂; Riffat, Surriya; 23 Aug. 2019; Mithi 24.7436°N, 69.8061°E.

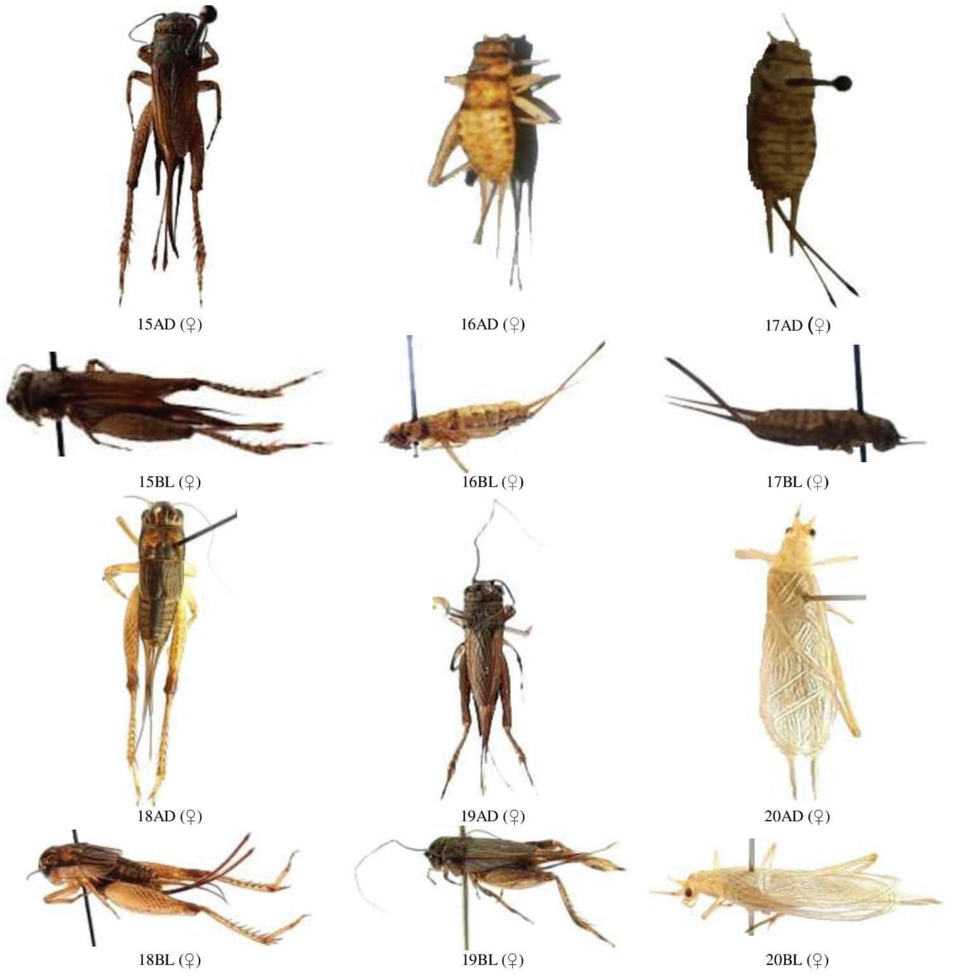

Figure 3. Male and female head dorsal view of Gryllidae species, subfamily Gryllinae: 11 *T. (Brachyteleogryllus) commodus* ♀, 12 *Modicogryllus sindhensis* sp. nov. ♀,13 *Svercus palmetorum* ♀, 14 *Miogryllus itaquiensis* ♀, 15 *Callogryllus saeedi* ♀, 16 *C. ovilongus* ♀, 17 *C. bilineatus* ♀, 18 *Lepidogryllus siamensis* ♀. Subfamily Oecanthinae: 19 *Oecanthus fultoni* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

Description. Rather large and robust, colouration brownish-yellow (Fig. 1C). Head blackish with shining occiput (Fig. 2C). Pronotum unicolourous, concave, very slightly widening; anterior and posterior margins almost straight with numerous spots (Fig. 1C). Elytra extending to the apex of abdomen, mirror small, obliquely transverse (Fig. 8C). Wings long. Legs pale yellowish with numerous hairs. Tibia with eleven pointed spines on either side (Fig. 6C). Abdomen yellow, pubescent. Cerci well developed, pointed.

Male: LH 2.17 (mm), LP 2.66 (mm), LT 13 (mm), LF 11 (mm), LT 08 (mm), LT 4.9 (mm), TBL 28 (mm).

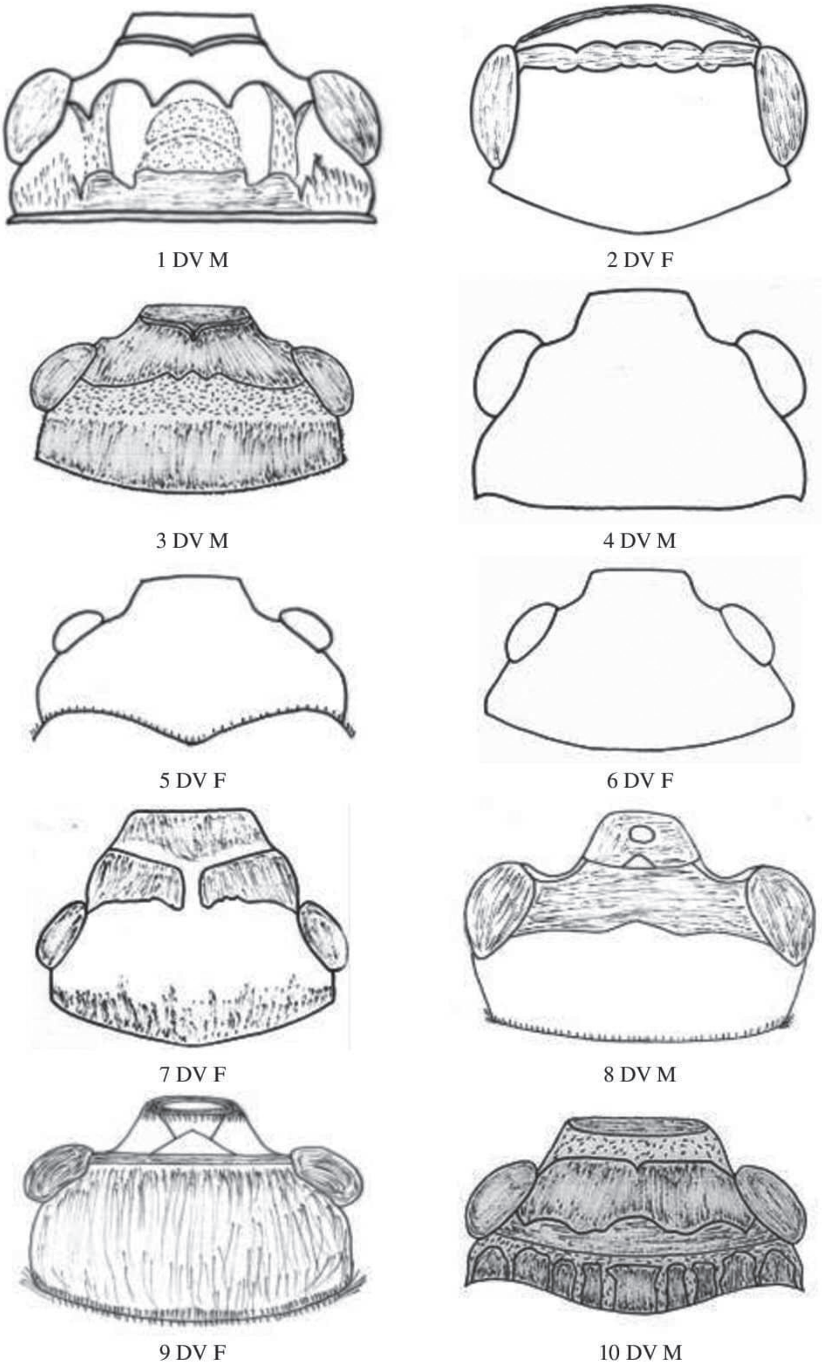

Figure 4. Male and female pronotum dorsal view of Gryllidae species, subfamily Gryllinae: 1, 2 *Acheta domesticus* ♂♀, 3 *A. hispanicus* ♂, 4, 5 *Gryllus (Gryllus) bimaculatus* ♂♀, 6 *G. (Gryllus) campestris* ♀, 7 *G. septentrionalis* ♀, 8 *Gryllodes sigillatus* ♂, 9 *Gryllodes supplicans* ♀, 10 *Teleogryllus (Brachyteleogryllus) occipitalis* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

Ecology. The species was recorded from Mithi. Usually, they are found in ditches of soil in rice fields. Weissman et al. (1980) reported that the adults seemed to appear in August but were abundant mid-August to September with a decline observed in October.

Global distribution. Portugal, Spain: Granada, India, Pakistan (Cigliano et al. 2020).

Remarks. This species is a new record from Sindh, Pakistan, and also for Asia. The body is wide and robust in structure compared to the more widely distributed *A. domesticus*. In our collection only a single male was captured, so more extensive collections are needed to establish its complete distribution.

Genus *Gryllus* Linnaeus (1758)

Gryllus (*Gryllus*) *bimaculatus* De Geer, 1773

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 5♂, 4♀; Surriya, Riffat; 21 Aug. 2019; Mithi 24.7436°N, 69.8061°E, 2♀; Riffat; Naushahro feroze 26.8463°N, 68.1253°E, 3♂, 4♀; Riffat, Surriya; 12 Sep. 2020; Chachro 25.1156°N, 70.2557°E, 4♂, 8♀; Surriya, Riffat; 19 Sep. 2020; Umerkot 25.3549°N, 69.7376°E, 2♀; Riffat; 20 Aug. 2020; Nara 34.6851°N, 135.8048°E, 6♂, 16♀; Surriya; 24 Aug. 2020; Nagarparkar 24.3572°N, 70.7555°E, 6♂, 11♀; Riffat, Surriya; 23 Aug. 2020; Tharparkar 24.8777°N, 70.2408°E, 1♂, 3♀; Riffat; 26 Aug. 2020; Sanghar 26.0436°N, 68.9480°E, 3♂, 8♀; Riffat, Surriya; 27 Aug. 2020; Islamkot 24.7014°N, 70.1783°E.

Description. Large size, stout. Colour blackish. Head curved feebly at anterior; wider at posterior (Fig. 1D, E). Pronotum concave with piriform impression on anterior disc (Fig. 4D, E). Elytra reach to the top of abdomen, wings much long (Fig. 8D, E). Legs dark brown and strongly pubescent (Fig. 1D, E). Posterior femora rather thick, dark brown with rufous base; posterior tibia with eight spines on superior margin (Fig. 6D, E). Ovipositor rather long and slender, feebly curved with very narrow, smooth, acute apical valves (Fig. 1D, E).

Male: LH 2.25 ± 0.15 (mm), LP 3.45 ± 0.057 (mm), LT 4.1 ± 1.5 (mm), LF 14.5 ± 0.57 (mm), LT 11.0 ±1.15 (mm), LT 4.2 (mm), TBL 22.5 ± 0.57 (mm) **Female:** LH 4.76 ± 0.74 (mm), LP 4.66 ± 0.35 (mm), LT 4.5 ± 1.63 (mm), LF 15.33 ± 0.57 (mm), LT 11.66 ± 0.816 (mm), LO 18.5 ± 0.57 (mm), TBL 16 ± 3.05 (mm).

Ecology. This species frequently occurred in the field. Plants affected by this species are *Tritium aestivum* (wheat), *Oryza sativa* (rice), *Sacharum officinarium* (sugarcane), and *Echinochloa colonum* (jungle rice). This species is hemimetabolous and moults 8–11 times to become adult (pers. obs.).

Global distribution. Ukraine, France, Spain, USA, India, West Bengal, Kashmir, Pakistan, Mali (Cigliano et al. 2020).

Remarks. *Gryllus bimaculatus* is variable in size with colour variations. During this study we collected this species from dry parts of Nagarparkar and confirm its presence in dry barren areas. Chopard (1969) reported that *G.* (*Gryllus*) *bimaculatus* causes severe damage to potato plants.

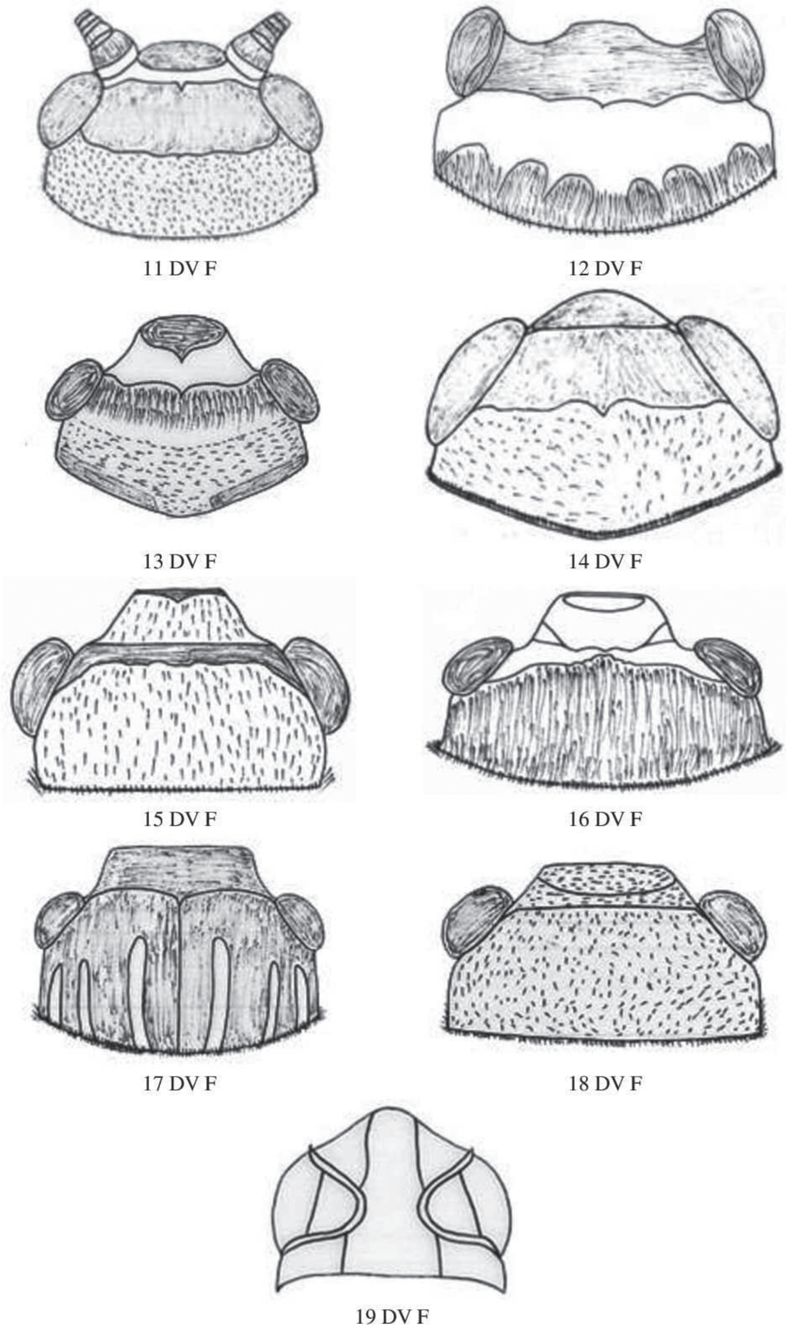

Figure 5. Male and female pronotum dorsal view of Gryllidae species, subfamily Gryllinae: 11, 12 *T. (Brachyteleogryllus) commodus* ♂♀, 13 *Modicogryllus sindhensis* sp. nov. ♀,14 *Svercus palmetorum* ♀, 15 *Miogryllus itaquiensis* ♀, 16 *Callogryllus saeedi* ♀, 17 *C. ovilongus* ♀,18 *C. bilineatus* ♀, 19 *Lepidogryllus siamensis* ♀, Subfamily Oecanthinae: 20 *Oecanthus fultoni* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

G*ryllus* (*Gryllus*) *campestris* Linnaeus, 1758
Figure 1–11, Table 1

Material examined. Pakistan, **Sindh Prov**. • 2♂, 6♀; Riffat; 12 Jul. 2019; Chachro 25.1156°N, 70.2557°E, 10♂, 23♀; Riffat, Surriya; 17 Jul. 2019; Umerkot 25.3549°N, 69.7376°E, 3♀; Riffat; 18 Aug. 2019; Nara 34.6851°N, 135.8048°E, 7♂, 12♀; Surriya, Riffat; 27 Aug. 2019; Nagarparkar 24.3572°N, 70.7555°E, 8♂, 15♀; Riffat, Surriya; 8 Jul. 2019; Tharparkar 24.8777°N, 70.2408°E, 4♂, 7♀; Surriya, Riffat; 3 Sep. 2020; Islamkot 24.7014°N, 70.1783°E.

Description. A large species, rather similar to *G.* (*Gryllus*) *bimaculatus*, but more rounded and curved (Fig. 1F). Head yellowish brown with patches and raised veins (Fig. 2F). Pronotum convex above, blackish brown with fine greyish pubescent; posterior margin sinuated; elytra extending to the apex of the abdomen (Fig. 4F), legs blackish testaceous with brown spots, pubescent. Posterior femora rather short and thick; posterior tibia armed with six spines on each margin (unfortunately broken of during photography). Abdomen brown, ovipositor long, slender with narrow, very acute apical valves (Fig. 1F).

Female: LH 4.6 (mm), LP 4.9 (mm), LT 18 (mm), LF 15, LT 13, TBL 29 (mm).

Ecology. *Tritium aestivum* (wheat), *Oryza sativa* (rice), *Sacharum officinarium* (sugarcane), *Echinochloa colona* (cultivated field) are all affected by this pest. It seems rare in numbers, and not widely occurring like other species of Gryllidae. These specimens were collected from rice fields whereas other plants such as sugarcane and wheat were also present, but with minor damage.

Global distribution. Denmark, Germany, Netherlands, Switzerland, UK, Pakistan (Cigliano et al. 2020).

Remarks. Due to its rare status and sporadic nature *G.* (*G.*) *campestris* is included in the red lists Hochkirch et al. (2007). It is flightless in its habitat of dune, short grasses, chalky soil, and light sandy porous soils. During our field survey we collected material from different districts. Our examination demonstrates that this species has morphological similarity to *G.* (*Gryllus*) *bimaculatus* but few differences in wing pattern and head morphology identifies each species.

***Gryllus septentrionalis* F. Walker, 1869**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♀; Riffat, Surriya; 21 Jul. 2019; Mahendrani, Umerkot 25.3549°N, 69.7376°E.

Description. Medium size, colouration rufous brown, rather strongly pubescent (Fig. 1G). Head long, rounded without any ornamentation. Face brown with yellow horizontal band; ocelli big, brown (Fig. 2G). Pronotum slightly enlarged in front, anterior margin feebly concave, posterior one pointed; disc convex, rufous with two

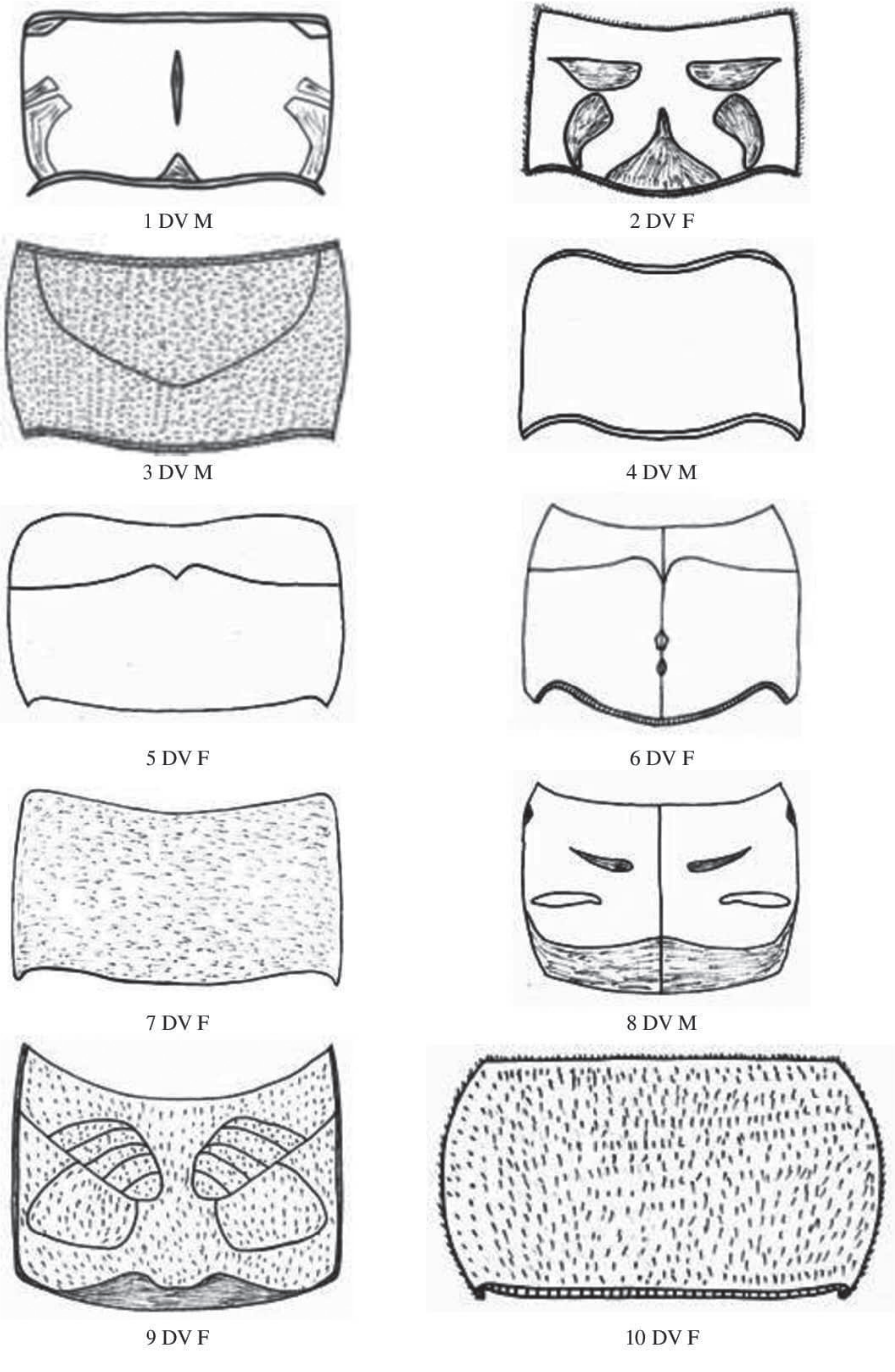

Figure 6. Femur and Tibia dorsal view of Gryllidae species, subfamily Gryllinae: 1, 2 *Acheta domesticus* ♂♀, 3 *A. hispanicus* ♂, 4, 5 *Gryllus (Gryllus) bimaculatus* ♂♀, 6 *G. septentrionalis* ♀, 7 *Gryllodes sigillatus* ♂, 8 *Gryllodes supplicans* ♀, 9 *Teleogryllus (Brachyteleogryllus) occipitalis* ♀, 10, 11 *T. (Brachyteleogryllus) commodus* ♂♀, 12 *Modicogryllus sindhensis* sp. nov. ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

large piriform impressions; lateral lobes with yellowish inferior part (Fig. 4G). Elytra brownish, reaching to apex of abdomen; dorsal fields with slightly oblique veins, rather projecting. Wings long (Fig. 9A). Legs pubescent; anterior and medium femora rufous brown; anterior tibia with large slender external tympanum; only internal face depressed. Posterior femora rather long, swollen. Tibia shorter than femora, armed with nine basal spines, four on joint of metatarsus (Fig. 6F). Abdomen brown; ovipositor moderately long, rather slender with very acute apical valves (Fig. 1G).

Female: LH 3.9 (mm), LP 4.2 (mm), LT 18 (mm), LF 12.5 (mm), LT 08 (mm), LT 05 (mm), TBL 26 (mm).

Ecology. *Gryllus septentrionalis* was collected from the village of Mahendrani, Umerkot in August.It was noted that this field was surrounded by *Citrus* (lemon) crops and other wild vegetation. This study suggests that extensive surveys are needed.

Global distribution. Argentina, Paraguay, Caribbean, Jamaica, Pakistan (Cigliano et al. 2020).

Remarks. This is the first record from the deserts of Thar, Sindh, Pakistan. According to Saeed (2000), this species of cricket occurs in terrestrial habitats throughout the world, and mostly damages cotton, rice, millet, and sugarcane crops. Due to their predatory nature, they are also helpful in biological control, but more detailed investigations are needed to identify this strategy in future.

Genus *Gryllodes* Saussure, 1874

G*ryllodes sigillatus* Walker, 1869

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 2♀; Riffat; 14 Jul. 2020; Mithi 24.7436°N, 69.8061°E, 1♂, 8♀; Surriya, Riffat; 19 Jul. 2020; Naushahro feroze 26.8463°N, 68.1253°E, 3♂, 15♀; Riffat; 2 Sep. 2019; Chachro 25.1156°N, 70.2557°E, 9♂, 12♀; Riffat, Surriya; 13 Aug. 2020; Umerkot 25.3549°N, 69.7376°E, 6♂, 7♀; Surriya, Riffat; 16 Aug. 2020; Nagarparkar 24.3572°N, 70.7555°E, 5♀; Riffat, Surriya; 4 Sep. 2020; Tharparkar 24.8777°N, 70.2408°E.

Description. Medium size, depressed, rather strongly pubescent (Fig. 1H). Head brown with wider, transverse yellowish bands on dorsal field; anterior narrow band curved between ocelli; face short, yellow; clypeus spotted with brown, front with feeble suture (Fig. 2H). Pronotum transverse with concave anterior margin; disc almost straight; yellowish with wide brown band along posterior margin and a more or less important spot of the same colour on the impressus (Fig. 4H). Elytra extending to 1/3 of abdominal tergite, truncated, rounded at apex; mirror quite apical, little wider than long, rounded posteriorly; wings reduced (Fig. 9B). Abdomen brown in the male (Fig. 1H).

Male: LH 2.8 ± 0.72 (mm), LP 3.25 ± 0.62 (mm), LT 4.1 ± 5.2 (mm), LF 11.5 ±1.0 (mm), LT 8.0 ± 0.57 (mm), TBL 14.5 ± 1.0 (mm) **Female:** LH 2.10 ± 0.8 (mm), LP 3.32 ± 0.72 (mm), LT 4.3 ± 5.7 (mm), LF 12.5 ± 1.2 (mm), LT 8.2 ± 0.62 (mm), TBL 18.6 ± 2.1 (mm).

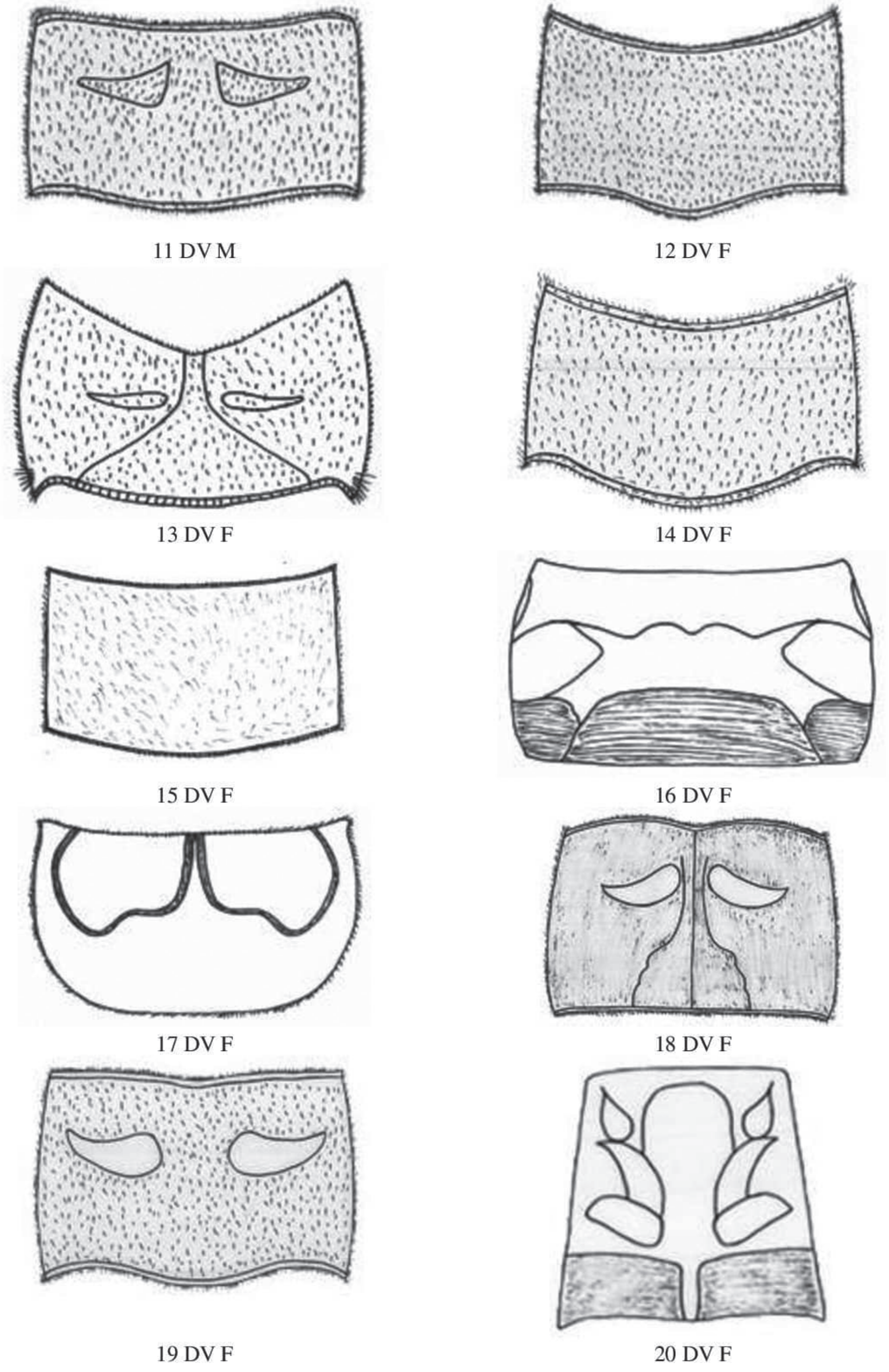

Figure 7. Femur and tibia dorsal view of Gryllidae species, subfamily Gryllinae: 13 *Svercus palmetorum* ♀, 14 *Miogryllus itaquiensis* ♀, 15 *Callogryllus saeedi* ♀, 16 *C. bilineatus* ♀, 17 *Lepidogryllus siamensis* ♀, Subfamily Oecanthinae: 18 *Oecanthus fultoni* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

Ecology. It commonly found everywhere but surprisingly, a single male only was reported during the present survey**.** Usually, this species is found in homes and lives under bricks and debris, and also in kitchens.

Global distribution. Australasia, Australia, Malaysia, West Bengal, USA, India, Pakistan (Cigliano et al. 2020).

Remarks. *Gryllodes sigillatus* is cosmopolitan in nature. This species is generally known as the tropical house cricket or Indian house cricket because they are found everywhere, domestic in all tropical countries. Khan (1954) reported that it caused huge damage to textiles mills in India. During our field survey we observed that this species moves at dusk from the holes of a termite mound. However, this species is not termitophilous in nature like other insects; it does not live with the termites.

Gryllodes supplicans **(Walker, 1859)**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 2♀; Riffat; 3 Jul. 2019; Nara 34.6851°N, 135.8048°E, 1♀; Surriya; 4 Jul. 2019; Umerkot 25.3549°N, 69.7376°E.

Description. Medium size, yellowish brown (Fig. 1I). Head small, narrow at the anterior, slightly curved at posterior. Face short, yellow with spotted clypeus. Frontal suture feebly arched (Fig. 2I). Pronotum transverse, feebly concave at anterior (Fig. 4I). Female elytra equilateral, reduced, extending to the extremity of abdomen. Wings caudate (Fig. 9C). Legs pubescent, yellowish, with few brown spots. Anterior tibia perforated on the external face with a rather long, oval tympanum (Fig. 6H). Abdomen brown with triangular median line on dorsal field. Ovipositor long, straight with narrow lanceolate apical valves (Fig. 1I).

Female: LH 3.15 (mm), LP 3.15 (mm), LT 4.2 (mm), LF 14 (mm), LT 10 (mm), LO 15 (mm), TBL 20 (mm).

Ecology. Annandale (1924) reported that this species lives in crevices, mostly occurring in wood and frequently in holes of bungalows. During the present study, we collected this species from a stack of wood from Umerkot.

Khan (1954) noticed that all females of Gryllidae deposit more than 150 eggs when temperatures are favourable, between 20–25 °C with the relative humidity of 80–82%. At present, only females were captured and is longer in total body length (20 mm) with the ovipositor ca. 15 mm compared to Chopards' (1969) report of total body length 12–15 mm and ovipositor 12–12.5 mm. This may be a geographical variant of the region; however, a detailed and comprehensive analysis of the taxa will be undertaken when more material will be collected.

Global distribution. America, Singapore, Berlin, Ceylon, India, Malaysia, China, Sri-Lanka, and Pakistan (Cigliano et al. 2020).

Remarks. Earlier, this species was collected by Chopard (1969) from various localities of India, but his specimens were smaller in size. The elytra of this species are

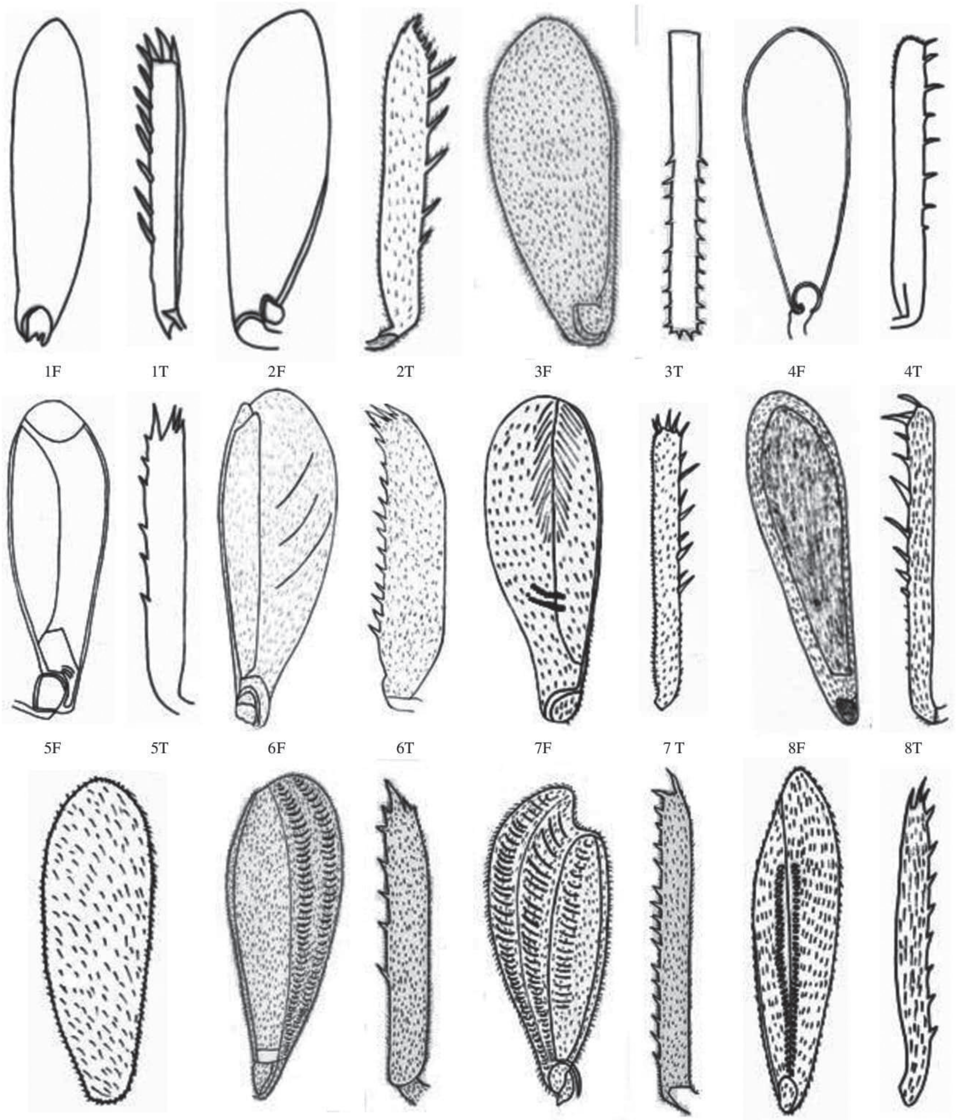

Figure 8. Male and female tegmen dorsal view of Gryllidae species, subfamily Gryllinae: 1, 2 *Acheta domesticus* ♂♀, 3 *A. hispanicus* ♂, 4, 5 *Gryllus (Gryllus) bimaculatus* ♂♀, 6 *G. (Gryllus) campestris* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

longer than those of *Sigillatus*, leading to the question of whether this species could be a macropterous form of the proceeding one. Considering the extreme reduction of the elytra of the female of *Sigillatus*, it seems difficult to admit the possibility of a return to fully winged form. However, future studies with more samples should resolve this problem.

Genus *Teleogryllus* Chopard, 1961

***Teleogryllus* (*Brachyteleogryllus*) *occipitalis* (Serville, 1838)**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♀; Riffat; 5 Sep. 2019; Mithi 24.7436°N, 69.8061°E.

Description. Medium to large size. Body pale brown (Fig. 1J). Head brown to dark with horizontal band at posterior margin. Ocelli dark brown (broken off while capturing photos). Pronotum dark brown, enlarged in front, its surface is rather strongly punctuated with numerous testaceous rufous spots (Fig. 4J). Female elytra extending to the apex of abdomen; elytral veins oblique, regularly spaced. Wings well developed with geometrical designs (Fig. 9D). Legs of the same colour as body; posterior femora moderately swollen, striated on external face; posterior tibiae armed with seven spines on each margin (Fig. 6I). Abdomen pale brown, yellowish beneath. Ovipositor long, slender (Fig. 1J).

Female: LH 2.1 (mm), LP 3.85 (mm), LT 08 (mm), LF 9 (mm), TBL 20 (mm).

Ecology. *Teleogryllus* is commonly known as black field cricket. Species of this genus are reported as a serious pasture pest in Australia and the warmer northern regions of New Zealand (Banfield and Cottier 1948; Reynolds and Langton 1973; Mill 1978). They reported that each year black field crickets cause considerable losses in pasture production over the dry summer period when stock feed is short. The resulting bare areas in the pasture are then opened to weed invasion because the black field crickets consume only pasture seed.

During the present study we captured only a single female from *Lolium perenne* grasses, which is considered as perennial ryegrass pasture, the main feed for dairy cows in temperate regions. This study suggests that preference of crickets for perennial ryegrass may lead high risk of damage to cultivated areas of Pakistan.

Global distribution. Sumatra, Java, Borneo, Philippines, Vietnam, Australia, Celebes, India, Bangladesh, Sri Lanka, Nepal, China, Burma, Malaysia, Singapore, Thailand, Pakistan (Cigliano et al. 2020).

Remarks. Until now 52 species of *Teleogryllus* were recorded by Cigliano et al. (2020). Gorochov (1985) reviewed the *Teleogryllus* species from Asia and established two subgenera. He moved *T. occipitalis* (Serville, 1838), *T. emma* (Ohmachi & Matsuura, 1951 *T. infernalis* (Saussure, 1877), *T. commodus* (Walker, 1869), and *T. oceanicus* (Le Guillou, 1841) into the subgenus *Brachyteleogryllus* with *T. occipitalis* as the type species, and he moved *T. mitratus* and *T. derelictus* into the subgenus *Macroteleogryllus* with the first as type species. Gorochov (1988) established another subgenus, *Afroteleogryllus*, with *T. clarus* as its type species from Africa, and added a further two new species in 1990. Otte (2006) downgraded genus *Cryncoides* as a subgenus under *Teleogryllus*. The remaining species are still in the pool of the subgenus *Teleogryllus* without having been studied again. In China, these crickets are often confused, and different species names have been used, until Ma et al. (2015) distinguished them by their genitalia. However, these changes are mainly based on morphological studies without molecular evidence.

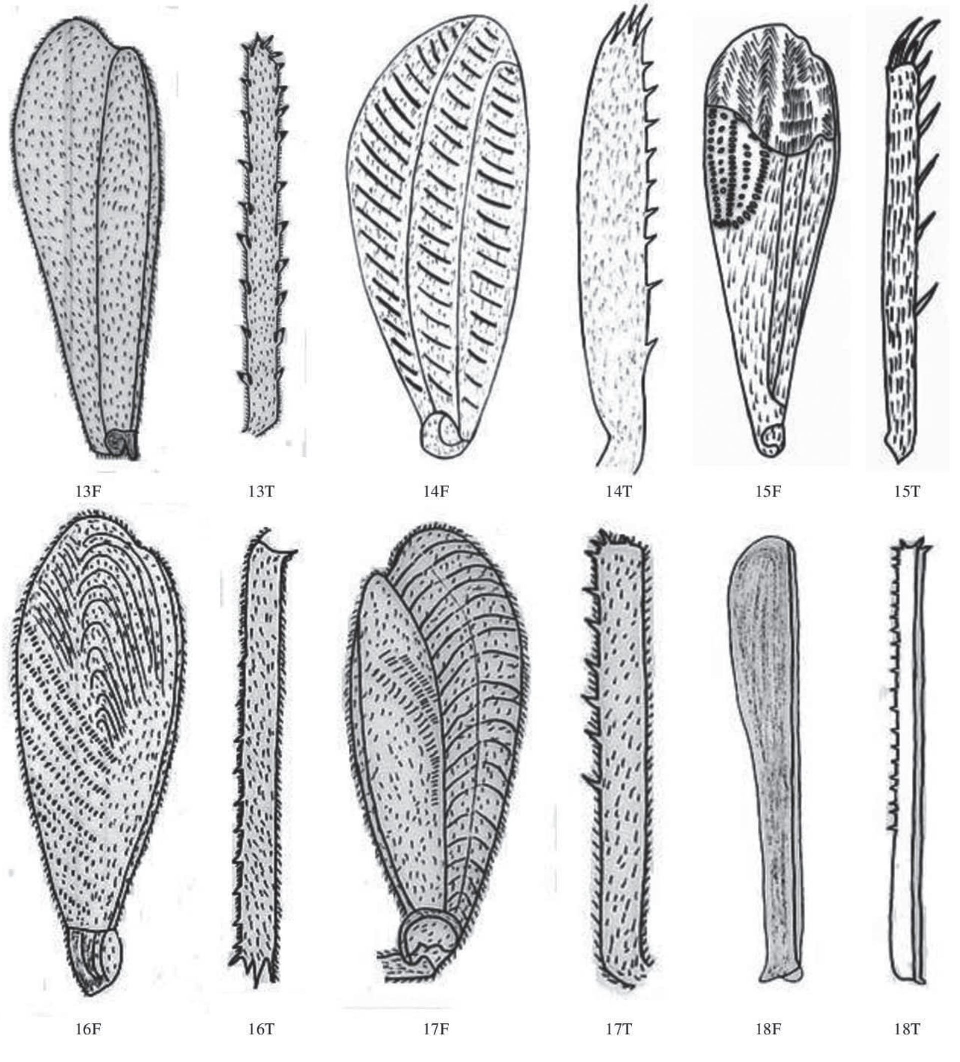

Figure 9. Male and female tegmen dorsal view of Gryllidae species, subfamily Gryllinae: 7 *G. septentrionalis* ♀, 8 *Gryllodes sigillatus* ♂, 9 *Gryllodes Supplicans* ♀, 10 *Teleogryllus (Brachyteleogryllus) occipitalis* ♀, 11, 12 *T. (Brachyteleogryllus) commodus* ♂♀, 13 *Modicogryllus sindhensis* sp. nov. ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

Teleogryllus (*Brachyteleogryllus*) *commodus* (Walker, 1869)

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♂, 1♀; Riffat, Surriya; 19 Aug. 2019; Nagarparkar 24.3572°N, 70.7555°E.

Description. Head short with vertical pale and dark bands at posterior margin (Fig. 1K, L). Ocelli dorsal field with dark horizontal band (Figs 2J, 3A). Pronotum dark brown, more or less varied fulvous, with black inferior margin (Fig. 5A, B). Elytra extending to the second last segment of abdominal tergite, a little rounded at apex; dorsal field shiny brown with a narrow yellowish band along external and apical margins; mirror reduced and somewhat broad. Wing long, extending to apex of abdomen (Fig. 9E, F). Legs rather short, widened, yellowish, mottled with brown and covered with abundant brown pubescence in which are mixed long bristles. Tibia rather thin, longer than femora, armed with seven internal spines (Fig. 6J, K). Abdomen pale brown with dark coloured. Ovipositor long, straight, with feebly flattened, acute apical valves, (Fig. 1K, L).

Male: LH 4.34 (mm), LP 4.06 (mm), LT 14 (mm), LF 12.6 (mm), LT 7.7 (mm), LT 07 (mm), TBL 21 (mm), **Female:** LH 2.5 (mm), LP 3.1 (mm), LT 11 (mm), LF 08 (mm), LT 7.4 (mm), LT 04 (mm), TBL 17 (mm).

Ecology. This species was reported from Nagarparkar. This area is surrounded by rock and fine sand. It was observed that due to burrowing habits this species uprooted many valued plants. This species is here reported from *Cymbopogon commutatus* which are perennial grasses and mostly used for medicinal purposes in the locality.

Global distribution. Australia, New Zealand, India, Pakistan (Cigliano et al. 2020).

Remarks. This species is commonly known as black field cricket. Its powerful legs are used for jumping. This species has numerous white strips on the abdomen which make it different from the other species. Zalitschek et al. (2012) reported that they are omnivores in nature. However, dietary requirements are similar but perform different functions depending upon the sex of the specimen: females take a protein-rich diet for the production of eggs while, male requires it for producing mating calls to attract females.

Genus *Modicogryllus* Chopard, 1961

Modicogryllus sindhensis sp. nov.

http://zoobank.org/E85E40CA-489A-41AA-9C18-94A8D0677CFC
Figures 1–11, Table 1

Material examined. ***Holotype.*** Pakistan, Sindh Prov. • 1♀; Riffat, Mohan leg.; 23 July 2019; Umerkot 25.3549°N, 69.7376°E. Reg. no.: 723 SEMJ.

Diagnosis. This species has a brightly coloured body along with a shiny pronotum. The tegmina and wing show different patches on their entire surface.

Description. Small size, covered in pubescence. Colour light brown (Fig. 1M). Head short, yellow, adorned with rufous spots, dorsal field of ocelli with pubescent horizontal dark bands (Fig. 3B). Pronotum depressed above with straight yellowish posterior margin on dorsal field (Fig. 5C). Elytra extending to apex of abdomen; veins of dorsal field rather irregular and condensed (Fig. 9G). Legs brownish. Pubescence rather thick, compressed. Anterior tibia bearing small, oval, external tympanum. Posterior tibia armed with ten external and one medio-internal spines (Fig. 6L). Abdomen brown. Ovipositor short, straight, slender with very small, lanceolate, acute apical valves (Fig. 1M).

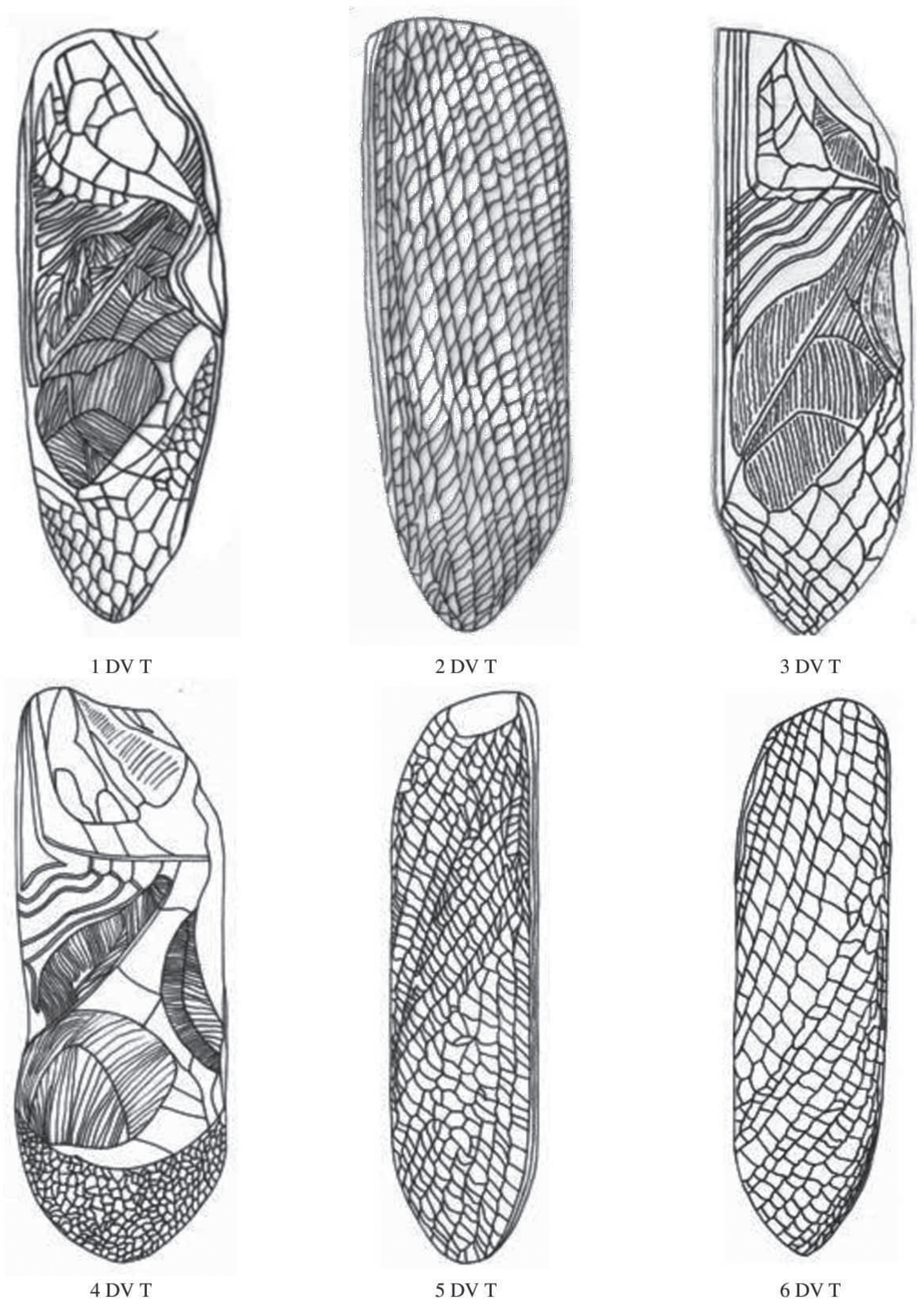

Figure 10. Male and female tegmen dorsal view of Gryllidae species, subfamily Gryllinae: 14 *Svercus palmetorum* ♀, 15 *Miogryllus itaquiensis* ♀, 16 *Callogryllus saeedi* ♀, 17 *C. ovilongus* ♀,18 *C. bilineatus* ♀, 19 *Lepidogryllus siamensis* ♀, Subfamily Oecanthinae: 20 *Oecanthus fultoni* ♀. Abbreviations: D, dorsal, L, lateral. Scale bars: 2 mm.

Female: LH 2.1 (mm), LP 2.45 (mm), LF 10 (mm), LT 11(mm), LO 10 (mm), TBL 15 (mm).

Habitat. The specimen was collected from *Sorghum vulgare* near Desert Thar (Umerkot) 25.3549°N, 69.7376°E.

Derivatio nominis. The specific epithet refers to collection of this species from Desert Thar of Sindh.

Depository. The type material (TN: 723 SEMJ) has been deposited in Sindh Entomological Museum, Department of Zoology, University of Sindh, Jamshoro.

Remarks. The genus *Modicogryllus* was erected by Chopard (1961), within which he described four species from north-east part of India viz: *M.semiobscurus* (Chopard), *M. ehsani* (Chopard), *M. rehni* (Chopard), and *M. minimus* (Chopard). Our collected species has a brightly coloured body along with a shiny pronotum. The tegmina and wing show different patches on their entire surface. However, the shape, length, and other characteristics of the ovipositor make it different from the other species in the genus.

Genus *Svercus* Gorochov, 1988

***Svercus palmetorum* (Krauss, 1902)**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 2♀; Surriya, Riffat; 22 Aug. 2020; Dahli, Tharparkar 24.8777°N, 70.2408°E.

Description. Medium size. Colouration rufous brown, shiny (Fig. 1N). Head little wider than pronotum in front; occiput convex with frontal rostrum narrow, ocelli united by a small oblique keel (Fig. 3C). Pronotum dark brown, slightly broader than long with concave anterior margin, posterior margin feebly convex (Fig. 5D). Elytra extending to the apex of abdomen, narrow posteriorly. Wing well developed (Fig. 10A). Legs testaceous brown, pubescent. Anterior tibia perforated on external face only. Posterior tibia armed with nine internal, 11 external, one medio-internal spines (Fig. 7A). Abdomen brown. Ovipositor rather long, straight with lanceolate apical valves (Fig. 1N).

Female: LH 1.8 (mm), LP 2.7 (mm), LT 9.6 (mm), LF 09 (mm), LT 6.6 (mm), LT 03 (mm), TBL 16 (mm).

Ecology. This species was collected from the village Dahli Taluka Tharparkar Sindh, Pakistan. This species was reported from *Larrea tridentate* called the creosote bush. It is a medium-sized evergreen shrub with pointed leaves and a waxy coating. This plant has great medicinal value, recommended to cure fever, colds, stomach, pains, arthritis, and as a general pain killer; it is also used for cuts, and bacterial and fungal infections.

Global distribution. Libya, Algeria, Pakistan (Cigliano et al. 2020).

Remarks. Reitmeier et al. (2012) reported this species from Corsica in humid places (except those that were recorded from Bonifacio and Filitosa in September 2010. They further identified the status of this species, its distribution, and life parameters. During our field survey we also noticed that this species occurs in humid places, but we were not able to study its life parameters.

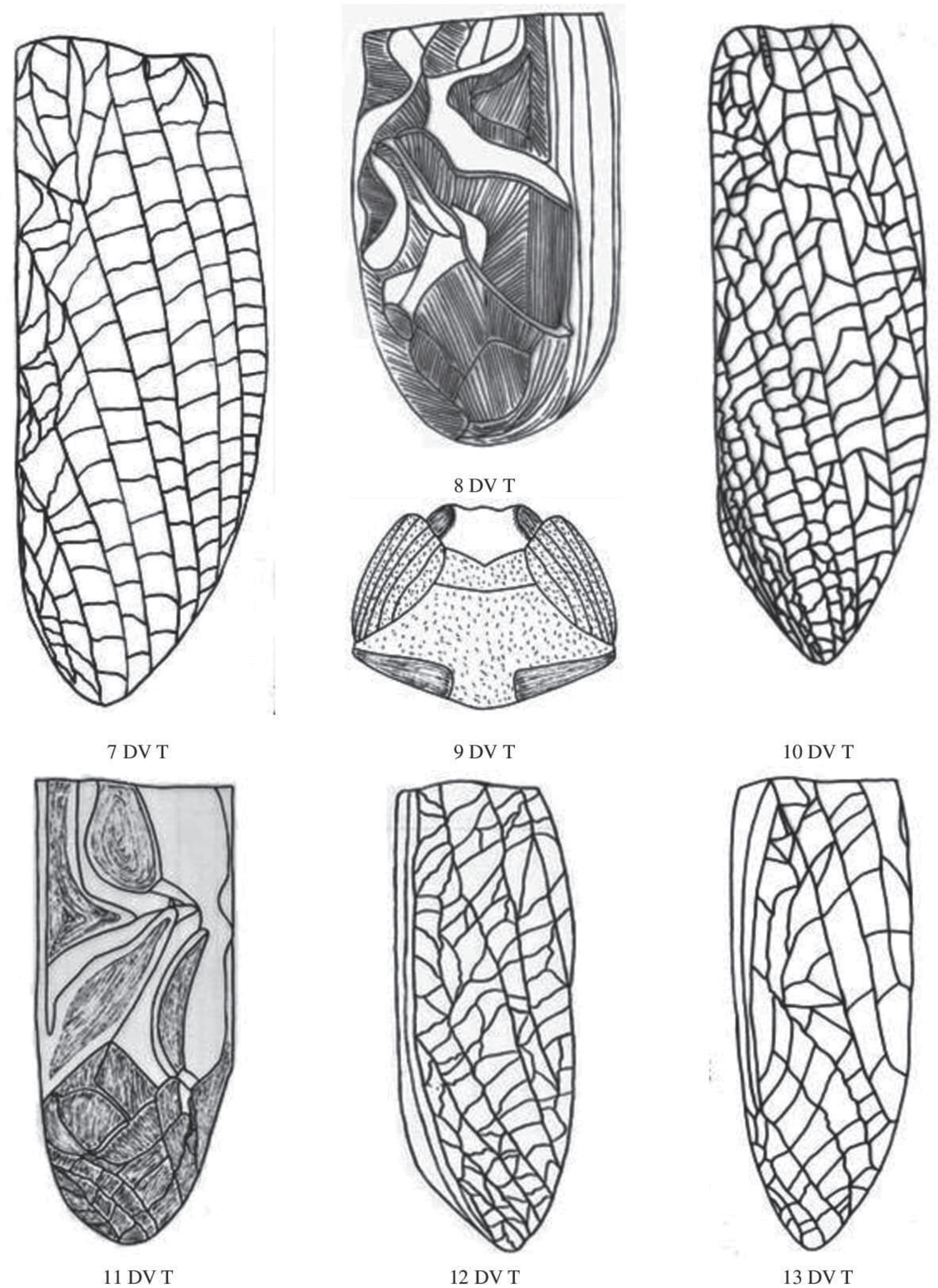

Figure 11. A map of Pakistan **B** map of Sindh province **C** areas within Sindh province. Maps reproduced by ArcGIS 10.5.

Genus *Miogryllus* Saussure, 1877

Miogryllus itaquiensis Orsini & Zefa, 2017

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♀; Riffat; 5 Sep. 2019; Chachro, Nagarparkar 24.3572°N, 70.7555°E.

Description. Medium size. Colouration brown (Fig. 1O). Head black bright and globous; whitish spot posteriorly containing scape and following inner margins of eyes, becoming punctuated with brown with white stripe before reaching occiput (Fig. 3D). Pronotum black with pubescence, dorsal disc wider than long, bristles on anterior and posterior margins; lateral lobes marked with antero-ventral whitish spot which becomes pale brown posteriorly (Fig. 5E). Elytra extending to two-thirds of abdomen, apical field well developed. Wing surpassing abdomen tip (Fig. 10B). Legs dark brown dorsally, whitish ventrally. Tibia armed with nine internal, four medio-internal spines (Fig. 7B). Abdomen black, sternites whitish. Cerci pale brown, short. Ovipositor long, slender, straight with lanceolate apical valves (Fig. 1O).

Female: LH 03 (mm), LP 3.1 (mm), LT 09 (mm), LF 10 (mm), LT 0.8 (mm), LT 4.2 (mm), TBL 12 (mm).

Ecology. This species was reported from Chachro, Nagarparkar on *Encelia farinose* roots. This plant is commonly known as the Brittle bush. It is a medium-sized, rounded shrub with long, oval, silvery grey leaves. The resin collected from this plant is used as glue (Hogan and Michael 2013); these authors also stated that Brittle bush treats toothaches. Some animals such as desert Bighorn sheep and Kangaroo rats eat its seeds.

Global distribution. Argentina, Brazil South, Rio Grande do Sul, Itaqui, Sindh, Pakistan (Cigliano et al. 2020).

Remarks. The pronotum of *M. itaquiensis* bears a whitish lateral lobe, while *M. tucumanensis* has the pronotum with uniform colouration. We collected a single female for the first time from Chachro, Sindh, Pakistan. However, more extensive surveys are needed to explore its distribution in the desert region.

Genus *Callogryllus* Sjöstedt, 1910

Callogryllus saeedi (Saeed, 2000)

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 5♀; Surriya, Riffat; 23 Aug. 2020; Sanghar 26.0436°N, 68.9480°E.

Description. Medium size. Colouration yellow (Fig. 1P). Head short, narrow, yellowish shiny, adorned on each side with dark brown line extending from occiput, along eye (Fig. 3E). Pronotum as wide as long, barely widening anteriorly with two dark spots on dorsal field (Fig. 5F). Elytra reduced. No wings (Fig. 10C).

Legs yellowish, strongly pubescent. Anterior tibia perforated with oval tympanum on external face. Posterior femora rather thick, brown with rufous base, posterior tibia armed with six long external, four various medio-internal spines (Fig. 7C). Abdomen yellow with dark spots on each tergite. Ovipositor long, straight, slender (Fig. 1P).

Female: LH 2.1 (mm), LP 2.8 (mm), LT 03 (mm), LF 12 (mm), LT 10 (mm), LO 14 (mm), TBL 17 (mm).

Ecology. This species was previously reported by Saeed (2000) from *Triticum aestivum* in Pakistan. We reported the female from *Dactyloctenium aegyptium* grasses.

Global distribution. India (this study), Pakistan (Saeed et al. 2000).

Remarks. During this study, we have reported five females from Sanghar District which are a new record for Sindh province. Our thorough examination shows that this species is similar to *C. ovilongus* with the exception of a dark slanting band between the compound eyes, and the size of ovipositor: *C. saeedi* has a smaller ovipositor which is ca. 14 mm while *C. ovilongus* has a longer ovipositor, ca. 18–20 mm. In addition, the elytra of this female are quite different from those of *C. ovilongus*.

Callogryllus ovilongus Saeed & Yousuf, 2000

Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 4♀; Riffat, Surriya; 16 Sep. 2020; Nagarparkar 24.3572°N, 70.7555°E.

Description. Medium size. Colouration yellow (Fig. 1Q). Head short, narrow, very neat. Eyes rounded, moderately projecting; ocelli small (Fig. 3F). Pronotum 1.5 × as wide as long, slightly concave at anterior margin, straight at posterior margin; one side rather strongly convex (Fig. 5G). Elytra yellow, reduced (Fig. 10D). No wings. Legs light yellow, hind femora thick at base and slightly narrow at posterior, armed with six internal spines. Hind tibiae small, narrow, and straight. Abdomen dark yellowish above, pubescent and pale yellow beneath. Ovipositor rather long, very slender with extremely narrow, acute apical valves (Fig. 1Q).

Female: LH 3.85 (mm), LP 3.5 (mm), LT 5.2 (mm), LF 4.1 (mm), LO 15 (mm), TBL 16 (mm).

Ecology. During the present study, females of this species are reported from Nagarparkar, Desert Thar, from xerophytic plants which were surrounded by sagebrush and saltbush trees.

Global distribution. China, India, Bangladesh, Nepal, Pakistan (Cigliano et al. 2020).

Remarks. This species was erected by Saeed (2000) from Peshawar, KPK based on a single female specimen; subsequently Malik et al. (2013) reported its male from the Hyderabad -Sindh. We have a single female from the rocky area of Nagarparkar and confirm its presence in the desert area.

***Callogryllus bilineatus* (Bolívar, 1900)**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 2♀; Riffat; 25 Aug. 2019; Islamkot 24.7014°N, 70.1783°E.

Description. Medium size. Colouration brown to yellowish (Fig. 1R). Head brown, short, dome-shaped with four yellowish vertical sutures (Fig. 3G). Pronotum brown, concave anteriorly while pubescent and convex posteriorly with longitudinal rufous bands on dorsal field (Fig. 5H). Elytra scarcely extending to apex of first abdominal tergite, slightly crossing at median line with internal oblique margin, apex rounded; dorsal field plain with straight veins at regular intervals; transverse veinlets very scarce; lateral field with four curved veins (Fig. 10E). Legs yellow, brownish at base, strongly pubescent, irregular bands on dorsal field. Posterior tibiae armed with eleven external, three medio-internal spines (Fig. 7D). Abdomen yellow to dark brown, longitudinal rufous bands on each side. Ovipositor very long, straight, apical valves with dark base (Fig. 1R).

Female: LH 3.6 (mm), LP 04 (mm), LT 05 (mm), LF 13.5 (mm), LT 10 (mm), LT 03 (mm), TBL 18 (mm).

Ecology. This species is recorded from wheat crops cultivated at Islamkot, Sindh. Weissman et al. (1980) observed that the hoppers emerged in the early days of June and continued to grow till mid-July. Adults were recorded from then to September. Peak period of species' occurrence was noted as mid-August to end of September. Thereafter, no individuals were observed in the field. High risk was reported to *Triticum* (wheat) crops from different areas of Islamkot, Sindh (reference).

Global distribution. India, Sindh, Pakistan (Cigliano et al. 2020).

Remarks. Chopard (1969) compiled a detailed account on this species: the head had the same pattern as *C. ovilongus*. The abdomen showed the longitudinal bands on both sides. The elytral length extended from the apex of the abdominal tergite. He calculated body length as 12 mm, pronotum 2.5 mm, elytra 2 mm, and ovipositor 9 mm. The collected specimens show variation in size as well as in other parameters, possibly due to geographical and feeding habitats. This species has unique characteristics, including the presence of a black band that runs from the pronotum where it makes a raised bulging cup-like structure; this black band covers the whole length of tegmen it follows a narrow straight line on the abdominal segments to the end of the last segment.

Tribe Modicogryllini
Genus *Lepidogryllus* Otte & Alexander, 1983

***Lepidogryllus siamensis* Chopard, 1961**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♀; Surriya; 27 Jul. 2019; Ramalani, Umerkot 25.3549°N, 69.7376°E.

Description. Medium size. Colouration dark brown (Fig. 1S). Head shiny brown, short, narrow, ocelli black, horizontal dark band between (Fig. 3H). Pronotum as long as head, 2 × wider than long on dorsal field, anterior and posterior margin pilose, truncated, dorsal surface brownish, mottled; lateral lobe of pronotum a little deeper than pronotal length (Fig. 5I). Elytra hardly reaching abdominal end. Wings well developed, with condensed veins (Fig. 10F). Legs brown, hind femora much longer than middle femora. Posterior tibia armed with seven external, three medio-internal spines, very wide at anterior, numerous patches on dorsal surface (Fig. 7E). Abdomen brown. Cerci long tapered. Ovipositor long, straight, with yellowish base (Fig. 1S).

Female: LH 1.96(mm), LP 2.03(mm), LT 9.5(mm), LF 5.6(mm), LT 07(mm), LT 04(mm), TBL 11(mm).

Ecology. This species was recorded for the first time from the village Ramalani, Umerkot, on the roots of *Acacia nilotia* locally known as "babul". This is a medium-sized, thorny, nearly evergreen tree found in the desert area. Generally, it grows to 20–25 mm but may remain shrubby in poor conditions. Our specimen was collected from a shrub. This tree provides limber, fuel, shade, food, dye, and gum, and it also impacts the environment positively through soil reclamation.

Global distribution. Korea, Japan, Taiwan, Thailand, India, Hawaii, China, Pakistan (Cigliano et al. 2020).

Remarks. *Lepidogryllus* has a very close morphological resemblance with *Velarifictorus*: the male has an enlarged round head with a swollen frons (Randell, 1964). Kim (2013) also reported the many similarities between these two genera. The species of these genera also have very significant variation in their morphometric parameters. Kim (2013) reported a body length of 14–15.2 mm in *L. siamensis*; we report a body length 11 mm.

Oecanthinae
Oecanthini
Genus *Oecanthus* Serville, 1831

***Oecanthus fultoni* Walker, 1962**
Figures 1–11, Table 1

Material examined. Pakistan, **Sindh Prov.** • 1♀; Riffat; 16 Aug. 2020; Umerkot 25.3549°N, 69.7376°E.

Description. Large size. Colouration light pale green to yellowish (Fig. 1T). Head short, narrow with dark brown ocelli (Fig. 3I). Pronotum flat, concave posteriorly (Fig. 5J). Elytra, transparent, extending to 2/3 of abdomen. Wings rounded, broad, with condensed irregular veins (Fig. 10G). Legs same colour as body. Femora long, thin, slightly wider at anterior and compressed at posterior. Posterior tibia thin, slender, armed with 21 external and three medio-internal spines (Fig. 7F). Abdomen pale yellowish. Ovipositor short. Cerci long with pointed ends (Fig. 1T).

Female: LH 1.96 (mm), LP 2.73 (mm), LT 14 (mm), LF 3.57 (mm), LT 3.85 (mm), TBL 22 (mm).

Ecology. *Oecanthus fultoni is* a new record from Umerkot, Desert Thar, Pakistan. This species is reported from *Cynadon dactylon* (common lawn grasses) surrounded by wild plants.

Global distribution. Ohio, Franklin, New Jersey, Washington, Pakistan (Cigliano et al. 2020).

Remarks. Walker and Gurney (1967) observed differences between populations of this species from the coasts of western and eastern USA showing that *O. fultoni* displays variations in the structure of the metanotal gland.

Key to the genera of *Gryllidae* of Sindh

1 Head brown with two variables extending testaceous bands (Fig. 2A, B). Pronotum adorned with two large brown bands (Fig. 4A, B)......................................**2**

_ Head curved feebly at anterior; wider at posterior (Fig. 1D, E). Pronotum concave with piriform impression on anterior disc (Fig. 4D, E)..............................**3**

2 Elytra extending to the apex of abdomen, mirror small, obliquely transverse (Fig. 8C). Wings long. Legs pale yellowish with numerous hairs. Tibia with eleven pointed spines on either side (Fig. 6C). Abdomen yellow, pubescent. Cerci well developed, pointed...***Acheta* Linnaeus**

_ Elytra extending to 1/3 of abdominal tergite, truncated, rounded at apex; mirror quite apical, little wider than long, rounded posteriorly; wings reduced (Fig. 9B). Legs pubescent, yellowish, with few brown spots. Anterior tibia perforated on the external face with a rather long, oval tympanum (Fig. 6H). Abdomen brown with triangular median line on dorsal field..**4**

3 Legs blackish testaceous with brown spots, pubescent. Posterior femora rather short and thick; posterior tibia armed with six spines on each margin (Fig. 1F). Abdomen brown, ovipositor long, slender with narrow, very acute apical valves (Fig. 1F)... ***Gryllus* Linnaeus**

_ Legs brownish, fuscous; posterior femora moderately swollen, striated on external face; posterior tibiae armed with seven spines on each margin (Fig. 6I). Abdomen pale brown, yellowish beneath. Ovipositor long, slender (Fig. 1J) **5**

4 Head small, narrow at the anterior, slightly curved at posterior. Face short, yellow with spotted clypeus. Frontal suture feebly arched (Fig. 2I). Pronotum transverse, feebly concave at anterior (Fig. 4I) ....***Gryllodes* Saussure**

_ Head short, yellow, adorned with rufous spots, dorsal field of ocelli with pubescent horizontal dark bands (Fig. 3B). Pronotum depressed above with straight yellowish posterior margin on dorsal field (Fig. 5C) **6**

5 Colour pale brown (Fig. 1J). Head brown to dark with horizontal band at posterior margin. Ocelli dark brown. Pronotum dark brown, enlarged in front, its surface is rather strongly punctuated with numerous testaceous rufous spots (Fig. 4J)..***Teleogryllus* Chopard**

_ Colour rufous brown, shiny (Fig. 1N). Head little wider than pronotum in front; occiput convex with frontal rostrum narrow, ocelli united by a small oblique keel (Fig. 3C). Pronotum dark brown, slightly broader than long with concave anterior margin, posterior margin feebly convex (Fig. 5D).......................................7

6 Legs brownish. Pubescence rather thick, compressed. Anterior tibia bearing small, oval, external tympanum. Posterior tibia armed with ten external and one medio-internal spines (Fig. 6L). Abdomen brown. Ovipositor short, straight, slender with very small, lanceolate, acute apical valves (Fig. 1M)......... ***Modicogryllus*** **Chopard**

_ Legs dark brown dorsally, whitish ventrally. Tibia armed with nine internal, four medio-internal spines (Fig. 7B). Abdomen black, sternites whitish. Ovipositor long, slender, straight with lanceolate apical valves (Fig. 10).............................. **8**

7 Elytra extending to the apex of abdomen, narrow posteriorly. Wing well developed (Fig. 10A). Ovipositor rather long, straight with lanceolate apical valves. Abdomen brown. (Fig. 1N) .... ***Svercus*** **Gorochov**

_ Elytra scarcely extending to apex of first abdominal tergite, slightly crossing at median line with internal oblique margin, (Fig. 10E). No wings. Ovipositor very long, straight, apical valves with dark base. Abdomen yellow to dark brown, longitudinal rufous bands on each side. (Fig. 1R).. **9**

8 Colour brown (Fig. 10). Head black bright and globous; whitish spot posteriorly containing scape and following inner margins of eyes, becoming punctuated with brown with white stripe before reaching occiput (Fig. 3D). Pronotum black with pubescence, dorsal disc wider than long, bristles on anterior and posterior margins; lateral lobes marked with antero-ventral whitish spot which becomes pale brown posteriorly (Fig. 5E) .... ***Miogryllus*** **Saussure**

_ Colour dark brown (Fig. 1S). Head shiny brown, short, narrow, ocelli black, horizontal dark band between (Fig. 3H). Pronotum as long as head, 2 × wider than long on dorsal field, anterior and posterior margin pilose, truncated, dorsal surface brownish, mottled; lateral lobe of pronotum a little deeper than pronotal length (Fig. 5I).. ***Lepidogryllus*** **Otte & Alexander**

9 Head brown, short, dome-shaped with four yellowish vertical sutures (Fig. 3G). Pronotum brown, concave anteriorly while pubescent and convex posteriorly with longitudinal rufous bands on dorsal field (Fig. 5H). Legs yellow, brownish at base, strongly pubescent, irregular bands on dorsal field. Posterior tibiae armed with eleven external, three medio-internal spines (Fig. 7D) ....
.... ***Callogryllus*** **Sjöstedt**

_ Head pale green, narrow with dark brown ocelli (Fig. 3I). Pronotum flat, concave posteriorly (Fig. 5J). Legs same colour as body. Femora long, thin, slightly wider at anterior and compressed at posterior. Posterior tibia thin, slender, armed with 21 external and three medio-internal spines (Fig. 7F)......................... ***Oecanthus***

Serville Keys to the species of *Gryllidae* of Sindh

1 Body colouration pale brown, fulvous, or testaceous (Fig. 1A). Head brown with 2 variably extending testaceous bands (Fig. 2A, B). Pronotum with 2 large brown spots (Fig. 4A, B). Posterior tibia armed with 11 spines on the basal side (Fig. 6A, B).. ***Acheta domesticus*** **Linnaeus**

_ Body colouration brownish yellow, rather large, robust (Fig. 1C). Head blackish with shiny occiput (Fig. 2C). Pronotum unicolourous, concave, very slightly wid-

_ ening anteriorly and posteriorly; posterior margins with numerous spots, without large brown spots, double line anteriorly and posteriorly (Fig. 1C). Tibia with 1 pointed spine on either side (Fig. 6C) **2**

2 Elytra extending to the apex of abdomen (Fig. 8C). Wings long. Legs pale yellowish with numerous hairs (Fig. 6C) **3**

_ Elytra reach to the top of abdomen, wings much long (Fig. 8D, E). Legs dark brown, strongly pubescent (Fig. 1D, E) **4**

3 Abdomen yellow, pubescent; ovipositor long, straight, serrated with numerous sutures. Cerci well developed, pointed at the terminus (Fig. 1C) .... ***A. hispanicus*** **Rambur**

_ Abdomen brown; ovipositor moderately long, rather slender with apical valves very acute (Fig. 1G). Cerci small, tapered at apex **6**

4 Body large, stout. Colour blackish. Head curved feebly anteriorly; wider at posterior (Fig. 1D, E). Pronotum concave with piriform impression on anterior disc (Fig. 4D, E) .... ***G.* (*Gryllus*) *bimaculatus*** **De Geer**

_ Body size medium to large. Colour brown. Head yellowish brown with patches and raised veins (Fig. 2F). Pronotum convex above, blackish brown with fine greyish pubescens; posterior margin sinuated (Fig. 4F) **5**

5 Elytra run beyond length of body, elytra with yellow patches on base................. .... ***G. campestris*** **Linnaeus**

_ Elytra equilateral reduced, extending to the extremity of abdomen, wings caudate (Fig. 9C) **7**

6 Fastigium of vertex black, shiny, flat, slightly curved at sides, large body size, elytra large with thick venation system along total body length .... ***G. septentrionalis*** **F. Walker**

_ Fastigium of vertex yellowish brown, curved at the anterior side; body elongated, elytra small, disjointed .... ***Gryllodes sigillatus*** **Walker**

7 Head small, brown, with narrow frontal rostrum, pronotum transverse, feebly concave anterior margin; elytra of female are moderately diverse **8**

_ Head wide at back and narrow in front, pronotum concave and slightly broad, Face blackish brown, right wing overlappintg on anterior wing .... ***T.* (*Brachyteleogryllus*) *occipitalis*** **Serville**

8 Femur thick at anterior but narrow at posterior, without spines. Tibia moderately thin, armed with 10 anterior spines, no spines on external side .... ***G. supplicans*** **Walker**

_ Femur thick, small, banded with vertical lines. Tibia thin with pointed spines with black bases, dorsal field of tegmina with several veins **9**

9 Head short with vertical light and dark bands at posterior margin. Ocelli dorsal field with dark horizontal band (Figs 2J, 3A). Pronotum dark brown, variably fulvous with black inferior margin (Fig. 5A, B) .... ***T.* (*Brachyteleogryllus*) *commodus*** **Walker**

_ Head larger, yellow, adorned with rufous spots, ocelli dorsal field with dark, horizontal, pubescent bands (Fig. 3B). Pronotum depressed above with straight yellowish posterior margin; dorsal field coarse (Fig. 5C) **10**

10 Femur wide with numerous patches and immovable spines, tibia has several spines on one side, tegmina dorsal field with 3 or 4 oblique veins **11**

_ Femur thick, small groove at anterior, small hairs on external and internal sides. Tibia armed with 9 external, 11 internal, and 2 medio-internal spines .... ***Svercus palmetorum*** **Krauss**

11 Elytra extending to the apex of abdomen; veins of the dorsal field rather irregular and condensed (Fig. 9G) **12**

_ Elytra extending to 2/3 of the abdomen, apical field well developed; wings surpassing abdomen tip (Fig. 10B) **13**

12 Abdomen brown. Ovipositor short, straight, slender with very small lanceolate, acute apical valves (Fig. 1M) .... ***Modicogryllus sindhensis*** **sp. nov.**

_ Abdomen yellow with dark spots on each tergite. Ovipositor long, straight, slender (Fig. 1P) **14**

13 Legs dark brown dorsally, whitish ventrally. Tibia armed with 9 internal and 4 medio-internal spines (Fig. 7B). Abdomen black, sternites whitish. Cerci pale brown, short. Ovipositor long, slender, straight with lanceolate apical valves (Fig. 1O) .... ***Miogryllus itaquiensis*** **Orsini & Zefa**

_ Legs brown, hind femora much longer than middle femora. Posterior tibia armed with 7 external, 3 medio-internal spines, much wider anteriorly, numerous patches on dorsal surface (Fig. 7E). Abdomen brown. Cerci long, tapered. Ovipositor long, straight with yellowish base (Fig. 1S) 15

14 Medium size. Colouration yellow (Fig. 1P). Head short, narrow, yellowish, shiny, adorned on each side with dark brown line extending from occiput, along eye (Fig. 3E). Pronotum as long as wide, feebly widening in front with two dark spots on dorsal field (Fig. 5F). Elytra reduced. No wings (Fig. 10C). Ovipositor slim and acute .... ***Callogryllus saeedi*** **Saeed**

_ Medium size. Colouration yellow (Fig. 1Q). Head short, narrow, very neat. Eyes rounded, moderately projecting; ocelli small (Fig. 3F). Pronotum 1.5 × as wide as long, slightly concave at anterior margin, straight posteriorly, one side rather strongly convex (Fig. 5G). Elytra yellow, small (Fig. 10D). With or without wings. Ovipositor small, very elongated, acute slim apical valve .... ***C. ovilongus*** **Saeed & Yousuf**

15 Eyes oval and brown, pronotum serrated overall and wide, abdominal part smaller than tegmen, wings large. Legs yellow, brownish at base, strongly pubescent, irregular bands on dorsal field. Posterior tibiae armed with 11 external and 3 medio-internal spines (Fig. 7D) .... ***C. bilineatus*** **Bolívar**

_ Eyes small, oval, bulging outwards, ocelli black, horizontal dark band present (Fig. 3H). Pronotum as long as head, dorsal field 2 × wider than long, anterior and posterior margins pilose, truncated, dorsal surface brownish, mottled (Fig. 5I). Wings with condensed veins (Fig. 10F). Legs brown, hind femora much longer than middle femora. Posterior tibia armed with 7 external, 3 medio-internal spines (Fig. 7E) **16**

16 Fastigium of vertex circular, brownish, shiny. Eyes small, dark brown. Head shiny brown, short, narrow; ocelli black, with horizontal dark band (Fig. 3H). Tegmen pointed at one end and curved at the other. Tibia with 10 spines. Cerci long tapered. Ovipositor long, straight, with yellowish base (Fig. 1S)............................ ....***Lepidogryllus siamensis*** **(Chopard)**

_ Fastigium of vertex small, tapered, green. Eyes black. Head short, narrow with dark brown ocelli (Fig. 3I). Tegmen snowy transparent extending to 2/3 tip of abdomen. Wings rounded, broad with condensed irregular veins (Fig. 10G). Tibia thin, slender armed with 21 external, 1 medio-internal spines (Fig. 7F). Cerci long with pointed ends. Ovipositor short (Fig. 1T) .... ***Oecanthus fultoni*** **Walker**

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RESEARCH ARTICLE

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Description of a new species of the genus *Neopseustis* Meyrick, 1909 from China, with a new classification of the genus (Lepidoptera, Neopseustoidea, Neopseustidae)

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Abstract
A new species of the genus *Neopseustis* Meyrick, 1909, *Neopseustis chentangensis* S.Y. Huang & Chen **sp. nov.,** which was confirmed by both morphological and molecular methods, is described from Xizang, China. This is currently the westernmost species in Asia of the primitive lepidopteran family Neopseustidae. The new species is externally reminiscent of *N. moxiensis* Chen & Owada, 2009; however, it can be easily distinguished from the latter by comparison of the male genitalia and is further distinguished by the large genetic distance in DNA barcodes (COI). The adult and genitalia of the new and similar species have been illustrated. Utilizing our new data, a new classification of the genus is provided, with its members subdivided into four species groups: the *meyricki*-group, the *moxiensis*-group, the *bicornuta*-group, and the *chentangensis*-group, which are supported by both molecular and morphological evidence. A checklist of the genus and a key to the species groups are also provided.

Keywords
Classification, Himalaya, India, *Neopseustina*, new species, Sichuan, Xizang

Introduction

The family Neopseustidae is a small and archaic lepidopteran family known only by four genera and 14 species and with a peculiar disjunct distribution. Ten of these species are found in Southeast Asia, and the rest are found in South America (Davis 1975; Davis and Nielsen 1980; Davis and Nielsen 1985; Liao et al. 2021). Kristensen (1999) listed several probable autapomorphies for the family, mainly taken from the head, thorax, and abdomen, including the facial scales being restricted to paired lateral and usually swollen patches, the prominent apodemal plate invaginated from the upper base of the propecoxal bridge, the male sternum VII with medial spinose process, etc. Faucheux et al. (2006) studied the antennal flagellum sensilla of several neopseustid species and stated that one sensillum type, called "multiporous large sensillum basiconicum" in their work, is unknown in other lepidopterans except Neopseustidae; thus, the presence of such a sensillum constitutes an autapomorphy of the family. Recent molecular studies have brought new knowledge concerning the phylogenetic position of the family. Mutanen et al. (2010) and Regier et al. (2013) recovered the clade Acanthopteroctetidae+Neopseustidae, but with weak support. Kristensen et al. (2015) found that with the discovery of Aenigmatineidae, the clade Acanthopteroctetidae+Aenigmatineidae+Neopseustidae (abbreviated as the AAeN clade in that work) was strongly supported, and Aenigmatineidae was found to be sister to Neopseustidae. The close relationship is supported by the sharing of a strong precoxal bridge between the prothoracic pleuron and sternum. Moreover, the AAeN clade was found to be sister to all the Heteroneura (Kristensen et al. 2015). Regier et al. (2015) also reported the grouping of Neopseustidae and Acanthopteroctetidae, and this clade was found to be supported by the presence of the smooth intercalary sclerotization and the alignment of antennal scale sockets in longitudinal rows in the antenna. Regier et al. (2015) also suggested that the former monotypic Neopseustoidea should include also the Acanthopteroctetidae and Aenigmatineidae, and that together they form the sister group to Heteroneura. Externally, Neopseustidae adults are small to medium-sized moths with long antennae and semitransparent, thin-scaled wings, and they resemble some families in the order Neuroptera. Little is known about the biology of Neopseustidae. Adults can be active during the day or night, flying above bushes or attracted to light traps (Liao et al. 2021; present study). As for the immature stages, Grehan (1991) suggested that the disjunct distribution of the plant family Lardizabalaceae fitted well with that of the family Neopseustidae, but no feeding had ever been recorded. Regier et al. (2015) reported an astonishing parasitoid immature stage on Limacodidae of *Neopseustis meyricki* Hering, 1925, but later the larvae of this Taiwanese species have been found to feed on *Ampelopsis brevipedunculata* var. *hancei*, family Vitaceae (DearLep 2021), suggesting that the former record was based on an error. For other Neopseustidae there is no information on immatures.

To date, two genera and seven species have been recorded from mainland China, which are distributed in Henan, Sichuan, Guizhou, Hunan, Guangxi, and Guangdong provinces (Davis 1975; Chen et al. 2009; Liao et al. 2021). Xizang Autonomous

Prefecture, also known as Tibet, is a biological hotspot region located in southwestern China and is well known for its various biotopes. Due to the diverse vegetation types found at different altitudes, this area is home to many families of Lepidoptera, and new discoveries are frequently reported. Neopseustidae are currently unknown for the Xizang fauna. During a survey conducted in May 2021, the first author unexpectedly captured a strange looking individual of this intriguing family from Chentang Town, Xigaze City, located in southern Xizang. After careful examination, this individual has been proven to be an unknown species, which is described herein. This is currently westernmost distribution record of the genus *Neopseustis* in Asia, and it is also the first record of the family in Xizang. Furthermore, we provide a new classification for the species in the genus *Neopseustis*, based both on molecular and morphological evidence.

Materials and methods

Morphological study.

Specimens examined were collected during daytime, using an insect net, or with a light trap at night and subsequently deposited in the collection of the South China Agricultural University (SCAU), Guangzhou. Photographs of the adult and the habitat of the new species were taken using a Sony DSC-RX100 v. 1.00 camera. The abdomens were removed and macerated in 10% NaOH for about 2 min at about 95 °C for dissection of the genitalia. The genitalia were removed from the abdomen and mounted in glycerin for photographing. Photographs of the genitalia of the new species were taken under a Keyence VHX-5000 digital microscope, and those of other taxa were taken under a Zeiss SteReo Discovery V.12 digital microscope. Photographs of adults and genitalia were processed using Adobe Photoshop CS5 software. The terminology for adults and genitalia follows Davis (1975) and Liao et al. (2021).

Molecular analysis.

Our molecular analysis comprised 19 samples, six of which are newly obtained COI sequences for DNA barcoding. Detailed information on these samples is provided in Table 1. Three COI sequences of three species of the genus *Apoplania* Davis, 1975, two sequences of the monobasic genus *Synempora* Davis & Nielsen, 1980, and two sequences of one species of the genus *Neopseustis* were downloaded from BOLDSystem (www.boldsystems.org). Five sequences belonging to three species of the genus *Neopseustis* and a sequence of *Endoclita davidi* (Poujade, 1886), which was used as the outgroup in our phylogenetic analysis, were downloaded from NCBI (www.ncbi.nlm.nih.gov). The details of protocols for DNA extraction, amplification, and sequencing have been provided in previous publications (Fan et al. 2016; Tang et al. 2017; Huang et al. 2019). The sequences were aligned using Clustal W (Thompson et al. 1997) implemented in

Table 1. Voucher information and GenBank accession numbers for COI sequences of the Neopseustidae specimens and outgroup in this study. Newly obtained sequences are indicated by an asterisk (*).

Taxon	Locality	Date	Voucher Number	Accession Number
Neopseustis chentangensis S.Y. Huang & Chen sp. nov.	Xizang, China	V.2021	CT1	OK148463*
Neopseustis rectagnatha Liao, Chen & Huang, 2021	Hunan, China	VIII.2020	HAUHL039474	MW804623
Neopseustis rectagnatha Liao, Chen & Huang, 2021	Hunan, China	VIII.2020	HAUHL039473	MW804622
Neopseustis rectagnatha Liao, Chen & Huang, 2021	Hunan, China	VI. 2020	HAUHL040282	MW804609
Neopseustis archiphenax Meyrick, 1928	Henan, China	VII. 2002	LNAUT030–14	N/A
Neopseustis archiphenax Meyrick, 1928	Henan, China	VII. 2002	LNAUT031–14	N/A
Neopseustis sinensis Davis, 1975	Sichuan, China	VII. 2009	BX1	OK148464*
Neopseustis sinensis Davis, 1975	Sichuan, China	VII. 2009	YJ1	OK148465*
Neopseustis meyricki Hering, 1925	Taiwan, China	N/A	LS-06–0068	GU828566
Neopseustis moxiensis Chen & Owada, 2009	Sichuan, China	VIII. 2004	MX1	OK148466*
Neopseustis fanjingshana Yang, 1988	Hunan, China	VIII. 2019	HAUHL041880	MW804624
Neopseustis fanjingshana Yang, 1988	Hunan, China	VIII. 2008	SZ1	OK148467*
Neopseustis bicornuta Davis, 1975	Sichuan, China	VII. 2009	YJ2	OK148468*
Apoplania valdiviana Davis & Nielsen, 1985	Cautin, Chile	XII. 1982	LNAUT029–14	N/A
Apoplania penai Davis & Nielsen, 1980	Chiloe Island, Chile	XII. 1981	LNAUT022–14	N/A
Apoplania chilensis Davis, 1975	Curico Las Tablas, Chile	II. 1985	LNAUT019–14	N/A
Synempora andesae Davis & Nielsen, 1980	Sagrario Puerto, Argentina	II. 1979	LNAUT041–14	N/A
Synempora andesae Davis & Nielsen, 1980	Aguas Calientes, Argentina	II. 1979	LNAUT042–14	N/A
Endoclita davidi (Poujade, 1886)	Hunan, China	XI. 2015	HN20170409020	KY928030

MEGA v. 7.0 (Kumar et al. 2016) with default parameters, and genetic distances were calculated using Kimura-2-parameter models implied by the same software. Maximum likelihood analyses were performed using IQ-tree v. 2.1.3 (Minh et al. 2020) with the branch support values evaluated by 1000 ultrafast bootstrap (UFBS) replicates (Minh et al. 2013) on the web server (http://iqtree.cibiv.univie.ac.at/). We considered the branch support strong when the UFBS was 95 or higher. Genetic distances were calculated using the Kimura-2-parameter models implied by the same software. All sequences were submitted to GenBank under the submission numbers OK148463 to OK148468. The specimens with voucher numbers CT1, BX1, YJ1, YJ2, MX1, and SZ1 were deposited in SCAU.

Taxonomy

Genus *Neopseustis* Meyrick, 1909

Neopseustis Meyrick, 1909: 436.

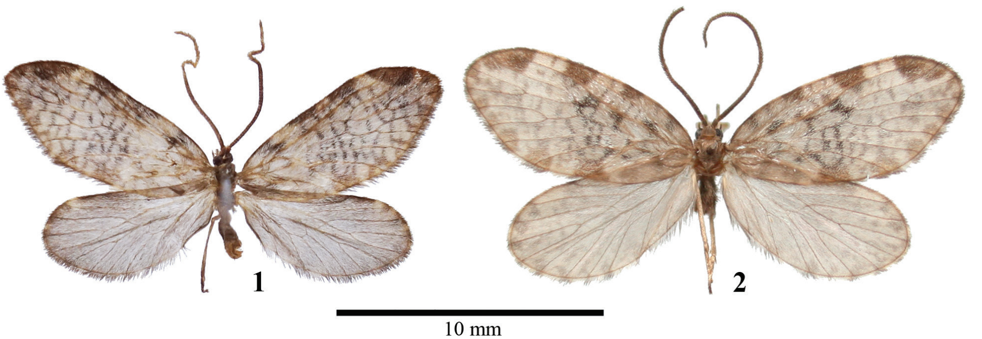
Type species. *Neopseustis calliglauca* Meyrick, 1909, by monotypy. [Type locality: Khasi Hills, Assam, India].

***Neopseustis chentangensis* S.Y. Huang & Chen sp. nov.**
http://zoobank.org/9E16636E-F0EE-4738-9DD9-4A6259EB96B6
Figures 1, 3–10

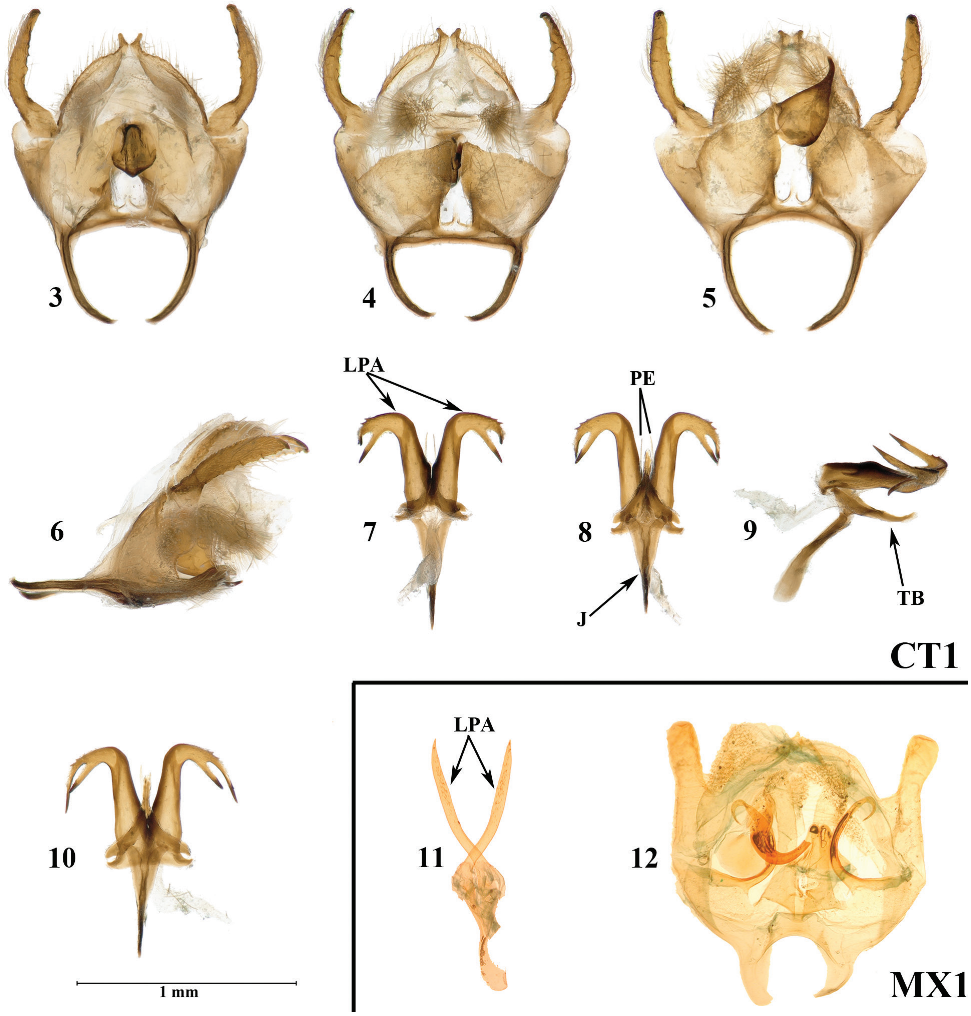
Type material. *Holotype:* male, altitude 2600 m, 23.V.2021, Chentang Town, Dingjie County, Xigaze City, Xizang Autonomous Prefecture, P.R. China, leg. Siyao Huang, voucher number and dissection number CT1 (SCAU).

Diagnosis. Externally, *N. chentangensis* resembles *N. moxiensis* Chen & Owada, 2009 (Fig. 2, 11–12) from Moxi, western Sichuan, share a fuscous ground colour on both wings. However, the new species can be immediately distinguished from *N. moxiensis* by the combination of the following characters: smaller size (length of forewing 8.7 mm vs 9 mm in holotype of *N. moxiensis*), narrower forewing (slightly broader in *N. moxiensis*), patches along forewing costa slenderer and darker (patches along forewing costa thicker and lighter in *N. moxiensis*), narrower hindwing and light fuscous ground colour (broader hindwing and light yellowish brown ground colour in *N. moxiensis*), and more uniform fringe in both wings (cilia clearly chequered, especially in hindwing in *N. moxiensis*). In the male genitalia, *N. chentangensis* can be easily distinguished from *N. moxiensis* by the shape of the latero-posterior process of anellus, which is long, robust, and L-shaped; the distal end is deeply bifurcated and forms two sharp processes bending anteriorly (in *N. moxiensis*, the latero-posterior process of anellus is not L-shaped and bent anteriorly at the tip.). The tegumenal lobe is significantly slenderer after it is flattened (in *N. moxiensis* the tegumenal lobe is much broader when it is flattened), the valvae lack the uncinate process apically, and long and thick processes ventrally (both processes present in *N. moxiensis*). The anterior arms of the vinculum are more slender (these arms are broader and shorter in *N. moxiensis*). From the other congeners, *N. chentangensis* can be simply distinguished by the shape of its latero-posterior process of anellus mentioned above.

Description. Adult: length of forewing 8.7 mm. Antennae brownish dorsally. Head, thorax, and abdomen uniformly brownish. Forewing nearly oval, apex slight-



Figures 1, 2. Males of *Neopseustis* spp. **1** *Neopseustis chentangensis* S.Y. Huang & Chen sp. nov., holotype, Chentang, Xizang, CT1 **2** *N. moxiensis*, holotype, Moxi, Sichuan, MX1.



Figures 3–12. Male genitalia of *Neopseustis* spp. **3–10** *Neopseustis chentangensis* sp. nov., holotype, dissection number CT1 **3** genitalia capsule in natural shape with anellus-juxta-parameres removed, dorsal view **4** same, in ventral view **5** genitalia capsule flattened with anellus-juxta-parameres removed **6** genitalia capsule in natural shape with anellus-juxta-parameres removed, in lateral view **7** anellus-juxta-parameres in natural shape, in dorsal view **8** same, in ventral view **9** same, in lateral view **10** anellus-juxta-parameres flattened, in ventral view **11, 12** *Neopseustis moxiensis*, holotype, dissection number MX1 **11** anellus-juxta-parameres flattened, in ventral view **12** genitalia capsule flattened with anellus-juxta-parameres removed. J = Juxta; LPA = lateroposterior process of anellus; PE = parameres; TB = transverse bar. Scale bar: 1 mm (Figures **3–10**).

ly pointed. Forewing ground color pale yellowish fuscous, with four fuscous patches along costa to apex. Several irregular black or brownish transverse lines present in the median and submarginal zones. A row of brownish spots extending from apex to anal angle along termen. Fringe fuscous from apex to anal angle, slightly checkered with creamy white in dorsum. Hindwing oval, ground color uniformly light fuscous. Hindwing apex with light yellowish spot at the marginal zone. Fringe generally fuscous from apex to anal angle and slightly checkered with creamy white around anal angle.

Male genitalia: Uncus fused with tegumen, bifurcate basally and forming two short and distally rounded lobes. Gnathos strongly sclerotized thoroughly, consisting of a medially curved, short, and robust distal process and a large and thick base. Socii rounded, densely setose. Tegumenal lobe slightly curved outwards beyond the base and gradually narrowing towards its tip. Valvae totally fused with vinculum, broad and nearly trapezoid in natural shape. Vinculum broad posteriorly, abruptly narrowing anteriorly and forming long and slender arms. Lateroposterior process of anellus generally L-shaped, thick, and robust, with the tip deeply bifurcate and forming two sharp processes bending anteriorly. Two denticles present at the upper margin of dorsal process. Paired processes of anellus absent. Transverse bar in lateral view obtuse-triangular and slightly bending upwards near tip, while in dorsal and ventral views generally triangular with the lower angles shallowly bifurcate. Juxta in lateral view slightly curved outwards and nearly broad Y-shaped in dorsal and ventral views. Parameres short and setose-like, weakly sclerotized, situated between the two lateroposterior processes of anellus.

Female. Unknown at present.

Bionomics. The holotype of *N. chentangensis* was spotted weakly flying above bushes during the daytime at an altitude about 2600 m. The collecting site (Fig. 13) is located at the edge of a forest along a road in a valley.

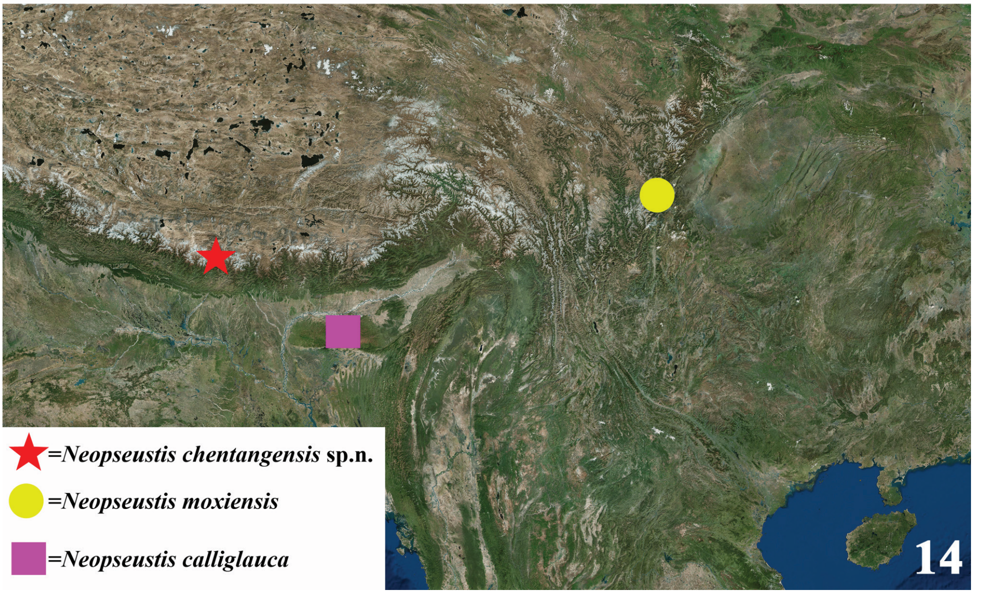
Distribution. Currently only known from the type locality, Chentang Town (Fig. 14).

Etymology. The specific epithet chentangensis is derived from the type locality, Chentang Town.

Molecular analysis. The Kimura-2-parameter distance of the genus *Neopseustis*, based on COI barcoding, is given in Table S1. The maximum interspecific divergence occurred between *N. chentangensis* and *N. moxiensis*, which was 11.7%, and the minimum interspecific divergence occurred between *N. fanjingshana* and *N. bicornuta*, which was 1.5%. According to the table, *N. chentangensis* is genetically distinct from its congeners, with the genetic divergence varying from 7.6 to 11.5%. Based on the ML tree (Fig. 15) constructed using the COI barcoding region, the genus *Neopseustis* was monophyletic (UFBS = 98), and subsequently diverged into four clades, with three of them receiving strong support (UFBS > 95). *Neopseustis chentangensis* was found to be sister to all the remaining taxa in the current study.

Discussion

Although Davis (1975) and Liao et al. (2021) considered that the genus *Neopseustis* should be subdivided into two groups based on the morphology of male genitalia and molecular phylogenetic analysis, we consider that this genus may actually comprise of at least four groups, after utilizing more data from previously unsampled taxa. The first group, as already recognized by Davis (1975) and Liao et al. (2021), consists of



Figures 13, 14. 13 collecting site of *Neopseustis chentangensis* in Chentang Town, Xizang **14** Distribution map of some *Neopseustis* spp. in Asia.

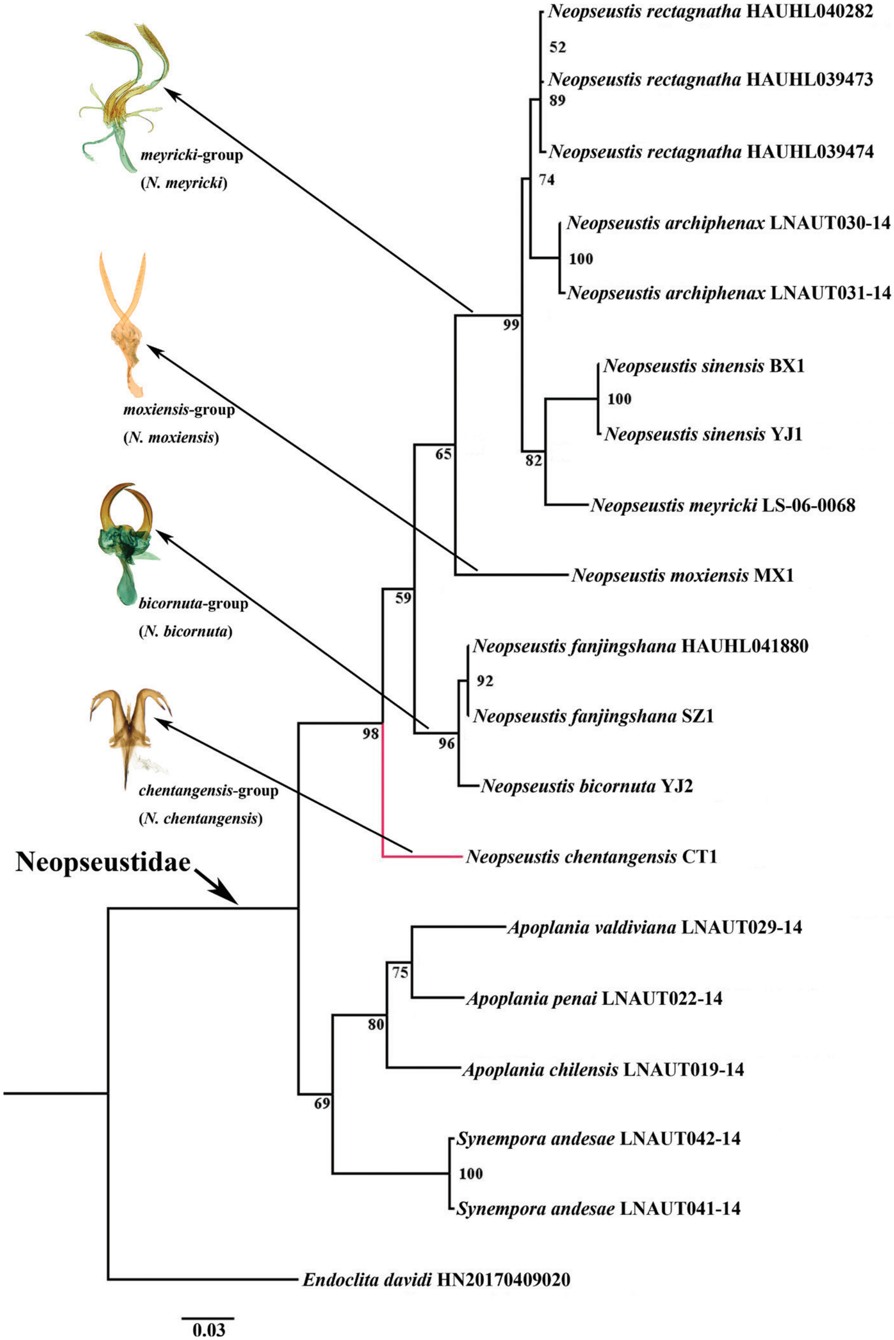


Figure 15. Phylogenetic tree of *Neopseustidae* based on an analysis of the COI barcoding region, using the maximum likelihood method. The genus *Neopseustis is* divided into four groups with their corresponding anellus-juxta-parameres illustrated on the left side.

N. rectagnatha Liao, Chen & G.H. Huang, 2021; *N. meyricki* Hering, 1925; *N. archiphenax* Meyrick, 1928; and *N. sinensis* Davis, 1975, and is called the *meyricki*-group. The second group consists only of *N. moxiensis* Chen & Owada, 2009, and is called the *moxiensis*-group. The third group, consisting of *N. fanjingshana* Yang, 1988 and *N. bicornuta* Davis, 1975, is called the *bicornuta*-group and probably also includes the unsampled type species, *N. calliglauca* Meyrick, 1909, based on the morphology of its anellus-juxta-parameres. The fourth group consists of only *N. chentangensis* S.Y. Huang & Chen and is called the *chentangensis*-group. Among these four groups, except for the *meyricki*-group which is unique in having well-developed parameres and a narrow, short, and forked lateroposterior process on the anellus, the *moxiensis*, *bicornuta*, and *chentangensis* groups all share ill-developed parameres, but they can be distinguished from each other by the combination of features in the male genitalia. The *moxiensis*-group is characterized by the latero-posterior process of the anellus covered by dense spinules from middle to distal end, in addition to the valvae which have an uncinate process apically and a long and thick process ventrally. The *bicornuta*-group is characterized by the latero-posterior process of the anellus smooth from middle to distal end and the absence of ventral process in the valvae. The *chentangensis*-group is characterized by latero-posterior process of anellus long, L-shaped with apex deeply bifurcating and bending anteriorly, and gnathos with a large and thick base.

It is rather intriguing that although *N. chentangensis* is similar externally only to *N. moxiensis* in *Neopseustis*, among the whole genus, they have the greatest genetic divergence. Their male genitalia structures are also considerably different from each other, suggesting that the relationship between them is distant. We believe that their external similarity may probably due to their parallel evolution under similar environments. Unlike their relatively whitish congeners inhabiting the mid- and lower-elevation mountainous areas in India, mainland China, and Taiwan, these two species all inhabit high mountainous areas above 2500 m, and the similar cool climate in high elevation areas in western Sichuan and southern Xizang. This probably may have led to the evolution of their dark wing coloration which can help them absorb heat faster. This assumption is also supported by the studies of Wu et al. (2019), Trullas et al. (2007), and Pereboom and Biesmeijer (2003), who produced similar conclusions.

The former westernmost record of the genus *Neopseustis* is the type species *N. calliglauca*, which is found in the Khasi Hills in India. The current record of this new species is situated about 520 km northwest of Khasi Hill, and thus is currently the westernmost record of the genus. The discovery of *N. chentangensis* in Chentang, on the southern slope of the Himalayas in Xizang, suggests that the investigation of the microlepidopteran fauna is still inadequate in remote areas along the Himalaya. The collection site of the new species is very close to the border of China and Nepal, and Neopseustidae are unknown in Nepal. It can be expected that this species or other new species will someday be discovered in Nepal or Bhutan. Moreover, Neopseustidae are also expected in the southeastern part of Xizang, where no species are currently found. It is possible that the absence of this family there is only due to a lack of surveys, as poor transportation conditions in past decades makes this paradise of moths difficult to access.

Checklist of the genus *Neopseustis* Meyrick, 1909

***meyricki*-group**

N. archiphenax Meyrick, 1928
Distribution. Myanmar, China (Sichuan, Henan)
N. meyricki Hering, 1925
Distribution. China (Taiwan)
N. rectagnatha Liao, Chen & G.H. Huang, 2021
Distribution. China (Hunan, Guangxi, Guangdong)
N. sinensis Davis, 1975
Distribution. China (Hunan, Sichuan)

***moxiensis*-group**

N. moxiensis Chen & Owada, 2009
Distribution. China (Sichuan)

***bicornuta*-group**

N. bicornuta Davis, 1975
Distribution. China (Sichuan)
N. calliglauca Meyrick, 1909
Distribution. India (Khasi Hills, Meghalaya)
N. fanjingshana Yang, 1988
Distribution. China (Guizhou, Hunan)

***chentangensis*-group**

N. chentangensis S.Y. Huang & Chen, sp. nov.
Distribution. China (Xizang)

Key to the species-groups of the genus *Neopseustis* based on male genitalia structures

1 Parameres well developed and narrow; lateroposterior process of anellus short, and forked .... ***Neopseustis meyricki*-group**
– Parameres poorly developed **2**
2 Latero-posterior process of anellus long, L-shaped with apex deeply bifurcate, bending anteriorly .... ***Neopseustis chentangensis*-group**
– Latero-posterior process of anellus apex not bifurcate and pointed posteriorly **3**
3 Latero-posterior process of the anellus covered by dense spinules from middle to distal end; valvae with an uncinate process apically and a long and thick process ventrally .... ***Neopseustis moxiensis*-group**
– Latero-posterior process of the anellus smooth from middle to distal end; valvae without a ventral process .... ***Neopseustis bicornuta*-group**

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Supplementary material 1

Table S1. The Kimura-2-parameter distance on COI sequences between different taxon of the genus *Neopseustis* sampled for the current study

Authors: Siyao Huang

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.1078.75461.suppl1

ZooKeys 1078: 49–55 (2021)
doi: 10.3897/zookeys.1078.75499
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RESEARCH ARTICLE

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Anormalous liu sp. nov.: a first record and a new species of the genus *Anormalous* Liu, 2011 (Orthoptera, Tettigoniidae, Phaneropterinae) from India

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Abstract

The Phaneropterinae, commonly known as the bush katydids, are among the most diverse tettigonids in the world. A new species *Anormalous liu* **sp. nov.** is described from Kashmir, India. This is the second species in the short-winged genus *Anormalous*. It is differentiated from the other species from China by the absence of posterior apical spurs on the fore and mid tibiae, the male subgenital plate with two long cylindrical lobes fused with each other and blunt at the apices, and the male stridulatory area longer than broad. We include a key to species in the genus *Anormalou*. The holotype has been deposited in the Museum of Zoology Department, Aligarh Muslim University, Aligarh Uttar Pradesh, India.

Keywords

Anormalous, India, Kashmir, new species, Phaneropterinae

Introduction

Katydids show an incredible diversity of forms and species (Heller et al. 2014), with many species reported from India. Some katydids sporadically become very obvious due to a sudden spurt in their population size due to weather conditions (Rentz 2010). Important work on the taxonomy and distribution of the Tettigoniidae (including Phaneropterinae) of India include those of Barman and Srivastava (1976), Shishodia (2000), Barman (2003) and Shishodia et al. (2010). The Phaneropterine occupy a wide range of open habitats (Kocarek and Holusa 2006). Recently, Nagar et al. (2014, 2015) and Farooqi et al. (2021) reported new species of Phaneropterinae from India.

The genus *Anormalous* most resembles the genera in the tribe Ducetiini in the lateral lobe of the pronotum, the tympanum structure, the fore tibiae, and the absence of styli in male subgenital plate, but differs by the particular tegminal structure (Liu 2011). The genus was established for the species *Anormalous zhangi* Liu, 2011 from southern China, with only male specimen reported. The new species described herein can be assigned to the genus *Anormalous* based on similarities of the tegminal structure, but differs in various morphological characters described below.

Materials and methods

During a field survey conducted in 2021 at different places in the Kashmir region, the specimens were collected by handpicking or with the help of sweep nets. Out of all collected samples, one male, and three females of the new species were found. They were preserved in alcohol and brought to the laboratory for identification. The specimens were examined under a stereo zoom binocular microscope. Genitalia were observed after cleaning with KOH. Photographic images were done using a DSLR camera with macro-lens. All body parts were measured using a vernier caliper. Both the holotype and paratype have been deposited in the Museum of Zoology Department, Aligarh Muslim University, Aligarh Uttar Pradesh, India.

Results and discussion

Anormalou Liu, 2011

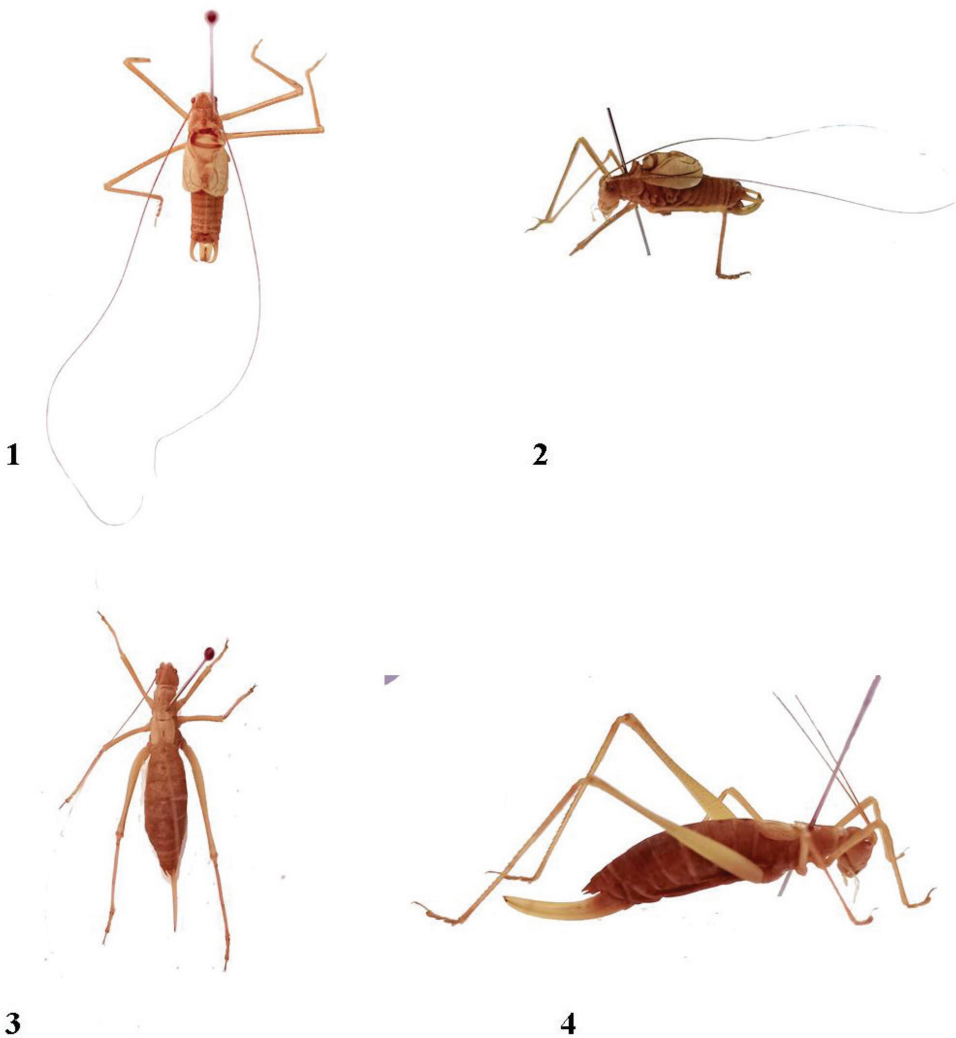
Description. Small sized body (Figs 1–4), light green, head more or less oval in shape (Fig. 5), fastigium dorsally sulcate with conical apex, narrower than first antennal segment (Fig. 8). Lateral lobe of pronotum distinctly longer than high (Fig. 6). Pronotal disc with prozona smooth and metazona flat, without lateral carinae (Fig. 7). Lateral lobe of pronotum with shallow humeral sinus. Eyes large and bulging outwards, antenna long (Figs 1–4), male subgenital plate elongate, notch at apical margin present or absent, devoid of distinct styli (Fig. 10), female tegmen comparatively shorter than male's with visible longitudinal veins (Figs 3, 4), last abdominal tergite rounded (Fig. 16), and ovipositor weekly curved (Fig. 18).

Distribution. China and India (Kashmir)

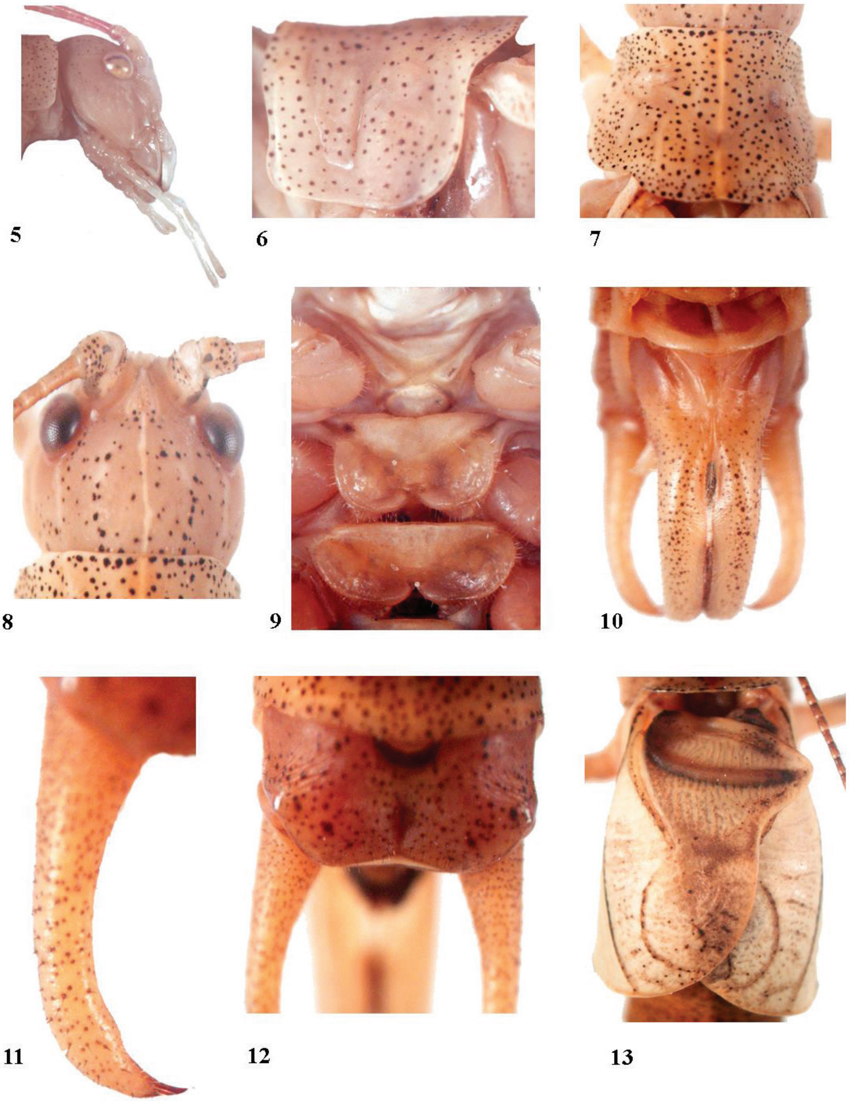
Key to species of the genus *Anormalous* (males only)

1 Posterior apical spurs on fore and mid tibiae present, male subgenital plate elongate, split from basal third into two triangular lobes, notch narrow triangular, lateral margin of lateral lobe tapering towards apices, male stridulatory area broader than long .... ***Anormalous zhangi*** **Liu, 2011**

– Posterior apical spurs on fore and mid tibea absent, male subgenital plate with two long cylindrical lobes fused with each other, blunt at the apices (Fig 10), with a small notch at anterior portion, male stridulatory area longer than broad (Fig 13) .... ***Anormalous liu*** **sp. nov.**



Figures 1–4. *Anormalous liu* sp. nov. **1–2** Holotype male and **2–3** paratype female.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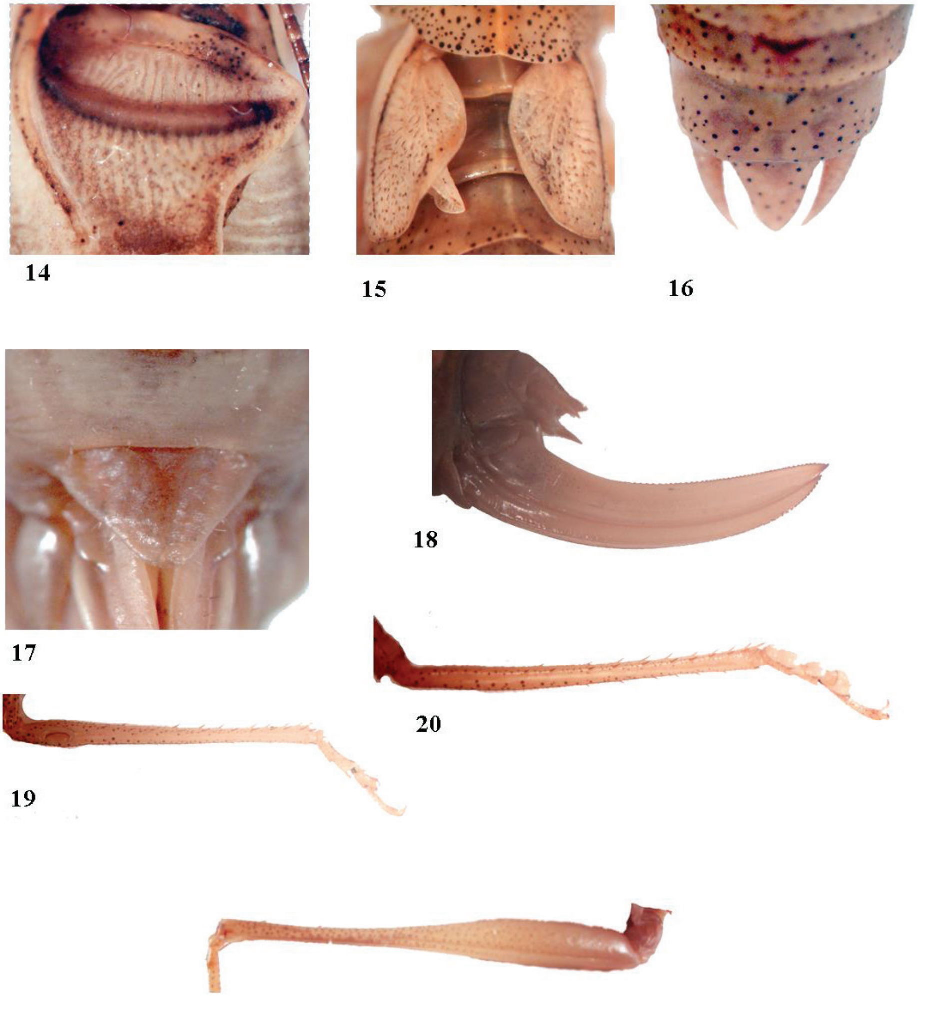
Figures 5–13. Characters of the holotype male *Anormalous liu* sp. nov. **5** head lateral **6** lateral view of pronotum **7** dorsal view of pronotum **8** fastigium **9** sternum **10** subgenital plate **11** cerci **12** male last tergite **13** tegmen.

***Anormalous liu* sp. nov**
http://zoobank.org/49E3B7CD-3911-4E83-9D04-133C29361A30
Figures 1–21

Description. Male: Small sized body, eyes large and bulging outwards, antenna long and flexible, light green, fastigium dorsally sulcate with conical apex, narrower than first antennal segment. Pronotum saddle shaped; lateral lobe of pronotum distinctly



Figures 14–21. *Anormalous liu* sp. nov. **14** male stridulatory file **15** female tegmen **16** female last tergite **17** female subgenital plate **18** ovipositor **19** fore tibia **20** mid tibia **21** hind femur.

longer than high. Pronotal disc with prozona smooth and metazona flat, without lateral carinae. Lateral lobe of pronotum with shallow humeral sinus. Tegmen short not surpassing the abdomen with longitudinal veins well developed, apex rounded; hind wings not well developed and shorter than tegmen. Fore tibia with two rows of 9 evenly- distributed spines ventrally; mid tibia with 12 spines ventrally and 6 dorsally; prosternum unarmed; mesosternum and metasternum with two more or less rounded lobes. Male last abdominal tergite rounded with a shallow depression; cerci long and cylindrical with pointed apex. Male subgenital plate elongated with two long lobes attached together; small notch at anterior end; apical end without distinct styli.

Female: Last abdominal tergite rounded without any incision; subgenital plate small, conical; epiproct long and tongue- shaped; cerci small, slender tapering toward the end; ovipositor long and weekly curved, with small teeth at distal end.

Remarks. The new species differs from the only other species, *Anormalous zhangi* Liu (2011), as follows: male subgenital plate with two long cylindrical lobes fused with each other, blunt at the apices (Fig. 10), male stridulatory area longer than broad (Fig. 13), and absence of posterior apical spurs on fore and mid tibiae.

Distribution. India, Kashmir

Etymology. The name of the species is given after Chun-Xiang Liu who described the genus *Anormalous.*

Material examined. *Holotype*: Male. India: Jammu and Kashmir; Kashmir, Kupwara, (34.5262°N, 74.2546°E), 01 male, 16.08.2021, on grass, collected by Muzamil Syed Shah deposited in Museum of Zoology Department, Aligarh Muslim University, Aligarh Uttar Pradesh, India.

***Paratype:* Female:** India: Jammu and Kashmir; Kashmir, Baramulla, Gulmarg (34.0484°N, 74.3805°E), two females, 20.08.2021, on grass, collected by Muzamil Syed Shah deposited in Museum of Zoology Department, Aligarh Muslim University, Aligarh Uttar Pradesh, India.

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The authors are very grateful to University Grants Commission (UGC), New Delhi India for providing financial assistance during the work. Thanks are also due to the Chairman, Department of Zoology, Aligarh Muslim University, for providing necessary instruments and facilities during the entire work.

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RESEARCH ARTICLE

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Two new species of *Helochares*, with additional faunistic records from China (Coleoptera, Hydrophilidae, Acidocerinae)

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Abstract

Two new species, *Helochares guoi* Yang & Jia, **sp. nov.** and *Helochares distinctus* Jia & Tang, **sp. nov.**, are described. Two species are recorded for the first time from China: *Helochares negatus* Hebauer, 1995 from Yunnan, and *Helochares minusculus* d'Orchymont, 1943 from Guangdong. Additional faunistic data from China are provided for the following species: *Helochares hainanensis* Dong & Bian, 2021, *Helochares nipponicus* Hebauer, 1995, *Helochares sauteri* d'Orchymont, 1943, *Helochares densus* Sharp, 1890, *Helochares lentus* Sharp, 1890, *Helochares neglectus* (Hope, 1854) and *Helochares anchoralis* Sharp, 1890. The Chinese fauna of *Helochares* comprises 16 species, 11 of which are illustrated in this contribution. *Helochares crenatus* Régimbart, 1921 is removed from the Chinese fauna.

Keywords

New records, Oriental Realm, species distribution, taxonomy, water scavenger beetle

Introduction

Helochares Mulsant, 1844 is one of the most diverse and widespread genera of Hydrophilidae, mainly distributed in the Afrotropical, Oriental and Australian realms, with a few species also present in the Palearctic, Neotropical and Nearctic realms. d'Orchymont (1919) recognized five subgenera within *Helochares*: *Hydrobaticus* MacLeay, 1871, *Chasmogenus* Sharp, 1882, *Helochares* Mulsan, 1844, *Helocharimorphus* Kuwert, 1890 and *Sindolus* Sharp, 1882. Hansen (1991) added *Batochares* Hansen, 1991 as a subgenus of *Helochares* although he recognized *Helochares* as a polyphyletic group at that time. Fernández (1986) separated subgenus *Chasmogenus* from *Helochares* and reinstated its generic status. Subgenera *Batochares* and *Sindolus* were elevated to generic status based on the molecular phylogeny by Short et al. (2021). The remaining three subgenera, *Helochares* (s. str.), *Hydrobacticus* and *Helocharimorphus* were synonymized with *Helochares* based on molecular phylogeny and morphological characters (Girón and Short 2021; Short et al. 2021).

So far, 159 species have been described worldwide (Hansen 1999; Short and Hebauer 2006; Short and Fikáček 2011; Girón et al. 2021), but there is no detailed revision of any continent although many species have been described since the end of the last century (e.g., Hebauer 1995, 1998, 2002; Matsui 1995; Hebauer et al. 1999) except for the revision of subgenus "*Hydrobaticus*" of the New World (Short and Girón 2018).

The fauna of Chinese *Helochares* is poorly known. The first Chinese species, *H. neglectus*, was described by Hope (1845) from Guangzhou, Guangdong Province. Since then, 16 species have been recorded (e.g., d'Orchymont 1919, 1925, 1940, 1943a, b; Pu 1963; Gentili et al. 1995; Fikáček et al. 2015; Jia and Tang 2018; Dong and Bian 2021). Of all known Chinese species, thirteen occur south of the Yangtze River, and the other three in the northwest, northeast and southwest China (Jia and Tang 2018; Dong and Bian 2021). Adult *Helochares* (s. str.) usually occur in ponds and at the edge of slow streams, or on surface of wet stones covered with leaves (*H. fuliginosus* d'Orchymont). In China, *Helochares* is the only hydrophilid genus in which adult females carry their egg cases beneath their abdomens.

Material and methods

Male genitalia were dissected in some specimens of each species. Dissected genitalia were transferred to a drop of absolute alcohol for removing membranes after 8–10 hours in 10% KOH at room temperature, and subsequently mounted in a drop of glycerine on a piece of transparent plastic slide attached below the respective specimens. Morphological characters of the male genitalia were examined using a Nikon SMZ800 compound microscope. Genitalia photographs were taken using a Zeiss Axioskop 40 compound microscopes and combined with AutoMontage software version 3.8. Photographs of habitus and external morphology were taken using a Leica M205C stereomicroscope and combined with AutoMontage software.

Detailed descriptions of *Helochares* were provided by Hansen (1991). Morphological terminology largely follows Hansen (1991) and Komarek (2004).

Examined specimens are deposited in the following collections:

IRSN Institute Royal de Sciences naturelles, Brussels, Belgium;
IZCAS Chinese Academy of Sciences, Institute of Zoology, Beijing, China;
SYSU Entomological Collection of Sun Yat-sen University, Guangzhou, China.

Specimens in which the depository is not indicated are deposited in SYSU.

Taxonomy

Helochares guoi **Yang & Jia, sp. nov.**
http://zoobank.org/2BAF353A-6A2D-439C-BBF0-08A84906B3E4
Figs 1–2, 6, 8–9, 24–26

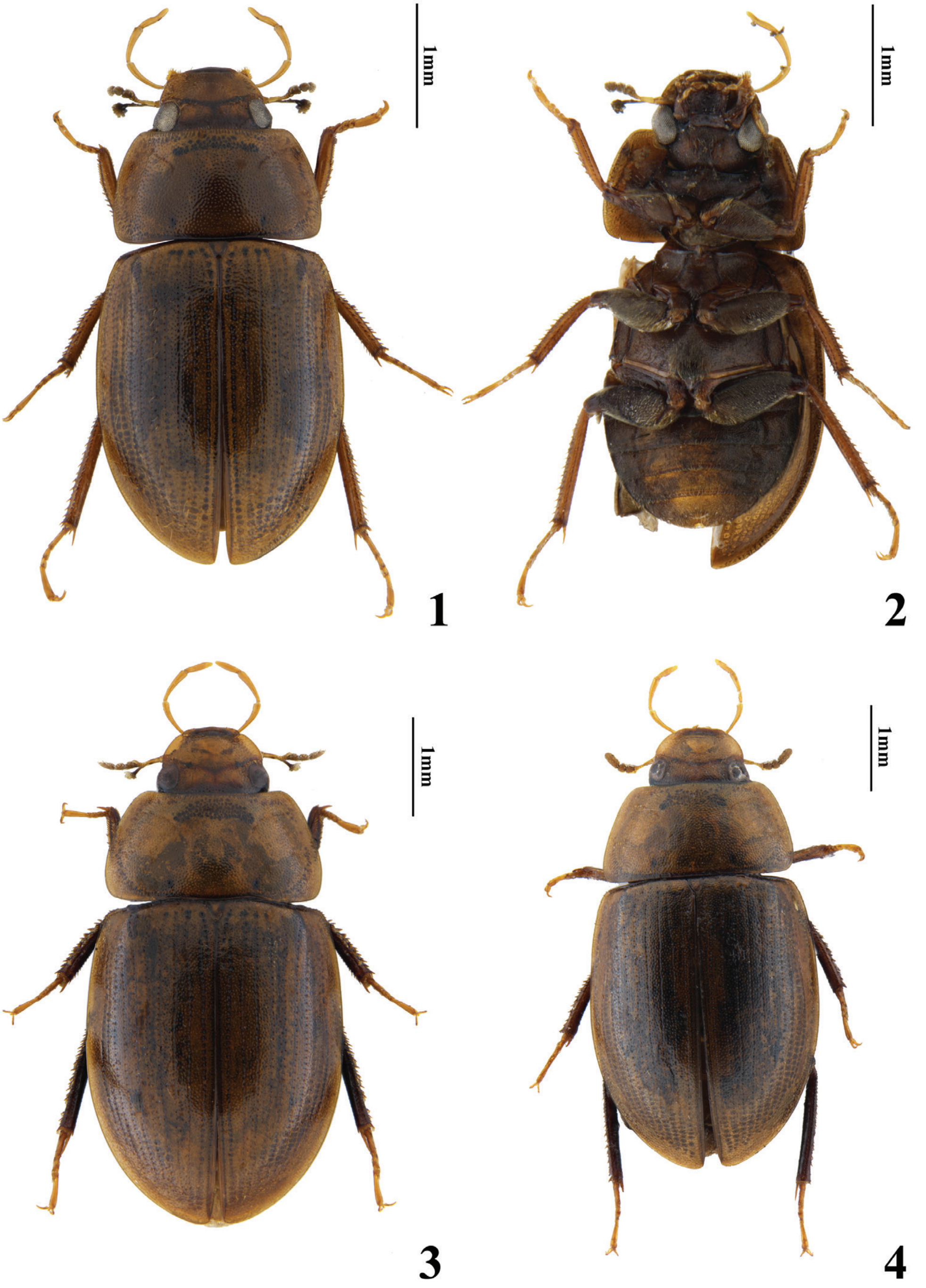
Material examined. ***Holotype*****:** Male, Guangdong, Shenzhen, Dapeng Peninsula, Getian village, 22.48175°N, 114.52643°E, 2.viii.2019, Fenglong Jia and Zuqi Mai leg. ***Paratype***: 1 female, same data as holotype.

Differential diagnosis. This species is very similar to *H. lentus* Sharp, 1890, *H. densus* Sharp, 1890, *H. sauteri* d'Orchymont, 1943 and *H. hainanensis* Dong & Bian, 2021 in size, form and other morphological characters. It can be distinguished based on aedeagus characters. Aedeagus: membranous inner sac with a cluster of strong sclerotized spines (Figs 24–26); median lobe with a lateroventral tooth subapically (Fig. 25).

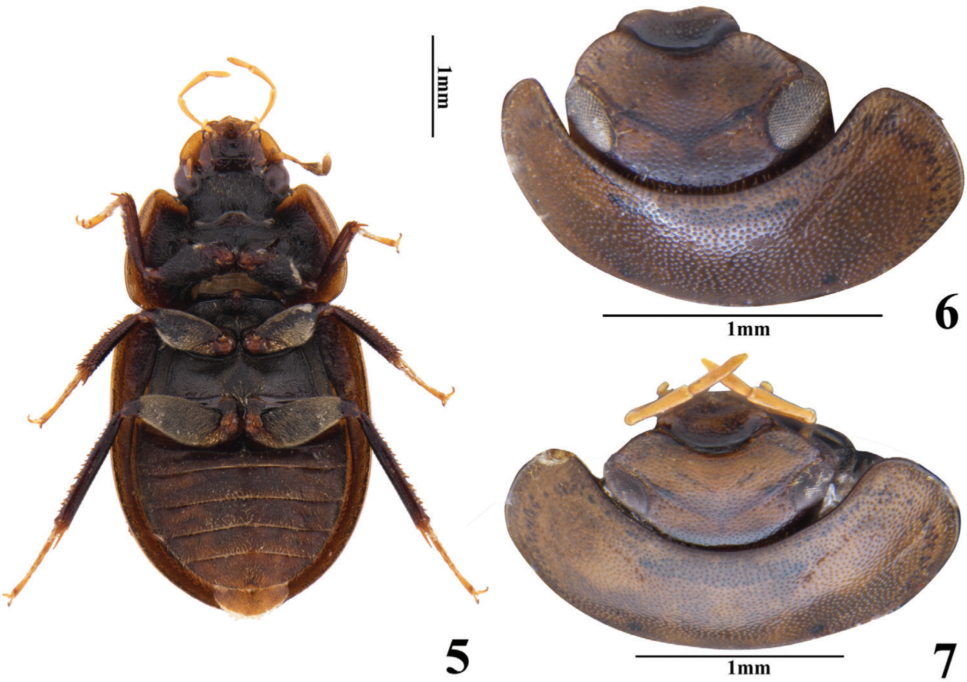
Description. ***Form and colour*** (Figs 1–2, 6). Body length 4.0 mm, body width 2.1 mm. Oval, moderately convex. Dorsum of head, pronotum and elytron yellow-brown, clypeus black. Antennae yellow-brown with club black. Maxillary palps uniformly yellow-brown. Labial palps yellow, not darkened apically. Venter, including legs, blackish brown, tarsomeres yellow-brown.

Head. Antenna with scape ca as long as antennomeres 2 and 3 combined (Fig. 8). Maxillary palps ca 1.25 × as long as width of head anterior to eyes; apical segment symmetrical, about same as penultimate in length (Fig. 9). Clypeo-labral margin curved medially. Labrum, frons and clypeus with systematic punctures (with setae) same size as ground punctures; frons and clypeus with ground punctation dense and coarse, distance between punctures 0.8–1.2× width of one puncture. Mentum subquadrate, with anterior margin strongly emarginate, slightly depressed medially, surface with some oblique wrinkles.

Thorax. Ground punctation on pronotum and elytron similar to that on head, distance between punctures 0.8–1.2× width of one puncture; anterior margin with very fine bead. Elytron with short scutellary series of punctures and 10 striae, punctures in striae distinctly coarser than surrounding ground punctation; systematic punctures (with setae) same size as coarse punctures in striae. Prosternum moderately elevated



Figures 1–4. Habitus **1–2** *Helochares guoi* Yang & Jia, sp. nov. **1** dorsal view **2** ventral view **3–4** *Helochares distinctus* Jia & Tang, sp. nov. **3** paratype, dorsal view **4** holotype, dorsal view.



Figures 5–7. Habitus **5** *Helochares distinctus* Jia & Tang, sp. nov.: ventral view **6–7** head, dorsal view **6** *Helochares guoi* Yang & Jia, sp. nov. **7** *Helochares distinctus* Jia & Tang, sp. nov..

medially, not tectiform or carinate medially, with a transverse groove anteriorly. Mesoventrite with small tubercle medially, not carinate medially. Metaventrite without glabrous area posteromedially. Femora densely pubescent, only glabrous at apex. Meso-, and metatarsomeres 1 to 4 with dense long setae ventrally, metatarsomeres with a fringe of long swimming-hairs dorsally. Protarsal claws in male somewhat stronger than in female and slightly angularly curved, bearing a blunt basal tooth; mesotarsal claws as protarsals, but only moderately curved with a blunt tooth; metatarsal claws only moderately curved, with a blunt basal tooth.

Abdomen. Ventrites uniformly and densely pubescent. Fifth abdominal ventrite with apical emargination fringed with stiff yellowish setae.

Aedeagus (Figs 24–26). Phallobase ca 0.12mm; paramere ca 0.76mm, obtuse apically, outer margin almost parallel in basal three quarters, apical quarter gradually narrowed and rounded apically; membranous inner sac with cluster of strong sclerotized spines (Figs 24–26); median lobe longer than parameres, ca 0.89 mm, apical fifth gradually narrowed apicad, with small latero-ventral tooth subapically, truncate apically (Fig. 25); basal apophyses about half as long as median lobe, ca 0.45 mm.

Remarks. The male holotype bears a long "branch" arising subapically from the antennal pedicel (Fig. 8), which is absent on the paratype (female). This structure is likely a fungus that parasitizes on the antenna.

Etymology. This species is named after Mr. Qiang Guo, the manager of the Shenzhen Wildlife Conservation Division, Guangdong, for his help when we collected in Shenzhen.

Distribution. China (Guangdong): known only from the type locality.

Habitat. This species was collected in the mud at the edge of a seasonal pond.

Helochares distinctus Jia & Tang, sp. nov.

http://zoobank.org/385B9F5A-3203-4B21-902C-9FBC74DC07F6

Figs 3–5, 7, 10–23, 27–28

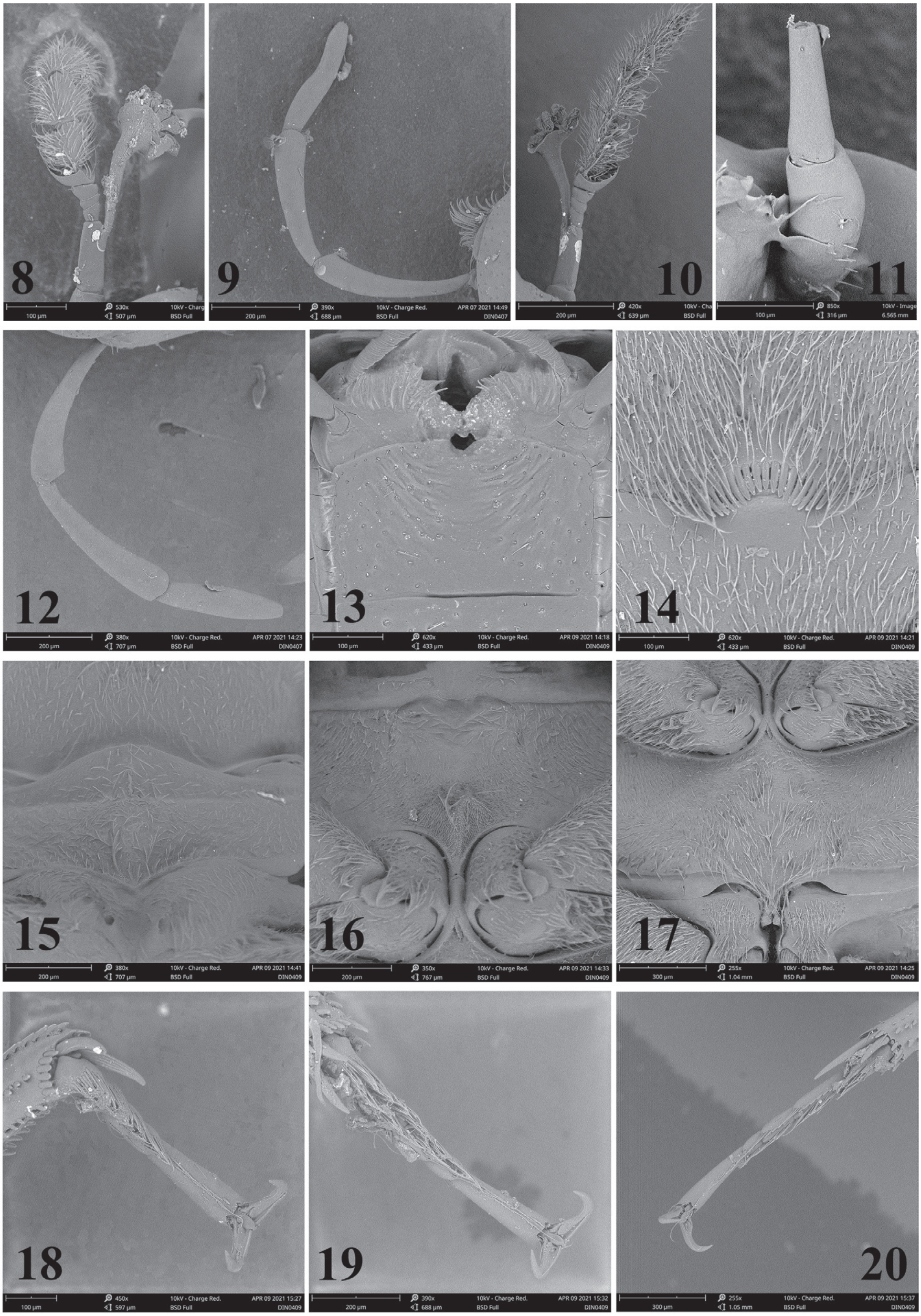
Material examined. ***Holotype*:** male, Jiangxi, Jing'an County, Zaodu town, Nanshan, 29°01'N, 115°16'E, 315m, 2.viii.2015, Renchao Lin and Yudan Tang leg. ***Paratype***: 1 male, Hunan, Guidong County, Bamianshan Nature Reserve, 25°58'21"N, 113°42'37"E, 973 m, 2015.vi.15, Renchao Lin and Yudan Tang leg.

Differential diagnosis. This species is very similar to *H. lentus* Sharp, 1890, *H. densus* Sharp, 1890, *H. sauteri* d'Orchymont, 1943 and *H. hainanensis* Dong & Bian, 2021 in size, form and other morphological characters, but it is very easy to distinguish from all known species by aedeagal features. Aedeagus (Figs 27–28) with median lobe slightly shorter than parameres, nearly rhombic, apex with a globular structure with a cluster of apical spines and with a long baseball-bat-shaped branch medially, membranous inner sac with some strong spinous protrusions. *Helochares distinctus* Jia & Tang, sp. nov. can easily be distinguished from *H. guoi* Jia & Yang, sp. nov. by its larger size, median lobe of the aedeagus with a globular structure with a cluster of spines apically and with a long baseball-bat-shaped branch medially; membranous inner sac with some less strongly spinous protrusions.

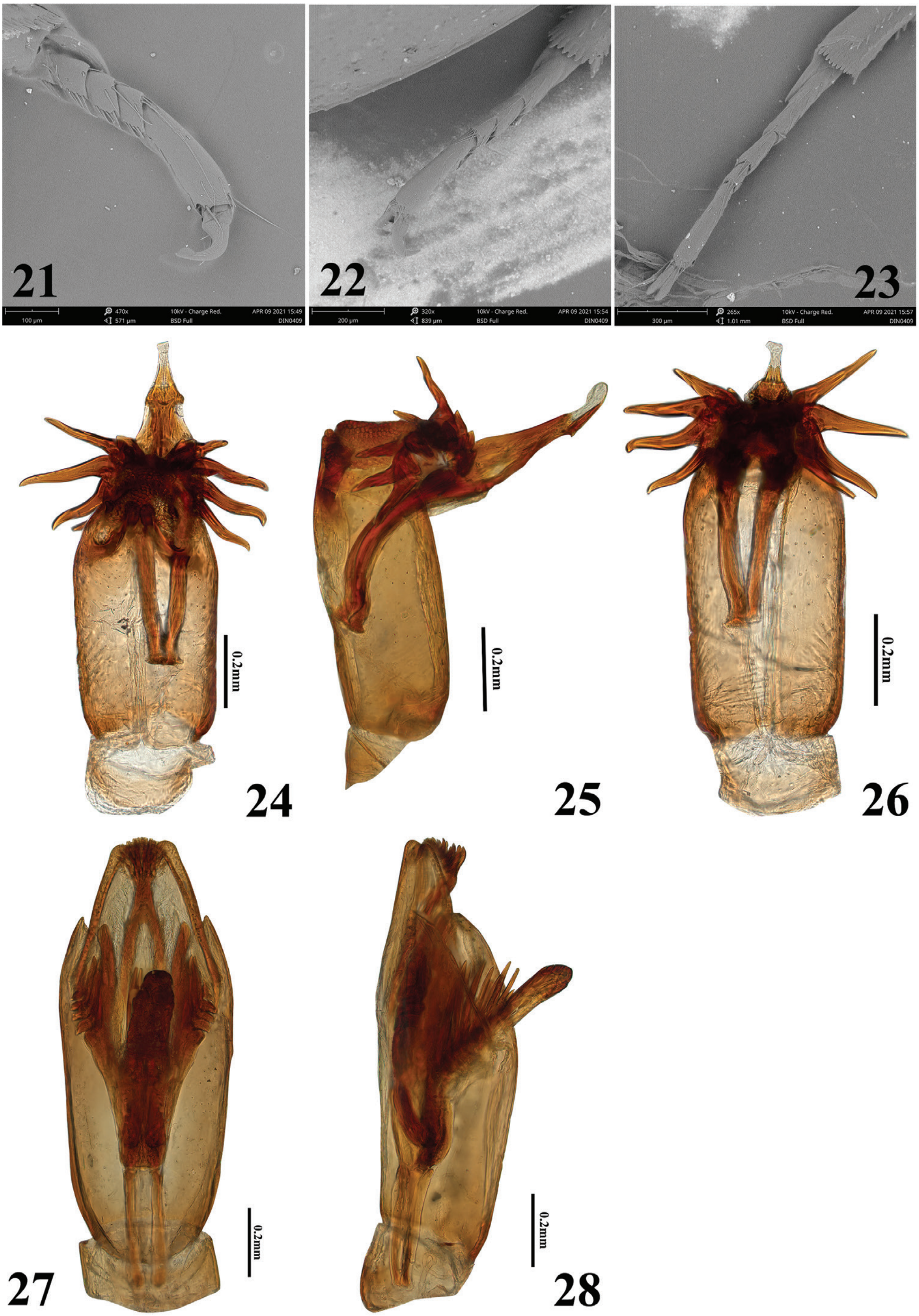
Description. ***Form and colour*** (Figs 3–5, 7). Body length 5 mm, body width 2.6 mm, oval, moderately convex. Dorsum of head, pronotum, elytra and clypeus yellow-brown, labrum dark brown. Antennae yellow-brown with club black. Maxillary palps uniformly yellow-brown. Labial palps yellow, not darkened apically. Venter, including legs, blackish brown, tarsomeres yellow-brown.

Head. Antennae with scape ca as long as antennomeres 2 and 3 combined (Figs 10–11). Maxillary palps ca 1.25 × as long as of width of head anterior to eyes; apical segment asymmetrical, slightly shorter than the penultimate in length (Fig.12). Clypeo-labral margin straight medially. Labrum, frons and clypeus with systematic punctures (with setae) same size as ground punctures; frons and clypeus with ground punctation dense and coarse, distance between punctures 0.5–1.2× width of one puncture. Mentum subquadrate, with anterior margin strongly emarginate, slightly depressed medially, surface with some oblique wrinkles (Fig. 13).

Thorax. Ground punctation on pronotum and elytron similar to that on head, distance between punctures 0.5–1.2× width of one puncture; anterior margin without very fine bead. Elytron with short scutellary series of punctures and 10 striae, punctures in striae distinctly coarser than ground punctures; systematic punctures as coarse as punc-



Figures 8–20. 8–9 *Helochares guoi* Yang & Jia, sp. nov. **8** antennae **9** maxillary palp **10–20** *Helochares distinctus* Jia & Tang, sp. nov. **10** the antennae of fig3 **11** the antennae of fig4 **12** maxillary palp **13** mentum **14** apex of fifth abdominal ventrite **15** prosternum **16** mesoventrite **17** metaventrite **18** protarsomeres **19** mesotarsomeres **20** metatarsomeres.



Figures 21–28. 21–23, 27–28 *Helochares distinctus* Jia & Tang, sp. nov. **24–26** *Helochares guoi* Yang & Jia, sp. nov. **21** protarsomeres **22** mesotarsomeres **23** metatarsomeres **24–28** aedeagus **24** ventral view **25** lateral view **26** dorsal view **27** dorsal view **28** lateral view.

tures in striae. Prosternum moderately elevated medially, not tectiform or carinate medially (Fig. 15), with a transverse groove anteriorly. Mesoventrite with a small posteromedial tubercle, not carinate medially (Fig. 16). Metaventrite without glabrous area posteromedially. Femora densely pubescent except at apex (Fig. 17). Meso-, and metasomeres 1 to 4 with dense long setae ventrally, posterior tarsomeres with a fringe of long swimming-hairs dorsally. Protarsal claws in male somewhat stronger and a little angularly curved, bearing a blunt basal tooth; mesotarsal claws as protarsals, but only moderately curved with a blunt tooth; metatarsal claws only moderately curved, with a blunt basal tooth (Figs 18–23).

Abdomen. Ventrites uniformly and densely pubescent. Fifth (apical) abdominal ventrite with apical emargination fringed with stiff yellowish setae (Fig. 14).

Aedeagus (Figs 27–28). Phallobase ca 0.24 mm; paramere ca 1.0 mm, widest at the apical quarter, apical quarter slightly narrowed towards to the apex, apex rounded; median lobe slightly shorter than the parameres, ca 0.91 mm, nearly rhombic, apex with a globular structure with a cluster of apical spines and with a long baseball-bat-shaped branch medially; membranous inner sac with some strong spinous protrusions; basal apophyses about one third as long as the median lobe, ca 0.35 mm.

Remark. The antennal pedicel of the male paratype also bears a long fungus as in *H. guoi* Yang & Jia sp. nov. (Fig. 10). However, the male holotype of this species lacks such a structure, although only the scape and pedicel remained on the right antenna (Fig. 11) and the left antenna was lost.

Etymology. Latin “distinctus”, referring to the antennae and aedeagus with clearly different characters from other known species.

Distribution. China (Jiangxi, Hunan).

Habitat. Living on edge of stagnant water pool.

Additional faunistic data. Fig. 65

Helochares hainanensis **Dong & Bian, 2021**

Figs 29, 38, 45, 46

Helochares (*Hydrobaticus*) *hainanensis* Dong & Bian, 2021:168. Type locality: China (Hainan).

Material examined. Guangdong: 6 males, 11 females, Shenzhen, Dapeng Peninsula, Kuichong, Paiyashan Mt., alt. 8 m, 22°38'59"N, 114°30'37"E, 5.xi.2018, Weicai Xie leg.

Distribution. China (Hainan, Guangdong). New for Guangdong.

Habitat. This species occurs in mud with aquatic grass at the edge of a pool.

Helochares nipponicus **Hebauer, 1995**

Figs 30, 47, 48

Helochares striatus Sharp, 1873: 60. Type locality: Japan (Kyushu).

Helochares nipponicus Hebauer, 1995: 6 (RN). Species name "striatus" was preoccupied by *Hydrobius striatus* Boheman, 1851 (= *Helochares striatus* (Boheman 1851)).

Material examined. Nei Mongol: 1 male, Tongliao, The source of Daqinggou, 235 m, 27.viii.2014, Weijie Sun leg. **Jiangxi:** 1 male, Shangrao, Sanqingshan, 15–20.iv.2007, Fenglong Jia leg.

Distribution. China (Jilin, Nei Mengol, Jiangxi), Japan, Korea. New for Jiangxi and Nei Mongol.

Habitat. This species occurs in mud with aquatic grass at the edge of a pool.

Helochares negatus Hebauer, 1995

Figs 31, 39, 49, 50

Helochares negatus Hebauer, 1995b: 5. Type locality: Bangladesh (Dinajpur).

Material examined. Yunnan: 2 males, 3 spec., Mengla, 4.viii.2007, Jiahui Li leg., 1 male, Mengla, Wangtianshu, 22.vii.2011, Yun Li leg., 1 male., Puer, 29.vii.2007, Fenglong Jia leg.; 5 males, 2 females, Yingjiang County, Tongbiguan village, Kaibangyahu, 24.58°N, 97.67°E, 1289 m, 25.v.2016, Yudan Tang and Ruijuan Zhang leg.

Distribution. China (Yunnan), Bangladesh. New for China.

Habitat. This species occurs in mud with aquatic grass at the edge of a pool. It is occasionally collected by light trap.

Helochares minusculus d'Orchymont, 1943

Figs 32, 40, 51, 52

Helochares minusculus d'Orchymont, 1943a: 10. Type locality: Indonesia (Sumatra).

Material examined. Guangdong: 3 males, 44 spec., Zhuhai, 24.xi.2007, Fenglong Jia leg.; 4 spec., Zhuhai, Qi'ao Island, 12.VII.2005, Fenglong Jia leg.; 1 male, Shaoguan, Danxiashan, 27.v.2010, Fenglong Jia leg.

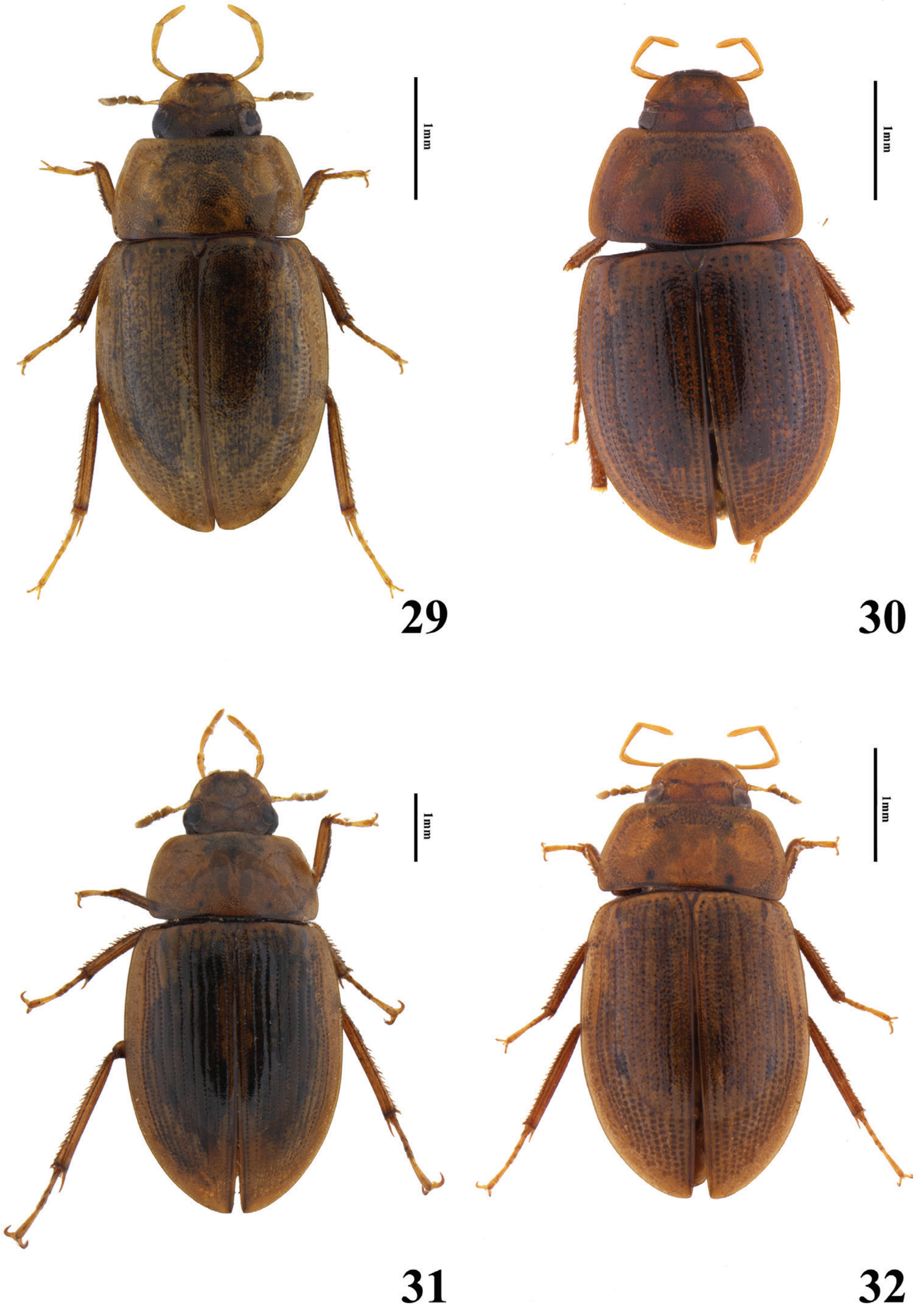
Distribution. China (Guangdong), Myanmar, Indonesia. New for China.

Habitat. This species occurs in mud with aquatic grass at the edge of pool or slow stream.

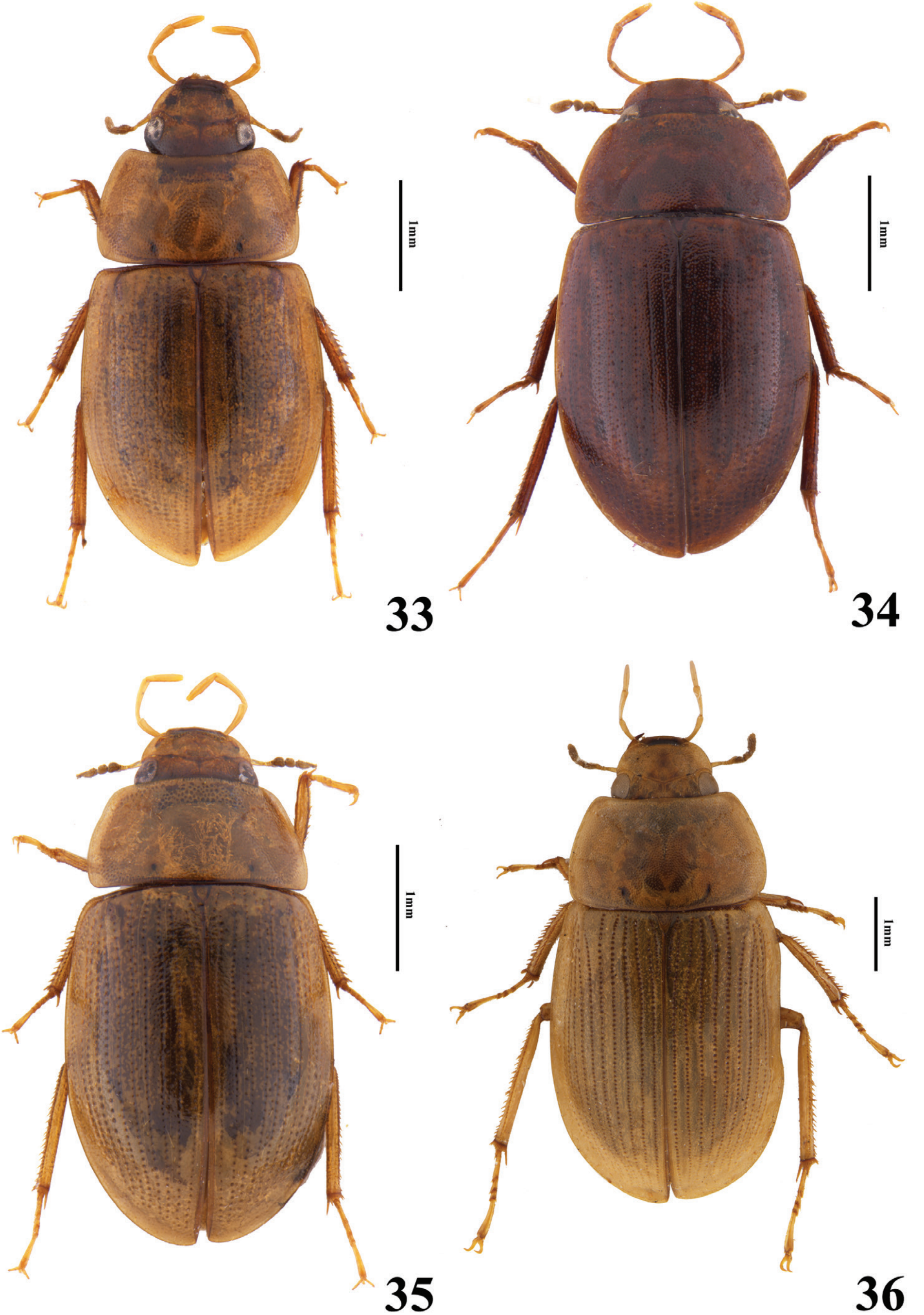
Helochares sauteri d'Orchymont, 1943

Figs 33, 41, 53, 54, 55

Helochares Sauteri d'Orchymont, 1943a: 6. Type locality: China (Taiwan).

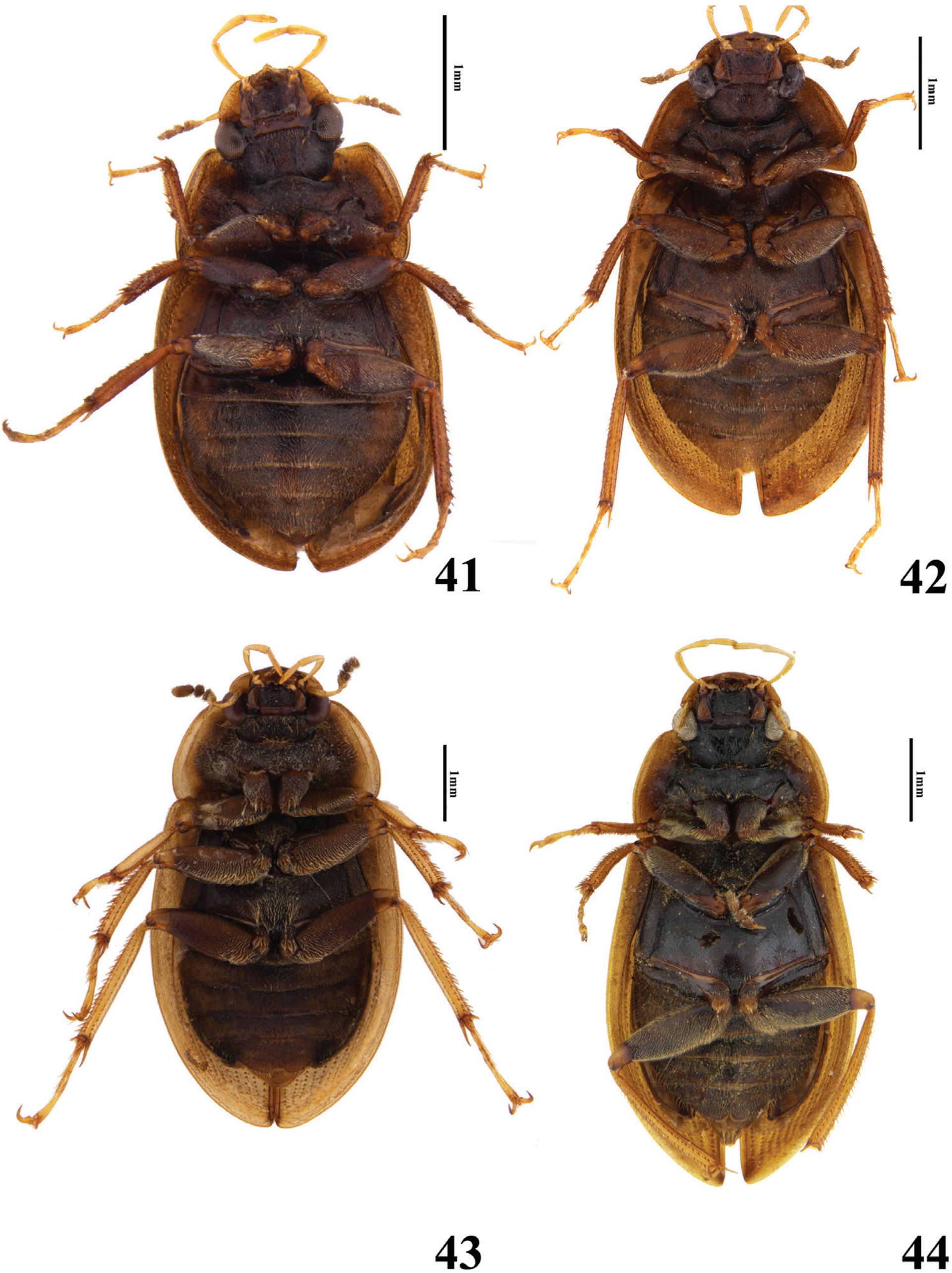


Figures 29–32. Habitus of *Helochares* spp., dorsal view **29** *H. hainanensis* Dong & Bian **30** *H. nipponicus* Hebauer **31** *H. negatus* Hebauer **32** *H. minusculus* d'Orchymont.



Figures 33–36. Habitus of *Helochares* spp., dorsal view **33** *H. sauteri* d'Orchymont **34** *H. densus* Sharp **35** *H. lentus* Sharp **36** *H. neglectus* (Hope).

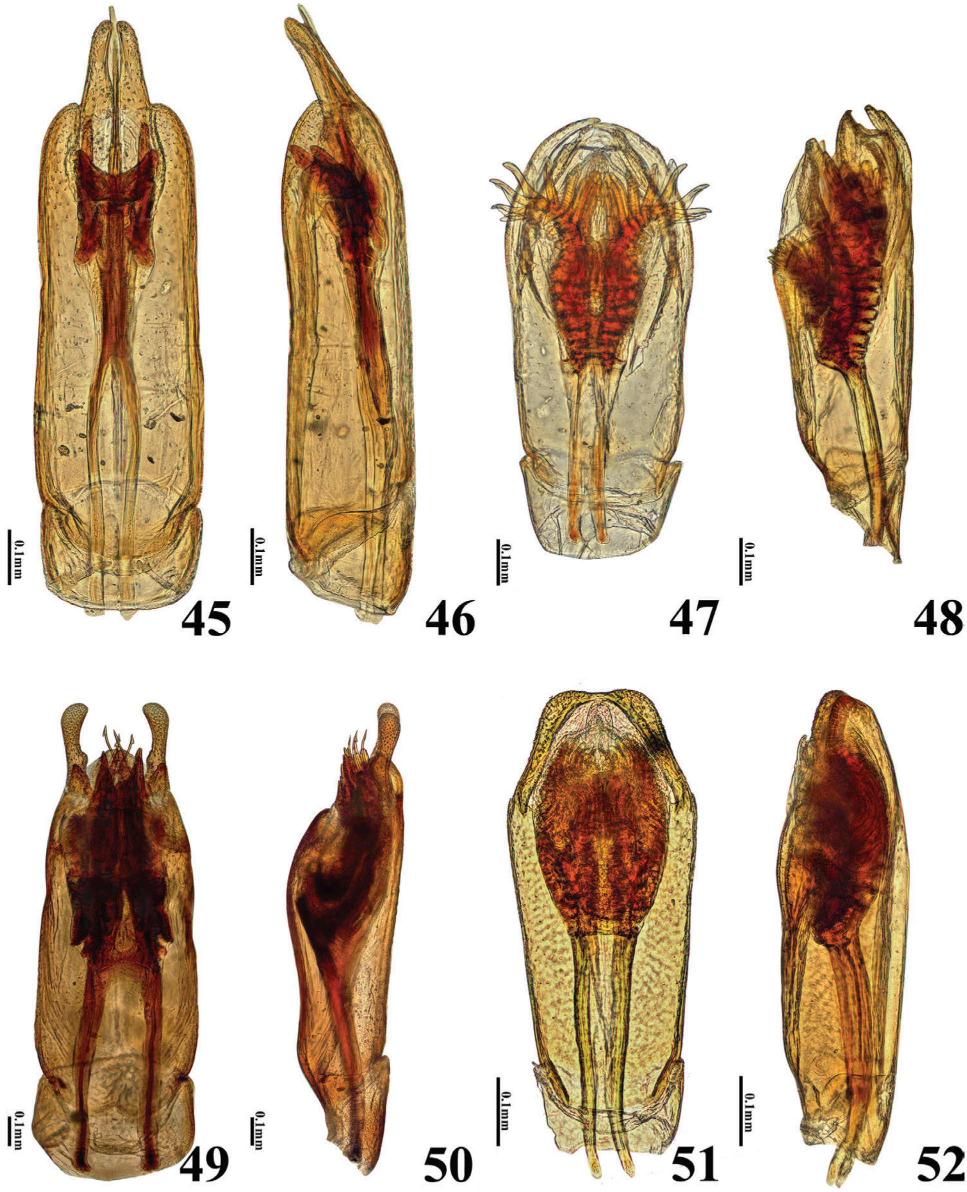
Figures 37–40. Habitus of *Helochares* spp. **37** *H. anchoralis* Sharp (dorsal) **38** *H. hainanensis* Dong & Bian (ventral) **39** *H. negatus* Hebauer (ventral) **40** *H. minusculus* d'Orchymont, (ventral).



Figures 41–44. Habitus of *Helochares* spp **41** *H. sauteri* d'Orchymont (ventral) **42** *H. lentus* Sharp (ventral) **43** *H. neglectus* (Hope) (ventral) **44** *H. anchoralis* Sharp (ventral).

Material examined. ***Paratype*** male (IRSN), Ta-maon Id. (II), 92–87. A. d'Orchymont det.: *Helochares* (*Hydrobaticus*) *sauteri* m., coll. A. d'Orchymont.

Additional material examined. Hubei: 1 spec., Wuchang, 17.v.1961, Zhelong Pu leg. **Zhejiang:** 55 spec., Tianmushan, 27.vii.-10.viii.2009, Fenglong Jia leg. **Jiangxi:** 1



Figures 45–52. Aedeagi of *Helochares* spp **45–46** *H. hainanensis* Dong & Bian **45** dorsal **46** lateral **47–48** *H. nipponicus* Hebauer **47** dorsal **48** lateral **49–50** *H. negatus* Hebauer **49** dorsal **50** lateral **51–52** *H. minusculus* d'Orchymont **51** dorsal **52** lateral.

male, 5 females, Yichuan City, Yifeng County, Guanshan nature reserve, 26°30'05.63"N, 114°00'53.19"E, 379 m, 17–18.vi.2016, Yudan Tang and Ruijuan Zhang leg.; 11 spec., Jiulianshan, 20.iv.2009, Fenglong Jia leg.; 42 spec., Shangrao, Sanqingshan, 15.viii.2006 & 15–20.iv.2007, Fenglong Jia and Haidong Chen leg.; 4 spec., Jinggangshan, Baiyinghu, 800m, 27.iv.2011, Fenglong Jia leg.; 6 spec., Jinggangshan, Shuangxikou, 3.x.2010, Shuang

Zhao and Fenglong Jia leg.; 2 spec., Jinggangshan, Dajing parkland, 19.ix.2010, Shuang Zhao leg.; 3 spec., Jinggangshan major peak, 2.x.2010, Yue Jia and Yuran Cao leg.; 1 spec., Jinggangshan, Jingzhushan, 4.x.2010, Fenglong Jia leg.; 21 spec., Jing'an County, Sanzhaolun village, Tangli, 260 m, 3.viii.2015, Renchao Lin and Yudan Tang leg.; 22 spec., Suichuan County, Nanfengmian nature reserve, 816 m, 18.vi.2015, Renchao Lin and Yudan Tang leg.; 17 spec., Jing'an County, Daqishan forestry centre, 350 m, 16.vii.2014, Renchao Lin leg.; 6 spec., Jing'an County, Zaodu town, Nanshan village, 315 m, 19.vii.2014, Renchao Lin leg.; 1 male, 5 spec., Shangyou County, Guanggushan, 25°55'11"N, 114°03'04"E, 846 m, 21.vi.2015, Renchao Lin and Yudan Tang leg. **Hunan:** 1 male, 3 females, Hunan, Zhuzhou City, Taoyuandong nature reserve, 28°33'16.73"N, 113°34'55.97"E, 394 m, 14–15.vi.2016, Yudan Tang and Ruijuan Zhang leg.; 2 spec., Nanyue, 4.ix.1941, Zhelong Pu leg.; 3 spec., Zhuzhou City, Yanling County, Taoyuandong, Jiashui, 19.v.2014, Renchao Lin and Xiaolin Liu leg.; 2 spec., Zhuzhou City, Yanling County, Taoyuandong, Mihua village, 25.v.2014, Renchao Lin and Xiaolin Liu and Chang Pan leg.; 1 spec., Zhuzhou City, Yanling County, Taoyuandong, 20.v.2014, Xiaolin Liu and Chang Pan and Weicai Xie leg. **Fujian**: 4 spec., Wuyishan, Daanyuanhe, 16.vii.2010, Fenglong Jia leg.; 14 spec., Nanjing, Hexi town pond, 13.vii.2010, Fenglong Jia leg.; 1 spec., Ningde City, Ningde normal college behind the mountain, 200 m, 3.x.2012, Zeyu Wang leg. **Guangdong**: 40 spec., Shaoguan, Danxiashan, 20.iv.2008 & 16.V.2009 & 27.V.2010 & 28.VIII.2012 & 23–26.IV.2013, Fenglong Jia and Keqing Song and Shuang Zhao leg.; 3 spec., Danxiashan, Zhanglaofeng, 8.vi.2012, Fenglong Jia leg.; 2 spec., Danxiashan, Yangyuanshan, 10.vi.2011, Fenglong Jia leg.; 1 spec., Danxiashan, Jinshiyan, 22.iv.2012, Fenglong Jia and Junlei Liao leg.; 2 spec., Fengkai, Heishiding, 20–22.vii.2007, Fenglong Jia and Lijun Yang leg.; 2 spec., Fengkai, Heishiding, 2.vii.2011, Fenglong Jia and Lijun Yang leg.; 4 spec., Nanling, Dadongshan, 24.vi.2009, Fenglong Jia leg.; 2 females, Guangzhou, Baiyunshan, 18.iv.1958, Zhelong Pu leg.; 8 spec., Huizhou, Longmen County, Nankunshan, 23.6538N 113.9469E, 239.6 m, 26.ix.2021, Zhuoyin Jiang and Zuqi Mai leg. **Guizhou**: 2 spec., Pingba, Machang, 13.viii.1982, Zhihe Huang leg.; 2 spec., Rong County, Pingyang village, Xiaodanjiang, 15.ix.2005, Shuang Zhao leg. **Sichuan**: 6 spec., Leshan City, Emeishan, Qingyin'ge, 750 m, 7.vi.2014, Renchao Lin leg.; 2 spec., Emeishan, 6.vii.1982, Zhihe Huang leg.; 3 spec., Qingchengshan, 8.viii.1982, Zhihe Huang leg.

Distribution. China (Fujian, Guangdong, Guizhou, Hubei, Hunan, Jiangxi, Sichuan, Taiwan, Zhejiang). New for Hunan.

Habitat. This species occurs in mud, or under root of waterside grass of pool or slow stream. It can occasionally be collected in mud without aquatic grass, and by light trap.

Helochares densus **Sharp, 1890**

Figs 34, 56, 57, 58

Helochares densus Sharp, 1890: 352. Type locality: Sri Lanka (Kandy; Dikoya; Bogawantalawa).

Figures 53–60. Aedeagi of *Helochares* spp **53–55** *H. sauteri* d'Orchymont **53** dorsal, **54** lateral, **55** ventral and data of specimen **56–58** *H. densus* Sharp **56** dorsal **57** lateral **58** ventral and data of specimen **59–60** *H. lentus* Sharp **59** dorsal **60** lateral.

Material examined. 1 male (IRSN), coll. R.I.Sc.N.B. N. Vietnam Tonkin, Hoa Bih, de Cooman leg.; A. d'Orchymont det.: *Helochar*. (*Hydrobaticus*) *densus* sb., with a handwriting label: *Helochares densus*.

Additional material examined. Guangdong: 4 males, 3 females, Shenzhen, Dapeng Peninsula, Kuaichong, Paiyashan Natural Park, 22°38'59"N, 114°30'37"E, alt. 8 m, 5.xi.2018, Fenglong Jia and Weicai Xie leg.; 1 male, Shenzhen, Dapeng Peninsula, Bantianyun, 22°31'16"N, 114°29'43"E, alt. 127.73 m, 7.viii.2019, Zhenming Yang, Zhuoyin Jiang, Guangyu Guo and Xinyuan Ji leg.; 4 spec., Shenzhen, Paiyashan, 17.v.2012, Fenglong Jia and Junlei Liao leg.; 3 spec., Shenzhen, 8–15.viii.2006, Fenglong Jia leg.; 1 male, 2 females, Shenzhen, Pingshan, Malanshan, 22°38'31"N, 114°19'41"E, alt. 284 m, 27.vii.2019, Zhenming Yang, Zhuoyin Jiang, Guangyu Guo and Xinyuan Ji leg. Shenzhen, Neilingding, 10.v.1998, Tongxu Peng leg.; 9 spec.; 9 spec., Zhuhai, 24.xi.2007, Fenglong Jia leg.; 3 spec., Zhuhai, the mountain behind of campus of Sun Yat-sen University, 5–8.vii.2011, Fenglong Jia leg.; 5 spec., Zhuhai, Hengqin Island, 10.vii.2006, Fenglong Jia leg.; 4 spec., Zhuhai, Qi'ao Island, 12.vii.2005, Fenglong Jia leg.; 2 spec., Danxiashan, Jinshiyan, 8.vi.2012, Fenglong Jia leg.; 1 spec., Danxiashan, the north of Yangyuanshi paddyfield, 23.iv.2012, Junlei Liao leg.; 1 spec., Xinhui, 6.iv.2006, Fenglong Jia leg.; 20 spec., Guangzhou, Baiyunshan, 23.1978N 113.2948E, 15.ix.2021, Zhuoyin Jiang and Zuqi Mai leg.; 1 male, 2 females, Guangzhou, Kangle, 24.vii.1964, Jiuru Zhang leg.; 1 spec., 1 spec., Shantou, 15.v.1964, Tongxu Peng leg. **Guangxi:** 1 female, Jingxi, Bangliang, 6.viii.2010, Jianhua Huang leg. **Hainan:** 4 spec., Jianfengling, 22.xi.1983, Zhihe Huang leg.; 2 spec., Jianfengling, Tianchi, 5–6.vii.1981, Guofeng He leg.; 3 spec., Wanning, 17.xii.1957, Cuiying Li leg.; 2 spec., Tongshi, 19.xii.1957, Cuiying Li leg.; 1 spec., Xinglong, 3.i.1964, Tongxu Peng leg.; 6 spec., Changjiang, Bawang town, 10.v.2007, Yibing Ba and Juntong Lang leg. **Macao**: 1 male, Ludangcheng, ecological preservation area, one area, 15–16.x.2016, Fenglong Jia and Weicai Xie leg. **Yunnan:** 2 spec., 1090 m, 30.vii.2010, Wangang Liu leg.; 1 female, Honghehekou, Binglangzhai Reservoir, 4.v.2011, Yun Li light trap.; 1 spec., Mengla, Wangtainshu, 6–7.viii.2007, Guodong Ren and Wenjun Hou and Yalin Li leg.; 1 male, Xishuangbanna Botanical Garden (west area), near Wanglian Hotel, 4–11.iv.2021, Huang Baoping leg.

Distribution. China (Fujian, Guangdong, Guangxi, Hainan, Hunan, Jiangxi, Macau, Sichuan, Yunnan, Zhejiang), Andaman Islands, India, Thailand, Vietnam. New for Macao.

Habitat. This species occurs in mud with aquatic grass at the edge of pool, under root of waterside grass, or slow stream. It never was collected by light trap.

Helochares lentus **Sharp, 1890**

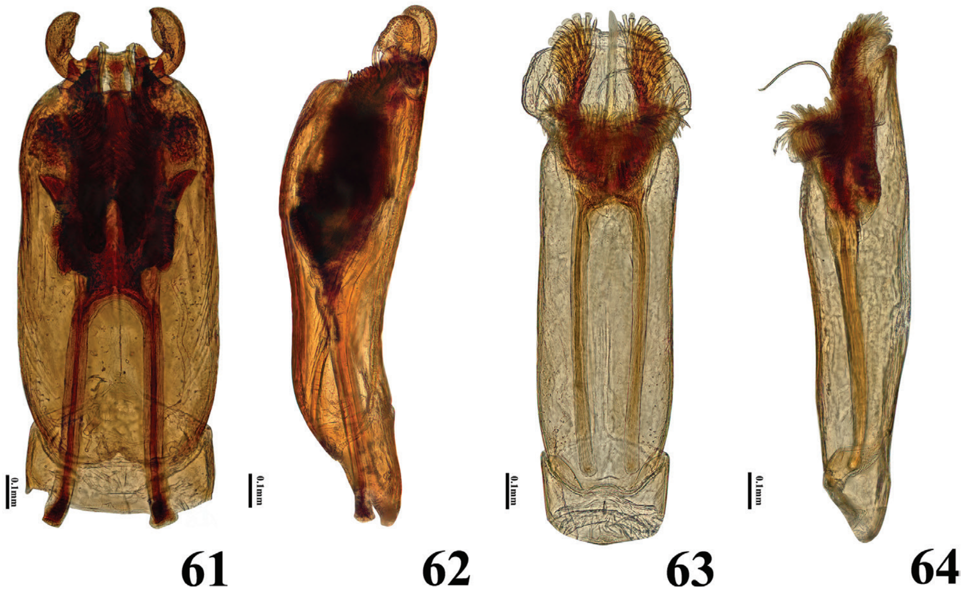
Figs 35, 42, 59, 60

Helochares lentus Sharp, 1890: 352. Type locality: Sri Lanka (Dikoya).

Material examined. Guangdong: 1 male, 1 spec., Xuwen, 27.ix.1985, Zhihe Huang leg.; 1 spec., Zhanjiang, Chikan, 25.ix.1985, Zhihe Huang leg.; 10 spec., Fengkai, Heishiding, 13.viii.2010, Fenglong Jia, Yue Jia, Bingjie Chen and Weilin Xu leg.; 12 spec., Fengkai, Heishiding, 4–6.x.2013, Fenglong Jia, Yue Jia, Bingjie Chen and Weilin Xu leg.; 8 spec., Fengkai, Heishiding, 20–22.ix.2014, Fenglong Jia, Renchao Lin and Yudan Tang leg.; 2 spec., Fengkai, Heishiding, 29.v.1984, Wu Wu leg.; 2 spec., Fengkai, Heishiding, 10.iv.1985, Zhihe Huang leg.; 2 males, Guangzhou, Conghua, Liuxihe, Xitou village, 23.7125N 113.8697E, 398.6 m, 28.ix.2021, Zhuoyin Jiang and Zuqi Mai leg.; 4 spec., Shenzhen, Futian mangrove salt-water fish pond, 30.v.2015–1.vi.2015, Fenglong Jia and Renchao Lin leg. **Guangxi**: 6 spec., Fangcheng, Fulong, 24.v.1999, Xin Ke leg.; 3 spec., Napo, Nonghua, 750 m, 18.viii.1998, Fusheng Huang leg.; 1 spec., Jinxiu, Luoxiang, 200 m, 15.V.1999, Xuezhong Zhang leg. **Hong Kong:** 21 spec., Qingkuai pond, 29.x.2013. Y.M. Lee and Eric and Rex Ch Shih and Alex Lee leg.; 32 spec., Rongshuao, 10 m, 11.vi.2014, Fenglong Jia and Weicai Xie and Jiahuang Chen leg.; 2 spec., Nanyong (before the dam), 21 m, Fenglong Jia and Weicai Xie and Alex leg.; 2 spec., Shaluodong, 185 m, 28.x.2013, Fenglong Jia and Weicai Xie and Alex light trap. **Jiangxi**: 1 spec., Jing'an County, Zaodu town, Nanshan village, 315 m, 2.viii.2015, Renchao Lin and Yudan Tang leg. **Xizang:** 1 male (IZCAS), IOZ(E)2056679, Motuo County, Beibeng, near Liberation Bridge, 2016.VI.17N [N = night], 773 m, 29.2432°N, 95.1673°E, Liang Hongbin leg.; 1 male, 3 spec. (IZCAS), Motuo County, Beibeng, 2015.VIII.23N [N = night], 799 m, light trap, 29.3431°N, 95.1700°E, Liang Hongbin and Huang Zhengzhong leg. **Yunnan:** 6 spec., Yingjiang County, Nabang town, 24.75°N, 97.56°E, 239 m, Yudan Tang and Ruijuan Zhang leg.; 50 spec., Jingdong County, Taizhong town, 1395 m, 15.iv.2015, Renchao Lin and Yudan Tang leg. ; 2 spec., Pohui, 2.ix.1939, Zhelong Pu leg.; 2 spec., Lufeng village, 26.iii.1940.; 1 female, Jinping, Mengla, 370 m, 30.iv.1956, Keren Huang leg.; 1 female, Jingdong, 1170 m, 24.vi.1956, Keleirangnuofusiji leg.; 1 female, Hekou, Xiaonanxi, 200m, 7.vi.1956, Keren Huang leg.; 1 female, Cheli, Damenglong, 640 m, 29.iv.1957, Shuyong Wang leg.; 1 female, Mangshi, 1000 m, 12.v.1956, Benshou Zhou leg.; 4 spec., Mengla, 2007.viii.2, Jiahui LI leg.; 2 spec., Mengla Shangyong, 2007.viii.2, Lei Shi leg.; 1 spec., Mengla, 6–7.viii.2007, Guodong Ren, Wenjun Hou and Yaping Li leg.; 1 spec., Wangting, 2011.iv.29, Wangang Liu leg.; 2 spec., Yingjiang, 820 m, 25.v.1983, Lizhong Hua leg.; 2 spec., Huijiang, i.1940, Zhelong Pu leg. 3 males, 1 female, Xishuangbanna Botanical Garden (west area), near Wanglian Hotel, 4–11.iv.2021, Huang Baoping leg.; 1 male, 1 female, Honghe, Hani Automatic prefecture of Yi Nationality, Lvchun County, Niukong town, in Terrance, 1336 m, 22.9872°N, 102.2675°E, Jiang Zuoyin, Yang Zhenming, Mai Zuqi and Huang Baoping leg.

Distribution. China (Fujian, Guangdong, Guangxi, Guizhou, Hong Kong, Hunan, Jiangxi, Sichuan, Taiwan, Xizang, Yunan), Bangladesh, Cambodia, India, Indonesia, Malaysia, Sri Lanka, Thailand, Vietnam. New for Fujian, Guangdong, Guizhou, Hunan, Jiangxi, Sichuan.

Habitat. This species occurs in mud with aquatic grass at the edge of a pool. It can occasionally be collected by light trap.



Figures 61–64. Aedeagi of *Helochares* spp **61–62** *H. neglectus* (Hope) **61** dorsal **62** lateral **63–64** *H. anchoralis* Sharp **63** dorsal **64** lateral.

Helochares neglectus **(Hope, 1854)**
Figs 36, 43, 61, 62

Hydrobius neglectus Hope, 1854: 16. Type locality: Guangzhou, China.
Helochares crenatus (Régimbart, 1903): Pu 1963: 79 (misidentification, Yunnan).

Material examined. Jiangxi: 30 spec., Jing'an County, Zaodu town, Nanshan village, 315 m, 29°01'N, 115°16'E, 2.viii.2015, Renchao Lin and Yudan Tang leg.; 5 spec., Jing'an County, Zaodu town, Nanshan village, 315 m, 29°01'N, 115°16'E, 2.viii.2015, Renchao Lin and Yudan Tang leg.; 32 spec., Jing'an County, Zaodu town, Nanshan village, 315 m, 29°01'N, 115°16'E, 19.vii.2015, Renchao Lin and Yudan Tang leg.; 1 spec., Lushan, Poyanghu, 10.viii.1963, Zhelong Pu leg.; 1 spec., Jiujiang, 24.viii.1941, Zhelong Pu leg. **Hunan**: 1male, 8 spec., Jishou City, Mayang County, Lancui village, 27°46'17"N, 109°51'41"E, 349 m, 15.ix.2016, Fenglong Jia and Ruijuan Zhang leg.; 3 spec., Yizhang, 8.x.1941, Zhelong Pu leg.; 2 spec., Tongdao, 19.viii.1982, Zhihe Huang leg.; 2 spec., Xianghuaihua, Yushuwan, 17.vi.1965, Zhenyao Chen leg.; 1 spec., Nanyue, 4.ix.1941, Zhelong Pu leg.; 1 spec., Chengyuan, 6.iii.1941, Zhelong Pu leg. **Fujian:** 1 spec., Fu'an, 20.ix.1963, Shanxiang Lin leg. **Guangdong:** 1 sepc., Shenzhen, Neilingding nature reserve, 22°24'44"N, 113°48'46"E, 6m, 23–26.viii.2016, Fenglong Jia, Weicai Xie, Ruijuan Zhang and Shishuai Wang leg.; 27 spec., Baiyunshan, 2.xi.1964, Jincai Bao leg.; 20 spec., Guangzhou, Luhu, 2.xi.1964, Zhenyao Chen leg.; 32 spec., Lianhe, 18.x.1964, Zhenyao Chen and Jincai Bao leg.; 20 spec., Guangzhou, Xinshi, 11.x.1964, Chengmu Chen and Zhengwei Huang leg.; 10 spec., Heshan, 22–24.iv.2002, Ruizhen Wen leg.; 7 spec., Heshan, 6.vi.2006, Guilin Liu leg.; 8 spec., Dongguan, Lianhuashan, 20.vi.2002, Guilin Liu leg.; 12 spec., Lianzhou, Dadongshan, 25.ix.2008, Yun Wang leg.; 2 spec., Dinghu, 10.v.1994, Fenglong Jia leg.; 1 spec., Dinghushan, 22–23.v.1964, Ping Lin and Yaoquan Li leg.; 1 spec., Xuwen, 27.ix.1985, Zhihe Huang leg.; 1 spec., Henan, Kangle, 30.vi.1964 & 13.vii.1964, Qiuquan Li, Jiuru Zhang, Shitian Li and Shunbang Liu leg.; 6 spec., Henan, Kangle, 2.vii.1965, Qiuquan Li, Jiuru Zhang, Shitian Li and Shunbang Liu leg.; 2 spec., Henan, Kangle, xii.1962, Qiuquan Li, Jiuru Zhang, Shitian Li and Shunbang Liu leg.; 1 spec., Xinhui, viii.2001, Xiaoli Tong leg.; 4 spec., Guangzhou, viii.1938, Zhelong Pu leg.; 6 spec., Guangzhou, Henan, 24.v.1957, Zhelong Pu leg.; 1 spec., Guangzhou, Xicun, 3.iv.1963, Yousheng Lai leg.; 1 spec., Sun Yat-sen University campus, 26.iv.1963, Youzheng Lai leg.; 2 spec., Sun Yat-sen University campus, vii.1985, Youzheng Lai leg.; 2 spec., Guangzhou, Shipai, 26.vi.1955, Zhaojian Liang leg.; 2 spec., Guangzhou, Chisha, 28.ix.1964, Zhenyao Chen leg.; 3 spec., Guangzhou, Shipai, 18.x.1964, Zhaojian Liang leg.; 1 spec., Guangzhou, Ruyuan, Longxi, 9.x.1964, Zhenyao Chen leg.; 1 female, Guangzhou, Shuzhugang, 3.v.1957, Zhelong Pu leg.; 1 female, Shenzhen, 12–15.viii.2006, Fenglong Jia leg.; 10 spec., Shenzhen, Futian Mangrove nature reserve, 2–4.iv.2015, Fenglong Jia, Renchao Lin, Zhenhua Liu and Kai Chen leg.; 7 spec., Shenzhen, Futian Mangrove nature reserve, 30.v-1.vi.2015, Fenglong Jia, Renchao Lin, Yudan Tang and Kai Chen leg.; 1 spec., Shenzhen, Luohu, Yinhu, 28.xi.1998, Fenglong Jia leg.; 2 spec., Lianxian, vi.1945, Zhelong Pu leg.; 1 spec., Fengkai, Heishiding, 1.vii.1987, Chen leg.; 1 female, Gaoming, Yangmei town, 23–26.iv.2006, Fenglong Jia leg.; 1 spec., Yingde, 5.viii.1962, Ping Lin leg.; 1 spec., Guangzhou, Xinzhou, 17.vi.1963, Youshen Lai leg.; 1 spec., Guangzhou, Shilangang, 9.v.1963, Youshen Lai leg. **Guangxi**: 98 spec., Yangshuo, 1985, Shoujian Chen leg.; 3 spec., Nanning, 19.vi.1977, Zhelong Pu leg.; 10 spec., Nanning, vi.1958, Zhihe Huang leg.; 11 spec., Shangsi, Hongqi forestry centre, 300 m, 27.v.1999, Xuezhong Zhang leg.; 5 spec., Fangcheng, Fulong, 23.v.1999. Xin Ke leg.; 3 spec., Napo, Nonghua, 750m, 18.viii.1998, Fusheng Huang and Wenzhu Li leg.; 1 spec., Napo, Beidou, 550 m, 12.iv.1998, Chunsheng Wu leg.; 3 spec., Shangsi, Hualan town, Hualan village, 204 m, in pool, 6.vii.2011, Keqing Song leg.; 1 spec., Hechi, 4.xi.1941, Zhelong Pu leg.; 4 spec., Jingxi, Bangliang, 6.viii.2010, Jianghua Huang leg. **Hainan:** 1 spec., Hainan, 16.XII.1957, Cuiying Li leg.; 1 spec., Xinglong, 24.xii.1957, Cuiying Li leg.; 1 spec., Yinggeling, 5.iv.2008, Yuxia Yang leg. **Yunnan**: 3 spec., Yingjiang, 25.v.1983, Lizhong Hua leg.; 1 spec., Jinping, Mengla, 500 m, 20.iv.1956, Keren Huang leg.; 1 spec., Pohui, 2.ix.1979, Zhelong Pu leg.; 1 male, Xishuangbanna Botanical Garden (west area), near Wanglian Hotel, 4–11.iv.2021, Huang Baoping leg.

Distribution. China (Fujian, Guangdong, Guangxi, Hainan, Hong Kong, Hubei, Hunan, Jiangsu, Jiangxi, Shanghai, Sichuan, Yunnan, Zhejiang), Cambodia, Malaysia, Thailand, Vietnam. New for Hong Kong.

Habitat. This species occurs in mud with aquatic grass at the edge of pool, or under root of waterside grass on the bank of slow stream. It can sometimes be collected by light trap.

Helochares anchoralis Sharp, 1890

Figs 37, 44, 63, 64

Helochares anchoralis Sharp, 1890: 352. - Sri Lanka [Colombo].

Material examined. Jiangxi: 1 female, Nanchang. **Guangdong:** 3 spec., Henan, 24.v.1957, Zhelong Pu leg.; 1 spec., Henan, Fenghuang, 25.xi.1957; 2 spec., Guangzhou, Lianxian, vi.1945, Zhelong Pu leg.; 2 spec., Dinghu, 10.v.1994, Fenglong Jia leg.; 2 sepc., Sun Yat-sen University campus, 15.iv.1958.; 1 spec., Guangzhou, viii.1938, Zhelong Pu leg.; 1 spec., Shaoguan, Yingde, 4.viii.1962, Ping Lin leg.; 1 spec., Shenzhen, Neilingding, 3.vii.1998, Haidong Chen leg.; 1 female, Shenzhen, Paiyashan, 17.v.2012, Fenglong Jia and Junlei Liao leg.; 1 spec., Zhuhai, Hengqin Island, 10.vii.2006, Fenglong Jia leg. **Guangxi**: 10 spec., Yangshuo, 1985, Shoujian Chen leg.; 7 spec., Guangxi, Nanning, 19.vi.1977, Zhihe Huang leg.; 2 spec., Nanning, vi.1958, Zhelong Pu leg. **Hainan**: 3 spec., Sanya, 24.xii.1963, Tongxu Peng leg.; 1 male, Lingshui, Diaoluoshan, 29.xii.1963, Zhenda Lin leg.; 1 female, Hainan, 19.XII.1963, Tongxu Peng leg. **Chongqing** : 1 spec., Chongqing, 8.iii.1942, Xiangzhi Chen leg. **Yunnan**: 3 spec., Jingdong, 1200m, 9.v.1957, A. Mengqiaciji leg.; 4 spec., Xiaomengyang, 850m, 4.v.1957, Qiuzhen Liang leg.; 1 female, Cheli, 500m, 7.iv.1955, Keleirangnuofusiji leg.; 1 female, Pohui, 2.ix.1939, Zhelong Pu leg.; 1 female, Jinping, Mengla, 370m, 22.iv.1956, Keren Huang leg.; 1 female, Hekou, 8.vii.1977, Zhihe Huang leg.

Distribution. China (Chongqing, Fujian, Guangdong, Guangxi, Hainan, Hubei, Jiangxi, Sichuan, Taiwan, Yunnan), Bangladesh, Cambodia, India, Indonesia, Laos, Philippines, Sri Lanka, Thailand, Japan. New for Chongqing, Jiangxi and Guangxi**.**

Habitat. This species occurs in mud with aquatic grass at the edge of pool. It can occasionally be collected by light trap.

Discussion

Helochares is a typical tropical group that is mainly known from the Oriental and Afrotropical realms. Of the 20 species known from China, 18 occur south of the Qinling-Huaihe Line, *Helochares obscurus* (Müller, 1776) occurs in Xinjiang and *H. nipponicus* Hebauer, 1995 in the Palearctic Realm from Jilin to Zhejiang (Fig. 65). It is very possible that *H. nipponicus* will be found in south China with further exploration.

Although over 160 species of *Helochares* are described in the world, it is likely that there is still enormous potential for more new species to be described in the Oriental

65

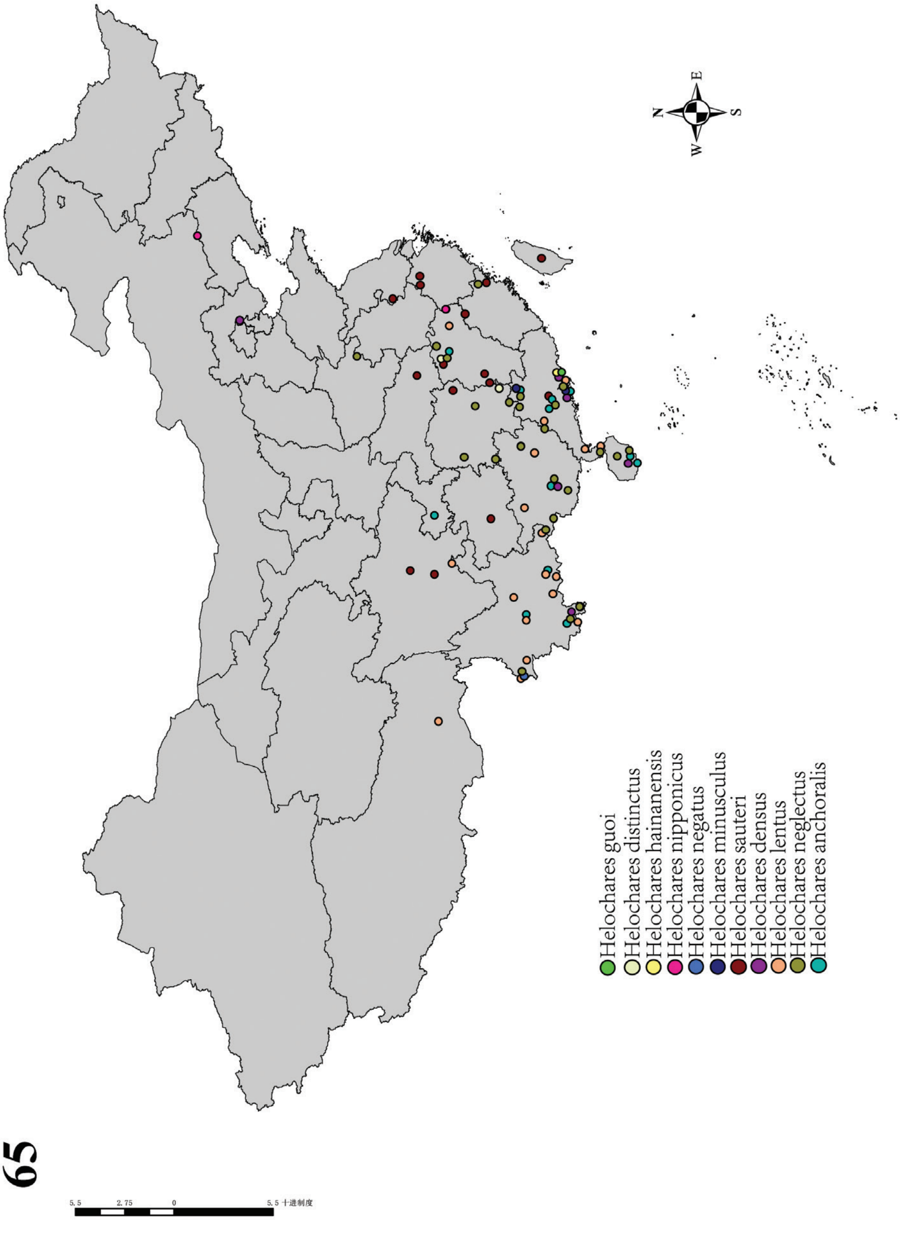


Figure 65. Distribution map of *Helochares* species in China.

Realm including the Chinese part. The discovery of new species and newly recorded species by Dong and Bian (2021) and us extends the known range of the genus in China. However, there are some dubious records.

d'Orchymont (1928) reported *H. crenatus* Régimbart, 1921 from Cambodia, Tokin, Bangladesh, Malaysia, Indonesia, Philippine, and India. d'Orchymont (1940) described *Helochares nebridius* d'Orchymont, 1940 and identified all the material of *H. crenatus* from Indonesia (Java, Sumatra) as *H. nebridius*. He also provided a table in which distribution information of all known species of *Helochares* in the Oriental Realm was given. Based on this table, *H. crenatus* only occurs in "Inde continentale" and other records of this species were excluded (d'Orchymont, 1943a). The distribution of this species outside India comprises Thailand (Hebauer 1995) and China (Pu 1963). Pu (1963) first reported *H. crenatus* from Yunnan, China based on one specimen, which was followed by Gentili et al. (1995), Hansen (1999) and Dong and Bian (2021). There has been no other report of the species from China. The specimen Pu checked is deposited in IZCAS. The second author visited IZCAS in 2018 and checked the specimens from Yunnan, but unfortunately the specimen was a female. After studying the specimen, the second author did not find any difference with *H. neglectus* (Hope), a very common species in Yunnan. The specimen of *H. crenatus* checked by Pu is probably *H. neglectus* (Hope). So, the report of *H. crenatus* from Yunnan is dubious. We suggest removal of *H. crenatus* from the Chinese fauna.

Dong and Bian (2021) described *Helochares tengchongensis* Dong & Bian, 2021 from Yunnan. The species was compared with *Helochares lentus* Sharp, 1890, a species that is common in China. However, based on the original description and photos, *H. tengchongensis* is much closer to *H. densus* Sharp, 1890. Therefore, it is necessary to compare the types of *H. tengchongensis* and *H. densus* to shed light on the status of *H. tengchongensis*.

Dong and Bian (2021) described *Helochares wuzhifengensis* Dong & Bian, 2021 from Wuzhifeng town, Jiangxi Province, China. This species was originally compared with *Helochares nipponicus* that is also distributed in eastern and northeastern China. We checked over 180 specimens from the Luoxiaoshan Mountain Range of which 147 specimens were from neighbouring areas of Wuzhifeng. All of the specimens we checked are *H. sauteri* d'Orchymont, 1943. Based on the photo of the aedeagus, *Helochares wuzhifengensis* is very similar to *H. sauteri* except for the apical process of the median lobe (see Dong and Bian, 2021: 170, fig. 7). We discussed with Bian the similarity between *H. sauteri* and *H. wuzhifengensis*. After carefully checking the holotype of *H. wuzhifengensis*, she told us that the aedeagus is very similar to that of *H. sauteri*, but the median lobe is much narrower apically (see Dong and Bian, 2021: fig. 7). However, the lateral membrane of the median lobe sometimes becomes nearly transparent after being treated with glacial acetic acid (Dong and Bian (2021) treated the aedeagus with this chemical). Therefore, we are not sure if the median lobes of the aedeagi of *H. wuzhifengensis* and *H. sauteri* are identical. This conflict may be solved by dyeing the aedeagus of the holotype of *H. wuzhifengensis*.

Acknowledgements

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Supplementary material 1

Two new species of Hydrophilidae were described and additional faunastic records of *Helochares* from China were provided

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Morphological and molecular characterisation of the Popijač's Yellow Sally, *Isoperla popijaci* sp. nov., a new stenoendemic stonefly species from Croatia (Plecoptera, Perlodidae)

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Abstract

A new species of the Yellow Sally genus (*Isoperla* Banks, 1906) is described, based on morphological (males and females adults, larval and egg) and molecular (the barcode region of the cytochrome c oxidase subunit I gene (*COI*)) features. Popijač's Yellow Sally, *I. popijaci* Hlebec & Sivec, **sp. nov.** inhabits two karstic sources of the Krasulja rivulet in Croatia. Male and female of the new species are characterised by colouration patterns of the head and pronotum; the dimensions of the female subgenital plate; the medial penial armature and oval-shaped egg without collar and anchor. The larvae differ from their congeners by the uniquely coloured head and pronotum. Based on morphological characteristics *I. popijaci* **sp. nov.** belongs to the *I. tripartita* species group. Phylogenetic and taxonomic relationships were reconstructed using three methods of phylogenetic inference and three species delimitation methods. As *I. popijaci* **sp. nov.** occurs at a narrow area of the Krasulja rivulet in Krbava field, the study puts emphasis on the conservation and hotspot importance of the temporary rivers in the Dinaric karst. Furthermore, the study accentuates the necessity for further research on the genetic diversity of Plecoptera in Croatia.

Keywords

Conservation, Dinaric karst, DNA barcoding, *Isoperla popijaci* sp. nov., karstic source, species delimitation

Introduction

Predominantly regarded as a biological indicator of well oxygenated water in freshwater ecosystems (Illies and Schmitz 1980; Hamid and Rawi 2017; Morinière et al. 2017; DeWalt and Ower 2019; Ferreira et al. 2020), stoneflies (Plecoptera) and their absence can indicate pollution, changes in habitat conditions, habitat destruction and climate changes (Urbanič and Toman 2007; Fochetti and Tierno de Figueroa 2008; Bálint et al. 2011). In total, 50 Plecoptera species are reported from Croatia and, due to the many suitable habitats, it is assumed that this number is higher (Popijač and Sivec 2009a, 2009b). Members of the subfamily Perlodinae are, in general, vividly coloured, medium to large-sized, show high genetic diversity and are often microendemic (Zwick 1973, 2004; Li and Murányi 2015). The genus *Isoperla* Banks, 1906 is represented by 188 species worldwide and 60 species in Europe (DeWalt et al. 2020). The genus has a Holarctic and Oriental distribution (Zwick 1973; Szczytko and Stewart 1979; Sandberg and Kondratieff 2013; Szczytko and Kondratieff 2015) and represents the most diverse genus of the family Perlodidae in Europe (Graf et al. 2009, 2018). Thereby, the area of the Balkan stands out as a diversity hotspot with 21 species, of which 12 are endemic to the Peninsula and often restricted to specific habitats (Murányi 2011; Murányi et al. 2016).

Basic characteristics by which the species within the genus *Isoperla* are distinguished are penial morphology, head and pronotal pattern, egg structures and drumming signals (Despax 1936; Illies 1952, 1954, 1966; Sivec and Stark 2002; Murányi 2011; Michalik et al. 2017). In the last few years, a considerable number of new Plecoptera taxa have been described, especially from China (Li et al. 2013; Ji et al. 2014; Li and Murányi 2015; Chen et al. 2019; Cao et al. 2020), but also in Europe, like *Isoperla pesici* Murányi, 2011; *I. autumnalis* Murányi, 2011; *I. citrina* Murányi, 2011 (Murányi 2011; Murányi et al. 2016); *I. vjosae* Graf et Vitecek, 2018 (Graf et al. 2018); *I. claudiae* Graf et Konar, 2014 (Graf et al. 2014) and *I. nagyi* Murányi, Kovács et Graf, 2020 (Murányi et al. 2020).

During fieldwork research since 2004, ten *Isoperla* species were recorded in Croatia. An additional one is here described as *Isoperla popijaci* sp. nov., which shares morphological characteristics of the penial armature with species from the *I. tripartita* species group.

The following study provides a morphological description of the new species: illustrations of the main taxonomical characters (in males, females, larvae and eggs); as well as its phylogenetic placement within the genus based on the mitochondrial cytochrome c oxidase subunit I (*COI*) barcode region as a marker. Moreover, the conservation importance of the intermittent Krasulja rivulet and its watercourse, as well as Dinaric karst (Western Balkan region) is discussed.

Materials and methods

Material collection and preparation.

Adults of *I. popijaci* sp. nov. were collected in June 2019 at the entrance to the Ševerova Cave (karstic source of the intermittent Krasulja rivulet in Krbava field).

A subsequent collecting trip upstream of the Krasulja rivulet (in June 2021), near the karstic source adjacent to the village of Mirići, resulted in finding more specimens of *I. popijaci* sp. nov.

A total of 42 specimens (34 adults and 8 larvae) belonging to *Isoperla popijaci* sp. nov., were collected. Adult specimens were collected using sweep nets, while larval specimens were collected by handpicking. The aedeagus was everted in the field and specimens were fixed and stored in 96% ethanol for morphological and molecular analysis. Morphological characteristics of male terminalia were examined after potassium hydroxide (KOH) treatment.

Type material depository and museum acronyms.

The holotype and part of the paratypes series are deposited in the Croatian Natural History Museum, Zagreb, Croatia (CNHM), Collection of Plecoptera Sivec & Hlebec, while other paratypes are kept in the Slovenian Museum of Natural History, Ljubljana, Slovenia (PMSL).

Photography and drawings.

Photographs, diagnostic characterisation and comparative morphological examination of specimens were made using a ZEISS SteREO DiscoveryV.20 stereomicroscope. Pencil drawings were produced with a camera lucida and then digitally edited and inked. Figures 3A, B, 4A–D (SEM images) were made using a JEOL JSM-7000F scanning electron microscope. The penis (one of paratype specimen) for the SEM study was critical-point dried (Figure 4A–D).

Nomenclature is in accordance with the International Code of the Zoological Nomenclature (ICZN 1999). The species is proposed by following the rules of the Code. Abbreviations for the type specimens are HT–holotype, PT–paratype and PTs–paratypes.

Comparative analysis.

Comparative study on the morphology of penial structures was conducted using ten species belonging to the genus *Isoperla*, collected in Croatia: *I. bosnica* Aubert, 1964; *I. inermis* Kaćanski et Zwick, 1970; *I. rivulorum* (Pictet, 1841); *I. lugens* (Klapálek, 1923); *I. illyrica* Tabacaru, 1971; *I. tripartita* Illies, 1954; *I. grammatica* (Poda, 1761); *I. difformis* (Klapálek, 1909); *I. oxylepis* (Despax, 1936) and *I. albanica* Aubert, 1964. Morphological taxonomic classifications follow the traditional system (Poda 1761; Pictet 1841; Klapálek 1909, 1923; Despax 1936; Illies 1952, 1954, 1966; Aubert 1964; Tabacaru 1971; Kaćanski and Zwick 1970, Murányi 2011; Murányi et al. 2016).

DNA extraction, amplification, and sequencing.

One male, one female and one larva of *Isoperla popijaci* sp. nov. were used in molecular analyses and mutually associated. DNA was extracted from the single leg of specimens using QIAamp DNA Micro Kit (Qiagen, Germany) according to the manufacturer's specifications and eluted in 50 μl of elution buffer. The 5' fragment of the mitochondrial cytochrome c oxidase subunit I gene (*COI*) was amplified using standard PCR-protocols and four sets of primers: LCO-1490/HCO-2198 (Folmer et al. 1994) or C_LepFolF/C_LepFolR (as was used in Hebert et al. 2004) or a combination of MLepF1/LepR1 and MLepR1/LepF1 (yielding two shorter, overlapping fragments as was used in Hajibabaei et al. 2006) in 20 μl reactions. Polymerase chain reactions (PCRs) for all primer sets were carried out using: 1 x DreamTaq reaction buffer with 2 mM $MgCl_2$ (Thermo Fisher Scientific Inc., US), 0.2 mM dNTPs, 0.4 μM of each primer, 0.025 U/μl of DreamTaq polymerase (Thermo Fisher Scientific Inc., US) and 1 μl of eluted DNA. For the first mentioned primers set (LCO-1490/HCO-2198) the following PCR cycling conditions were applied: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min, followed by a final extension step at 72°C for 10 min. PCR products were purified using Exonuclease I (0.05 U/μl), FastAP Thermosensitive Alkaline Phosphatase (0.025 U/μl) enzymatic system (Thermo Fisher Scientific Inc., US). The reaction was carried using the protocol: 1 h at 37°C followed by 20 min at 80°C. Sequencing was performed by Macrogen Inc. (Amsterdam, The Netherlands) using the amplification primers. Sequences obtained in the study were deposited in the BOLD database (Ratnasingham and Hebert 2007) and GenBank (under the accession numbers MW907977–MW907980, MW907982–MW907988 and MW907990–MW907993).

Sequence data and phylogenetic analysis.

In total, 15 obtained *Isoperla* sequences were checked, edited, assembled from both directions and inspected manually for base-pair ambiguities, as well as stop codons, indels or double peaks in chromatograms (as indicators for the possible erroneous amplification of nuclear mitochondrial pseudogene) in Geneious R6 (https://www.geneious.com). All available *Isoperla* sequences were retrieved from the GenBank and BOLD databases (accessed 10/01/2021) and aligned with sequences from this study using MAFFT v.7 (Katoh and Standley 2013). Any length variants were excluded from the final alignments. Sequences were collapsed into 456 unique *COI* haplotypes using the online tool FaBox v.1.5 (Villesen 2007) and, from all species, the most diverse haplotypes from *I. tripartita* and *I. rivulorum* species group, as well as species *I. lugens*, were retained for further analysis. The final dataset for phylogenetic analysis and species delineation comprised 27 sequences, including 10 haplotypes observed in this study (see Table 1). *Isoperla obscura* (INTAP055-17) and *Taeniopteryx burksi* (08INHSP-002) were selected as outgroups according to the North American Plecoptera phylogeny published by South et al. (2020). Amongst morphologically-defined species, evolutionary divergence was estimated using the pairwise comparison of the uncorrected genetic distances (*p*-distances) in MEGA-X (Kumar et al. 2018). For *p*-distances, a colour heat map was drawn using the Python data visualisation library Seaborn (version 0.11.1, Waskom 2021). Phylogenetic relationships were estimated

Table 1. Collection details and geographical origin of the specimens used in phylogenetic analysis. Haplotypes obtained in this study, marked with asterisk. Paratypes of *Isoperla popijaci* sp. nov. used in molecular analysis marked in bold (male, female and larval). Abbreviations: AL (Albania), AT (Austria), C (Croatia), F (France), G (Germany), M (Montenegro), S (Switzerland). Outgroups (INTAP055-17 and 08INHSP-002) are not shown. Specimen identifier: I. Sivec.

Specimen ID	BOLD/GenBank Process ID	Taxon	Locality	Legit	Coordinates	Publication
*DH71	CROPL066-21	*Isoperla rivulorum*	C: Kupa River, spring	I. Sivec	45°29.47'N, 14°41.36'E	this study
GBOL01391	GBCOU1198-13	*Isoperla rivulorum*	F: Rhone-Alpes, Hauteville	Balke, Morinière, Toussaint, Taenzler, Bellanger, Hoch	45°29.52'N, 6°35.04'E	Morinière et al. (2017)
PE219	INTAP187-17	*Isoperla rivulorum*	AT: Flexenpass	W. Graf	47°09.17'N, 10°09.91'E	–
PE268	INTAP226-17	*Isoperla rivulorum*	AT: Flexenpass	W. Graf	47°09.17'N, 10°09.91'E	–
GBIFCH00280047	PLEAA237-20	*Isoperla rivulorum*	S: Effluent, Pont de Nant	Sartori Michel & Derleth Pascale	46°15.07'N, 7°06.43'E	–
GBOL01390	GBCOU1197-13	*Isoperla rivulorum*	F: Rhone-Alpes, Hauteville	Balke, Morinière, Toussaint, Taenzler, Bellanger, Hoch	45°29.52'N, 6°35.04'E	Morinière et al. (2017)
*DH107	CROPL097-21	*Isoperla illyrica*	C: Trilj, Grab, spring	I. Sivec	43°38.93'N, 16°45.74'E	this study
*DH482	CROPL197-21	*Isoperla illyrica*	C: Trilj, Grab, spring	I. Sivec	43°38.93'N, 16°45.74'E	this study
*DH123	CROPL109-21	*Isoperla tripartita*	C: Cetina River, spring	I. Sivec	43°58.54'N, 16°25.81'E	this study
*DH478	CROPL195-21	*Isoperla tripartita*	C: Cetina River, spring	B. Horvat	43°58.54'N, 16°25.81'E	this study
*DH551	CROPL225-21	*Isoperla tripartita*	C: Papuk, Gospin potok	I. Vučković	45°34.47'N, 17°41.76'E	this study
*DH137	CROPL122-21	*Isoperla tripartita*	C: Trilj, Grab, spring	I. Sivec	43°38.93'N, 16°45.74'E	this study
Itri0101M	VJOSA001-17	*Isoperla tripartita*	AT: Lainzer Tiergarten	O. Zweidick	48°09.57'N, 16°12.83'E	Graf et al. (2018)
Itri0102M	VJOSA002-17	*Isoperla tripartita*	AT: Lainzer Tiergarten	O. Zweidick	48°09.57'N, 16°12.83'E	Graf et al. (2018)
MT348738	GBMNC47893-20	*Isoperla tripartita*	Macedonia	D. Murányi	41°16.07'N, 20°31.24'E	Murányi et al. (2020)
MT348735	GBMNC47896-20	*Isoperla tripartita*	Macedonia	D. Murányi	42°03.14'N, 20°46.92'E	Murányi et al. (2020)
MT348732	GBMNC47899-20	*Isoperla tripartita*	Macedonia	D. Murányi	40°58.78'N, 21°15.22'E	Murányi et al. (2020)
DH129	CROPL115-21	*Isoperla popijaci* sp. nov.	C: Ševerova Cave	I. Sivec	44°40.78'N, 15°37.87'E	this study
DH130	CROPL116-21	*Isoperla popijaci* sp. nov.	C: Ševerova Cave	I. Sivec	44°40.78'N, 15°37.87'E	this study
***DH926**	CROPL249-21	*Isoperla popijaci* sp. nov.	C: Ševerova Cave	D. Hlebec	44°40.78'N, 15°37.87'E	this study
DH142	CROPL127-21	*Isoperla* PL	C: Plitvice Lakes, Drakulića River	I. Sivec	44°46.87'N, 15°39.47'E	this study
DH143	CROPL128-21	*Isoperla* PL	C: Plitvice Lakes, Drakulića River	I. Sivec	44°46.87'N, 15°39.47'E	this study
*DH538	CROPL214-21	*Isoperla* PL	C: Plitvice Lakes, Drakulića River	M. Kučinić, I. Vučković	44°46.87'N, 15°39.47'E	this study
DH541	CROPL217-21	*Isoperla* PL	C: Plitvice Lakes, Drakulića River	M. Kučinić, I. Vučković	44°46.87'N, 15°39.47'E	this study
*DH629	CROPL230-21	*Isoperla* PL	C: Plitvice Lakes, Drakulića River	M. Kučinić, I. Vučković	44°46.87'N, 15°39.47'E	this study
Itri0201M	VJOSA003-17	*Isoperla vjosae*	AL: Vjosa River, Kutë	S. Vitecek, W. Graf	40°28.35'N, 19°44.94'E	Graf et al. (2018)
Itri0202M	VJOSA004-17	*Isoperla vjosae*	AL: Vjosa River, Kutë	S. Vitecek, W. Graf	40°28.35'N, 19°44.94'E	Graf et al. (2018)
Itri0301M	VJOSA005-17	*Isoperla vjosae*	AL: Vjosa River, Kutë	S. Vitecek, W. Graf	–	–
Itri0302L	VJOSA006-17	*Isoperla vjosae*	AL: Vjosa River, Kutë	S. Vitecek, W. Graf	40°28.35'N, 19°44.94'E	Graf et al. (2018)
Ipe0101M	VJOSA007-17	*Isoperla pesici*	M: Redice	W. Graf	42°53.02'N, 19°18.95'E	Graf et al. (2018)
Ipe0102F	VJOSA008-17	*Isoperla pesici*	M: Redice	W. Graf	42°53.02'N, 19°18.95'E	Graf et al. (2018)
GBOL17507	GBMIX2517-15	*Isoperla lugens*	G: Nationalpark Berchtesgaden	R. Gerecke	47°33.48'N, 12°48.24'E	Morinière et al. (2017)
PE031	INTAP025-17	*Isoperla lugens*	AT: Koerbersee	W. Graf	47°16.09'N, 10°07.66'E	–
PE269	INTAP227-17	*Isoperla lugens*	AT: Flexenpass	W. Graf	47°09.17'N, 10°09.91'E	–

by three different optimality criteria: Neighbour Joining (NJ), Maximum Likelihood (ML) and Bayesian Inference (BI). NJ and ML were performed in MEGA-X (Kumar et al. 2018), while BI in MrBayes 3.2.7. (Ronquist et al. 2012). For ML and BI, the optimal model of nucleotide evolution (Hasegawa-Kishino-Yano model with gamma distributed rate variation amongst sites and a significant proportion of invariable sites: HKY+I+G) was selected under the Bayesian Information Criterion (BIC) using jModelTest 2.1.5 (Darriba et al. 2012). Nodes in the phylogenetic trees with bootstrap values P ≥ 70 in NJ and ML and posterior probabilities values pp ≥ 0.90 in BI were considered well supported. NJ was made using the Kimura-2-parameter (K2P) model of nucleotide substitution with the pairwise deletion option. Bootstrap support was inferred using the fast bootstrap algorithm, based on 5000 replicates. Nearest-Neighbour-Interchange (NNI), a heuristic method using the fast bootstrap algorithm, was used in ML with 1000 replicates.

For BI, the dataset was partitioned by codon positions. Two separate runs with four Metropolis-coupled Monte Carlo Markov chains (MMCM) were performed for 10 million generations while trees were sampled every 1000 generations with the first 25% of sampled trees discarded as burn-in. The remaining trees were used to create a 50% majority rule consensus tree. TRACER v.1.7.1 (Rambaut et al. 2018) was used to check the convergence between the two runs. The phylogenetic trees were visualised using FigTree v.1.4.3. (Rambaut 2009) and iTOL v.5 (Letunic and Bork 2021). Several methods of species delimitation were applied: the Automatic Barcode Gap Discovery (ABGD) method (Puillandre et al. 2012), the Bayesian implementation of the Poisson Tree Processes (bPTP) method (Zhang et al. 2013) and the multi-rate Poisson Tree Process (mPTP) method (Kapli et al. 2017). The ABGD was performed at the web server by using the K2P model. All values were set to default, except the value of relative gap width, which was set to 1, while the default gap width of 1.5 resulted in a single group. The bPTP method was performed on the web server at http://species.h-its.org, while the mPTP method was run on the web server at http://mptp.h-its.org/. Both methods were applied using default parameters, outgroups have been removed from the analysis and the same ML input tree was used.

Results

Taxonomic part

New species description

Isoperla popijaci **Hlebec & Sivec sp. nov.**
http://zoobank.org/60B76C3E-14C2-4D5D-9587-C1931C87952B
Figures 1A–E, 2A–G, 3A, B, 4A–D

Material examined (1♂ HT, 10♂♂ PTs, 23♀♀ PTs and 8 larvae PTs): 1♂HT (96% ethanol) Original label: Croatia, Lika, Krbava field, Krasulja rivulet, karstic source Ševerova Cave; 44°40.78'N, 15°37.87'E, 21 June 2019, I. Sivec leg. (CNHM: CPSH);

6♂♂ **PTs** and 11♀♀ **PTs** (96% ethanol) same data as for the holotype; 5 larvae **PTs** (96% ethanol) 09 April 2015, I. Sivec leg.; 3 larvae **PTs** (96% ethanol) 22 February 2021, D. Hlebec leg.; 1♂**PT** and 3♀♀ **PT**s (96% ethanol) 2 June 2021, I. Sivec leg.; 3♀♀ **PTs** (96% ethanol) 18 June 2021, D. Hlebec leg.; 3♂♂ **PTs** and 6♀♀ **PTs** (96% ethanol) karstic source nearby village Mirići, 44°43.14'N, 15°38.09'E, 2 June 2021, I. Sivec leg.

Type material depository. HT (1♂) and 31 PTs (7♂♂+18♀♀+6 larvae) in Zagreb, Croatia (CNHM), Collection of Plecoptera Sivec & Hlebec, under accession number CPSH 1–32; and 10 PTs (3♂♂+5♀♀+2 larvae) in Ljubljana, Slovenia (PMSL).

Type locality. Croatia, Lika, Krbava field, Krasulja rivulet, karstic source Ševerova Cave, 44°40.78'N; 15°37.87'E; 640 m a.s.l.

Diagnosis. The new species *I. popijaci* sp. nov. belongs to the *I. tripartita* species group, with divided medial penial armature into upper and lower coloured portions. It has, however, a specific penial armature on the ventral lobe of the penis, different from all known *Isoperla* species. The upper medial armature is subdivided, and the lower medial armature is present in two scale spike-like areas. The proximal part has a pair of drop-shaped areas armoured with spines, longer at the tip and shorter at the base. The medial penial armature with a field of shorter spines as in Figure 4C. Only a few irregular spines on the lateral side of the penis in the area of the upper armature of the penis.

Description. Macropterous in both sexes, medium-sized species with yellow head and pronotum.

Adult. Body length: **HT** male 18.5 mm; **PTs**: males 17–19 mm (n = 10), females 16.5–18 mm (n = 23).

Forewing length: **HT** male 12 mm; **PTs**: males 11–13.5 mm, females 11.5–14 mm.

Colouration. General colour uniformly brownish (Figure 1C), slightly paler ventrally and laterally.

Head. The central part of the head pale yellowish; darker at the lower part and between ocelli; slightly darker in the frontal and lateral part. M-line and tentorial callosities weakly expressed and inconspicuous. Pale spot positioned centrally between the ocelli, paler in the central distal part of the head. Eyes slightly smaller than the area delimited by the three ocelli. Scape and pedicel dark brown. Palpi uniformly cream coloured. The distal part of the antennae pale and the proximal segments darker (Figures 1A, 2A).

Wings. Wings translucent brownish, venation dark brown.

Pronotum. Pronotum yellowish, rectangular with angled edges. Medial and lateral parts of the pronotum pale; central part on both sides slightly darker and with dark brown textured surface (Figures 1A, 2A).

Mesothorax and metathorax. Ventral surface of thorax uniformly brownish; dorsal side slightly darker, lateral part lighter. Mesonotum and metanotum predominantly dark brown.

Legs. Femora and tibia brownish, same as body colouration. Tarsi slightly darker than femora and tibia on the dorsal side and pale ventrally.

Male abdomen. Mesobasisternum and metabasisternum brown in the middle and darker laterally. Ventral surface of male abdomen uniformly brownish, slightly darker dorsally. A few proximal segments of cerci pale, with rest dark brown.

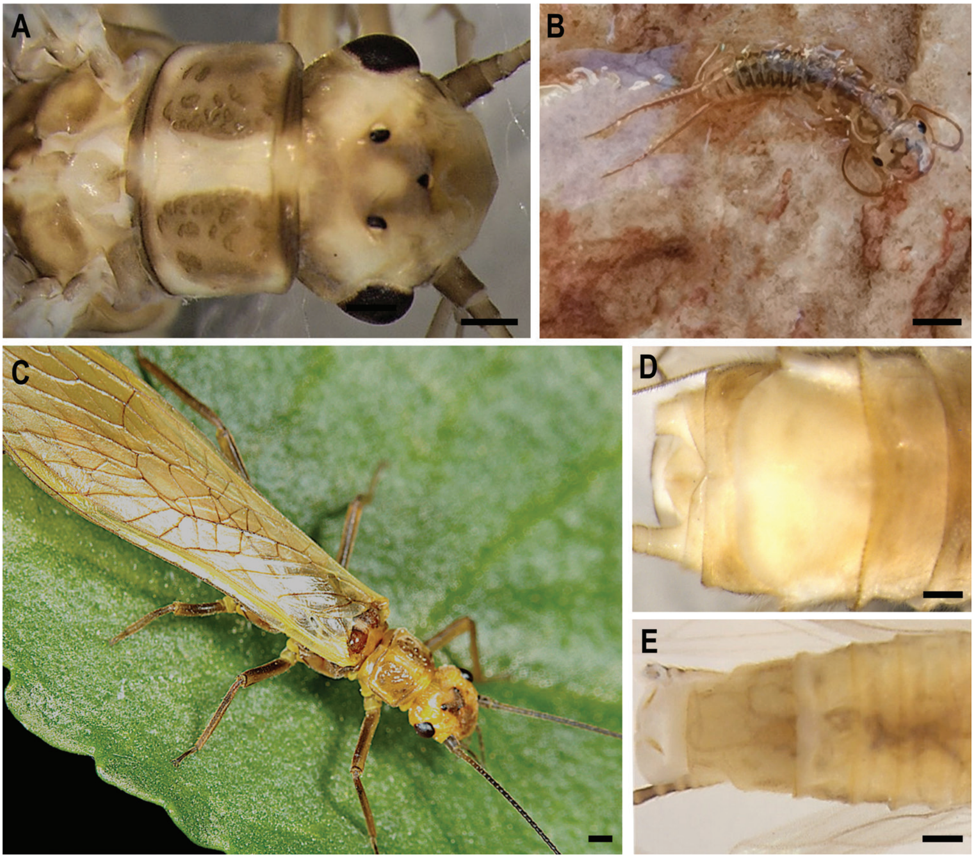

Figure 1. Morphology of *Isoperla popijaci* sp. nov. **A** head and pronotum in dorsal view (adult male HT) **B** habitus (larval PT) **C** habitus (adult male PT) **D** female terminalia in ventral view (PT) **E** everted male copulatory organ (HT). Scale bar: 0.5 mm **A–E**.

Penis (everted). Divided into four lobes, with a basal section in everted position. The medial penial armature on the ventral surface of the penis divided into an upper and a lower part, both are coloured (Figures 2C, G, 4A), upper part rather pale. The upper medial penial armature is further subdivided into left and right arms, elongated, delimited from scales of the lateral lobes (Figure 4C). Length of the arms is 200–250 µm, width 100–120 µm. Scales of the upper medial penial armature forming a drop-shaped area, spike-like, with longer scales at the tip and shorter ones at the base. Length of the scales 25–37 µm, width 7–9 µm at the base. The lower part of the medial penial armature subdivided, with an irregular upturned V-shaped area and bearing very short spines (Figure 4D). Length of the areas 220–250 µm, width 100–140 µm. The scales are spike-like, thinner than in the upper medial armature. The ventral lobe hemispherical, covered with hair-like scales, in some places ciliated scales. The medial lobe small with diverse scales. Lateral penial armatures located on the lateral lobes, above the basal section, small and indistinct with only a few spines. Detail of the lateral lobe as in Figure 4B.

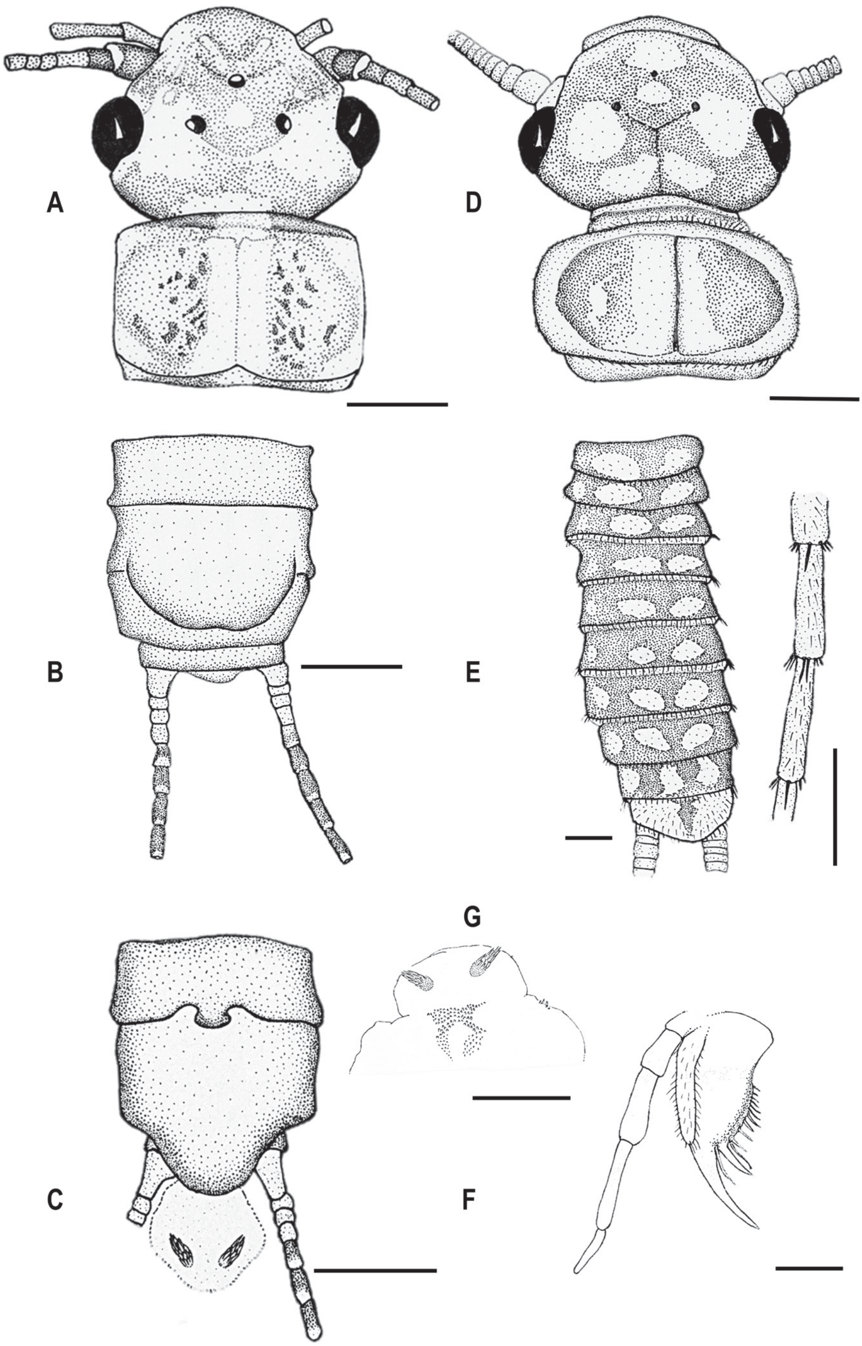

Figure 2. Morphology of *Isoperla popijaci* sp. nov. **A** head and pronotum in dorsal view (adult female PT) **B** terminalia in ventral view (adult female PT) **C** terminalia in ventral view (adult male HT) **D** head and pronotum in dorsal view (larval PT) **E** abdomen in dorsal view and detail of a distal segment of a cercus (larval PT) **F** right maxilla in dorsal view (larval PT) **G** penial armature (adult male HT). Scale bars: 1 mm (**A–D**); 0.5 mm (**E–G**).

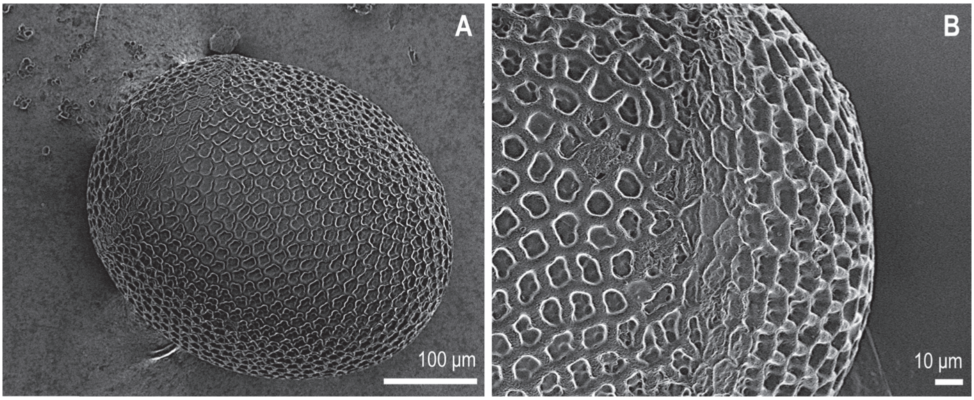

Figure 3. Egg of *Isoperla popijaci* sp.nov. **A** whole egg, lateral view **B** detail of hatching line, lateral view

Female abdomen. All tergites uniformly brownish. Sternites slightly paler brownish. A few basal segments of cerci pale, rest of cerci dark brown. Subgenital plate large and wide reaching near the end of sternite IX (widely concave in the middle) (Figure 2B).

Egg. Chorion light brown, 0.34–0.38 mm long and 0.29–0.33 mm wide (n = 22). Chorion with marked ornamentation of irregular round shape. Follicular cell impressions with finer inner punctations. Hatching line distinct. Micropyles not well recognisable. Collar and anchor missing (Figure 3A, B).

Larva. Body length of not-completely-mature larva 14–16 mm (n = 8). General colour pale brownish; with darker markings on head and abdomen. Body and legs typically pilose. Swimming hairs present on femora, tibiae and tarsi. Posterior abdominal fringe short and cercal fringe no longer than width of cercal segment. General colour of the head brownish, with a darker transversal mask connecting eyes and ocelli (Figure 2D). M line indistinct. Eyes well developed. Mouth parts and basal parts of antennae pale coloured; distal part of antennae dark brownish. Lacinia bidentate; inner margin with 4–5 stout setae and a row of short thin setae below subapical tooth. Pronotum rounded; brownish; with indistinct darker pattern centrally and distinctly paler laterally (Figure 2D). Pronotal setal fringe with short bristles and bearing only a few longer setae at posterior margin. Ventral side of the body and leg pale coloured. Abdominal tergites darker, brown with a pair of relatively large drop-shaped pale spots in the middle of the abdomen (Figure 2E). Paraprocts and cerci uniformly pale. Setation on distal section of cercal segments with rather uniform setae and single larger dorsal setae.

Etymology. The specific name is the genitive singular of the Latinised version of the surname Popijač (Popiacus, -i, m.), given in honour of colleague Dr Aleksandar Popijač and his achievements in field research and knowledge of the Plecoptera fauna in Croatia.

Distribution and ecology. The species was collected at the entrance to the Ševerova Cave, occasional karstic source of the intermittent Krasulja rivulet in Krbava field and two year later (on 2 June 2021) near the karstic source of the same rivulet,

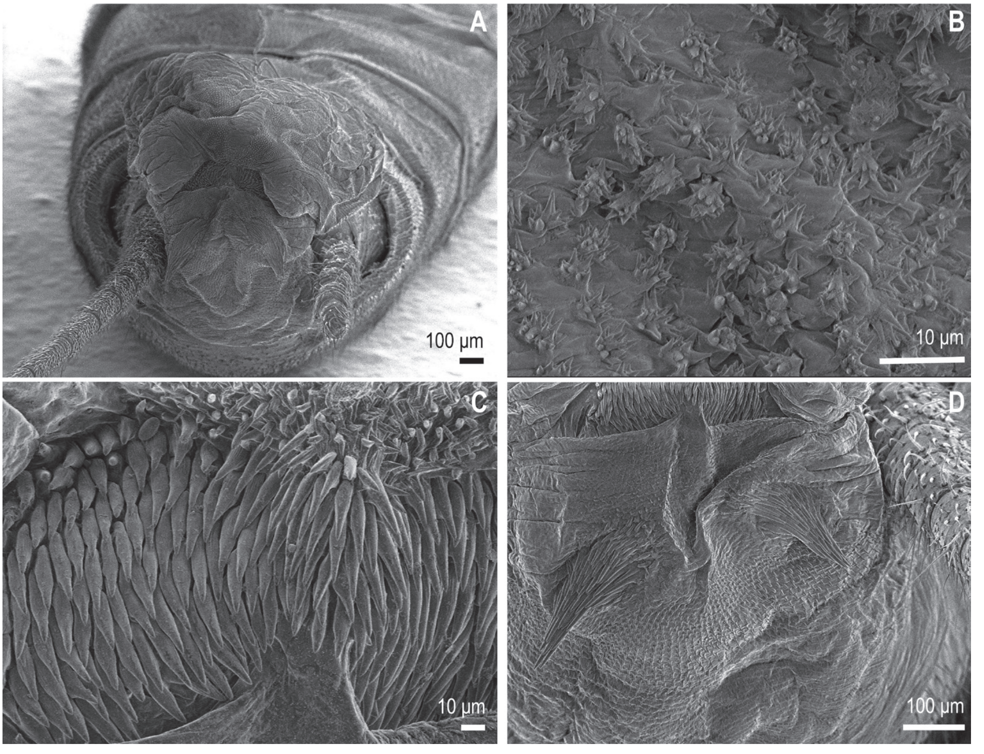

Figure 4. Extruded penis of *Isoperla popijaci* sp. nov. **A** male abdomen with extruded penis, ventral view **B** detail of penial armature on the lateral lobe, dorsal view **C** scales of the upper medial penial armature, dorsal view **D** pair of the scales spike-like on the ventral lobe, dorsal view.

near the village of Mirići. The Ševerova Cave (old name Hrnjakova Cave) is located on the northern edge of the Krbava field (karst field located near settlement Krbavica in the vicinity of the Plitvice Lakes National Park). The temporary Krasulja rivulet is part of the hydrogeological system of the Krbavica River (Figure 5E). For several months a year, the water runs from the cave and forms the Krasulja rivulet, which flows into the Krbavica River and sinks on the south side of the field. When the discharge of the Krasulja falls below 60 l/sec, the water-flow ceases from Ševerova Cave (Malinar and Čepelak 2009). The stream does not have a rich stonefly fauna and the species found at this locality, except the newly-described species of *Isoperla*, are *Amphinemura standfussi* (Ris, 1902) and *Nemoura cinerea* (Retzius, 1783). The substrate at the collection site of larvae was mainly composed of larger fractions.

Conservation status

The new species should probably be regarded as Critically Endangered (CR) or Vulnerable (VU) by the IUCN Criteria. Up to now, it is known only from the areas nearby two karstic sources.

Figure 5. Type locality of the Popijač's Yellow Sally, *Isoperla popijaci* sp. nov.**:** Ševerova Cave in Croatia **A** and **B** photographs in wet phase **C, D** photographs in dry phase **E** map (blue circle indicates type locality).

Phylogenetic part

The alignment of *COI* gene sequences was 658 bp in length and comprised of 202 variable sites, of which 139 were parsimony informative. Three implemented criteria of phylogenetic reconstruction (NJ, ML and BI) resulted in congruent topologies with highly similar support values (Figure 6), characterised by the presence of two deeply divergent lineages, *I. popijaci* sp. nov. and "*Isoperla* PL", which did not cluster with any of the currently defined taxa.

Mitochondrial *COI* sequences, obtained from *I. popijaci* sp. nov. (adults and larva), were identical (a single unique haplotype). The monophyly of the newly-described species is highly supported (Figure 6). This species represents the first branch-off within the clade comprised of monophyletic *I. lugens* and *I. rivulorum* subclades, as well as another tentative new taxon obtained in this study (clade designated as "*Isoperla* PL" with representatives CROPL214-21 and CROPL230-21). The designation "PL" denotes the abbreviation Plitvice Lakes, nearby where a specimen was found. Five sequences of "*Isoperla* PL" represent 2 haplotypes (CROPL214-21 and CROPL230-21) with low intraspecific uncorrected *p*-distance (0.0096).

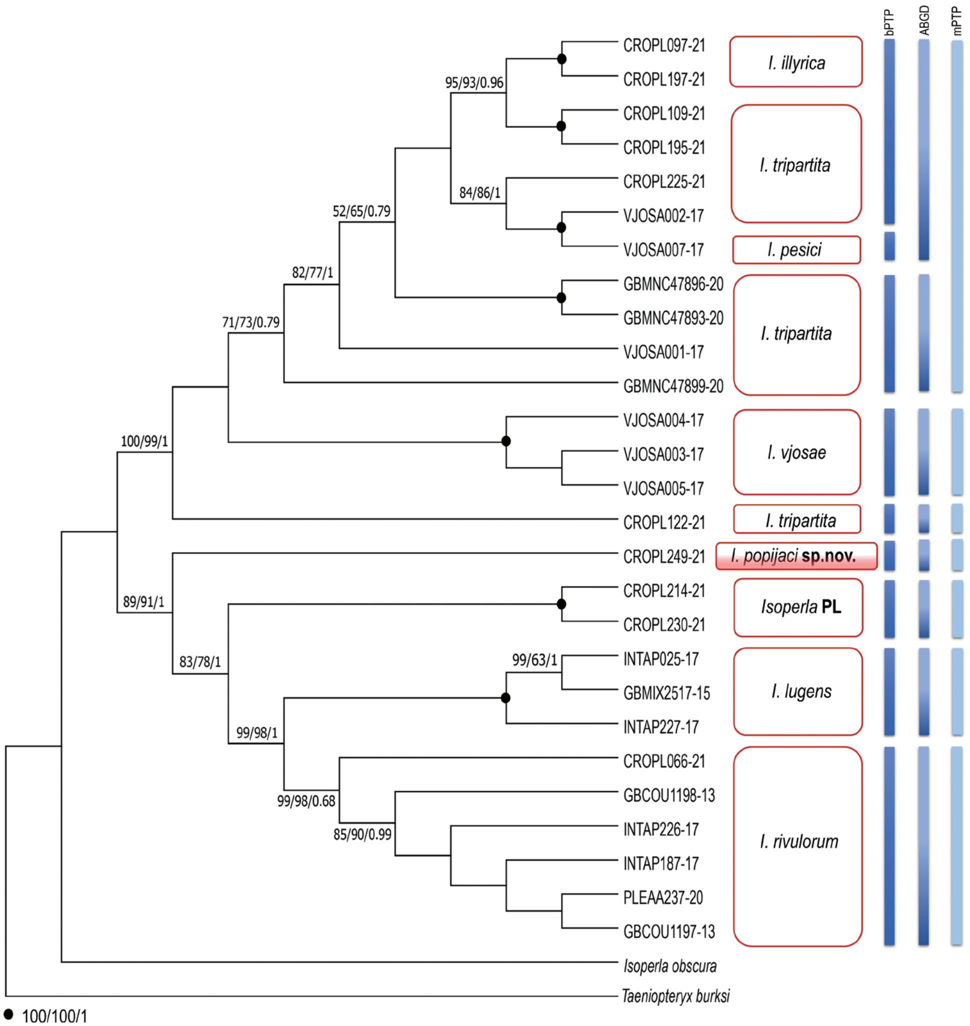

Figure 6. Maximum Likelihood cladogram, based on the analysis of the *COI* haplotypes of *Isoperla* species. Numbers at the nodes indicate Neighbour-Joining (NJ), Maximum Likelihood (ML) bootstrap support values (BS) and Bayesian posterior probabilities (BPP), respectively. The results of species delimitations are represented with the vertical bars, from left to right, indicate the OTUs inferred by bPTP, ABGD and mPTP. "*Isoperla* PL" indicates additional separate lineage obtained in this study. Terminal codes present BOLD/GenBank Process ID, as in Table 1.

Intraspecific uncorrected *p*-distances are as follows for the following species: 0.32–1.59% in *I. rivulorum*, 0.16–0.48% in *I. lugens*, 0.01–7.82% in *I. tripartita*, 0.32% in *I. vjosae* and 0.16% in *I. illyrica*. Interspecific uncorrected *p*-distances for *I. popijaci* sp. nov. ranged from 6.69–12.59%; specifically, 6.69–7.17% to *I. rivulorum*, 8.15–8.45% to *I. lugens*, 9.99–10.22% to *I. vjosae*, 10.4–12.6% to *I. tripartita*, 10.38–12.61% to *I. illyrica*, 10.69% to *I. pesici* and 8.12% to the "*Isoperla* PL" (Figure 7). Overall, observed intraspecific genetic distances within the genus ranged from 0.01–7.82%.

Within the *I. rivulorum* clade, Croatian sample CROPL066-21 appeared as a separate lineage, subdivided from Alpine specimens (Figure 6). The uncorrected *p*-distances for sample CROPL066-21 are in the range 1.28–1.59% to other *I. rivulorum* samples.

A well-supported clade comprised two newly-discovered lineages (*Isoperla popijaci* sp. nov. and "*Isoperla* PL"), together with *I. lugens* and *I. rivulorum*, and was recovered in all three tree-building algorithms.

According to the results of the first molecular characterisation of *I. illyrica* obtained in this study, specimens clustered in a within the monophyletic clade with intraspecific uncorrected *p*-distances of 0.16%. Interspecific *p*-distances between *I. illyrica* and *I. tripartita* ranged from 0.96–5.91%.

All species delimitation analyses (bPTP, ABGD and mPTP) for mtDNA (*COI*) have delineated two well-separated lineages *Isoperla popijaci* sp. nov. and "*Isoperla* PL" as tentative species. Applied methods resulted in various numbers of delineated groups. In the ABGD analysis, initial partitioning identified eight, while recursive partitioning showed the existence of nine putative species for the majority of prior intraspecific divergence values (P). The mPTP method delimited seven operational taxonomic units (OTUs) and, according to these results, is the most conservative approach, while the bPTP recognised 9 OTUs.

Contrary to ABGD and bPTP, the mPTP analysis shows *I. illyrica*, *I. tripartita* and *I. pesici*, morphologically assigned to *I. (tripartita)* species group, as a single OTU. These species are completely separated into three OTUs in the bPTP analysis. The separation of sample CROPL122-21 as a distinct species (*I. tripartita*) was supported by all three species delimitation methods.

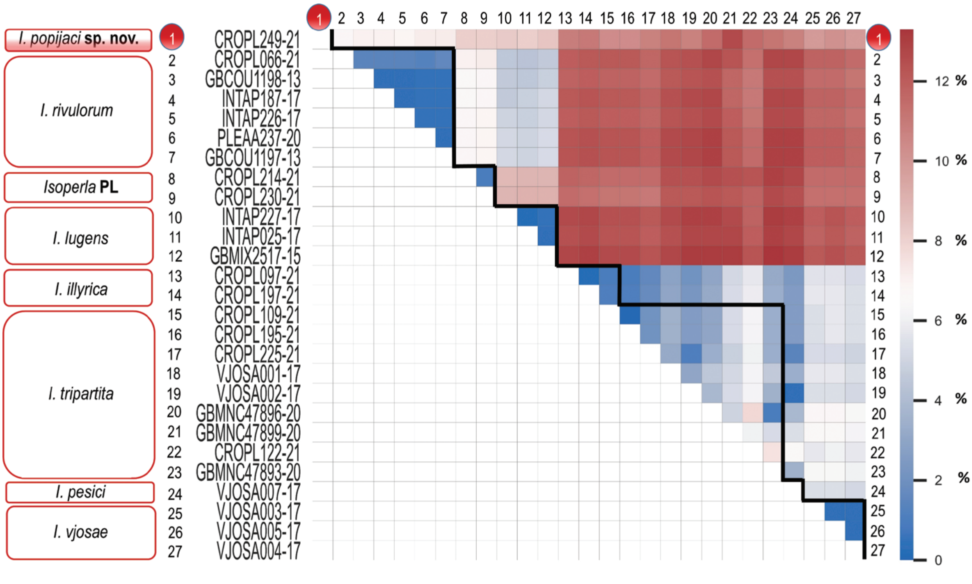

Figure 7. Colour heat map showing inter- and intraspecific uncorrected *p*-distances of the mitochondrial cytochrome oxidase subunit I (*COI*) barcode region. *Isoperla popijaci* sp. nov. and *Isoperla* PL appear as highly divergent. Intraspecific *p*-distances are outlined by the black line.

Discussion

Phylogeny and genetic diversity

The lowest interspecific *p*-distance between *I. popijaci* sp. nov. and *I. rivulorum* was found to be 6.69%, indicating distinct species. This exceeds intraspecific divergences (ISD ≥ 2%) commonly used as one of the criteria for a delimitation of closely-related species in aquatic insects: Ephemeroptera, Plecoptera and Trichoptera (Ball et al. 2009; Zhou et al. 2009). Values above 2% have already been reported amongst Plecoptera (Zhou et al. 2010, Gill et al. 2015), which was probably caused by poor mobility of some Plecoptera species (Boumans and Baumann 2012) and, consequently, geographical isolation among populations.

The finding of the second well-separated lineage ("*Isoperla* PL"), most closely related to species *I. rivulorum* (interspecific *p*-distance from 6.54–7.19%) implies existence of another new species of the genus *Isoperla* (unpublished data). Taxa obtained in this study (*Isoperla popijaci* sp. nov. and "*Isoperla* PL") are separated by a large interspecific *p*-distance of 8.12%. Future research will seek to determine whether this value has repercussions to the geographical isolation and specificity of the (micro-) habitats in which the taxa were found.

Based on the occurrence of *I. lugens* (alpine species) and *I. rivulorum* (alpine, central European species) in the Dinaric karst and their appearance as the most recently diverged lineages within *I. popijaci* + "*Isoperla* PL" + *I. lugens* + *I. rivulorum* clade (Figure 6), it can be assumed that the Dinaric karst might represent the area of origin of those alpine species as well as the diversification centre from where they spread northwards. However, to test this hypothesis, data across the whole distributional range and use of other molecular markers (mitochondrial and nuclear as well) are necessary.

To establish a final phylogenetic relationship in the monophyletic *I. tripartita* species group, it is necessary to collect specimens from its entire range and use a multigene molecular approach as well.

Previous research showed the wide range of variability in intraspecific divergence within the order Plecoptera (Zhou et al. 2009; Gill et al. 2015; Stark et al. 2015) and uncorrected intraspecific *p*-distances from our study (0.01–7.82%) are consistent with the previously reported values.

Systematic implications

Based on the morphological characteristics, the new species can be assigned to the *Isoperla tripartita* species group. The *I. tripartita* species group is characterised by the divided medial penial armature (into upper and lower coloured portions, divided or subdivided) and lateral penial armatures (Illies 1954; Murányi 2011; Murányi et al. 2016). Popijač's Yellow Sally is characterised by divided medial penial armature, with the distal part bearing short spines, but with indistinct lateral penial armature. The genetic distinction, in combination with morphological features, is significantly different from all other species and promotes *I. popijaci* sp. nov. as a new species.

Phylogenetic reconstructions support the monophyly of the *I. tripartita* species group, which is, together with *I. grammatica*, notable by the high morphological variability of certain species (Zwick 1978; Murányi 2011). In Croatia, significant morphological variability has been also observed in *I. inermis* from different localities (personal observation), of which some are very similar to *I. difformis* (Central European species) in the penial armature. Therefore, future studies should investigate relationships between and within *Isoperla* populations from the Balkan Peninsula (e.g. Cetina River, National Park Plitvice Lakes, Kupa River and nearby springs in Slovenia) by applying a multi-gene approach.

Other species are somewhat less variable and occupy smaller distributional areas (as recently described species from Europe and Asia). Those endemics are of special interest to our study because it is assumed that more endemics species are likely to be discovered, especially in poorly-explored areas with high biodiversity like the Balkans. More new species are expected to be found in Croatia, as the majority of the country's territory has not been studied yet regarding Plecoptera.

Anthropogenic activities have already resulted in the reduction of population size (especially larger species from the genera *Perla*, *Dinocras* and *Perlodes*) (personal observation). All the above-mentioned calls for more detailed studies of species distributional patterns, as well as of genetic diversity of populations. Emphasis should also be put on the isolated habitats (karst areas) as they can have the highest conservation value as refugium and the maintenance of genetic diversity.

Cave-dependent stoneflies?

Until now, Popijač's Yellow Sally is known to inhabit the parts of the rivulet close to two karstic sources, of which one is a cave entrance. Although there are no true troglobionts within the order Plecoptera, several species have been found to inhabit stream sources around the openings of caves (for example *I. inermis*) and there are no records of these species from the downstream part of the same stream. Another example is *Brachyptera tristis* (Klapálek, 1901), a species that spends its entire life cycle underground (the stream of Krupa River) (personal observations). It is, hence, important to pay special attention to the research of caves, pits, underground and temporary rivers and streams that abound in the Dinaric karst geology. These habitats host some of the most complex and diverse faunas (Culver and Sket 2000) as a consequence of composite geological history and the intensive process of karstification (Sket 1999). The Balkan Peninsula is known for its high biodiversity (Sket et al. 2004), especially of aquatic species (Kryštufek et al. 2007, Previšić et al. 2009, 2014; Murányi 2011; Vitecek et al. 2015; Kučinić et al. 2017). It can be expected that future research will contribute to the discovery of biodiversity patterns as well as new species, especially microendemic species (Graf et al. 2009, 2012; Kučinić et al. 2013; Vitecek et al. 2017). Karst habitats, such as Ševerova Cave, represent some of the most dynamic freshwater habitats, especially in terms of biological-geological interactions (Ridl et al. 2018). With the alternation of wet and dry phases and temporal dynamics of water flow, temporary rivers have a great influence on local ecological interactions, both in aquatic and terrestrial habitats (Larned et al. 2010). It is a significant assumption that climate change will increase the duration and frequency of dry phases, so it is expected that this will lead to the disappearance of taxa whose entire life cycle (or at least part of it) is related to aquatic environments (Larned et al. 2010).

Conclusions

Isoperla popijaci sp. nov. is probably a stenoendemic Yellow Sally species found at two karstic sources of the intermittent Krasulja rivulet in Lika (Croatia), which has morphological characteristics similar to species from the *I. tripartita* species group. Phylogenetic analysis revealed the well-supported sister-group relationship of *I. lugens* and *I. rivulorum* and a basal position of *I. popijaci* sp. nov. relative to this clade. Considering its restricted distribution, *Isoperla popijaci* sp. nov. should have the highest priority in conservation efforts.

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Incongruent molecular and morphological variation in the crab spider *Synema globosum* (Araneae, Thomisidae) in Europe

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Abstract

Establishing species boundaries is one of the challenges taxonomists around the world have been tackling for centuries. The relation between intraspecific and interspecific variability is still under discussion and in many taxa it remains understudied. Here the hypothesis of single versus multiple species of the crab spider *Synema globosum* (Fabricius) is tested. The wide distribution range as well as its high morphological variability makes this species an interesting candidate for re-evaluation using an integrative approach. This study combines information from barcoding, phylogenetic reconstruction based on mitochondrial CO1 and ITS2 of more than 60 specimens collected over a wide range of European localities, and morphology. The findings show deep clades with up to 6% mean pairwise distance in the CO1 barcode without any biogeographical pattern. The nuclear ITS2 gene did not support the CO1 clades. Morphological assessment of somatic and genital characters in males and females and a morphometric analysis of the male palp uncovered high intraspecific variation that does not match the CO1 or ITS2 phylogenies or biogeography either. Screening for endosymbiotic *Wolbachia* bacteria was conducted and only a single infected specimen was found. Several scenarios might explain these inconsistent patterns. While the deep divergences in the barcoding marker might suggest cryptic or ongoing speciation or geographical isolation in the past, the lack of congruent variation in the nuclear ITS2 gene or the studied morphological character systems, especially the male palp, indicates that *S. globosum* might simply be highly polymorphic both in terms of its mtDNA and morphology. Therefore, more data on ecology and behaviour and full genome sequences are necessary to ultimately resolve this taxonomically intriguing case.

Keywords
DNA barcoding, genital organs, MRA, multivariate ratio analysis, PCA, principal component analysis, species delimitation, spider taxonomy

Introduction

To assess species richness is an urgent duty to manage and conserve biodiversity. Estimation of species richness using various extrapolation methods is one way to tackle the issue. One influential work of this kind was carried out by Erwin (1982), who estimated from canopy fogging in Panama that the number of extant arthropod taxa may be as high as 30 million. The only way to verify such estimates is to approach real numbers by describing new species. However, assessing species numbers is often difficult, especially in the case of hyper-diverse groups like arthropods (Colwell 1994).

Traditional taxonomy, which is mainly based on morphological traits, was the most effective way to describe new species before molecular techniques became widely applicable. Nowadays, combining these two approaches has become the most powerful method taxonomists use to identify, delimitate, and describe new species (Dayrat 2005; Schlick-Steiner et al. 2010; Gokhman 2018). However, there are numerous ways in which morphological and molecular data can disagree which poses challenges to taxonomists (Funk and Omland 2003; Hebert et al. 2003; Fleck et al. 2006). This became especially clear with the advent of DNA barcoding in the last years (Blagoev et al. 2009).

DNA barcoding is nowadays a very common method for species identification, based on the analysis of a short genetic fragment (Coddington 1996; Hebert et al. 2003; Čandek et al. 2013). Usually, this fragment is the cytochrome c oxidase 1 gene (CO1) located in the mitochondrial DNA (mtDNA). To identify a specimen, the sequenced barcoding fragment is compared with an existing library. There were intensive sampling and sequencing efforts to build a universal barcode library, i.e., Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007, 2013; Blagoev et al. 2016), that provides a cheap tool to identify specimens quickly with a single-locus approach. Beside this, DNA barcoding can also aid species discovery (Dayrat 2005; Tyagi et al. 2019), but the suitability of this method in closely related species or within a species complex is still under discussion (Whitworth et al. 2007; Slowik and Sikes 2015; Spasojevic et al. 2016; Gibbs 2018). Potential obstacles for barcoding as the sole tool for species discovery include incomplete lineage sorting (Galtier et al. 2009), recent species divergence in big population size organisms (Maddison 1997), and endosymbiont-mediated introgressive hybridisation (Hurst and Jiggins 2005; Goodacre et al. 2006; Klopfstein et al. 2016). Another constraint is that barcodes sometimes reflect biogeographical patterns instead of species specificity (Nicholls et al. 2012; Collins and Cruickshank 2013).

There are two major ways in which barcoding, and morphology can disagree (Funk and Omland 2003; Hogner et al. 2012). The first scenario concerns morphologically clearly separable species, but the interspecific variability of the barcode is low or even zero. The second scenario includes morphologically cryptic species that can only be identified with molecular data, or with additional data that demonstrate mating barriers between the species (e.g., behavioural data or chemical volatiles) (Töpfer-Hofmann et al. 2000; Kunz et al. 2012). Both patterns have been reported in a diverse array of organisms, including spiders (Slowik and Sikes 2015; Ivanov et al. 2018). When providing equivocal results, barcoding studies should be evaluated carefully, i.e., by multilocus approaches or by analysis of morphological variation (Lefébure et al. 2006; Dellicour and Flot 2018).

With almost 50'000 described species (World Spider Catalog 2021), spiders are the second largest group of arachnids after mites. From the mid of the 18th century (Clerck et al. 1757) until today, their copulatory organs have been successfully used for species delimitation and description. Unfortunately, that means that juveniles are mostly impossible to identify, and females may also cause problems in certain groups. Therefore, the barcoding approach is especially popular among arachnologists (Hebert et al. 2003; Blagoev et al. 2016).

The crab spider *Synema globosum* (Fabricius, 1775) shows a Palaearctic distribution, ranging from Western Europe to Eastern Asia (Ono 1988, World Spider Catalog 2019). Juvenile individuals show some ballooning behaviour (Blandenier and Fürst 1998) that can explain the wide distribution range. Next to the wide distribution, *S. globosum* shows high polymorphism in colour pattern and in the morphology of copulatory organs; this is reflected in many synonyms and subspecies names (World Spider Catalog 2021). Integrative taxonomy could help in structuring this variation and may even lead to the discovery of previously overlooked, cryptic species.

Here, we present the results of a combined molecular (based on the two markers CO1 and ITS2), morphological and morphometrical study on the variation in *S. globosum* over a wide range of sampling localities. Additionally, we perform a screening for the bacterial endosymbiont *Wolbachia* to examine its potential influence on intraspecific variation in the mitochondrial CO1 gene.

Methods

Data collection

Seventy-two adult *S. globosum* individuals were collected across the species range within Europe, including Portugal, France, Italy, Cech Republic, North Macedonia, Turkey, and Greece (Fig. 1; Suppl. material 1). Portuguese specimens were provided from the University of Nottingham. The distance between the two most distant sample sites was 3'100 km. Calculation of geographical distance was done with QGIS (QGIS Development Team, 2018) and the map shown in Fig. 1 was created in R version 4.0.3, using the packages maps v. 3.3.0 and sp v 1.4.5 (Pebesma et al. 2005; Becker et al. 2018; RCore team 2020). After capture, the specimens were preserved in 100% ethanol and stored at -80 °C. Specimens were identified using Levy (1985), Utochkin (1960b), Mcheidze (1997) accessed via World Spider Catalog 2021 and araneae, Version 02.2021 (Nentwig et al. 2021). From each spider, two legs were used for DNA extraction. The extracted DNA was then stored at -20 °C at the Natural History Museum of Bern, Switzerland.

Laboratory protocols

For DNA extraction, 180 µl buffer and 20 µl protease K according to Qiagen Easy Cube digestion protocol were used to digest the two legs. Digestion duration was 14–16 h at 56 °C. After digestion, DNA was purified with the Qiagen Easy Cube following the rodent blood and tissue protocol.

The PCR mixture was composed as follows: 12.5 µl GoTaq Hot Start Green Master Mix (Promega, Switzerland), 6.5 µl nuclease free water, 2 µl DNA and 2 µl forward and reverse primer (10 µM). The PCR conditions were an initial denaturation for two minutes at 94 °C, 35 cycles with a denaturation phase for 30 sec at 94 °C, an annealing phase for 30 seconds at adequate temperature for each primer (Table 1), and an elongation phase for 45 seconds at 72 °C. For the CO1 primer pair LCO1490/Chelicerata2R, five pre-cycles with the higher annealing temperature were included.

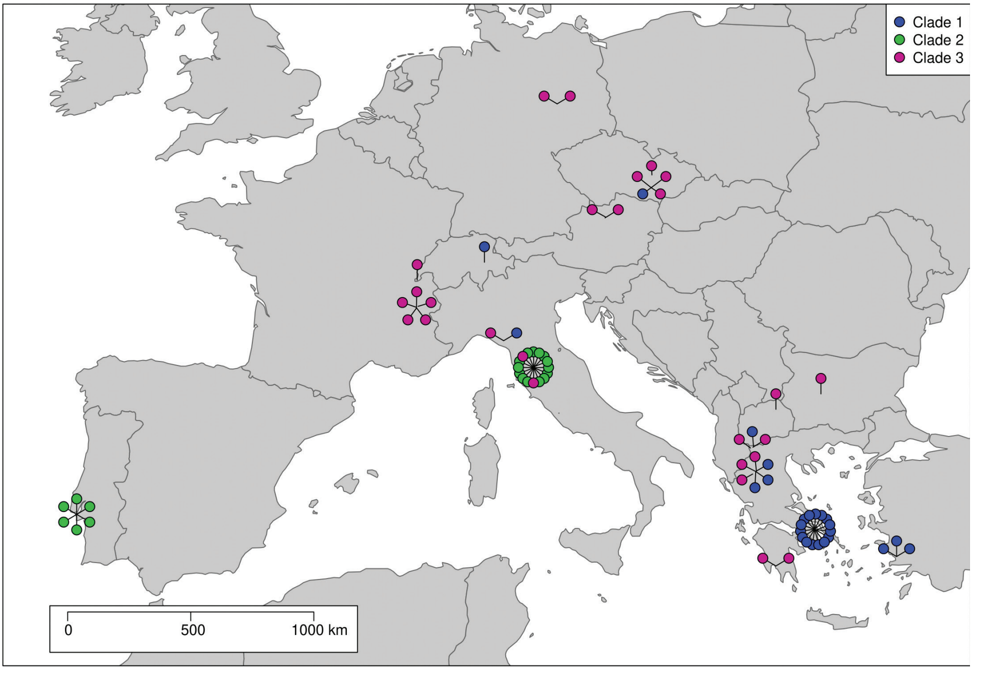

Figure 1. Map of localities of 72 Synema globosum individuals used for the CO1 phylogeny. The specimens were collected in Portugal, France, Italy, Czech Republic, North Macedonia, Greece, and Turkey. Sequences of specimens from Switzerland, Austria, Germany, and Bulgaria were obtained from BOLD. The colours correspond to the three clades in the CO1 phylogeny of S. globosum.

Table 1. Primer sequences with references and annealing temperatures.

Gene Primer	Forward (F) Reverse (R)	Sequence 5'–3'	Reference	Annealing °C
CO1	–	–	–	–
LCO1490	F	GGTCAACAAATCATAAAGATATTGG	(Folmer et al. 1994)	50/48 °C
ChelicerataR2	R	GGATGGCCAAAAAATCAAAATAAATG	(Barrett and Hebert 2005)	–
C1-J- 2183	F	CAACATTTATTTTGATTTTTTGG	(Folmer et al. 1994)	47 °C
C1-N-2778	R	GGATAATCAGAATATCGTCGAGG	(Simon et al. 1994), (Barrett and Hebert 2005)	–
ITS2	–	–	–	–
ITSf	F	TCCTCCGCT TATTTATATGC	(Agnarsson 2010)	50 °C
ITSr	R	GGGTCGATGAAGAACGCAGC	–	–
Wolbachia	–	–	–	–
wspF	F	TGGTCCAATAAGTGATGAAGAAACTAGCTA	(Jeyaprakash and Hoy 2000)	53 °C
wspR	R	AAAATTAAACGCTACTCCAGCTTCTGCAC	–	–

The final elongation cycle was for 10 minutes at 72 °C. The quality of the PCR product was tested on a 1% agarose gel where 1.6 µl of the dye Midori Green (Nippon Genetics, Europe) was added. The obtained PCR products were sequenced in both directions by LGC Genomics in Berlin, Germany.

The CO1 alignment consisted of 64 successfully sequenced *S. globosum* specimens and 1239 bp of CO1, which included the original "barcode region" amplified by the Folmer primers (first 648 bp) and the CO1 terminal region obtained with an additional primer set (remaining 591 bp) (Table 1). All CO1 sequences were without double peaks and overall, of good quality. We added eight *S. globosum* specimens (from Germany, Austria, Switzerland and Bulgaria) with the original barcode from BOLD (Ratnasingham and Hebert 2007, 2013) to this alignment (Suppl. material 2), which resulted in a final CO1 alignment of 72 *S. globosum* sequences. The outgroup was built of eight sequences also obtained from BOLD, which corresponded to seven different species from the family of crab spiders (Thomisidae) and from two closely related families (Philodromidae and Sparassidae). For the nuclear ITS2 gene, we obtained 379 bp from 64 *S. globosum* specimens and added two Genbank (Benson et al. 2007) sequences of the outgroup taxon *Cymbacha* (Thomisidae) (Suppl. material 2). All specimens (N=64) were tested for *Wolbachia* by trying to amplify specific gene of *Wolbachia* DNA in spider samples using the primer pair wspF/wspR (for conditions see Table 1). Positive amplifications were visualised on an electrophoresis gel.

Molecular data analysis

All sequences were prepared for analysis with MEGA7 (Kumar et al. 2016). Both CO1 and ITS2 alignments were constructed with the MUSCLE package as implemented in MEGA7 under default parameters. As a quality control, we checked CO1 sequences for stop codons and gaps, none of which were found.

Bayesian phylogenies were reconstructed in MrBayes version 3.2.6 (Ronquist et al. 2012). We used PartitionFinder 2.1.1 (Lanfear et al. 2012) to infer the partitions and whether the substitution models should include among character rate variation and proportion of invariable sites (settings: search = greedy, branchlengths = linked, model_selection = AICc). While the results suggest that all three codon positions should be analysed as different partitions, we combined first and second positions because the second positions showed almost no variation at all and thus should not be used to infer substitution rates. No such partitioning was applied for ITS2 since it is not a protein coding gene. Finally, a mixed substitution model was used to sample over the complete model space (Huelsenbeck 2004), while the among character rate variation and proportion of invariable sites were modelled according to the results of PartitionFinder. Markov Chain Monte Carlo (MCMC) sampling was conducted with one cold and three heated chains for 20 million generations, sampling every 1000th. The starting tree was not specified. For summary statistics, the first 50% of samples were discarded as a burn-in. We assumed convergence of the analyses when the average standard deviation of split frequency (ASDSF) was below 0.01, effective sample size was above 200 and likelihood graphs indicated stabilisation. For maximum likelihood (ML) estimation, we used RAxML (Stamatakis 2014). We performed 1000 bootstrap replicates under the GTRCAT model with a rapid search for bootstrap support and an exhaustive search for the ML tree. For the input files of MrBayes and RAxML and for the ITS2 consensus tree, see Suppl. material 4, 5. Trees were edited in FigTree Version 1.4.2 (Rambaut 2019) and additionally with Affinity publisher.

To quantitatively assess potentially overlooked species within *S. globosum*, the Bayesian Poisson tree processes (bPTP) method was applied, as a single marker method for species delimitation (Zhang et al. 2013). For the analysis, the Bayesian majority rule consensus tree with outgroup was used to delimit species. The analysis was ran on the bPTP server (https://species.h-its.org/ptp/) for 100'000 MCMC iterations with a burn in of 0.1. The number of generations was enough to reach convergence. For ITS2 and CO1, a haplotype network with PopArt (Leigh and Bryant 2015) implemented TCS network (Clement et al. 2002) was made to trace potentially different haplotypes. Because the PopArt software excludes all the sites with ambiguous nucleotides and gaps, for the haplotype reconstruction of ITS2, we excluded the sequences that introduced ambiguities at all but one parsimony informative sites. The exempted site contained too many ambiguities across alignment, thus we preferred that the analysis excludes this site to loosing substantial number of sequences and geographical information.

Morphological analysis

In total, 61 specimens were successfully used for the morphological analysis. For each of the 34 adult females, four or five photographs were taken showing the dorsal and ventral views of the habitus, the ventral view of the opisthosoma, epigyne and vulva. For each of the 28 adult males, six pictures were made, showing the dorsal and ventral view of the habitus and the ventral, prolateral, dorsal, and retrolateral view of palps. Habitus and palp pictures were stacked from multi-focus records under a LEICA M205 C stereomicroscope with the corresponding IMS client software package. Body size measurements were also performed with the IMS client software. The pictures of epigynes and vulvae were taken on the digital microscope Keyence VHX -500F. If necessary, pictures were edited (i.e., corrected for brightness and contrast) with paint. NET (Brewster 2017) and Adobe Photoshop CS4. Additionally, all epigynes, vulvae and palps in ventral and retrolateral view were drawn in 122 sketches. Palps were fixed in glycerole gelatine and drawings were made under the Leica MZ 16 stereomicroscope with a 1.6× Planapo objective. The vulvae were embedded in Hoyer's medium and drawn with a Zeiss Axioplan 2 compound microscope. Damaged (N = 1) and juvenile (N = 3) specimens were excluded from the morphological analysis. The following traits were examined: continuity of the black pattern on the dorsal side of opisthosoma; red, yellow or white colouration of the female opisthosoma; variation of the white stripe on the ventral side of opisthosoma; number of teeth on the prolateral and retrolateral claw of leg I; number of spines on metatarsus I; colouration pattern of male femora III and IV; cymbium of male palps, size and overall shape of the retrolateral tibial apophysis (RTA), the base of the RTA (BRTA), tibial apophysis (TA) and the embolus tip; on removed epigyne, shape of copulatory duct, receptaculum seminis, fertilisation duct and vulva hood. A morphological data matrix with all mentioned data was made, see Suppl. material 6. The four most promising out of these 12 measurements (colour, teeth of the prolateral claw on leg one, percentage of the white colour in male femora VI and if the vulval hood extends over the entrance) were then plotted on the CO1 Bayesian consensus tree, to see the evolution of morphology patterns according to CO1 clades (Fig. 3). The version R 4.1.1 (Rcore Team 2021) and the packages "ape" (Paradis and Schliep 2019) and "ctv" (Zeileis 2005) were used.

Multivariate ratio analysis

For the morphometric data analysis, we used multivariate ratio analysis (MRA) by Baur and Leuenberger (2011). MRA comprises a commonly applied set of tools for explorative data analysis. It is especially useful for addressing questions in systematics and evolutionary biology (Baur et al. 2014; Petrović et al. 2017; Huber and Schnitter 2020; Selz et al. 2020; Nagy et al. 2021; Schmidt et al. 2021). Here we calculated a shape PCA and plotted the resulting shape PCs against isometric size (i.e., the geometric mean of all variables). We furthermore computed the PCA ratio spectrum for finding the most important character ratios with respect to a particular shape PC.

Reliability of variables used in the morphometric analysis followed the procedure described by Bailey and Byrnes (1990). However, calculation was done following Wolak et al. (2012) and by using their implementation in the R package ICC. Note, that we apply here reliability (R) in relation to measurement error (M) as: R = 1-M.

The morphometric data set contained a few missing values. There were imputed with the help of the R package MICE (Buuren and Groothuis-Oudshoorn 2011) by using the default settings of the function mice(). Four measurements of palp were taken (see Table 2 and Fig. 2). Concerning the male palp, the images showed the palp from the ventral side. The structures were chosen because of the good defined start and endpoints. Difficulties to pose the palp in alcohol led to different positions in the pictures and hindered to take measurements of the bulb, RTA and TA. All four measurements were repeated 4×, so that we could perform the reliability analysis. One male specimen was excluded (AR10769). The measurements were done with IMAGEJ (Schneider et al. 2012). The morphometric analysis was performed with R Studio (RCore Team 2020) and the R-script from Baur and Leuenberger (2020). The results were visualised using package ggplot2 (Wickham 2016). Measurements on the female genitalia were not conducted because of the structural instability. Images of palps used for the morphometric analysis as well all R-scripts used for the MRA and reliability analysis are available on Zenodo (Urfer and Baur 2021).

Table 2. Measurements of the male palp.

Abbreviation	Character name	Definition
cym.l	Cymbium length	Distance of the anterior margin to the tip of the cymbium
cym.b	Cymbium breadth	widest breadth of the cymbium
bul.b	Bulb breadth	widest breadth of the genital bulbus
tib.b	Tibia breadth	breadth of the tibia base at the patella joint

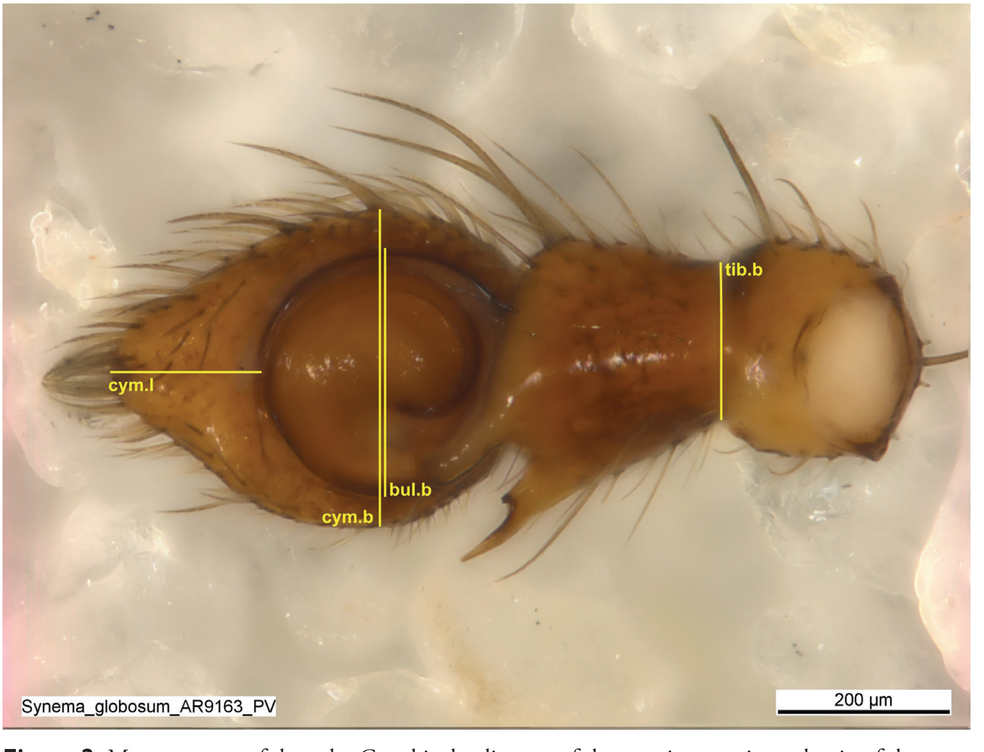

Figure 2. Measurements of the palp: Cym.l is the distance of the anterior margin to the tip of the cymbium, cym.b is the maximum breadth of the cymbium, bul.b is the maximum breadth of the genial bulbus and tib.b is the breadth of the tibia at the base of the patella joint.

Results

Molecular analysis

The 1239 bp long CO1 alignment contained 113 polymorphic sites. The CO1 tree showed clear support for the monophyly of *S. globosum*. The Bayesian and ML analyses of CO1 both inferred three clades (Fig. 3). These clades were not geographically separated and occurred sometimes within the same sample location (Figs 1, 3). The mean uncorrected p-distance between clade one and clade two was approximately 6% and between clade one and clade three 5.5% (Table 3). We recovered the outgroup as expected, except for *S. parvulum* (Hentz, 1847) from America which grouped closer to *Diaea dorsata* (Fabricius, 1777) and rendered the genus *Synema* Sundevall 1833 paraphyletic. In the combined CO1 and ITS2 Bayesian analysis, we had poor convergence according to the ASDSF value (> 0.03). Therefore, we excluded the concatenated tree from our study.

For the CO1 tree with the outgroups, the bTPT analysis suggested between nine and 13 species. The best ML and Bayesian solution considered seven outgroup species and three highly supported (pp > 0.7) species within *Synema globosum*. The haplotype network of *S. globosum* showed a slight geographic pattern with two main haplotypes: one dominantly containing Greek specimens plus one specimen from Switzerland and the second from Turkey with another haplotype containing Italian and Portuguese specimens (Fig. 5). The other haplotypes showed no clear geographical pattern and contained individuals from a few countries.

In ITS2 16 out of 379 positions were variable according to the PopArt setting based on the reduced dataset; ten of these 16 were parsimony informative. The network showed two dominant haplotypes with no clear geographic pattern (see haplotype network, Fig. 4). According to SeqStat four nucleotide indels were found. There were three gaps of one nucleotide and one indel of two nucleotids. One of the two nucleotide indels was heterozygous in an individual from Italy. The uncorrected pairwise distance in ITS2, calculated with MEGA7 ranged from 0.00 to 0.05 with mean of 0.01. In the endosymbiont screening all individuals were tested for *Wolbachia* but in only one individual, we had a positive amplification on the electrophoresis gel. Since there was a proper positive control, the positive specimen was not sent for sequencing.

Morphological analysis

The morphology of *S. globosum* showed extensive variation in both sexes in almost every structure that was examined (Figs 6–8), but none of this variation showed any correspondence to the CO1 clades geography. The number of teeth on the claws of leg I varied from five to eleven and the difference between the prolateral and the retrolateral claw was small (± 1 tooth). The number of spines on metatarsus I and metatarsus II varied from three to four.

Female morphology. The average body size in females was 5.5 mm (3.48 mm to 7.83 mm). The female opisthosoma had a red, yellow or white ground colour. The

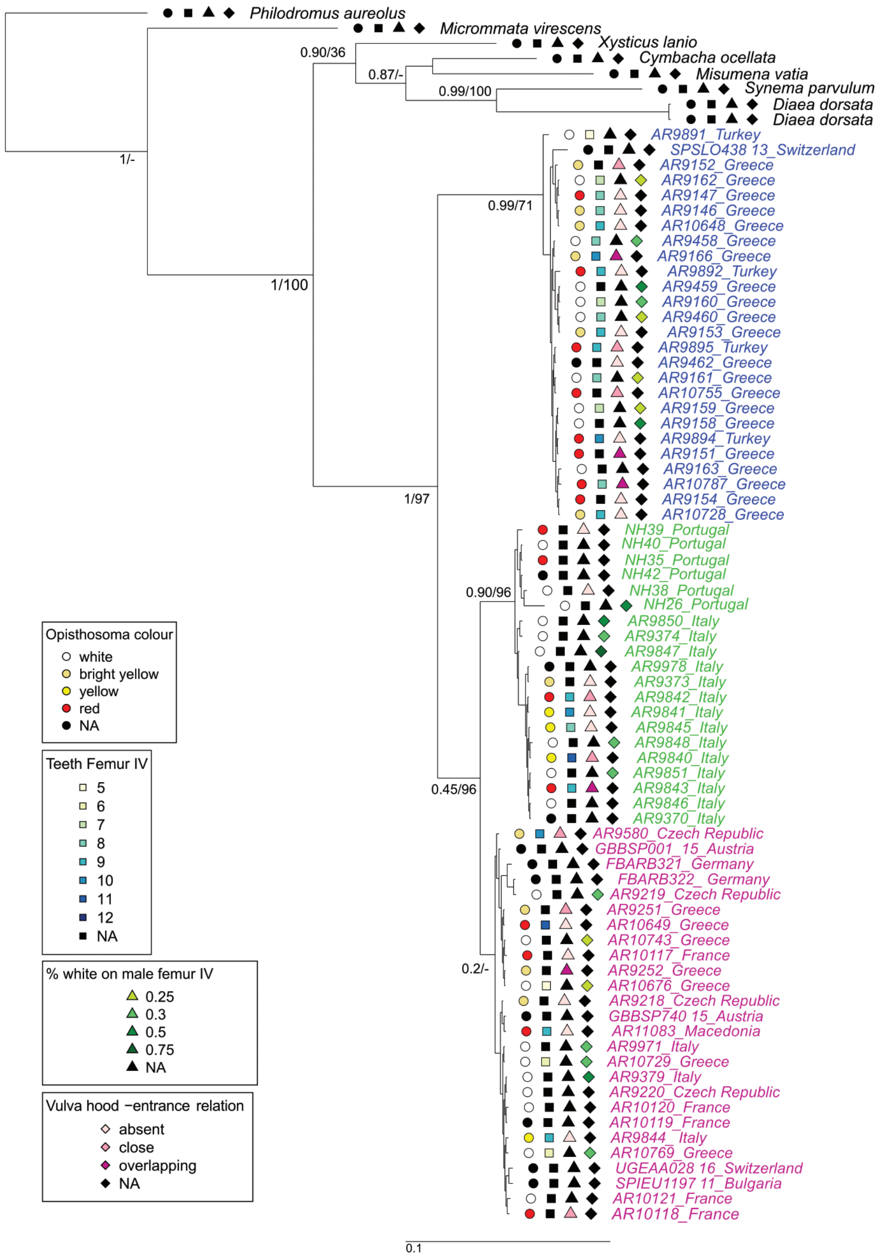

Figure 3. Bayesian majority rule consensus tree for CO1. The analysis included 72 individuals of *Synema globosum* and eight outgroup sequences. Node supports represent Bayesian posterior probabilities/ML bootstrap support based on 1,000 replicates; colours correspond to the three distinct clades. The specimen labels contain country information after the specimen number. Four different symbols before each specimen correspond to the states of four scored morphological traits; circles indicate the colour of the opisthosoma, squares the number of teeth on the prolateral claw of leg one, upside triangles the percentage of white colour starting at the base of leg IV in males, downside triangles the entrance state of the vulval hood; black filled symbols indicate a not applicable state (NA).

Table 3. Mean uncorrected p-distances between and within the CO1 clades of *S. globosum*.

CO1 clades	Clade 1	Clade 2	Clade 3
Clade 1	0.002	–	–
Clade 2	0.061	0.004	–
Clade 3	0.053	0.029	0.004

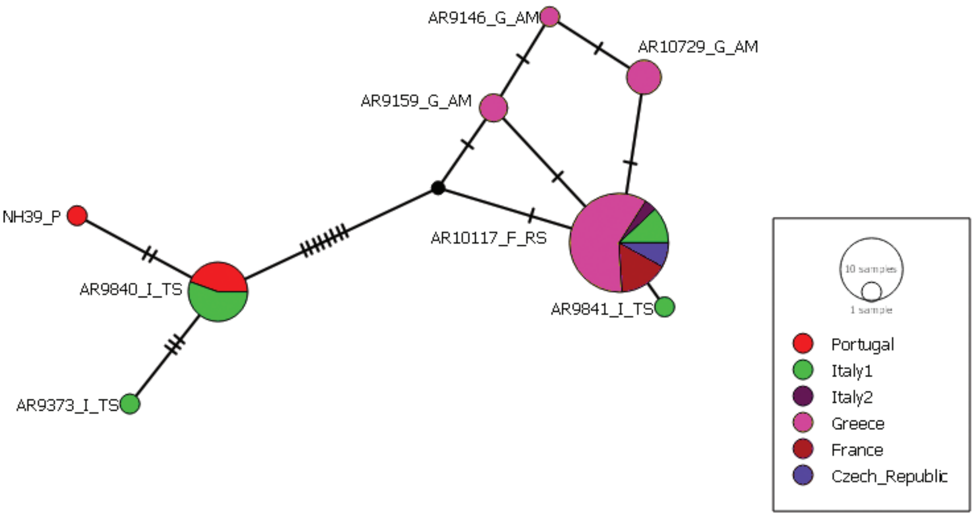

Figure 4. ITS2 haplotype network of *Synema globosum*. Nodes represent haplotypes with the size corresponding to the frequency of the haplotype. The short black lines represent mutations. The colours represent the countries of origin of sequences and have no relation with the CO1 clades.

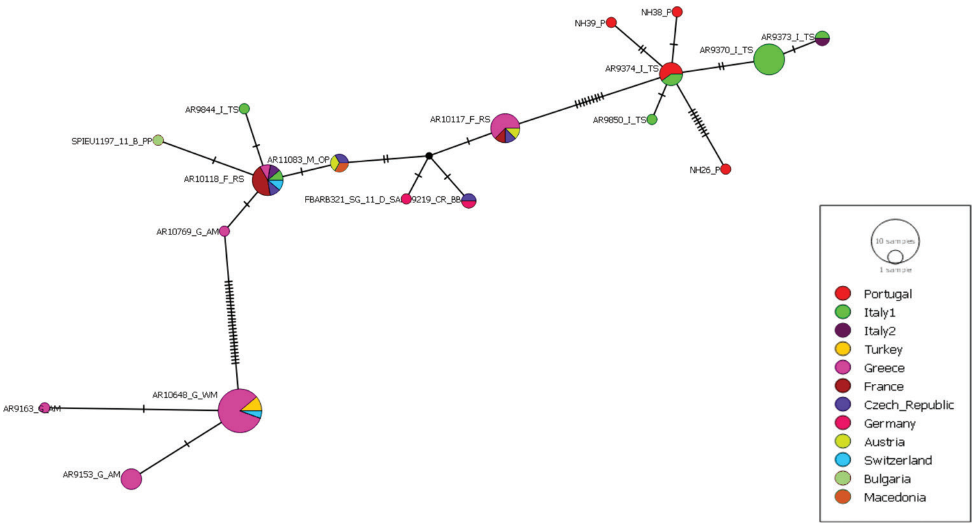

Figure 5. CO1 haplotype network of *Synema globosum*. Nodes represent different haplotypes with the size corresponding to the frequency of the haplotype. The short black lines represent mutations. The colours represent the countries of the origin of the sequences.

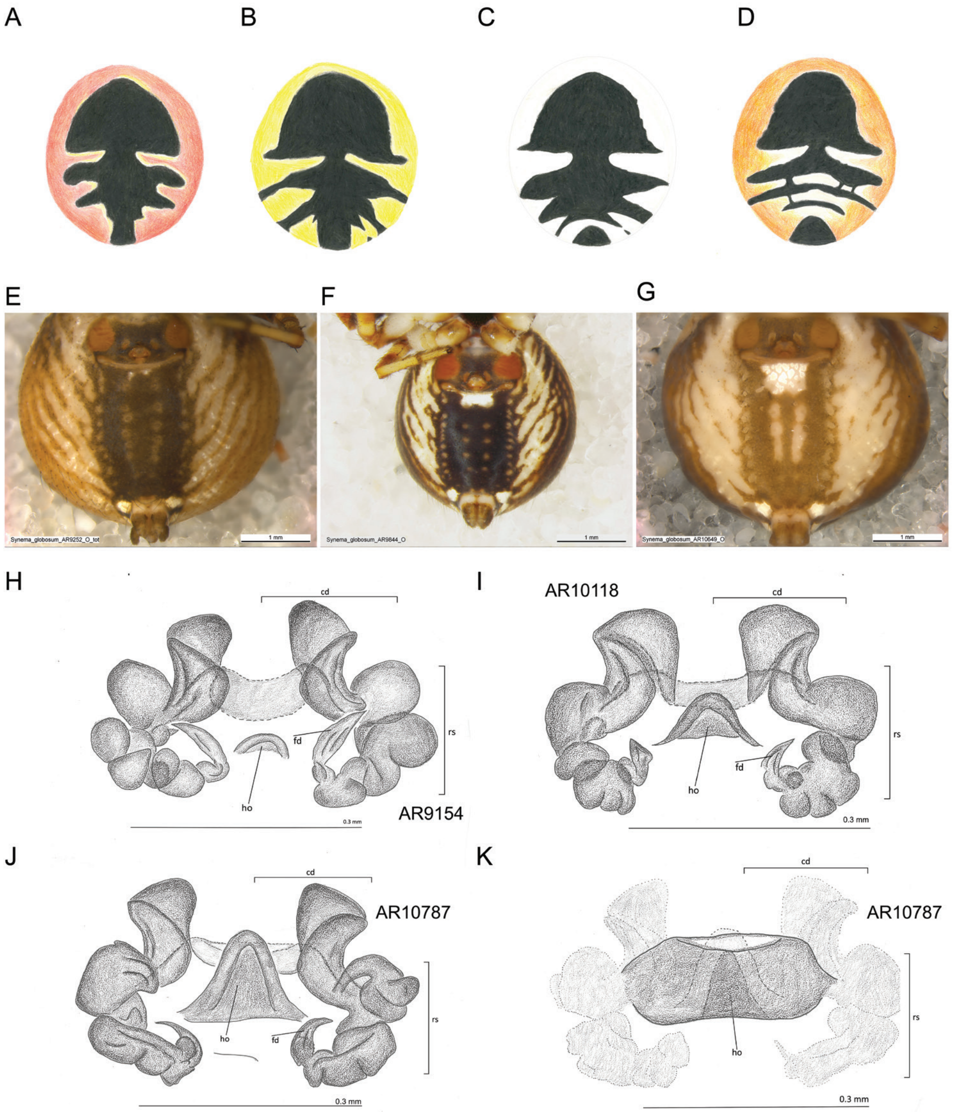

Figure 6. Variation in morphology in the female. **A–D** opisthosoma, dorsal view, colour and black pattern variation **E–G** white stripe on the ventral side of opisthosoma **E** Greece, Peloponnese **F** Italy, Tuscany **G** Greece, west Macedonia **H–J** variation in the vulva **H** Greece, Marathonas **I** France, Savoy **J** Greece, west Macedonia **K** epigyne of the specimen from **J** with very deep hood. Abbreviations: cd = copulatory duct, rs = receptaculum seminis, fd = fertilisation duct, ho = hood.

black colour pattern on the dorsal side of the opisthosoma was unique to each specimen, in some being continuous and in others interrupted in various ways (Fig. 6A–D). Ventrally on the opisthosoma, there was often a white stripe behind the epigyne, but this could also be entirely absent (Fig. 6E–G). Neither the colour nor the colour patterns covaried with the CO1 clades. The vulvae showed large variation (Fig. 6H–K).

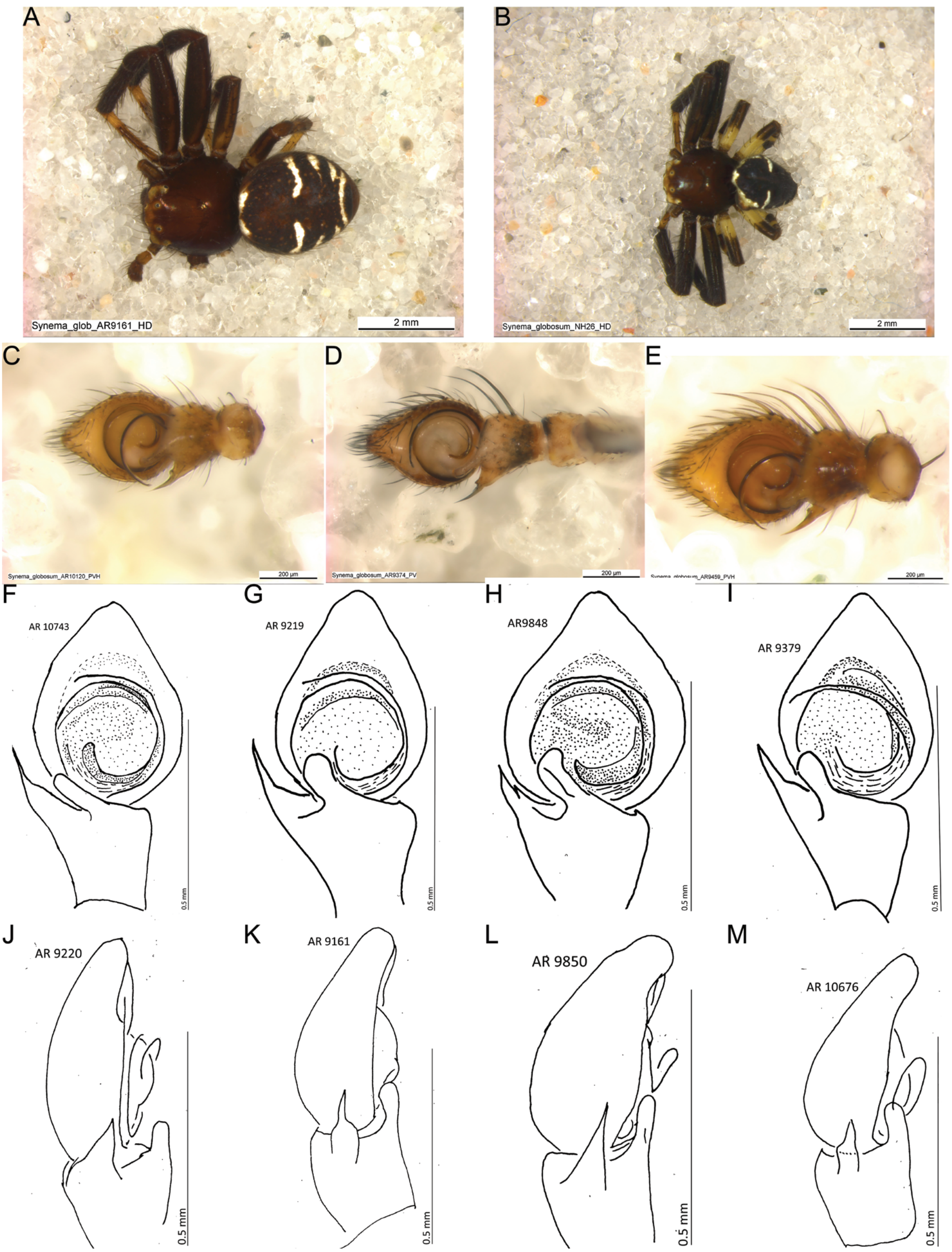

Figure 7. Variation in morphology in the male I. **A, B** habitus males with different colour pattern on femora III and IV **A** Greece, Marathonas **B** Portugal **C–E** Different sizes of palp in ventral view **C** France, Savoy **D** Italy, Siena **E** Greece, Marathonas **F–I** palp, ventral view, the variation of the retrolateral tibial apophysis and the tibial apophysis **F** Greece, West Macedonia **G** Czech Republic, Brno **H, I** Italy, Tuscany **J–M** retrolateral view of the palp, variation in the retrolateral tibial apophysis **J** Czech Republic, Brno **K** Greece, Attiki **L** Italy, Siena **M** Greece, west Macedonia.

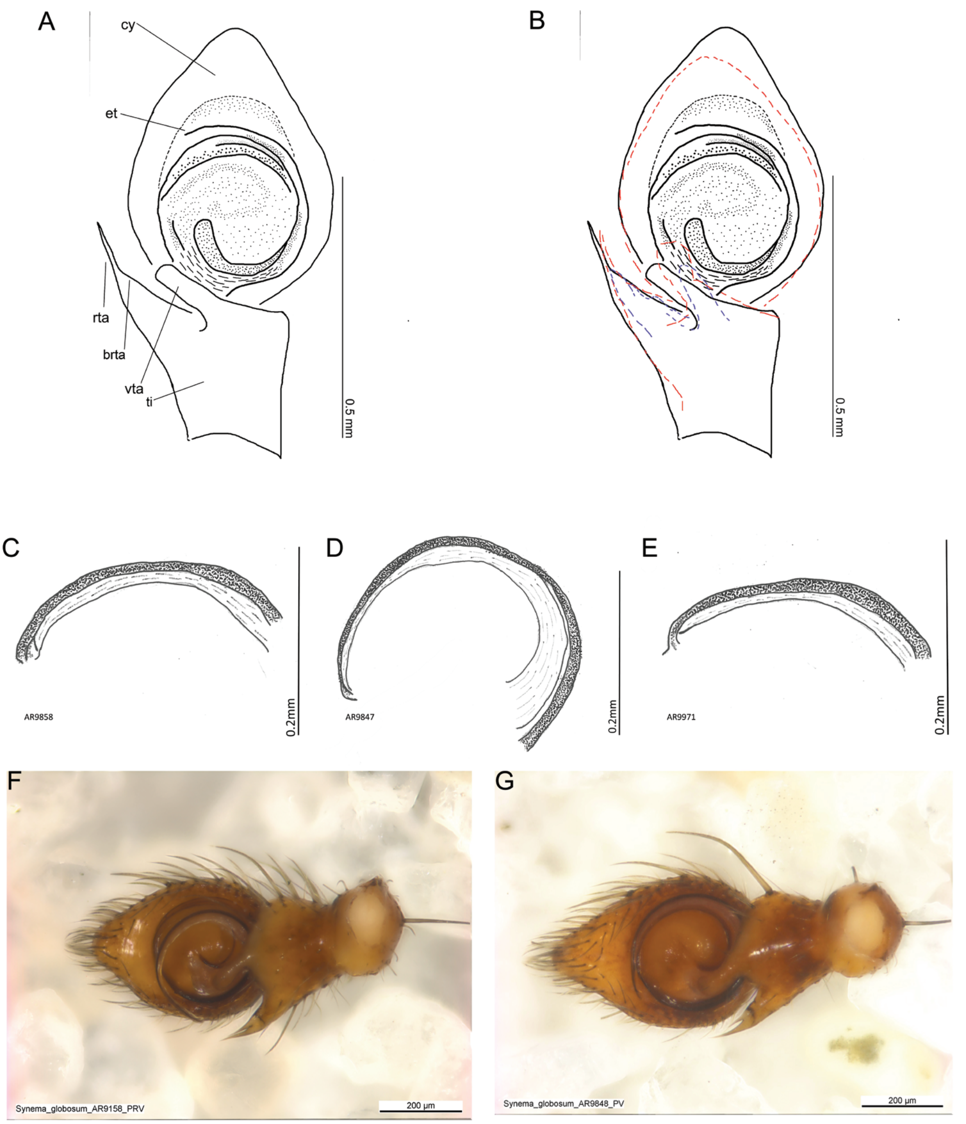

Figure 8. Variation in morphology in the male II. **A** palp with all variable structures **B** outlines of the palps from two additional males that showadditional variation **C–E** tips of embolus **C** Italy, Siena **D, E** Italy Toskana **F–G** two out of three individuals where the rta shows a second tip **F** Greece, Marathonas **G** Italy, Tuscany. Abbreviations: cy cymbium, et embolus tip, rta retrolateral tibial apophysis, brta base of the retrolateral tibial apophysis, ti tibia, vta ventral tibial apophysis

The copulatory duct contained cashew nut-shaped structures with various degrees of bending. The position of the *receptaculum seminis* varied, as does the position of the fertilisation duct. The two structures were closely connected and may influenced each other's position. The vulva hood showed large depth variation ranging from almost absent to the point where the hood overlapped the copulatory opening (Fig. 6H–K). No correspondence between these structures and the CO1 clades was found.

Male morphology. The average body size in males was 3.8 mm (2.94–4.56 mm). In contrast to females, they showed only black and white opisthosoma colour, with a much higher amount of black than white, sometimes small white coloured females could be confused with males. The black pattern on the opisthosoma was not always continuous (Fig. 7A, B). The ventral white stripe was short (usually) or absent (only in few individuals). Notable variation occurred in femora III and IV, which were either brownish or black or they were basally bright and then darker towards the apical part (Fig. 7A, B).

One trait used for identifying *S. globosum* males was the tibia of the palp, which was longer than wide (Levy 1985). This was confirmed in every examined male. However, the palps differed strongly in size (Fig. 7C–E), the height and shape of the cymbium, and the shape and size of the RTA. The TA showed less variation in shape and more in its position (Figs 7F–M, 8A, B). The embolar duct twisted 1.5× and ended distally with the embolus tip (Fig. 8A; Levy 1985). The tip of the embolus was without a thickened end (Fig. 8C–E). The tip of the RTA was needle-like. In three specimens from Italy and Greece, the base of the RTA is extended, seemingly forming a second, shorter tip (Fig. 8F, G). None of the examined structures matched the CO1 clades or showed geographical clustering.

Morphometric data analysis

Body measurements were first inspected concerning reliability (R). The latter was generally high to very high, with only a single character showing R = 89%. All other character had R > 95%. A table with confidence intervals together with a bar plot were available in the Zenodo repository (Urfer and Baur 2021).

Only shape PC1 was significant, which explained 72.2% of the variation. It showed only very slight differentiation among the clades, which overlapped strongly (Fig. 9A).

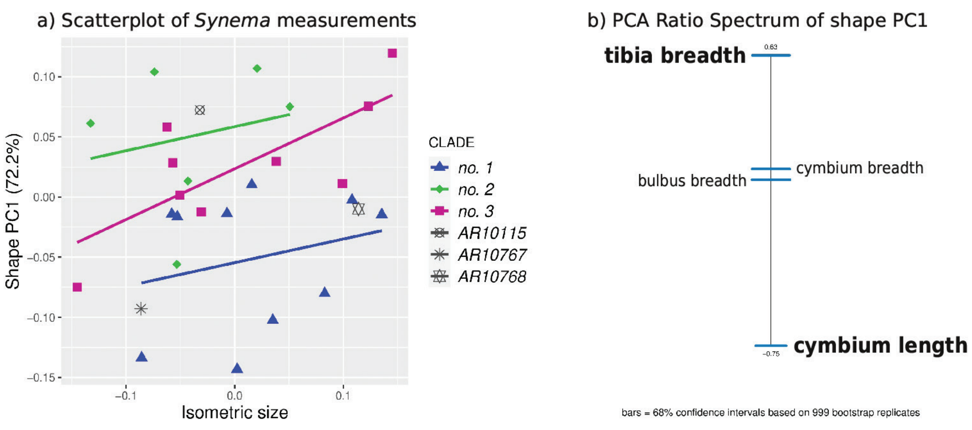

Figure 9. A Shape PC1 plotted against isometric size of 28 males. Colours correspond to the CO1 clades. **B** PCA Ratio Spectrum for shape PC1. The three specimens with grey symbols could not be included in the molecular analysis and therefore could not be attributed to a clade. Regression lines follow a least-squares model.

The PCA ratio spectrum revealed that shape variation was mostly related to the ratio of tibia breadth to cymbium length (tib.b/cym.l), and the importance of the other ratios must be considered negligible.

Discussion

The analysis of 64 *S. globosum* specimens showed an astonishingly high variation in morphological traits as well as in the mitochondrial gene CO1 and, to a much less extent, in the nuclear gene ITS2. While this could indicate overlooked species within *S. globosum*, the lack of a clear relationship between the groups delimited by molecular data and morphological variation or geographical distribution is not in favour of the cryptic species hypothesis or of an ongoing speciation process. The results rather suggest a single, highly variable species. However, for a final solution of this problem, more molecular data are needed, for example obtained with whole genome or ddRAD sequencing together with testing for mating barriers in *S. globosum*.

CO1 gene with three distinct clades

Barcoding can be used to accurately distinguish higher taxonomic groups, e.g., genus and family level in spiders (Čandek and Kuntner 2015; Coddington et al. 2016; Kennedy et al. 2020) and is nowadays commonly used as a helpful tool to support red list assessments and species inventories (Blagoev et al. 2013; Astrin et al. 2016; Crespo et al. 2018; Gregorič et al. 2020). Finding such deep CO1 clades within one species was thus unexpected. Deep CO1 mitochondrial divergence without speciation has been reported for several organisms such as the common redstart *Phoenicurus phoenicurus* (Hogner et al. 2012), gall wasps (Nicholls et al. 2012) and in the butterfly genus *Heliconius* (Muñoz et al. 2011). In arachnids, similar findings concern groups with a low dispersal ability such as mygalomorphs (Arnedo and Ferrández 2007) or species with major geographical barriers in their distribution area (Chamberland et al. 2020). Since there is evidence that *S. globosum* is able to balloon (Blandenier and Fürst 1998) and it is quite common in Europe, the low dispersal ability should not be the reason of the deep CO1 divergence.

The bTPT analysis suggested three species that correspond to the three CO1 clades identified in the phylogenetic analyses. It is a helpful tool for single-locus species delimitation, however Blair and Bryson (2017) suggested to treat the results with caution and to analyse at least a second nuclear marker, as single markers (and especially uniparentally inherited ones) might provide an incomplete picture.

Discordant patterns of nuclear and mitochondrial phylogenies in *S. globosum*

The ITS2 phylogeny of 64 *S. globosum* specimens did not reflect the CO1 clade pattern. ITS2 is a nuclear rRNA marker that is assumed to mutate via concerted evolution (Elder and Turner 1995). The number of ITS2 copies is very high in the genome, and the individual copies usually show no variation among each other because DNA repair mechanisms homogenise their sequences within the genome (Zimmer et al. 1980; Elder and Turner 1995; Álvarez 2003). Ortiz et al. (2021) found the pattern of over-splitting CO1 when testing ddRAD sequencing in an ant-eating *Zodarion* species. The species was assumed to consists of two cryptic lineages based on ecological traits. They found over-splitting in number of species when only the CO1 barcode gap was analysed. This gap was not supported by the variation in ITS2.

On the other hand, it is a plausible assumption, that CO1 barcodes can reflect cryptic speciation and ITS2 has low substitution rate which is too low to catch the interspecific distances. However, this should always be verified with a larger molecular study.

Haplotype analyses based on mtDNA markers can indicate ancient geographic structures (Nicholls et al. 2012). Thus, we could suggest that the slight geographic pattern identified in the CO1 haplotype network may reflect isolated populations in glacial refugia. However, ITS2-based networks do not support such an interpretation. A constraint to testing this hypothesis is the biased sampling towards eastern Europe, leading to an incomplete representation of west European haplotypes. To assess population structuring more appropriately in *S. globosum*, we suggest additional taxon sampling, especially of populations on the Iberian Peninsula, and application of more suitable molecular markers for population-level genetic analyses.

Introgression from a related species by past hybridisation events is a second scenario that could explain deep CO1 clades (Galtier et al. 2009). However, it does not explain the morphological variation to the extent we found it. Alternatively, despeciation after secondary contact might explain the CO1 clades as well as the high genetic and morphologic variation in *S. globosum.* Finding traces of despeciation could thus explain the patterns observed here. However, de-speciating lineages are very difficult to trace, even with large amounts of genetic data and are beyond the scope of our study (Taylor et al. 2006; Kearns et al. 2018).

Infection with endosymbiotic bacteria that may alter the mitochondrial structure of species (Hurst and Jiggins 2005; Goodacre et al. 2006) is an additional possible scenario. It offers a possibility for hybridisation and introduction of haplotypes into a species, in this way distorting single locus CO1 barcoding (Narita et al. 2006; Klopfstein et al. 2016). Since we found only a single infection, the probability of this scenario is also unlikely. But it should be kept in mind that an endosymbiotic infection has occurred very early and no traces of it are left today.

High morphological variation in *S. globosum*

The morphometric analysis of the male palp showed only a very slight differentiation among clades in the first shape PC, but in general the clades overlapped strongly. We found high and continuous variation in the colour pattern and the shape of the epigyne, vulva and palp in all examined populations of *S. globosum* (Fig. 3). Unusually high morphological variation is reflected by the number of described subspecies (Dahl 1907; Franganillo 1913, 1926b; World Spider Catalog 2021), which are nowadays considered unfounded and were probably described due to insufficient knowledge of the extent of intraspecific variation in this species. This shows that the assessment of intraspecific variation is underestimated and should be included more in taxonomic studies.

The main characters used to delimit species of spiders are found in the genitalia (e.g., Huber 2004). However, intraspecific genitalic variation in spiders is largely understudied and rarely accounted for in identification keys (e.g., Levy 1985; Mcheidze 1997; Utochkin 1960b). Genitalic variation, especially in male copulatory organs, is predicted by the theory of cryptic female choice, which states that parts of the male's genital bulb are supposed to serve as copulatory courtship devices enabling the female to evaluate the male's quality during mating (e.g., Eberhard 1996; Kuntner et al. 2009). Cryptic female choice could therefore play a role in shaping the morphological diversity of *S. globosum* palps. However, for a deeper understanding of these problems, further studies on the mating behaviour and mechanic coupling of the copulatory organs are necessary.

In our study we had a biased sample size mostly towards easter Europe. In this region, the species *Synema caucasicum* Utochkin, 1960, occurs regularly. The separation of *S. caucasicum* from *S. globosum* is based on the colour pattern on the ventral side of the opisthosoma, where *S. caucasicum* shows five brighter marks. The palp of *S. caucasicum* looks almost identical to that of *S. globosum*, and the epigyne structure lies within the variation that we recorded in *S. globosum*. *S. caucasicum* is endemic to Georgia and Azerbaijan (Khasayeva and Huseynov 2019; Utochkin 1960b) and it remains unclear if it just represents a local morph of *S. globosum*, as there are only insufficient drawings, sketches and descriptions of *S. caucasicum* available. Because a precise examination of the *S. caucasicum* taxonomy was beyond the scope of this study, this problem should be addressed in further investigations.

Conclusions

Based on a large set of specimens of *S. globosum* from a wide geographical range, we found three deep clades in the CO1 gene tree and large variation but no resolution in the ITS2 gene tree. We also found remarkable intraspecific morphological variation in sexual organs and in other characters that are commonly used for species delimitation. However, this variation does not show any geographical pattern or correspondence with the CO1 clades. In order to better understand the high morphological variability in *S. globosum*, we suggest looking at a larger molecular dataset, such as multilocus phylogeny based on restriction-site associated DNA markers (Peterson et al. 2012) or whole genome sequences conducted on a broader geographic range, which can capture processes at or below the current species level of *S. globosum.*

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Supplementary material 1

Coordinates of the collected specimens

Authors: Karin Urfer , Tamara Spasojevic , Seraina Klopfstein , Hannes Baur , Liana Lasut , Christian Kropf

Data type: occurences

Link: https://doi.org/10.3897/zookeys.1078.64116.suppl1

Supplementary material 2

Genbank and Bold numbers of the *Synema globosum* specimens that were obtained from these databases

Authors: Karin Urfer , Tamara Spasojevic , Seraina Klopfstein , Hannes Baur , Liana Lasut , Christian Kropf

Data type: genomic

Link: https://doi.org/10.3897/zookeys.1078.64116.suppl2

Supplementary material 3

GenBank accession numbers

Authors: Karin Urfer , Tamara Spasojevic , Seraina Klopfstein , Hannes Baur , Liana Lasut , Christian Kropf

Data type: Genomic

Link: https://doi.org/10.3897/zookeys.1078.64116.suppl3

Supplementary material 4

Input file Mr Bayes and RAxML

Authors: Karin Urfer , Tamara Spasojevic , Seraina Klopfstein , Hannes Baur , Liana Lasut , Christian Kropf

Data type: Phylogenetic

Explanation note: The exact input scripts that we used to run RAxML and MrBayes analysis.

Link: https://doi.org/10.3897/zookeys.1078.64116.suppl4

Supplementary material 5

Bayesian majority rule consensus tree of ITS2

Authors: Karin Urfer , Tamara Spasojevic , Seraina Klopfstein , Hannes Baur , Liana Lasut , Christian Kropf

Data type: Image

Explanation note: The analysis included 64 *S. globosum* specimens and two outgroup sequences. Node supports represent Bayesian posterior probabilities/ML bootstrap support based on 1,000 replicates. Colours correspond to the CO1 clades, black indicates the absence of a CO1 sequence. The last two letters are the country code according to ISO 3166.

Link: https://doi.org/10.3897/zookeys.1078.64116.suppl5

Supplementary material 6

Morphomatrix of the examined *Synema globosum* individuals

Authors: Karin Urfer , Tamara Spasojevic , Seraina Klopfstein , Hannes Baur , Liana Lasut , Christian Kropf

Data type: Morphological

Link: https://doi.org/10.3897/zookeys.1078.64116.suppl6

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RESEARCH ARTICLE

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Checklist and keys to Deltocephalinae leafhoppers (Hemiptera, Cicadellidae) from Pakistan

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Abstract

Keys to all levels of the subfamily Deltocephalinae (Hemiptera: Cicadellidae) of Pakistan are provided based on published records and original data from recent research. Checklists to the genera and species of Deltocephalinae are also given. A total of 49 genera with more than 100 species are now known from Pakistan. Two new synonyms are proposed, i.e., *Cicadulina striata* Ahmed, 1986 a junior synonym of *Cicadulina chinai* Ghauri, 1965, **syn. nov.** and *Macrosteles parafalcatus* Naveed & Zhang, 2018 a new junior synonym of *Macrosteles indrina* (Pruthi, 1930), **syn. nov.**

Keywords

Auchenorrhyncha, distribution, key, morphology, synonyms

Introduction

Cicadellidae, the largest family of Hemiptera, comprises 26–40 subfamilies (depending on the classification used, e.g., Dietrich 2005 and Oman et al. 1990, respectively). Included are nearly 22,000 species of which more than 200 species are known from

Pakistan (Khatri and Webb 2010). The largest leafhopper subfamily, Deltocephalinae, is found in all geographical regions and comprises more than 38 tribes and 923 genera (Zahniser and Dietrich 2013). The earliest Deltocephalinae to be recorded from Pakistan were by Pruthi (1930, 1936) who recorded several species from Indian localities which are now in Pakistan, e.g., Lyallpur, Changla Gali and Murree Hills. Thirty-one genera and 57 species of the subfamily were recorded from Pakistan by Khatri and Webb (2010); these authors also provided a checklist to Pakistan Deltocephalinae and illustrated the species, some new. Subsequently, Khatri and Rustamani (2011) provided a key to tribes and genera known at that time from Pakistan and, due to the revised classification of Zahniser and Dietrich (2013), some genera have been transferred from one tribe to another (see Remarks under Deltocephalinae). In this paper we add a further 18 genera and 51 species records, provide checklists and keys to species and include two new species synonymies; a total of 49 genera with more than 100 species is now known from Pakistan.

Much taxonomic work needs to be done for the fauna of Cicadellidae in various countries and this is particularly true for Pakistan. Such studies are not only important to discover the leafhopper diversity but also for pest management in agriculture and forestry as leafhoppers being one of the most important groups of vectors of plant pathogens (Claridge and Wilson 1991; Wilson and Turner 2010).

Materials and methods

All specimens were examined with a Leica ZOOM2000 stereomicroscope. Drawings were made using an Olympus drawing tube. Photos were taken by a ZEISS SteREO Discovery.V20 stereomicroscope equipped with a ZEISS AxiocamICc 5 camera that also provided measurements. Adobe Photoshop CS was used to compile photographs. Specimens from Pakistan are deposited in the various collections as indicated in the published records and additional specimens, examined and figured for this study, are deposited in the Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China.

Taxonomy

Deltocephalinae Fieber

The subfamily Deltocephalinae includes small-to-large, mainly wedge-shaped leafhoppers diagnosed as follows: head with ocelli on anterior margin near to eyes; frontoclypeus not swollen, carinae on anterior margin of head usually absent; lateral frontal sutures reaching to ocelli; antennal ledges reduced or absent; gena large, usually covering proepisternum, with a fine erect seta laterad of lateral frontal suture. Forewing macropterous to brachypterous; if macropterous, with apices usually overlapping at rest (except *Gurawa*); with two or three anteapical cells and often with one or more crossveins between A1 and claval suture; inner apical cell narrowed distally, not reaching to wing apex. Profemur AM1 seta distinct; row AV with short stout setae extending from base to 1/2–2/3 length of femur; intercalary row with various thin setae arranged in one row. Mesotrochanter with apical posteroventral stout seta. Metafemur macrosetal formula usually 2+2+1 with penultimate pair close-set. Metatibia usually anteroposteriorly compressed, ventrally with a median ridge. Male pygofer usually with a membranous cleft at basolateral margin. Valve produced posteriorly, lateral margins short, articulated with pygofer laterally. Subgenital plates articulated with each other and with valve rarely fused to each other and valve (*Goniagnathus*); usually triangular, normally somewhat flattened; with dorsal slot or fold articulating with style. Connective Y-shaped or linear, rarely T-shaped; devoid of anteromedial lobe or process. Style broad at base, bilobed basally; apophysis not elongate. First valvula convex to relatively straight; dorsal sculpturing pattern reaching the dorsal margin or not; sculpturing pattern striate, concatenate, reticulate, imbricate, maculate, or granulose. Second valvula with basal fused section as long as distal paired blades or longer; median dorsal tooth present or not; usually with small to large, regularly or irregularly shaped dorsoapical teeth on apical 1/3 or more; teeth sometimes restricted to apical 1/4, or absent.

Remarks. We treat Deltocephalinae here in its wider sense, following Zahniser and Dietrich (2013) to include Selenocephalini, Mukariini and Penthimiini. We also follow Zahniser and Dietrich (2013) for the placement of genera in tribes; this has particular implications for *Bampurius* placed in Athysanini by Khatri and Webb (2010), here placed in Scaphoideini and the genera placed in Scaphytopiini by Khatri and Webb (2010), i.e., *Grammacephalus* placed here in Scaphoideini, *Masiripius* placed here in Opsiini and *Varta* placed here in Vartiini.

Key to tribes and genera of Deltocephalinae from Pakistan

If genera are represented by a single species in Pakistan the species name is given.

1	Crown with transverse striations or carinae on anterior margin	**2**
–	Crown with anterior margin smooth or shagreen	**9**
2	Clypellus narrow, extending beyond margin of genae, tapered towards apex	**Koebiliini (Grypotina) 3**
–	Clypellus broader, not extending beyond margin of genae	**4**
3	Crown medially longer than next to eyes; aedeagus simple, without processes	***Sohipona webbi*** (p. 161)
–	Crown with uniform length; aedeagus with lateral processes	***Pinopona minuta*** (p. 161)
4	Antennae arising near upper corner of eyes	**Drabescini 5**
–	Antennae arising distinctly below upper corner of eyes	**6**

5 Dark robust species; crown similar in length throughout width (Fig. 1); antennal ledges strong; antennae similar in width to head; forewing appendix broad .... **Drabescina** (***Drabescus angulatus***) (p. 156)

– Pale narrow species; crown distinctly longer medially than next to eyes; antennal ledges weak or absent; antennae much longer than width of head; forewing appendix narrow .... **Paraboloponina** (***Dryadomorpha pallida***) (p. 157)

6 Crown slightly longer medially than next to eye.. .... **Athysanini (in part)** ***Tambocerus bulbulus*** (p. 143)

– Crown distinctly longer medially than next to eye **7**

7 Head depressed anteriorly, if not depressed then ocelli on crown close to foremargin; forewing venation reticulate (Fig. 2); aedeagus with single shaft....... .... **Penthimiini 8**

– Head not so depressed, ocelli on anterior margin; forewing venation not reticulate; aedeagus with two shafts..........**Mukariini** (***Mukaria splendida***) (p. 165)

8 Ocelli on anterior margin of crown........***Neodartus acocephaloides*** (p. 170)

– Ocelli on crown near anterior margin .... ***Penthimia compacta*** (p. 170)

9 Robust and squat species (Fig. 3); forewing with appendix extending aroundwing apex (Fig. 57); subgenital plates fused to each other and to valve; connective fused with aedeagus (Fig. 41) .... **Goniagnathini (*Goniagnathus*)**

– Without this combination of characters... **10**

10 Crown produced, pointed anteriorly; genae visible behind eyes in dorsal view; forewing truncate apically ....**Vartini (*Varta rubrofasciata*)** (p. 175)

– Without this combination of characters... **11**

11 Aedeagal shaft moveably hinged basally or if not hinged (*Gurawa*) forewing without appendix; connective loop-shaped with arms closely appressed anteriorly; first valvula dorsal sculpturing maculate to granulose not reaching dorsal margin; second valvula with uniform-shaped teeth ....**Chiasmini 12**

– Without this combination of characters... **17**

12 Male pygofer with caudal marginal darkly sclerotised dentate crest....***Aconurella***

– Pygofer not as above **13**

13 Head spatulate, foremargin sharply angled in lateral view, carinate (Fig. 67)....**14**

– Head not spatulate, foremargin rounded in lateral view (Fig. 68)..............**15**

14 Forewing lacking appendix; ocelli near anterior margin of head (Fig. 67)...... ....***Gurawa***

– Forewing when fully developed with appendix (Fig. 59); ocelli on vertex some distant from anterior margin.. ***Chiasmus***

15 Opaque green (rarely blue) species with black markings............. ***Nephotettix***

– Pale brown species with or without markings... **16**

16 Crown with or without transverse black band; male pygofer with few apical stout setae (Fig. 28)... ***Exitianus***

– Crown without transverse black band; male pygofer without apical stout setae (Fig. 27) ....***Leofa***

17 Ocelli closer to eyes than laterofrontal sutures; body dorsoventrally flattened; aedeagus with pair of apical processes..**Hecalini 18**

– Ocelli and laterofrontal sutures equidistant from eyes; body not dorsoventrally flattened; aedeagus with or without apical processes.........................**21**

18 Brown species; male pygofer with caudal marginal stout setae...................... .... ***Glossocratus***

– Pale to green species; male pygofer without caudal marginal stout setae....**19**

19 Crown with bold orange or yellow inverted V-shaped band, pronotum with two bold arcuate orange bands (Fig. 72); forewing with claval vein A1 merging with claval suture .... ***Linnavuoriella arcuata*** (p. 160)

– Crown without coloured bands or with bands subparallel or converging, but not very bold and not broadly contiguous at median line; pronotum with or without bands; forewing with A1 not merging with claval suture, but with two separate claval veins..**20**

20 Crown without orange or yellow colour pattern; tegmina unmarked (Fig. 8) ... .***Hecalus***

– Crown with pair of orange or yellow longitudinal bands subparallel or converging, but not contiguous anteriorly, sometimes faint or absent; tegmina invariably with apical brown patch with white spots (Fig. 74) ....***Thomsonia porrecta***

21 Aedeagus with two shafts .... **Opsiini 22**

– Aedeagus with one shaft..**26**

22 Aedeagus with shafts fused in basal half of the length, apically divergent, forming a circle (Fig. 53) .... ***Neoaliturus* (*Circulifer*)**

– Aedeagal shaft fused basally but well separated throughout........................**23**

23 Aedeagal shaft with apical or preapical processes (Fig. 44)............................ ....***Hishimonus phycitis*** (p. 165)

– Aedeagal shaft without apical or preapical processes..................................**24**

24 Aedeagal shaft with pair of ventral processes .... ***Opsius***

– Aedeagal shaft without pair of ventral processes **25**

25 Crown, thorax and forewing with irregular brown maculation, pronotum and scutellum without red markings (Fig. 10)..................................***Orosius***

– Crown sprinkled with fine dark brown spots, pronotum and scutellum with irregular red markings....................................***Masiripius lugubris*** (p. 165)

26 Connective fused to aedeagus .... **Deltocephalini 27**

– Connective articulated with aedeagus **29**

27 Crown with transverse black stripe; male pygofer with appendage on dorsal margin...***Paramesodes lineaticollis*** (p. 156)

– Crown without transverse black stripe; male pygofer without appendage on dorsal margin...**28**

28 Aedeagal shaft short, robust, strongly curved dorsally, with apical gonopore (Fig. 45)... ***Deltocephalus***

– Aedeagal shaft long, slightly curved dorsally, with gonopore indistinct (Fig. 46).. ***Maiestas***

29 Forewings with two anteapical cells; preatrium of aedeagus without long processes (Fig. 60) .... **Macrostelini 30**
– Forewings with three anteapical cells, if with two anteapical cells then preatrium of aedeagus with two long processes **32**
30 Head with crown of uniform length throughout width, more than four times broader than long (Fig. 12) .... ***Balclutha***
– Crown distinctly longer medially than next to eyes, two times or less broader than median length...**31**
31 Pale yellow to brown or black in colour; male pygofer processes absent, caudal margin with comb-like serrations (Fig. 29) .... ***Macrosteles***
– Golden yellow in colour, vertex with a pair of rounded dark brown spots; male pygofer with process present, caudal margin without comb-like serrations ....***Cicadulina***
32 Male segment X elongate and sclerotised dorsally (Fig. 38) .... **Cicadulini (*Pseudosubhimalus*)**
– Male segment X not as above **33**
33 Aedeagus with dorsal connective (Fig. 47)... ....**Limotettigini (*Limotettix*(*Scleroracus*) *cacheolus*)** (p. 161)
– Aedeagus without dorsal connective...**34**
34 Connective with arms parallel (Fig. 54) ....**Stenometopiini (*Stirellus*)**
– Connective with arms not parallel...**35**
35 Frontoclypeus long and narrow (except *Monobazus*) (Fig. 65); male or female pygofer with dense tufts of either long fine or regular setae .... **Scaphoideini 36**
– Frontoclypeus broad (Fig. 66); male or female pygofer without dense tufts of long fine setae **42**
36 Crown with distinct black spot near posterior margin (Fig. 75) .... ***Phlogotettix indicus***(p. 173)
– Crown without distinct black spot near posterior margin..........................**37**
37 Brown species, forewing with whitish costal area (Fig. 15) .... ***Grammacephalus***
– Brown to yellowish brown species, forewing without whitish costal area **38**
38 Forewing with 3 or 4 crossveins extending to costal margin from outer apical cell (Fig. 61) **39**
– Forewing with at most 2 crossveins in costal region **40**
39 Connective with paraphysis (Fig. 55); aedeagal shaft very short .... ***Scaphoideus harlani*** (p. 173)
– Connective without paraphysis; aedeagal shaft elongate, cylindrical............. .... ***Bampurius pakistanicus*** (p. 171)
40 Male subgenital pl. with mesal sclerotised process (Fig. 48).......................... .... ***Neolimnus egyptiacus*** (p. 172)
– Male subgenital pl. without mesal sclerotised process **41**

41 Aedeagal shaft with processes arising on dorsal surface .... ***Monobazus dissimilis*** (p. 172)
– Aedeagus with ventro-lateral processes .... ***Osbornellus*(*Mavromoustaca*) *macchiae*** (p. 172)
42 Connective arms closely appressed anteriorly .... **Paralimnini 43**
– Connective arms not closely appressed anteriorly, divergent......................... .... **Athysanini (in part) 47**
43 Crown with pair of black anterior markings (Fig. 18) .... ***Changwhania***
– Crown without pair of black markings..**44**
44 Anterior margin of crown with transverse black stripe (Fig. 19); connective V-shaped...***Paralimnus cingulatus***
– Anterior margin of crown without transverse black stripe; connective Y-shaped **45**
45 Subgenital plates short ....***Psammotettix emarginatus***
– Subgenital plates long **46**
46 Anal tube with long process (Fig. 49); aedeagus with dorsal connective well-developed (Fig. 50) ....***Jilinga***
– Anal tube without process; aedeagus with dorsal connective absent.............. ....***Soractellus nigrominutus*** (p. 169)
47 Crown pointed anteriorly; aedeagus without apical lateral processes ....***Platymetopius***
– Crown rounded anteriorly; aedeagus with apical laterally directed small processes (Fig. 52) ....***Euscelidius cornix***

Checklists and keys to species of Pakistani Deltocephalinae

Keys to all species of Pakistan Deltocephalinae are given for each genus containing more than one species. We follow Zahniser and Dietrich (2013) for most of the tribal diagnostic characters.

Athysanini Van Duzee

Diagnosis. It is impossible to provide a set of characters to easily diagnose this large tribe due to its morphological diversity. However, most members have the connective Y-shaped and lack the distinctive features of other tribes.

Euscelidius Ribaut

E. cornix **Naveed & Zhang**
Figs 23, 36, 52

Euscelidius cornix Naveed & Zhang, 2020c: 470, fig. 1A–G (Pakistan).

Platymetopius Burmeister

Platymetopius sp.

Remarks. From the figure (code number DW 50A, unidentified) given by Mahmood (1969) this genus is present in Pakistan.

Figures 1–15. (habitus, dorsal view) **1** *Drabescus angulatus* **2** *Neodartus acocephaloides* **3** *Goniagnathus* (*Tropicognathus*) *nepalicus* **4** *Aconurella prolixa* **5** *Gurawa minorcephala* **6** *Chiasmus* sp. **7** *Leofa* (*Prasutagus*) *pulchellus* **8** *Hecalus ghaurii* **9** *Hishimonus phycitis* **10** *Orosius aegypticus* **11** *Maiestas albomaculata* **12** *Balclutha punctata* **13** *Pseudosubhimalus pakistanicus* **14** *Limotettix* (*Scleroracus*) *cacheolus* **15** *Grammacephalus raunoi.*

Figures 16–24. (habitus, dorsal view) **16** *Neolimnus egyptiacus* **17** *Scaphoideus harlani* **18** *Changwhania terauchii* **19** *Paralimnellus cingulatus* **20** *Jilinga truncata* **21** *Soractellus nigrominutus* **22** *Tambocerus bulbous* **23** *Euscelidius cornix* **24** *Stirellus mankiensis.*

Tambocerus Zhang & Webb

Remarks. *Tambocerus* is one of the few Athysanini with transverse striations on the fore margin of the head.

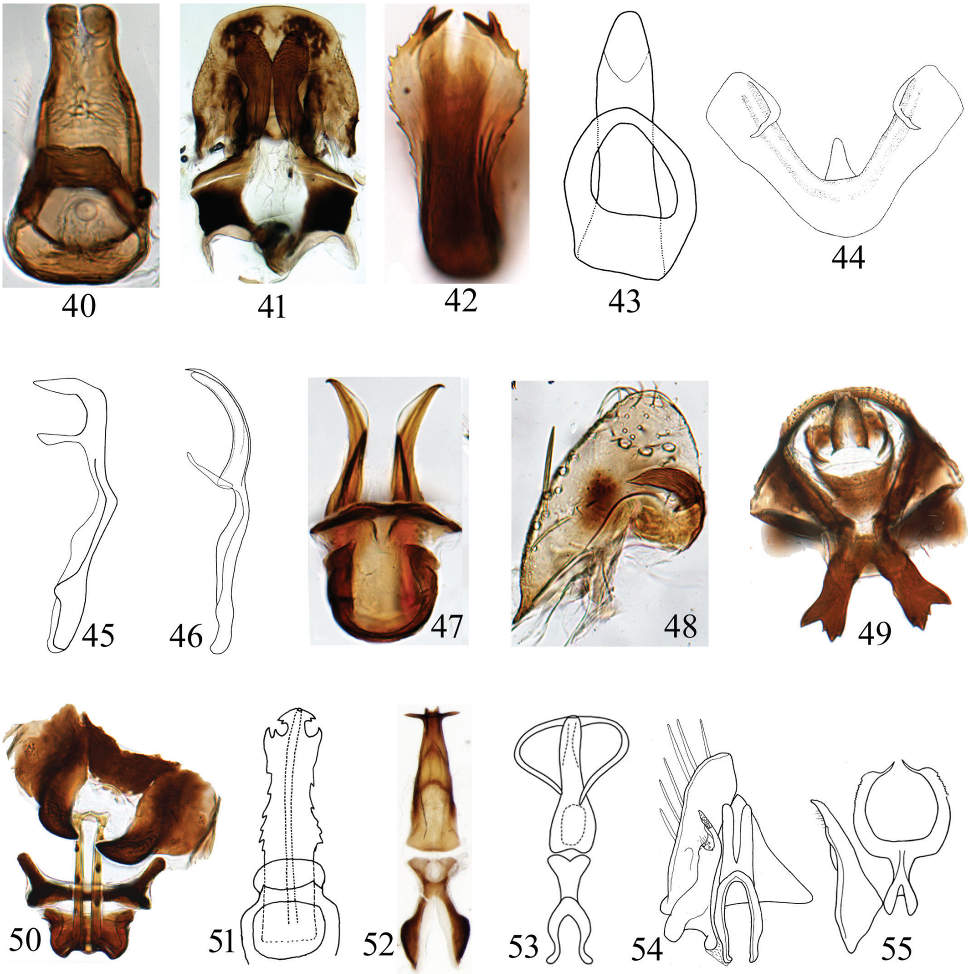
T. bulbulus Naveed & Zhang

Figs 22, 39, 51

Tambocerus bulbulus Naveed & Zhang, 2018i: 240, figs 3A–D, 4A–I (Pakistan).

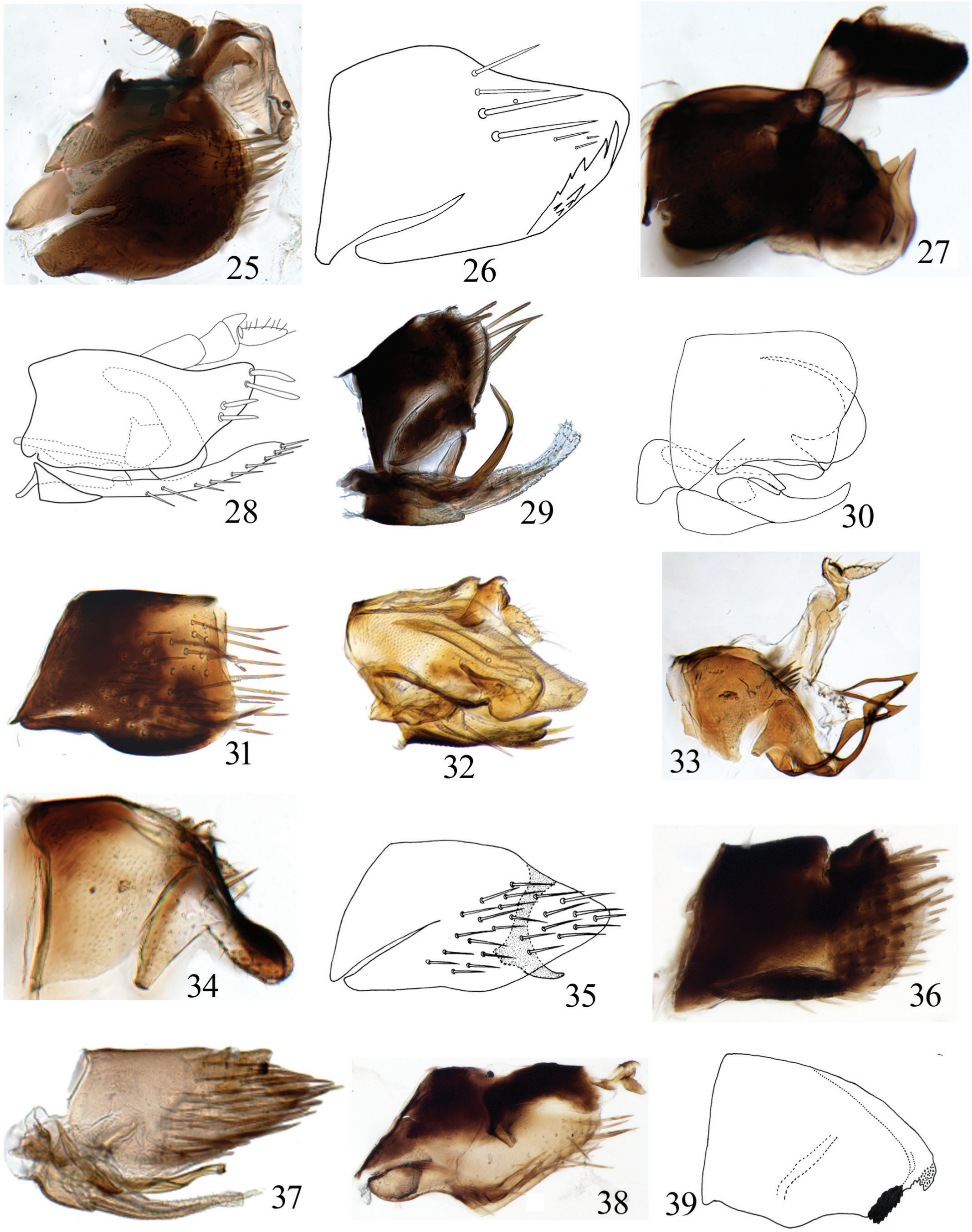
Chiasmini Distant

Diagnosis. These are small to medium sized leafhoppers, usually white, stramineous, green, brown, grey, or black in colouration, and sometimes iridescent. They can be identified by the tapering or parallel sided clypellus, aedeagus hinged at the base (hinge

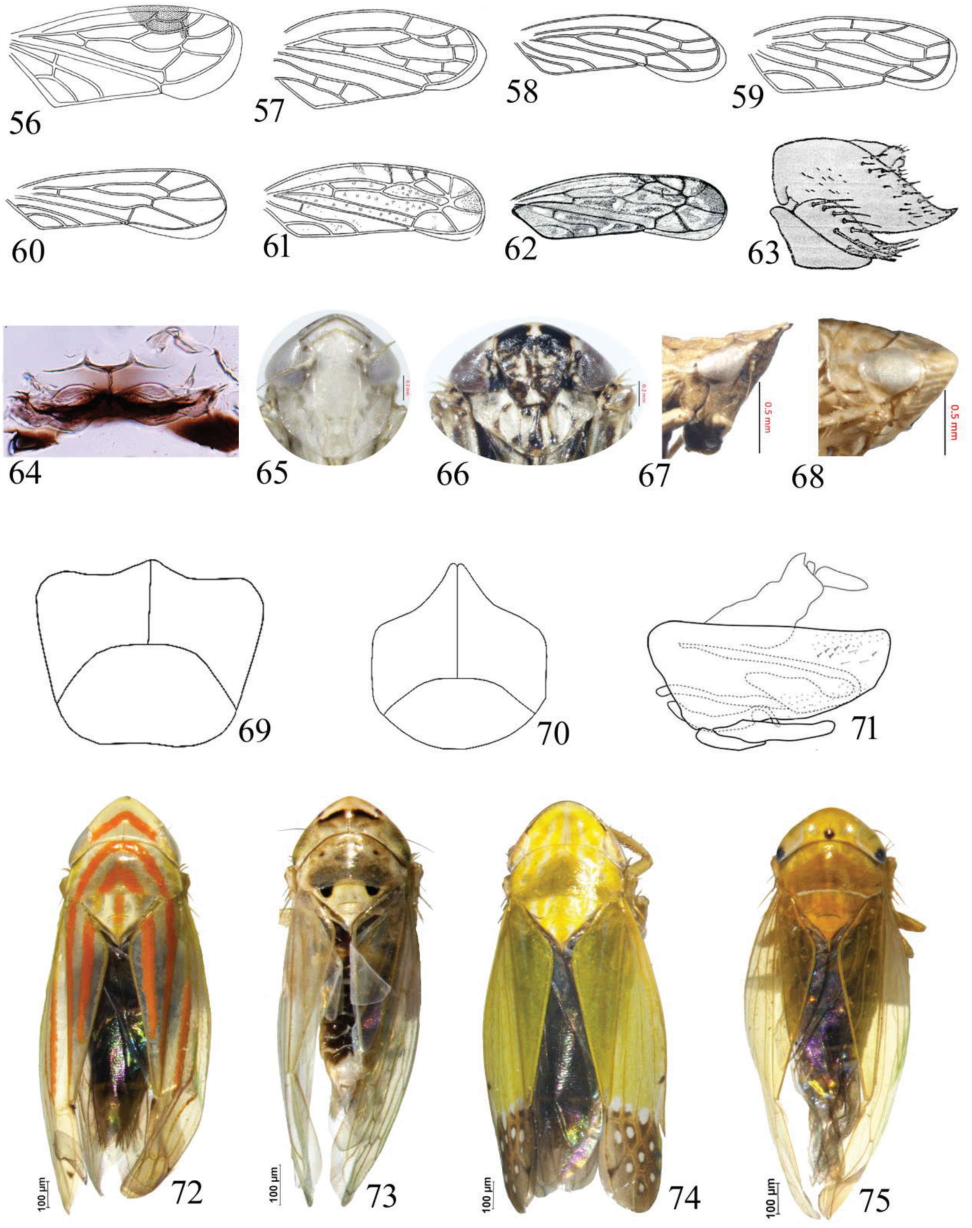


Figures 40–55. (male genitalia) **40** *Neodartus acocephaloides* aedeagus, dorsal view **41** *Goniagnathus* (*Tropicognathus*) *nepalicus* fused subgenital plates and valve, styles and base of connective **42** *Gurawa minorcephala* aedeagus, caudal view **43** *Chiasmus* sp. aedeagus, dorsal view **44** *Hishimonus phycitis* aedeagus, posterior view **45** *Deltocephalus vulgaris* aedeagus and connective, lateral view **46** *Maiestas* sp. aedeagus and connective, lateral view **47** *Limotettix* (*Scleroracus*) *cacheolus* aedeagus, dorsal view **48** *Neolimnus egyptiacus* subgenital plate **49** *Jilinga truncata* annal tube, ventral view **50** *Jilinga truncata* aedeagus and dorsal connective, ventral view **51** *Tambocerus bulbulus* aedeagus, posterior view **52** *Euscelidius cornix* aedeagus and connective, dorsal view **53** *Neoaliturus (circulifer) tenellus* aedeagus and connective **54** *Stirellus lahorensis* valve, style, and connective, dorsal view **55** *Scaphoideus harlani* connective and style.

usually but not always present), ovipositor usually extending far beyond the pygofer, first valvula dorsal sculpturing pattern maculate to granulose and usually submarginal, first valvula without distinctly delimited ventroapical sculpturing, and second valvula teeth obliquely triangular and serrated.



Figures 25–39. (male pygofer, lateral view) **25** *Neodartus acocephaloides* **26** *Aconurella prolixa* **27** *Leofa* (*Prasutagus*) *pulchellus* **28** *Exitianus nanus* **29** *Macrosteles parafalcatus* **30** *Balclutha punctata* **31** *Jilinga truncata* **32** *Stirellus mankiensis* **33** *Grammacephalus raunoi* **34** *Neolimnus egyptiacus* **35** *Paralimnellus cingulatus* **36** *Euscelidius cornix* **37** *Hecalus rawalakotensis* **38** *Pseudosubhimalus pakistanicus* **39** *Tambocerus bulbulus.*



Figures 56–75. 56–62 (forewings) **56** *Drabescus nitens* **57** *Goniagnathus* (*T.*) *quadripinnatus* **58** *Aconurella prolixa* **59** *Chiasmus* sp. **60** *Macrosteles indrinus* **61** *Bampurius pakistanicus* **62** *Scaphoideus immistus* **63** *Stirellus thattaensis*, pygofer, lateral view **64** *Macrosteles parafalcatus*, male 2nd abdominal tergites, dorsal view **65** *Scaphoideus harlani*, face **66** *Euscelidius cornix*, face **67** *Gurawa longispina*, head, lateral view **68** *Leofa naga*, head, lateral view **69** *Neoaliturus* (*C.*) *tenellus*, subgenital plates **70** *Neoaliturus* (*C.*) *opacipennis*, subgenital plates **71** *Stirellus viridulus*, pygofer, lateral view **72** *Linnavuoriella arcuata,* habitus, dorsal view **73** *Exitianus nanus*, habitus, dorsal view **74** *Thomsonia porrecta,* habitus, dorsal view; **75** *Phlogotettix indicus,* habitus, dorsal view.

***Aconurella* Ribaut**

***A. choui* Naveed & Zhang**

Aconurella choui Naveed & Zhang, 2018a: 72, fig. 5; pl. II, figs A–D (Pakistan).

***A. erebus* (Distant)**

Deltocephalus erebus Distant, 1908: 385 (India).
Aconurella erebus: Ghauri, 1974: 553–555, figs 14–17 (India).
Aconurella erebus: Naveed and Zhang 2018a: 68, fig. 2; pl. I, figs D–F (Pakistan).

***A. naranensis* Naveed & Zhang**

Aconurella naranensis Naveed & Zhang, 2018a: 71, fig. 4; pl. I, J–L (Pakistan).

***A. paraerebus* Naveed & Zhang**

Aconurella paraerebus Naveed & Zhang, 2018a: 68, fig. 3; pl. I, G–I (Pakistan).

***A. prolixa* (Lethierry)**
Figs 4, 26, 58

Thamnotettix prolixa Lethierry, 1885: 102 (Europe).
Thamnotettix minutes Haupt, 1917: 254. Synonymised by Dlabola 1963: 324.
Thamnotettix sanguisuga Lindberg, 1927: 88. Synonymised by Metcalf 1967a: 1597.
Cicadula indica Pruthi, 1930: 54. Synonymised by Khatri and Webb 2010: 9 (India).
Deltocephalus obtusus Metcalf, 1955: 266. (nom. nov. for *Deltocephalus simplex* Haupt, 1927, non *D. simplex* Van Duzee, 1892: 304).
Chiasmus karachiensis Ahmed et al., 1988: 13, fig. 3A–J. Synonymised by Khatri and Webb 2010: 9 (Pakistan).
Chiasmus lobata Ahmed et al., 1988: 14, fig. 4A–J. Synonymised by Khatri and Webb 2010: 9.
Aconurella neosolana Rao & Ramakrishnan, 1990a: 268, fig. 1 (India). Synonymised by Khatri and Webb 2010: 9.
Aconurella prolixa Khatri & Webb, 2010: 4, pl. 1, fig. g; fig. 9; Naveed and Zhang 2018a: 67, fig. 1; pl. I, A–C (Pakistan).

Key to *Aconurella* species (male) modified from Naveed and Zhang (2018a)

1 Pygofer side with many spinules at dorsoapical margin, some large............**2**
– Pygofer side dorsoapical margin without or with sparse small spinules........**4**

2 Subgenital plates as long as pygofer; with two macrosetae at apex .... ***A. paraerebus***
– Subgenital plates subequal to pygofer; with more than two macrosetae at apex **3**
3 Subgenital plates longer than pygofer; style apophysis smooth .... ***A. erebus***
– Subgenital plates shorter than pygofer; style apophysis serrate with enlarged preapical tooth .... ***A. naranensis***
4 Pygofer dorsal margin without spinules (Fig. 26); connective arms close together distally .... ***A. prolixa***
– Pygofer dorsal margin with small spinules; connective arms widely separate from each other .... ***A. choui***

Chiasmus **Mulsant & Rey**

C. alatus **Pruthi**

Chiasmus alatus Pruthi, 1930: 23, pl. II, figs 6, 6a, text figs 32–34 (India); Khatri and Webb 2010: 4 (Pakistan).

C. niger **Pruthi**

Chiasmus niger Pruthi, 1936: 108, pl. VIII, fig. 8, text fig. 122 (India); Khatri and Webb 2010: 4 (Pakistan).

Remarks. The identification key of this species has not been possible due to the uncertainty of the differences between very similar species. The previously described forms may prove to be synonyms.

Exitianus **Ball**

E. indicus **(Distant)**

Athysanus indicus Distant, 1908: 344 (India).
Athysanus atkinsoni Distant, 1908: 345 (India). Synonymised by Ross, 1968: 12.
Exitianus indicus: Ross 1968: 12, figs 9, 10, 26–30, 69.
Exitianus major Ahmed et al., 1988: 10, fig. 1 (Pakistan). Synonymised by Khatri and Webb 2010: 10.
Exitianus indicus: Duan and Zhang 2013: 36, pl. II, figs 3–6; Khatri et al. 2014: 3, pl. 1 (China).

E. nanus **(Distant)**
Fig. 73

Athysanus nanus Distant, 1908: 345 (India).

Athysanus insularis Distant, 1909: 47, pl. 4, figs 10, 10a. Synonymised by Ross 1968: 7.
Athysanus fasciolatus Melichar, 1911: 107 (East Africa). Synonymised by Linnavuori 1975: 626.
Athysanus simillimus Matsumura, 1914: 185 (Japan). Synonymised by Ross 1968: 7.
Athysanus vulnerans Bergevin, 1925: 42, figs 5–9 (East Africa). Synonymised by Ross 1968: 7.
Limotettix albipennis Haupt, 1927: 25, pl. II, figs 20a–c (Palestine). Synonymised by Dlabola 1963: 325.
Limotettix unifasciata Haupt, 1930: 159, fig. 9. Synonymised by Dlabola 1963: 325.
Athysanus digressus Van Duzee, 1933: 32 (USA). Synonymised by Linnavuoriand De-Long 1978: 237.
Exitianus nanus: Ross, 1968: 7, figs 1–3, 15–18, 76; Duan and Zhang 2013: 33, pl.pl. I, figs 1–2 (China); Khatri et al. 2014: 4; Duan and Zhang 2013: 33, pl. I, figs 1, 2; Khatri et al. 2014: 3, pl. 2 (Pakistan).
Exitianus karachiensis Ahmed, 1986: 59, fig. 5. Synonymised by Khatri and Webb 2010: 10.
Exitianus peshawarensis Ahmed & Rao, 1986: 76–77, fig. 1. Synonymised by Khatri and Webb 2010: 10.
Exitianus minor Ahmed et al., 1988: 12, fig. 2. Synonymised by Khatri and Webb 2010: 10.
Exitianus fulvinervis Li & He, 1993: 27; Li et al. 2011: 68, fig. 55. Synonymised by Duan and Zhang 2013: 33 (China).

Key to *Exitianus* species from Pakistan (male)

1	Crown with transverse brown band usually interrupted medially (Fig. 73); pygofer side with 2–6 apical brown or black macrosetae	***E. nanus***
–	Crown with transverse brown band usually complete; pygofer side with 2 or 3 apical brown or black macrosetae..	***E. indicus***

***Gurawa* Distant**

***G. minorcephala* Pruthi**

Fig. 5

Gurawa minorcephala Pruthi, 1930: 29, pl. II, fig. 10a, b, text figs 41,42 (Pakistan); Zahniser 2008: 22, figs 77–85; Dai et al. 2011: 38, fig. 1; Duan and Zhang 2012: 42–44, pl. I, fig. 1 (China); Viraktamath and Gnaneswaran 2013: 199–200, figs 22–29, 41, 55–58 (India); Naveed and Zhang 2018b: 482, figs 1E–H, 2A–G, 4A–E, 5B (Pakistan).

***G. longispina* Naveed & Zhang**

Gurawa longispina Naveed & Zhang, 2018b: 486, figs 1A–D, 3A–F, 5A (Pakistan).

Key to *Gurawa* species from Pakistan (male) modified from Naveed and Zhang 2018b

1	Crown with dorsal constriction at level of ocelli; aedeagal shaft with lateroapical spines long in posterodorsal view	***G. longispina***
–	Crown without dorsal constriction at level of ocelli; aedeagal shaft with lateroapical spines short in posterodorsal view	***G.minorcephala***

Leofa Distant

Key to subgenera of *Leofa* from Pakistan modified from Naveed and Zhang (2018c)

1	Submacropterous; pygofer with a well-developed dorsal appendage	***Leofa* (*Prasutagus*)**
–	Brachypterous; pygofer without dorsal appendage	***Leofa* (*Leofa*)**

L. (*L.*) *mysorensis* Distant

Leofa mysorensis Distant, 1918: 86; Viraktamath and Viraktamath 1992: 5, figs 10–19 (India); Naveed and Zhang 2018c: 46, figs 5–8 (Pakistan).

Leofa affinis Distant, 1918: 87. Synonymised by Viraktamath and Viraktamath 1992: 5 (India).

Leofa sanguinalis Distant, 1918: 87. Synonymised by Viraktamath and Viraktamath 1992: 5 (India).

Leofa unicolor Distant, 1918: 88. Synonymised by Viraktamath and Viraktamath 1992: 5 (India).

Leofa pedestris Distant, 1918: 88. Synonymised by Viraktamath and Viraktamath 1992: 5 (India).

Leofa parwala Pruthi, 1930: 26. Synonymised by Viraktamath and Viraktamath 1992: 5 (India).

L. (*L.*) *naga* Viraktamath & Viraktamath

Leofa naga Viraktamath & Viraktamath, 1992: 9–10, figs 31–40 (India); Naveed and Zhang 2018c: 46, figs 9–13 (Pakistan).

L. (*Prasutagus*) *pulchellus* Distant

Figs 7, 27

Prasutagus pulchellus Distant, 1918: 53–54, fig. 57 (India).

Leofa(*Prasutagus*) *pulchellus*: Zahniser, 2008: 18; Duan et al. 2012: 39 (China); Naveed and Zhang 2018c: 46, figs 1–4 (Pakistan).

L. (L.) *truncata* Viraktamath & Viraktamath

Leofa truncata Viraktamath & Viraktamath, 1992: 4, figs 1–9 (India); Naveed and Zhang 2018c: 47, 14–19 (Pakistan).

Key to *Leofa* species from Pakistan (male)

1 Subgenital plates rounded caudally; pygofer with or without shallow lateral furrow; aedeagal shaft with caudal hood, basal process short, narrower than width of shaft.. **2**

– Subgenital plates truncate caudally; pygofer deeply furrowed laterally; aedeagal shaft without caudal hood, basal process long, broader than width of shaft...***L. truncata***

2 Aedeagal shaft tubular, without lamellate expansion; gonopore slightly asymmetrically placed on left side; caudal hood not strongly developed................ ....***L. mysorensis***

– Aedeagal shaft hood-like with lateral lamellate expansion; caudal hood strongly developed; gonopore symmetrically placed ....***L. naga***

***Nephotettix* Matsumura**

***N. nigropictus* (Stål)**

Thamnotettix nigropictus Stål, 1870: 740 (India).

Nephotettix apicalis Distant, 1908: 360 (India); Ishihara 1964: 42; Ishihara and Kawase 1968: 123.

Nephotettix nigropictus yapicola Ghauri, 1971: 495.

Nephotettix nigropictus: Ghauri, 1971: 491; Vilbaste 1975: 233; Ramakrishnan and Ghauri 1979; Mahmood and Aziz 1979: 61, figs 1b, 3a–f (Pakistan); Duan and Zhang 2014: 219, pl. III; pl. VI: I–L; figs 14, 15 (China).

***N. parvus* Ishihara & Kawase**

Nephotettix parvus Ishihara & Kawase, 1968: 121 (Japan); Duan and Zhang 2014: 221, pl. IV, pl.VIIA–C; fig. 16 (China).

Nephotettix olivacea Mahmood & Aziz, 1979: 65 (Pakistan). Synonymised by Wilson 1989: 136.

***N. virescens* (Distant)**

Selenocephalus virescens Distant, 1908: 291 (India).

Phrynomorphus olivacescens Distant, 1918: 52. Synonymized by Wilson 1989: 135.

Nephotettix bipunctatus (Fabricius), Distant, 1908: 359.

Nephotettix impicticeps Ishihara, 1964: 42. Synonymized by Ghauri,1971: 484.
Nephotettix virescens: Ghauri, 1971: 484; Ramakrishnan and Ghauri 1979: 357; Duan and Zhang 2014: 223, pl. V; pl. VII: D–F; figs 17–18 (China).
Nephotettix oryzii Mahmood & Aziz, 1979: 63 (Pakistan). Synonymized by Wilson 1989: 135.

Key to species of *Nephotettix* (male)

1	Crown without traces of marginal and submarginal black transverse bands in both sexes	***N. virescens***
–	Crown with black submarginal transverse band markedly and fully developed	**2**
2	Anterior margin of pronotum marked with black transverse band	***N. nigropictus***
–	Anterior margin of pronotum without black markings	***N. parvus***

Cicadulini Van Duzee

Diagnosis. Cicadulini, following Zahniser and Dietrich (2013: 56), is a rather poorly defined tribe. It was defined by these authors in the following way: "small to medium sized, slender, stramineous, yellow, or greenish leafhoppers, sometimes with the anterior margin of the head marked with black spots. They can be identified by the male segment X often long and strongly sclerotised, and subgenital plates sometimes with a row of macrosetae near the middle and with long fine setae laterally" and additionally in their key: "male pygofer incised dorsally nearly to base". Clearly, this definition is not ideal as you may not be able to identify a taxon (for example in a key) based solely on "often" and "sometimes" characters and also in their figure 15 of *Cicadula* Zetterstedt, segment X is moderately long (although the dorsal pygofer incision is very long and therefore the dorsal bridge very short). In addition, the genus *Pseudosubhimalus* Ghauri, placed in Athysanini by Zahniser and Dietrich (2014), was subsequently placed in Cicadulini based on molecular evidence and (in its type species) segment X is long and well sclerotised (Meshram and Niranjana 2019) However, in the genus the subgenital plate macrosetae are marginal, and in one of its species, *P. katraini* Meshram and Niranjana, segment X is very short. Similarly, segment X is not elongate in the Nearctic *Knullana* DeLong. The following three species of this genus occur in Pakistan.

Pseudosubhimalus Ghauri

P. bicolor (Pruthi)

Ophiola bicolor Pruthi, 1936: 123 (India).
Pseudosubhimalus bicolor: Ghauri, 1974: 553; Meshram and Niranjana 2019: 7–9, figs 1A, 1B, 1E, 1G–1L, 2A–2F, 3A–3H (India, Pakistan).

***P. trilobatus* Meshram & Niranjana**

Pseudosubhimalus trilobatus Meshram & Niranjana, 2019: 7, 11–12, figs 1C, 1D, 4A–4F (India).
Pseudosubhimalus bicolor (Pruthi): Menghwar et al. 2015: 142, pl. 1, figs a-h (misidentification) (Pakistan).

***P. pakistanicus* Naveed & Zhang**
Figs 13, 38

Pseudosubhimalus pakistanicus Naveed et al., 2020a: 194, fig. 1A–H (Pakistan).

Key to *Pseudosubhimalus* species from Pakistan (male) modified from Naveed et al. (2020a)

1	Greyish green to pale yellow species, disc of crown without black or dark brown spots; pygofer lobe with weak ventral process (Fig. 38)	***P. pakistanicus***
–	Dark brown in colour, disc of crown with black or dark brown spots; pygofer lobe without ventral process	**2**
2	Pygofer ventral margin with dentations	***P. bicolor***
–	Pygofer ventral margin without dentations, smooth	***P. trilobatus***

Deltocephalini Fieber

Diagnosis. The members of this tribe are small to medium sized leafhoppers and are variable in colour. They can be identified by the tapering or parallel-sided clypellus, narrow lorum, linear connective with anterior arms closely appressed, connective fused to the aedeagus, and first valvula dorsal sculpturing imbricate (Scale-like).

***Deltocephalus* Burmeister**

***D. vulgaris* Dash & Viraktamath**
Fig. 45

Deltocephalus(*Deltocephalus*) *vulgaris* Dash & Viraktamath, 1998: 4, figs 1–11 (India); Zhang and Duan 2011: 3, fig. 3A–H (China); *Deltocephalus* (*Deltocephalus*) *vulgaris*: Naveed et al. 2019a: 285, figs 1A, B, 3A–D (Pakistan).

***D. infirmus* Melichar**

Deltocephalus infirmus Melichar, 1903: 203, pl. V, fig. 11 (Sri Lanka).
Jassargus infirmus: Ishihara, 1961: 244, figs 53–58 (misidentification).
Deltocephalus infirmus: Webb and Viraktamath 2009: 13, fig. 10; Naveed et al. 2019a: 285, figs 1C, 3D–G (Pakistan).

Key to *Deltocephalus* species from Pakistan (male) modified from Naveed et al. (2019a)

1 Crown with six brown spots on anterior margin; aedeagal shaft with shallow apical notch .... ***D. vulgaris***
– Crown with single brown spot on anterior margin adjacent to eyes; aedeagal shaft without apical notch.. ***D. infirmus***

Maiestas **Distant**

M. albomaculata **(Dash & Viraktamath)**
Fig. 11

Deltocephalus (*Recilia*) *albomaculatus*: Dash and Viraktamath 1998: 12, figs 29–34 (India).
Maiestas albomaculata: Webb and Viraktamath 2009; Naveed et al. 2019a: 287, figs 1E–1I, 3H–3I; Shah et al. 2021: 403, figs 1A–D (Pakistan).

M. indica **(Pruthi)**

Allophleps indica Pruthi, 1936: 120–121, pl. IX, fig. 3, text fig. 132 (Pakistan); Rao and Ramakrishnan 1990: 111 (India).
Deltocephalus (*Recilia*) *indicus*: Dash and Viraktamath 1998: 35–36, fig. 305 (India).
Maiestas indica: Webb and Viraktamath 2009: 22; Shah et al. 2021: 403, fig. 1E (Pakistan).

M. maculata **(Pruthi)**

Cicadula maculata Pruthi, 1930: 58–59, figs 80–81, pl. V, fig. 2 (India).
Thamnotettix prabha Pruthi, 1930: 62, figs 85, 86, pl. V, figs 6, 6a (India). Synonymized by Webb and Viraktamath 2009: 41.
Recilia prabha: Ghauri, 1980: 166–169, figs 1, 3–11.
Deltocephalus(*Recilia*) *maculata*: Dash and Viraktamath 1998: 32, figs 260–269 (India).
Maiestas maculata: Webb and Viraktamath 2009: 22, comb. nov.; Zhang and Duan 2011: 37–39, figs 33–35, pl. IV: E, pl. V: P, pl. VI: P (China); Shah et al. 2021: 404, fig. 2A–I (Pakistan).

M. pruthii **(Metcalf)**

Deltocephalus notatus Pruthi, 1936: 128–129, text fig. 139, pl. IX, fig. 10 (Pakistan). Preoccupied, not Melichar 1896.
Deltocephalus pruthii (Metcalf, 1967b: 1173, new name).
Maiestas pruthii: Webb and Viraktamath 2009: 20; Naveed et al. 2019a: 288, figs 2A–2C, 3J–3K; Shah et al. 2021: 4F–L (Pakistan).

***M. setosa* (Ahmed, Murtaza & Malik)**

Recilia setose Ahmed et al., 1988: 412–414, fig. 2 (Pakistan).
Maiestas setosa: Webb and Viraktamath 2009: 20 (Pakistan).

***Maiestas sinuata* Shah & Duan**

Maiestas sinuata Shah & Duan, 2021: 406, fig. 3A–H (Pakistan).

***M. subviridis* (Metcalf)**

Stirellus subviridis Metcalf, 1946: 125. Synonymized with *S. hopponis* (Matsumura) by Linnavuori, 1975: 617, in error;
Deltocephalus(*Recilia*) *subviridis*: Dash and Viraktamath 1998: 24, figs 166–172 (India);
Maiestas subviridis: Webb and Viraktamath 2009: 19, fig. 40; Khatri and Webb 2010: 11, pl. 2b, c, fig. 12 (Pakistan); Zhang and Duan 2011: 19 (China); Shah et al. 2021: 408, fig. 4A–E (Pakistan).

***M. tareni* (Dash & Viraktamath)**

Deltocephalus(*Recilia*) *tareni* Dash & Viraktamath, 1995: 74–76, figs 1–15; Dash and Viraktamath 1998: 16, figs 78–84 (India).
Maiestas tareni: Webb & Viraktamath, 2009: 22; Khatri and Webb 2010: 11, pl. 2d, fig. 11 (Pakistan); Zhang and Duan 2011: 20 (China); Naveed et al. 2019a: 288, figs 2G–I, 3N–3O; Shah et al. 2021: 408, fig. 5A–H (Pakistan).

***Maiestas trispinosa* (Dash & Viraktamath)**

Deltocephalus (*Recilia*) *trispinosus* Dash & Viraktamath, 1998: 35, figs 296–304 (India).
Maiestas trispinosa: Webb and Viraktamath 2009: 38; Shah et al. 2021: 408, fig. 6A–I (Pakistan).

Key to *Maiestas* species from Pakistan (male). *Maiestas setosa* is excluded from the key due to the poor original description and figures.

1	Overall colour dark brown; forewing with sub-basal and subapical irregular white transverse band (Fig. 11)	***M. albomaculata***
–	Colour not as above	**2**
2	Crown, face and thorax with black patches	***M. maculata***
–	Crown, face and thorax without black patches	**3**
3	Forewing with extra cross-veins, at least in clavus	**4**
–	Forewing without extra cross-veins	**5**

4	Aedeagus with a large subapical ventral process	***M. indica***
–	Aedeagus with a short apical ventral process	***M. pruthii***
5	Aedeagus with pair of short lateral processes	***M. trispinosa***
–	Aedeagus without lateral processes	**6**
6	Aedeagus in lateral view similar in width in distal half	***M. subviridis***
–	Aedeagus in lateral view evenly tapered from base to apex	7
7	Style apophysis broadest sub-basally; aedeagal shaft in lateral view not sinuate	***M. tareni***
–	Style apophysis broadest at base; aedeagal shaft in lateral view slightly sinuate	***M. sinuata***

Paramesodes **Ishihara**

P. lineaticollis **(Distant)**

Paramesodes lineaticollis (Distant, 1908: 294, *Paramesus*) (India); Wilson 1983: 21–22, figs 23–29.
Paramesodes ishurdii Mahmood & Meher, 1973: 135 (Pakistan). Synonymised by Wilson 1983: 21.

Drabescini Ishihara

Diagnosis. Drabescini are medium sized to large leafhoppers, variable in colour and shape. They can be identified by the following combination of characters: antennae long situated near upper part of face; antennal pits large, often encroaching onto frontoclypeus; anterior margin of head smooth, irregularly textured, or with one to many carinae or striae; nymph often with apical process on head. Two subtribes are present (see key and below).

Drabescina

Drabescus **Stål**

D. angulatus **Signoret**
Fig. 1

Drabescus angulatus Signoret, 1880: 210; Ghauri 1965: 688; Zhang and Webb 1996: 24, figs 380–384, 525.

Paraboloponina Ishihara

Dryadomorpha **Kirkaldy**

Remarks. See Zhang and Webb (1996: 6) for full synonymy.

D. pallida Kirkaldy

D. pallida Kirkaldy, 1906: 336; Webb 1981: 50–53, figs 41–56.

Remarks. See Zhang and Webb (1996: 14) for full synonymy.

Goniagnathini Wagner

Diagnosis. These are medium sized to large, squat, robust leafhoppers. They can be identified by the short and broad head, anterior margin of head glabrous, large forewing appendix (in macropterous individuals), subgenital plates fused to each other, valve apparently absent or fused to subgenital plates, style with broad basal part articulated with linear or modified apical part, and connective fused to the aedeagus.

Goniagnathus Fieber

G. (*Epistagma*) *guttulinervis* (Kirschbaum)

Jassus(*Athysanus*) *guttulinervis* Kirschbaum, 1868: 116 (Europe).
Thamnotettix putoni Lethierry, 1874: 444.
Goniagnathus ocellatus Jacobi, 1910: 133.
Goniagnathus guttulinervis: Dash and Viraktamath 2001: 64, figs 1–5 (India); Naveed and Zhang 2018j: 1805, fig. 1C; Shah and Duan 2020b: 16–17, figs 1A, B, 2A–H (Pakistan).

G. (*Tropicognathus*) *nepalicus* Viraktamath & Gnaneswaran
Fig. 3

Goniagnathus(*Tropicognathus*) *nepalicus* Viraktamath & Gnaneswaran, 2009: 56–57, figs 5, 6, 19–24 (Nepal); Naveed and Zhang 2018j: 1806, figs 1E–G; Shah and Duan 2020b: 16, 20, figs 1E, 1F, 5A–D (Pakistan).

G. (*Tropicognathus*) *punctifer* (Walker)

Bythoscopus punctifer Walker, 1858: 104.
Goniagnathus elongatus Lethierry, 1892: 209.
Goniagnathus spurcatus: Melichar 1903: 181.
Goniagnathus punctifer: Distant 1908: 311; Zhang 1990: 91; Dash and Viraktamath 2001: 71 (India).
Goniagnathus(*Tropicognathus*) *punctifer*: Duan and Zhang 2009: 53, figs 2A–E, 7E, 7K, 8D (China); Shah and Duan 2020b: 19, figs 6–8 (Pakistan).

***G.* (*Tropicognathus*) *quadripinnatus* Dash & Viraktamath**

Goniagnathus(*Tropicognathus*) *quadripinnatus* Dash & Viraktamath, 2001: 74–76, figs 45–50 (India); Naveed and Zhang 2018j: 1806, fig. 1D; Shah et al. 2020b: 16, figs 1C, 1D, 3A–G (Pakistan).

Key to subgenera and species of *Goniagnathus* from Pakistan (male) modified from Shah et al. (2020)

1	Male pygofer with dorsal appendage absent; aedeagus with pair of ventral processes exceeding aedeagal shaft	***G.* (*Epistagma*) *guttulinervis***
–	Male pygofer with dorsal appendage present; aedeagus with pair of ventral processes not exceeding aedeagal shaft	***G.* (*Tropicognathus*) 2**
2	Aedeagus with one pair of long processes present at mid-length, subgenital plates fused with truncate margin caudally	***G.* (*Tropicognathus*) *nepalicus***
–	Aedeagus with two pairs of processes	**3**
3	Aedeagal shaft with a pair of apical and a pair of median asymmetrical processes	***G.* (*Tropicognathus*) *punctifer***
–	Aedeagal shaft with two pairs of processes present near apex, having lateral processes longer and stouter than the dorsal processes	***G.* (*Tropicognathus*) *quadripinnatus***

Hecalini Distant

Remarks. A revision of Oriental Hecalini was given by Morrison (1973).

Diagnosis. The members of this tribe are medium sized to large, somewhat to strongly dorsoventrally flattened, stramineous, yellow, green, or brown leafhoppers, sometimes with bright orange or reddish markings. They can be identified by the produced and parabolically shaped head, dorsoventrally flattened body, lateral margin of pronotum as long as or longer than the basal width of eye, ocelli closer to eyes than laterofrontal sutures, apodemes of male sternite I long and relatively narrow, apodemes of male sternite II broad and well-developed, male pygofer often produced or pointed posterodorsally, segment X withdrawn into pygofer, ventral margins of male pygofer often lobate, aedeagus often with one or two pairs of apical processes, first valvula dorsal sculpturing granulose to maculate and submarginal, first valvula often with distinctly delimited ventroapical sculpturing, second valvula usually without teeth, humpbacked dorsally, and concave ventrally.

***Glossocratus* Fieber**

***Glossocratus* sp.**

Remarks. From the figure (unidentified) given by Mahmood (1979) this genus is present in Pakistan. No information is given by Mahmood on examined specimens.

***Hecalus* Stål**

***H. erectus* Naveed & Zhang**

Hecalus erectus Naveed & Zhang, 2018d: 581, fig. 1A–H; pl. IA–C (Pakistan).

***H. ghaurii* Rao & Ramakrishnan**
Fig. 8

Hecalus ghaurii Rao & Ramakrishnan, 1990b: 388, figs 1–11 (India); Naveed and Zhang 2018d: 584, fig. 2A–K; pl. ID–G (Pakistan).

***H. muzaffarabadensis* Naveed & Zhang**

Hecalus muzaffarabadensis Naveed & Zhang, 2018d: 585, fig. 3A–D; pl. I, figs H–J (Pakistan).

***H. prasinus* (Matsumura)**

Parabolocratus prasinus Matsumura, 1905: 48 (Japan); Morrison 1973: 417, figs 154–159 (Thailand); Mahmood 1979: 93 (Pakistan).

***H. rawalakotensis* Naveed & Zhang**

Hecalus rawalakotensis Naveed & Zhang, 2019c: 596, figs 1A–I, 2A–D (Pakistan).

***H. snipus* Naveed and Zhang**
Hecalus snipus Naveed & Zhang, 2018d: 386, fig. 4A–G; pl. II, figs A–C (Pakistan).

***H. umballaensis* Distant**

Hecalus umballaensis Distant, 1908: 274; Morrison 1973: 431, fig. 190; Rao and Ramakrishnan 1990b: 390, figs 31–38 (India); Naveed and Zhang 2018d: 587, fig. 5A–I; pl. II, figs D–F (Pakistan).

***H. veracious* Naveed & Zhang**

Hecalus veracious Naveed & Zhang, 2018d: 587, fig. 6A–H; pl. II, figs G–I (Pakistan).

Key to *Hecalus* species from Pakistan (male) modified from Naveed and Zhang (2018d) and Naveed et al. (2019c)

1	Greenish brown to dark in colouration on face and thorax	**2**
–	Yellowish green to pale yellow in colouration on face and thorax	**3**

2 Aedeagal shaft with long, leaf-like, pointed apical processes................................ ....***H. umballaensis***
– Aedeagal shaft with short, truncate apical processes.........................***H. snipus***
3 Aedeagal shaft with subapical dorsal flares and bifurcated apical processes ....***H. muzaffarabadensis***
– Aedeagal shaft without apical bifurcated processes **4**
4 Aedeagal shaft without lateral serrations.......................................***H. ghaurii***
– Aedeagal shaft with lateral serrations...**5**
5 Aedeagal shaft with lateral serrations throughout ....***H. erectus***
– Aedeagal shaft with lateral serrations limited to basal 2/3............................***6***
6 Aedeagal shaft nearly parallel sided throughout length in dorsal view............ ....***H. veracious***
– Aedeagal shaft broad in basal half, narrowed apically in dorsal view ....***H. rawalakotensis***

***Linnavuoriella* Evans**

***L. arcuata* (Motschulsky)**
Fig. 72

Platymetopius arcuatus: Motschulsky, 1859: 115.
Tetigonia kalidasa Kirkaldy, 1900: 294.
Parabolocratus citrinus Evans, 1941: 36.
Varta moshiensis Rao, 1973: 96 (India).
Hecalus arcuatus: Morrison 1973: 426.
Linnavuoriella arcuata: Hamilton 2000: 454; Catanach and Dietrich 2017; Naveed and Zhang 2019b: 619, fig. 2A–H (Pakistan); He et al. 2019: 267, figs 52–68 (China).

***Thomsonia* Signoret**

***T. porrecta* (Walker)**
Fig. 74

Acocephalus porrectus Walker, 1858: 362.
Platymetopius lineolatus Motschulsky, 1859: 114.
Hecalus kirschbaumii Stål, 1870: 737.
Thomsoniella albomaculata Distant, 1908: 278, fig. 178.
Parabolocratus merino Capco, 1959: 333.
Thomsoniella porrecta: Hamilton 2000: 454.
Thomsonia porrecta: He et al. 2019: 269, figs 69–85 (China).

Koebeliini Baker

Diagnosis. These are small to medium sized, yellow, light green or brown leafhoppers. They can be identified by the combination of following characters: ocelli distant from eyes, clypellus long, narrow and extending well beyond normal curve of gena, and metatarsomere I with platellae on plantar surface.

***Pinopona* Viraktamath & Sohi**

***P. minuta* Viraktamath & Sohi**

Pinopona minuta Viraktamath & Sohi, 1998: 114, figs 1–15 (India, Nepal).

***Sohipona* Ghauri & Viraktamath**

***S. webbi* Ghauri & Viraktamath**

Sohipona webbi Ghauri & Viraktamath, 1987: 50, figs 11–29 (Pakistan).

Limotettigini Baker

Diagnosis. These are small to medium sized ivory, greyish, or black leafhoppers, often with dark markings. They can be identified by the parallel-sided or tapering clypellus, pygofer dorsal margin with spine-like process and aedeagus articulated with plate-like "dorsal connective" at dorsal margin of socle.

***Limotettix* Sahlberg**

***Limotettix* (*Scleroracus*) Van Duzee**

***L.* (*S.*) *cacheolus* (Ball)**
Fig. 14

Ophiola stratula var. *cacheola* Ball, 1928: 189.
Limotettix (*Scleroracus*) *cacheolus*: Oman 1947: 205; Hamilton 1994: 122; McKamey 2001: 705 (USA); Naveed and Zhang 2018f: 79, figs 15–26 (Pakistan).

Macrostelini Kirkaldy

Diagnosis. Macrostelini are small to medium sized, slender, often stramineous, yellow, or greenish leafhoppers, with or without dark markings. They can be identified by their long, slender shape, forewing with two anteapical cells, subgenital plates usually with membranous digitate apical lobe, and male pygofer macrosetae sometimes plumose.

Balclutha Kirkaldy

B. incisa (Matsumura)

Gnathodus incisa Matsumura, 1902: 360 (Japan).
Balclutha indica Pruthi, 1930: 48, pl. IV, figs 4, 4a, 4b, text figs 67, 68 (*Eugnathodus*), India. Synonymised by Knight 1987: 1206.
Balclutha incisa: Knight 1987: 1206, figs 138–145; Webb and Vilbaste 1994: 72, figs 10–17; Chiang 1996: 67, fig. 3; Dai, Li and Chen 2004: 749 (China); Naveed and Zhang 2018e: 259, fig. 2A–E (Pakistan).

B. punctata (Fabricius)
Fig. 12

Cicada punctata Fabricius, 1775: 687.
Balclutha punctata: Blocker 1967: 7; Knight 1987: 1188, figs 32–38; Webb and Vilbaste 1994: 64, figs 44–54; Chiang 1996: 64, fig. 2; Dai, Li and Chen 2004: 749 (China); Naveed and Zhang 2018e: 261, figs 1A–C, 2F–K (Pakistan).

B. pararubrostriata Rao & Ramakrishnan

Balclutha pararubrostriata Rao & Ramakrishnan, 1990a (India): 106; Webb and Vilbaste 1994: 64, fig. 130; Naveed and Zhang 2018e: 262, figs 1D–G, 3A–G (Pakistan).

B. rubrostriata (Melichar)

Gnathodus rubrostriatus Melichar, 1903: 208.
Balclutha rubrostriata: Knight 1987: 1211, figs 160–166; Webb and Vilbaste 1994: 66, figs 123–129; Chiang 1996: 69, fig. 5; Dai, Li and Chen 2004: 749 (China).

B. sujawalensis Ahmed

Balclutha sujawalensis Ahmed, 1986: 54, fig. 2 (Pakistan).
Balclutha knighti Rao & Ramakrishnan, 1990a: 106, figs 1–8 (India). Synonymised by Webb and Vilbaste 1994: 67, figs 55–60.

A. viridinervis Matsumura

Balclutha viridinervis Matsumura, 1914: 166; Knight 1987: 1190, figs 46–51; Webb and Vilbaste 1994: 69, figs 75–82; Khatri and Webb 2010: 13 (Pakistan).

Key to Pakistan species of *Balclutha* (male) modified from Naveed and Zhang (2018e)

1 Crown, pronotum and forewings with orange red longitudinal bands......... **2**
– Crown, pronotum and forewings without orange red longitudinal bands; aedeagus with basal processes **3**
2 Pygofer with branches of posteroventral appendages only slightly divergent, extended posterad; distal part of aedeagal shaft distinctly curved in lateral view .... ***B. rubrostriata***
– Pygofer with branches of posteroventral appendages widely divergent, one extended dorsad, the other ventrad; distal part of aedeagal shaft straight in lateral view... ***B. pararubrostriata***
3 Sordid brown with brown markings (Fig. 12); aedeagal shaft short, C-shaped, curved dorsally and anteriorly to near level of basal apodeme..... ***B. punctata***
– Yellowish green; aedeagal shaft not extending to near level of basal apodeme.....**4**
4 Aedeagus with three or more pairs of processes, shaft not curved basally....... .... ***B. incisa***
– Aedeagus without ventral processes, shaft curved basally............................ **5**
5 Aedeagus with basal apodeme finger-like in lateral aspect, shaft slightly sinuate apically .... ***B. viridinervis***
– Aedeagus with basal apodeme not finger-like in lateral aspect, shaft not sinuate apically .... ***B. sujawalensis***

Cicadulina China

C. bipunctata (Melichar)

Gnathodus bipunctata Melichar, 1904: 47.
Cicadula bipunctella Matsumura, 1914: 173 (Taiwan).
Cicadulina bipunctata: Webb 1987a: 236; Webb 1987b: 694, figs 70–77; Naveed and Zhang 2018e: 269, fig. 8A–E (Pakistan).

C. chinai Ghauri

Cicadulina chinai Ghauri, 1964: 205 (India).
Cicadulina striata Ahmed, 1986: 57, fig. 4, syn. nov.
Cicadulina chinai: Naveed and Zhang 2018e: 269, figs 7A–C, 8F–M (Pakistan).

Remarks. Original figures of *C. striata* show similarity to *C.chinai* in the shape of the pygofer process and aedeagus in lateral view but the aedeagus in posterior view (if drawn correctly) is a bit narrower. Described from the holotype male and several paratypes from Gharo, Thatta district, Sindh province, Pakistan maize, 11.x.85, Ahmed (ZMUK); no type specimens could be found.

Key to Pakistan species of *Cicadulina* (male) modified from Naveed and Zhang 2018e)

1 Pygofer with slender, hook-like process ending in triangular apex .... ***C. bipunctata***

6 Pygofer with thick and sinuate process, bifurcate at apex .... ***C. chinai***

Macrosteles **Fieber**

M. indrina **(Pruthi)**
Figs 29, 64

Cicadula indrina Pruthi, 1930: 61–62, pl. V fig. 5, text figs 83–84. N (India).
Macrosteles indrina. New combintion by Khatri and Webb 2010: 14, fig. 17.
Macrosteles parafalcatus Naveed & Zhang, 2018e: 266, figs 5A–J, 6A–C (Pakistan), syn. nov.

Remarks. A re-examination of the material identified and figured as *M. indrina* by Khatri and Webb (2010) and original figures of *M. parafalcatus* shows that there is insufficient evidence to separate the two species. The two species differ only very slightly in the separation of the long apodemes of the second abdominal sternite (fig. 64). Other differences seen in their respective original figures, i.e., of the aedeagus and style, are due to differences of orientation. Therefore, we consider the two species to be synonyms.

M. shahidi **Ahmad**

Macrosteles shahidi Ahmed, 1986: 55, fig. 3 (Pakistan).

Remarks. The identity of this species is uncertain (see Khatri & Webb 2010: 14).

Mukariini Distant

Diagnosis. These are small to medium sized, often dorsoventrally depressed or ventrally flattened, brown, black, whitish, yellow, or green, leafhoppers, sometimes marked with orange or red. They can be identified by the produced head, often with frontoclypeus tumid distally, ventral part of face flat, lying nearly horizontally or concave, and ocelli distant from eyes.

Mukaria Distant

M. splendida Distant

Mukaria splendida Distant, 1908: 270 (India); Khatri and Webb 2011: 19, figs 3a–k (Pakistan); Viraktamath and Webb 2019, figs 3A–D, 5R–S, 7D, 10A–D, 13E–I, 27A–J (India).

Opsiini Emaljanov

Diagnosis. Opsiini are small to large, stramineous, yellow, green, or brown leafhoppers. They can be identified by the bifurcate aedeagus with two shafts and gonopores. Some Mukariini and *Ascius* (Scaphytopiini) have a similarly divided aedeagus but Opsiini lack the other characters that define those groups.

Hishimonus Ishihara

H. phycitis (Distant)
Figs 9, 44

Eutettix phycitis Distant, 1908: 363–364, fig. 231 (India).
Eutettix lugubris Distant, 1918: 60. Synonymised by Knight 1970: 128.
Hishimonus orientalis Emeljanov, 1969: 1102. Synonymised by Knight 1970: 128.
Hishimonus phycitis: Knight, 1970: 128–130, figs 10, 11, 13; Viraktamath and Murthy 2014: 114, figs 23–26, 161–176; Naveed and Zhang 2018j: 1805, figs 1A–B, 2A–J (Pakistan).

Masiripius Dlabola

M. lugubris (Distant)

Mahalana lugubris Distant, 1918: 64 (India).
Ziziphoides punctatus: Rao, 1967: 239, figs 1–6.
Masiripius lugubris: Webb and Godoy 1993: 424; Viraktamath and Murthy 1999: 44, 47, figs 27–39 (India).

Neoaliturus Distant

N. (*Circulifer*) *tenellus* (Baker)

Thamnotettix tenella Baker, 1896: 24.
Eutettix tenellus: Uzel 1911: 287.
Circulifer tenellus ambiguosus Young & Frazier, 1954: 34, fig. 3.
Neoaliturus tenellus: Nast 1972: 331.

Neoaliturus (*Circulifer*) *tenellus* Mozaffarian & Wilson, 2016: 24 (Iran).

***N.* (*Circulifer*) *opacipennis* (Lethierry)**

Cicadula opacipennis Lethierry, 1876: 83.
Cicadula vittiventris Lethierry, 1876: 84.
Cicadula nausharensis Pruthi, 1936: 113–114, fig. 127, pl. VIII, fig. 15 (Pakistan). Synonymised by Bindra et al. 1970: 664, figs 1–11.
Neoaliturus opacipennis: Mozaffarian and Wilson 2016: 24 (Iran).

Key to Pakistan species of *Neoaliturus* (male)

1 Subgenital plates widely truncated (Fig. 69) .... ***N.* (*C.*) *tenellus***
– Subgenital plates acuminate (Fig. 70) .... ***N.* (*C.*) *opacipennis***

***Opsius* Fieber**

***O. smaragdinus* (Distant)**

Eutettix smaragdinus Distant, 1908: 364 (India).
Cestius triradiatus Ahmed & Sultana, 1994: 129, fig. 2 (Pakistan).
Opsius smaragdinus: Khatri and Webb 2010: 6.

***O. versicolor* (Distant)**

Cestius versicolor Distant, 1908: 310, fig. 198 (India).
Opsius dissimilis Vilbaste, 1961: 43.
Cestius sakroensis Ahmed & Sultana, 1994: 126, fig. 1 (Pakistan). Synonymised by Khatri and Webb 2010: 6.
Opsius versicolor: El-Sonbati et al. 2020: 8, figs 13–18, 32–34, 47–49, 65–69.

Key to Pakistan species of *Opsius* (male)

1 Aedeagal shaft with ventral process directed away from aedeagal shaft dorsally .... ***O. versicolor***
– Aedeagal shaft with ventral process close to aedeagal shaft dorsally................ .... ***O. smaragdinus***

***Orosius* Distant**

***O. aegypticus* Ghauri**
Fig. 10

Orosius aegypticus Ghauri, 1966: 251, fig. 11 (Egypt).

O. albicinctus Distant

Orosius albicinctus Distant, 1918: 85 (India); Ghauri 1966: 236–239, fig. 3.

Key to Pakistan species of *Orosius* (male)

1	Aedeagal base bulbous	***O. aegypticus***
–	Aedeagal base not bulbous	***O. albicinctus***

Paralimnini Distant

Diagnosis. These are small to medium sized leafhoppers. They can be identified by the combination of the following characters: clypellus tapering apically or parallel-sided, lorum narrower than clypellus at base; connective with anterior arms closely appressed, articulated with aedeagus; female first valvula sculpturing imbricate or rarely maculate or granulose. The tribe is very similar morphologically to the closely related Deltocephalini, from which it can be distinguished by the articulation between the connective and aedeagus (fused in Deltocephalini), although a few species of *Flexamia* (Paralimnini) have the connective fused to the aedeagus.

Remarks. Khatri and Rustamani (2011) pointed out that the paralimnine *Hengchunia pakistanica* Asche and Webb (1994) was erroneously recorded from Pakistan as it is known from the Indian state of Gujarat (spelt as Gudjarat).

Changwhania Kwon

C. ceylonensis (Baker)

Deltocephalus bimaculatus Melichar, 1903: 204 (Sri Lanka); Kuoh 1966: 128 (China).
Deltocephalus ceylonensis Baker, 1925: 537. Replacement name for *Deltocephalus bimaculatus* Melichar.
Cicadula bipunctatus Pruthi, 1930:59, pl. V, fig. 3 (India). Synonymised by Webb and Heller 1990: 8.
Changwhania changwhani Kwon, 1980: 99, figs 1–8 (Korea). Synonymised by Webb and Heller 1990: 8.
Changwhania ceylonensis: Webb and Heller 1990: 452; Zhang et al. 2009: 22 (China); Naveed and Zhang 2018f: 77, figs 1–14 (Pakistan).

C. terauchii (Matsumura)
Fig. 18

Aconura terauchii Matsumura, 1915: 163, Table 1, fig. 8; Matsumura 1931: 1250; Esaki and Ito 1954: 175.

Changwhania terauchii Kwon, 1980: 97–99, figs 1 (1–3), 2 (1–8) (Korea); Webb and Heller 1990: 452; Cai, Sun and Jiang 2001: 93; Zhang et al. 2009: 21 (China); Naveed and Zhang 2019b: 619, fig. 1 A–I (Pakistan).

Key to species of *Changwhania* from Pakistan (male) modified from Naveed et al. (2019b)

1	Crown with pair of round black anterior markings; aedeagus with subapical processes and truncate apex	***C. terauchii***
–	Crown with pair of oval black anterior markings; aedeagus with apical processes and apically rounded	***C. ceylonensis***

Jilinga **Ghauri**

J. gopii **(Pruthi)**

Deltocephalus gopii Pruthi, 1936: 127, pl. IX, fig. 9, text fig. 138 (Pakistan).
Jilinga gopii (Pruthi), comb. nov. by Webb & Heller, 1990: 8; Webb and Viraktamath 2009: 34; Khatri and Webb 2010: 15.

J. neelumensis **Naveed & Zhang**

Jilinga neelumensis Naveed & Zhang, 2018g: 569, figs 1A–C, 3A–H, 4A–B (Pakistan).

J. truncata **Naveed & Zhang**
Fig. 20

Jilinga truncata Naveed & Zhang, 2018g: 571, figs 1D–F, 2A–C, 5A–I (Pakistan).

Key to *Jilinga* species of Pakistan (male) modified from Naveed and Zhang 2018g

1	Anal tube ventral processes with fused section longer than distal branches, branches with only small denticuli present; aedeagal shaft broad in posterior view, no more than three times longer than wide	***J. gopii***
–	Anal tube ventral processes with fused section shorter than distal branches, branches with large teeth; aedeagal shaft narrow in posterior view, more than four times longer than wide	**2**
2	Dorsal connective less than twice as wide as distance between dorsal and ventral arms; anal tube appendage ventral branches with smaller teeth evenly distributed between pair of large teeth in posterior view	***J. neelumensis***
–	Dorsal connective more than twice as wide as distance between dorsal and ventral arms; anal tube appendage ventral branches with smaller teeth concentrated on large medial tooth	***J. truncata***

Paralimnellus Emeljanov

P. cingulatus (Dlabola)
Figs 19, 35

Paralimnus cingulatus Dlabola, 1960: 2.
Paralimnus (*Bubulcus*) *cingulatus* Dlabola, 1961: 320.
Paralimnellus cingulatus: Emeljanov 1972: 107.
Bubulcus cingulatus: Hamilton 1975: 487; Webb and Heller 1990: 8.
Paralimnus (*Dlabolasia*) *cingulatus*: Nemesio 2007: 143.
Paralimnellus cingulatus: Xing and Li 2011: 54–56, figs 1–11 (China); Naveed and Zhang 2019b: 619, fig. 3A–J (Pakistan).

Psammotettix Haupt

P. emarginata Singh

Psammotettix emarginata Singh, 1969: 356, figs 51–55 (India).
Psammotettix swatensis Ahmed, 1986: 52, fig. 1.
Psammotettix quettensis Ara & Ahmed, 1988: 292, fig. 2.
Psammotettix emarginata: Khatri and Webb 2010: 15, pl. 2f; figs 18, 19 (Pakistan).

Soractellus Evans

S. nigrominutus Evans
Fig. 21

Soractellus nigrominutus Evans, 1966: 225–226, fig. 35H (Australia); Chalam and Subba Rao 2005: 234, figs 6–10 (India); Stiller 1988 (Africa); Xing and Li 2014: 298; Naveed and Zhang 2018k: 596 (Pakistan); Webb et al. 2019: 586, figs 1–5.
Soractellus jianfengensis Xing & Li, 2014: 297–300, figs 1–14, (China). Synonymised by Webb et al. 2019.
Soractellus lalianensis Naveed & Zhang, 2018k: 595–599 (Pakistan). Synonymised by Webb et al. 2019.

Penthimiini Kirschbaum

Diagnosis. Penthimiini are small to medium, squat, robust, often black or brown leafhoppers; often with ventral part of face and/or entire ventral side flattened and dorsal side convex. They can be identified by the ocelli on crown and often distant from eyes, strong antennal ledge, dorsally flattened and carinate protibia, and forewing with appendix large and extending around wing apex.

Neodartus Melichar

N. acocephaloides Melichar

Fig. 2

Neodartus acocephaloides Melichar, 1903: 163; Distant 1908: 246, fig. 155; Distant 1918: 25; Rao 1993: 81–82 (India).

Penthimia Germar

P. compacta Walker

Penthimia compacta Walker, 1851: 842; Distant 1908: 242; Shobharani et al. 2018: 7, figs 5–9, 42, 56–60, 62, 69, 79–92, 172–175, 210–223 (India).
Penthimia subniger Distant, 1908: 243–244, fig. 154.
Penthimia scapularis Distant, 1908: 244.
Penthimia maculosa Distant, 1908: 244–245, in part.

Scaphoideini Oman

Diagnosis. Scaphoideini, following Zhaniser and Dietrich (2013: 148), is a rather poorly defined tribe. It was defined by these authors in the following way (with wording from their key to tribes in square brackets and added characters from Viraktamath and Yeshwanth (2020) in bold): "None of the following characters are present in all taxa, but some combination of [most of] these characters is present in all and a few (*) appear to be unique to this tribe: head narrower than pronotum, produced; **genae sometimes wide and visible dorsally**; frontoclypeus long and narrow; antennae long [longer than width of head]; body slender; head and wings often with brown, orange, ochraceous, or ivory markings; forewing with one or more darkly pigmented reflexed veins in vicinity of outer anteapical cell; profemur row AV setae absent or reduced (without stout setae); metatibia macrosetae in row PD long, as long as or longer than 0.5x length of protibia*; male or female pygofer with dense tufts of long fine or regular [macro] setae*; subgenital plate apex membranous or long, digitate, and somewhat membranous or weakly sclerotised; subgenital plates with long fine setae laterally and/or dorsally (also occurs in other deltocephaline tribes); basal processes of aedeagus or connective sometimes present, connected or articulated to base of aedeagus or apex of connective stem; **aedeagus sometimes fused to connective".** The last mentioned character is found in *Sikhamani* Viraktamath and Webb and *Thryaksha* Viraktamath and Murthy.

Bampurius **Dlabola**

B. pakistanicus **Khatri & Webb**

Bampurius pakistanicus Khatri & Webb, 2010: 18, pl. 1a; figs 1, 2 (Pakistan).

Grammacephalus **Haupt**

G. genoicus **Dlabola**

Grammacephalus genoicus Dlabola, 1984: 52; Khatri and Webb 2010: 16, pl. 2g; fig. 22 (Pakistan).

G. indicus **Viraktamath & Murthy**

Grammacephalus indicus Viraktamath & Anantha Murthy, 1999: 42 (india); Khatri and Webb 2010: 16, pl. 2h; figs 20–21; Naveed and Zhang 2018h: 1816, fig. 1A–I (Pakistan).

G. pallidus **Linnavuori**

Grammacephalus pallidus Linnavuori, 1978: 479; Viraktamath 1981: 8, figs 10–17 (Indicus); Khatri and Webb 2010: 16, pl. 2i; fig. 23 (Pakistan).

G. punjabensis **Shah & Duan**

Grammacephalus punjabensis Shah & Duan, 2019: 82, figs 11, 12 (Pakistan).

G. rahmani **(Pruthi)**

Platymetopius rahmani Pruthi, 1930: 33, pl. III, figs 2, 2a, text figs 45–46 (Pakistan, India).
Grammacephalus rahmani (Pruthi, 1930: 33), Mahmood 1979; Viraktamath 1981: 7, figs 1–9; Khatri and Webb 2010: 16.

G. raunoi **Viraktamath**
Figs 15, 33

Grammacephalus raunoi Viraktamath, 1981: 9, figs 30–36 (India); Naveed and Zhang 2018h: 1816, fig. 2A–J (Pakistan).

Key to species of *Grammacephalus* from Pakistan (male) modified from Naveed and Zhang (2018h)

1	Male pygofer process absent	***G. genoicus***
–	Male pygofer process present	**2**
2	Pygofer process with an appendage; aedeagal shaft with median expansion laterally	***G. raunoi***
–	Pygofer process without appendage; aedeagal shaft without median expansion laterally	**3**
3	Pygofer process with bifurcated apex	***G. punjabensis***
–	Pygofer process without bifurcated apex	**4**
4	Aedeagal shaft tubular	***G. rahmani***
–	Aedeagal shaft not tubular	**5**
5	Aedeagal shaft strongly reflexed basally, rather incrassate	***G. pallidus***
–	Aedeagal shaft not strongly reflexed basally, not incrassate	***G. indicus***

Monobazus **Distant**

M. dissimilis **(Distant)**

Xestocephalus dissimilis Distant, 1918: 55 (India).
Deltocephalus fuscovarius Distant, 1918: 83. Synonymised by Webb and Viraktamth 2009: 29
Monobazus dissimilis: Khatri and Webb 2010: 7, pl. 1d; fig. 4 (Pakistan).

Neolimnus **Linnavuori**

N. egyptiacus **(Matsumura)**
Fig. 16

Scaphoideus egyptiacus Matsumura, 1908: 29.
Neolimnus egyptiacus Linnavuori, 1953: 114; Khatri and Webb 2010: 7, pl. 1c; fig. 7.
Scaphoideus karachiensis Ahmed et al., 1988: 410 (Pakistan). Synonymised by Khatri and Webb 2010: 7.

Osbornellus **(*Mavromoustaca*) Dlabola**

O. **(*M.*)** ***macchiae*** **Lindberg**

Circulifer macchiae Lindberg, 1948: 160.
Osbornellus(*Mavromoustaca*) *consanguineus* Dlabola, 1967: 38. Synonymised by Kartel 1982: 27.
Osbornellus (*Mavromoustaca*) *macchiae* Khatri & Webb, 2010: 8, pl. 1e; fig. 3 (Pakistan).

***Phlogotettix* Ribaut**

***P. indicus* Rao**
Fig. 75

Phlogotettix indicus Rao, 1989: 77; Meshram et al. 2015: 234, figs 22–36 (India).

***Scaphoideus* Uhler**

***S. harlani* Kitbamroong & Freytag**
Fig. 17, 55

Scaphoideus harlani Kitbamroong & Freytag, 1978: 11; Khatri and Webb 2010: 8, pl. 1f; fig. 8 (Pakistan).

Stenometopiini Baker

Diagnosis. These are small to medium sized, rarely brightly coloured but iridescent leafhoppers when alive. They can be identified by the narrow crown, shagreen texture of crown, clypellus parallel-sided or tapering apically, forewings often submacropterous to brachypterous, male pygofer sloping caudoventrally and with few macrosetae and often with a distinct lateral tooth, female ovipositor protruding far beyond the pygofer apex, first valvula dorsal sculpturing granulose to maculate and submarginal, first valvula with distinctly delimited ventroapical sculpturing, and second valvula without dorsal teeth.

***Stirellus* Osborn & Ball**

***S. kumratensis* Naveed & Zhang**

Stirellus kumratensis Naveed & Zhang, 2020b: 481, figs 5, 6, 9–15 (Pakistan).

***S. lahorensis* (Distant)**
Fig. 54

Volusenus lahorensis Distant, 1918: 72 (Pakistan).
Stirellus peshawarensis Mahmood, Sultana & Waheed, 1972: 80. Synonymised by Khatri and Webb 2010.
Paternus jhokensis Ahmed & Aziz, 1988: 805. Synonymised by Khatri and Webb 2010.
Stirellus lahorensis: Khatri and Webb 2010: 17, pl. 2j; fig. 24; Naveed and Zhang 2020b: 480, figs 1, 2 (Pakistan).

S. mankiensis Shah & Duan
Figs 24, 32
Stirellus mankiensis Shah & Duan, 2020a: 198, figs 9, 10 (Pakistan).

S. neoconvexus Naveed & Zhang

Stirellus neoconvexus Naveed & Zhang, 2020b: 481, figs 7, 8, 16–20 (Pakistan).

S. thattaensis Mahmood, Sultana & Waheed
Fig. 63

Stirellus thattaensis Mahmood, Sultana & Waheed, 1972: 82, fig. 2 (Pakistan).

S. viridulus (Pruthi)
Fig. 71

Paternus viridula Pruthi, 1930: 42, pl. IV, figs 1, 1a, text figs 57–59 (India).
Paternus viridulus Metcalf, 1967a: 2350.
Stirellus viridulus: Khatri and Webb 2010: 1–47; Naveed and Zhang 2020b: 481, figs 3, 4 (Pakistan).

S. tolla (Pruthi)

Aconura tolla Pruthi, 1930: 39, pl. III, figs 7, 7a, text fig. 54 (India); Shah and Duan 2020a: 196, figs 6–8 (Pakistan).

Key to species of the genus *Stirellus* from Pakistan (male) modified from Shah et al. (2020)

1	Crown 1.5 × longer than breadth between eyes	***S. lahorensis***
–	Crown less than 1.5 × or equal to breadth between eyes	**2**
2	Species yellowish green in colour	**3**
–	Species ochraceous to brownish in colour	**5**
3	Crown anterior margin very slightly angulate	***S. tolla***
–	Crown anterior margin acutely angled	**4**
4	Male pygofer long, with rounded apex (Fig. 71)	***S. viridulus***
–	Male pygofer short with pointed apex (Fig. 63)	***S. thattaensis***
5	Subgenital plate with macrosetae uniseriate laterally	***S. kumratensis***
–	Subgenital plate with macrosetae not uniseriate laterally	**6**
6	Connective stem shorter than anterior arms, aedeagal shaft with blunt apex	***S. neoconvexus***
–	Connective stem longer than anterior arms, aedeagal shaft with pointed apex	***S. mankiensis***

Vartini Zahniser & Dietrich

Diagnosis. Vartini are medium sized to large, somewhat elongate, greenish or bluish leafhoppers, usually with red or orange longitudinal stripes. They can be identified by the produced and pointed head, gena visible behind eye in dorsal view, elongate frontoclypeus, lorum distant from genal margin, profemur intercalary row setae thick and extending to or beyond middle of profemur, forewings truncate apically, apodemes of male sternite II long, subrectangular, flared apically, and pointed posterolaterally, connective with anterior arms appressed, and male segment X tube-like and protruding from pygofer and often well sclerotised.

***Varta* Distant**

***V. rubrofasciata* Distant**

Varta rubrofasciata Distant, 1908: 321, fig. 205 (India); Viraktamath 2004: 13, figs 33, 49, 50 (India, Taiwan).

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