

Identification guide to larvae of Caucasian *Epeorus* (*Caucasiron*) (Ephemeroptera, Heptageniidae)

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Abstract

The Caucasus and adjacent areas are inhabited by fifteen species of mayflies of the genus *Epeorus*, subgenus *Caucasiron* Kluge, 1997 (Heptageniidae). This identification guide aims to facilitate an accurate species identification of their larvae and sum up all available information on their taxonomy and distribution. An identification key is provided, and the important diagnostic characters of all species are described and illustrated. The larva of *E. (C.) insularis* (Braasch, 1983) is described for the first time. This study enables the routine identification of *Caucasiron* larvae necessary for biomonitoring and hydrobiological research in the Caucasus region.

Keywords

aquatic insects, mayflies, morphology, identification key

Introduction

The knowledge facilitating the identification of mayflies inhabiting the Caucasus biodiversity hotspot (Myers et al. 2000) is limited to checklists (e.g., Bojková et al. 2018: Iran; Gabelashvili et al. 2018: Georgia; Hrivniak et al. 2018: Armenia) and alpha taxonomic

papers focused mostly on the delimitation of newly described species/taxa. The available identification keys deal with selected genera only (Sinitshenkova 1976, 1979: *Epeorus* Eaton, 1881 and *Rhithrogena* Eaton, 1882, respectively; Jacob and Zimmerman 1978: *Baetis* Leach, 1815) or mayfly fauna of the wider region without sufficient information on Caucasian species specifically (Kluge 1997a). These keys are largely outdated, because the number of species newly described from the Caucasus has been steadily increasing in recent years (e.g., Hrivniak et al. 2017; 2018; 2019; 2020a; Martynov and Godunko 2017; Bojková et al. 2018). Therefore, the identification of larvae to the species level is complicated due to the necessity of compiling information from original descriptions and requires advanced experience with the taxonomy of mayflies and comparative collections. Modern identification keys are needed especially for researchers implementing biomonitoring programmes and routine hydrobiological surveys in the region. They often use data on the generic or family level only (e.g., Hakobyan et al. 2010; Asatryan et al. 2016; Hovhannisyan and Shahnazaryan 2016; Sharifinia et al. 2016) and often include numerous misidentifications (cf. Bojková et al. 2018). This study aims to partly fill this gap by providing a complex identification guide for the larvae of the genus *Epeorus*, subgenus *Caucasiron* Kluge, 1997 (Heptageniidae) (hereinafter *Caucasiron*) occurring in the Caucasus and adjacent areas. The *Epeorus* s. l. larvae are known to be sensitive to pollution, are relatively stenotopic, restricted to lotic habitats, and form an ecologically important component in macroinvertebrate assemblages (Morisi et al. 2003). *Caucasiron* species, together with the remaining representatives of Heptageniidae, can, therefore, be used as indicators in water quality assessments and hydrobiological surveys in the Caucasus region.

Caucasiron ranks among the most diverse mayfly groups in the Caucasus region, together with the genera *Rhithrogena*, *Electrogena* Zurwerra & Tomka, 1985, and *Ecdyonurus* Eaton, 1868. It is a monophyletic subgenus, sister to the subgenus *Iron* Eaton, 1885 distributed in North, Central and East Asia and the Nearctic Region (Hrivniak et al. 2020b). Kluge (1997b) defined *Caucasiron* based on the unique apomorphy among Heptageniidae (and mayflies in general), a projection on the costal margin of the gill plates II–VII (see Fig. 5G, arrow). Other morphological characters of *Caucasiron* include: gill plates forming a “suction disc” (i.e., a structure consisting of enlarged gill plate I and overlapping gill plates II–VII, and gill plate VII with a longitudinal fold allowing it to be bent ventrally under the abdominal segments; Fig. 1B–D) and medio-dorsally directed hair-like setae along the anterior margin of the head (Kluge 2015: 346, fig. 178). Imagines are characterized by tubular penis lobes without dorso-lateral denticles and well developed median titillators (Fig. 1A). For the morphological comparison of *Caucasiron* with other related subgenera of the genus *Epeorus* s. l. see Braasch (2006; *Alpiron* Braasch, 2006, *Ironopsis* Traver, 1935) and Kluge (2004; *Iron*).

The global diversity of *Caucasiron* comprises 17 species (Hrivniak et al. 2020b). This identification guide deals with 14 species inhabiting the Caucasus and neighbouring mountain ranges, such as the Zagros and Taurus Mountains, and one species

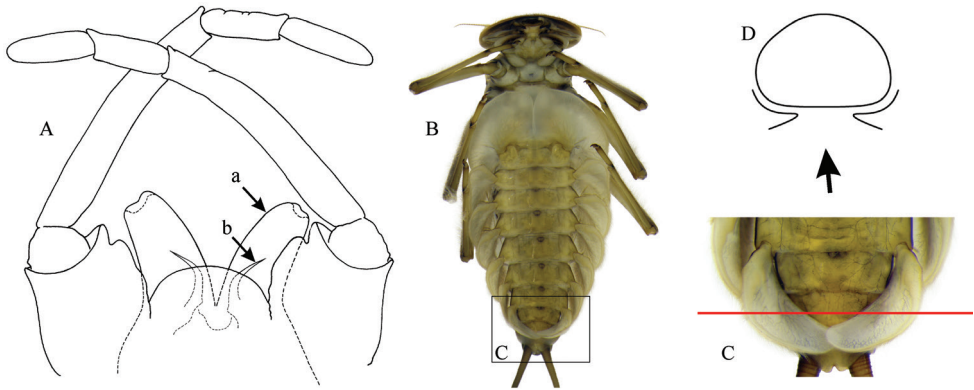


Figure 1. General morphology of *Epeorus* (*Caucasiron*): **A** male genitalia (a, penis lobus; b, titillator) **B** larva in ventral view **C** gills VII (in natural position from ventral view) **D** cross section of gills VII showing longitudinal fold.

inhabiting Samos Island. The extralimital species *E. (C.) guttatus* (Braasch, 1979) from Central Asia, *E. (C.) extraordinarius* Chen et al., 2010 from south-western China, and other Central Asian species presumably belonging to the genus *Caucasiron* (Hrivniak et al. 2017) are not included. All 15 species included in the guide are easily distinguishable based on both morphology and molecular data (Hrivniak et al. 2017, 2019, 2020a, b). Additionally, Hrivniak et al. (2020b) identified seven other distinct lineages based on molecular data only. Most of these lineages likely represent cryptic species or as yet have no formal morphological description. The distribution of possible cryptic lineages is to be found in the guide remarks of the respective morphotypes.

Individual species of *Caucasiron* have different distribution patterns in the Caucasus. Some species are local endemics to the Greater Caucasus, Pontic, Zagros, or Alborz Mountains. Others are widely distributed throughout the Caucasus and the adjacent areas of Anatolia, Cyprus, Iran, and Iraq (Hrivniak et al. 2017, 2019, 2020a, b). Their distribution and diversity patterns can be explained by geological and climatic history, and land development in the region that have significantly affected the diversification of *Caucasiron* in the Caucasus (Hrivniak et al. 2020b).

We aim to provide information necessary for the accurate species identification of *Caucasiron* to the professional public in order to allow the integration of *Caucasiron* into the hydrobiological surveys and biodiversity monitoring in the Caucasus. The main objectives of this study are to (i) form an identification key based on the reliable morphological characters of larvae, (ii) make an inventory of records of all species, and (iii) describe their geographic and altitudinal distribution based on our extensive data and available literature data. *Caucasiron* imagines are not described because of the lack of unambiguously associated specimens. Only information about whether the subimagines or imagines of a given species are described, how they were associated, and who described them, is provided.

Material and methods

Sampling

Larvae of *Caucasiron* were collected at 293 localities in Turkey, Georgia, Russia, Armenia, Azerbaijan, Iran, and Samos and Cyprus in 2008–2019 (Fig. 2). They were sampled by a hand net or a metal strainer and fixed in 96% ethanol in the field. Sampling sites fully covered Caucasus region and the geographical distribution of all known Caucasian *Caucasiron* species.

Morphological examination

Original descriptions of individual species were used for the initial species identification based on morphology (Sinitschenkova 1976; Braasch 1978, 1979, 1980; Braasch and Zimmerman 1979; Braasch and Soldán 1979; Hrivniak et al. 2017, 2019, 2020a). Due to insufficient details given in several of these descriptions, newly collected specimens (both larvae mounted on slides and larvae stored in ethanol) were compared with the type material (holotypes and/or paratypes) to accurately identify the species. Type series were studied in species recently described by us: *E. (C.) bicolliculatus* Hrivniak, 2017,

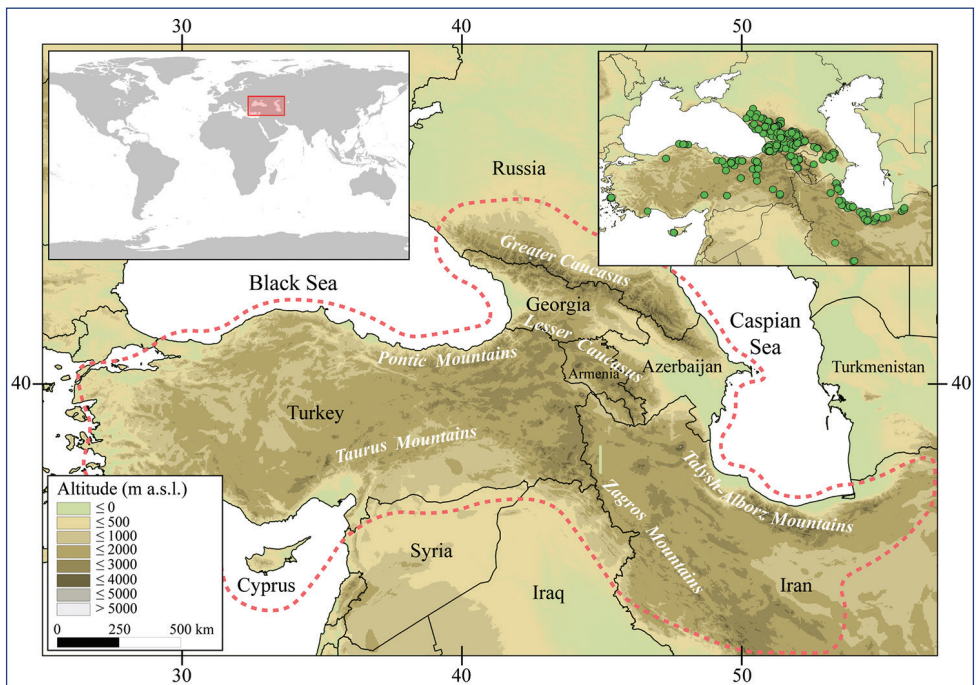


Figure 2. Topographic map of the Caucasus and adjacent mountain ranges with the position of the study area (upper left part) and distribution of our sampling sites (upper right part). Geographical coverage of identification guide of *Epeorus (Caucasiron)* larvae is defined by red dashed line.

E. (C.) turcicus Hrivniak, Türkmen & Kazancı, 2019, *E. (C.) alborzicus* Hrivniak & Sroka, 2020, *E. (C.) shargi* Hrivniak & Sroka, 2020, and *E. (C.) zagrosicus* Hrivniak & Sroka, 2020. Type specimens were also studied in following species: *E. (C.) iranicus* (Braasch & Soldán, 1979), *E. (C.) insularis* (Braasch, 1983), *E. (C.) magnus* (Braasch, 1978), *E. (C.) alpestris* (Braasch, 1979), *E. (C.) soldani* (Braasch, 1979), *E. (C.) longimaculatus* (Braasch, 1980), and *E. (C.) sinitschenkova* (Braasch & Zimmerman, 1979). Topotypes were collected and studied in several species: *E. (C.) insularis*, *E. (C.) alpestris*, *E. (C.) soldani*, *E. (C.) longimaculatus*, *E. (C.) iranicus*. The extent of morphological variability in each species was mostly determined based on specimens, which species identity was proved by molecular species delimitation (Hrivniak et al. 2017, 2019, 2020a, b).

Body parts with morphological structures requiring microscopical examination (i.e., mouthparts, femora, abdominal terga) were mounted on slide using HydroMatrix® (MicroTech Lab, Graz, Austria) mounting medium. In order to remove the muscle tissue for an investigation of the cuticular structure, the specimens were left overnight in a 10% solution of NaOH prior to slide mounting. Drawings were made using a stereomicroscope Olympus SZX7 and a microscope Olympus BX41, both equipped with a drawing attachment. Photographs were obtained using Leica DFC450 camera fitted with macroscope Leica Z16 APO and folded in Helicon Focus version 5.3. All photographs were subsequently enhanced with Adobe Photoshop™ CS5. The terminology was used mostly according to Kluge and Novikova (2011) and Kluge (2004, 2015).

Results and discussion

Larval morphological diagnostic characters

A set of larval diagnostic characters used in the identification guide (listed below) was derived from Braasch and Soldán (1979), who proposed them for the distinguishing larvae of the genus *Iron*. In the concept of Braasch and Soldán (1979), *Iron* included currently recognized taxa *Iron*, *Ironopsis*, *Caucasiron*, and *Alpiron* (see Hrivniak et al. 2020b for the revised concept and phylogeny of these taxa). Individual diagnostic characters are briefly described and figured for all species.

Morphological characters for the larval identification of *Caucasiron*:

- i) **coloration of abdominal terga:** shape of medial macula (Fig. 4I, arrow) and length of lateral stripes (extended dorso-posteriorly or not; Figs 13H, I, 16G respectively). Sometimes the medial macula is visible only partly being concealed by a preceding abdominal segment due to the telescoping contraction of the abdomen. The abdomen must be sufficiently extended manually to expose all length of individual segments to recognize the shape of the medial macula.

- ii) **coloration of abdominal sterna:** presence/absence and shape of pattern.

The coloration pattern of abdominal terga and/or sterna is often species-specific and valuable in the species identification of *Caucasiron* larvae. It is easily visible

and, thus, valuable for the routine identification. However, it often fades in older material or in inadequately fixed larvae, and the intensity of coloration, especially of abdominal sterna, varies among specimens and instars, and may be poorly expressed in some specimens. Therefore, the combination of all characters provided in the guide should be considered for an accurate species identification. The coloration pattern is usually present on terga II–IX (X) and sterna II–VIII (IX). However, patterns vary among segments, therefore, for the purpose of the key we compare terga V–VII and sterna II–VI, which are more species-specific.

- iii) **surface of abdominal terga:** presence/absence of outgrowths (protuberances, spines, etc.), shape of sensory setae (hair-like/wide at base; Figs 5E, 20E respectively), density, shape, and sclerotization of denticles along posterior margin of tergum VII (mounting on microscopic slide required).

Except *E. (C.) bicolliculatus* with a pair of postero-medial protuberances on abdominal terga II–IX (Hrivniak et al. 2017: figs 11, 31, 32; Fig. 34H, arrows), dorsal surface of abdominal terga of Caucasian *Caucasiron* species do not bear any outgrowths or spines.

Denticles along posterior margin of abdominal terga are pointed and irregular in size in all Caucasian *Caucasiron* species. However, the denticles of some species are denser and more sclerotized, e.g., in *E. (C.) znojtkoi* Tshernova, 1938 (Fig. 8E) and *E. (C.) nigripilosus* (Sinitshenkova, 1976) (Fig. 14E), strongly sclerotized, elongated and curved, e.g., in *E. (C.) magnus* (Fig. 11E) or less sclerotized and narrowed, e.g., in *E. (C.) longimaculatus* (Fig. 29E). The pattern of denticles slightly varies among terga and depends on a lateral distance from the midline of a given tergum. Thus, the reference part for the description of denticulation along the posterior margin of terga is used in the key. It is represented by tergum VII, the section from its midline to approximately half distance to the lateral margin.

- iv) **medial hypodermal femur spots:** presence/absence and shape (rounded/elongated). The character is relatively stable and usually present on dorsal surface of femora of all leg pairs. Variability was observed in *E. (C.) caucasicus* (Fig. 4F–H) and includes absence on all or at least some of the legs.
- v) **mouthparts:** setation on dorsal surface of labrum (sparse hair-like setae/dense bristle-like setae; Figs 5A, 11A respectively) and shape of mandibular incisors (blunt/pointed) (mounting on microscopic slide required).

Mouthparts of Caucasian *Caucasiron* species are generally without distinct diagnostic characters in most of the species. The only exception is labrum, mandibles and maxillae of *E. (C.) magnus*. This species differs from all others by setation of dorsal surface of labrum (dense bristle-like setae; Fig. 11A), pointed mandibular incisors (Fig. 11B, C), and thickened maxillary dentisetae (not figured). The shape of labrum is generally variable in most of the Caucasian *Caucasiron*. Exceptions are *E. (C.) magnus*, *E. (C.) alpestris*, and *E. (C.) sinitshenkovae*, the shape of labrum of which can be considered as one of the diagnostic characters. However, it should be noted that the shape of labrum is often distorted during the slide preparation and should be observed in natural position (not flattened), as well as suggested for other Heptageniidae (e.g., *Ecdyonurus*) (Bauernfeind 1997). Therefore, the shape

of labra figured in the guide are not flattened on slide but drawn from dorsal view in natural position. Drawings of the shape of mandibular incisors were based on flattened incisors on slides. Despite mandibular incisors are not considered as distinct character in the species identification, they are figured in the guide for comparison with *E. (C.) magnus*, and for purposes of further taxonomy, in case some new species with different incisors will be found in the future.

- vi) gill plates:** size of a projection on costal margin of gill plates III (with/without distinct projection; Fig. 5G, arrow and Fig. 29G respectively) and shape of gill plates VII in natural position from ventral view (narrow/wide).

The shape of gill plates I–VI is more or less identical between individual species. However, the gill plate VII is specific for some species; e.g., narrow, banana-shaped plate in *E. (C.) soldani* (Figs 19L, 20H–K), *E. (C.) longimaculatus* (Figs 28L, M, 29H–L), or *E. (C.) sinitschenkova* (Figs 25J; 26H–K); wider and rounded shape in *E. (C.) nigripilosus* (Figs 13K, 14H–J) or *E. (C.) alborzicus* (Figs 40J, K, 41H–J). Importantly, the shape of the gill plate VII must be observed in natural position from ventral view, without flattening on a slide (as shown e.g., in Fig. 7L–P). As a part of the gill plate VII is longitudinally bent under the abdomen (Fig. 1C, D), its shape is often deformed during the slide preparation by straightening of its inner margin.

- vii) tarsal claw denticulation:** number of denticles.

Denticulation of tarsal claws was omitted in the guide, due to its high overlap among species and frequent abrasion. Tarsal claws of all species usually possess 2–4 denticles.

- viii) shape of head in fully grown larvae:** ellipsoid/oval trapezoidal/sharply trapezoidal.

The shape of head (in dorsal view) may be used as one of the diagnostic characters in some species; e.g., *E. (C.) znojko* is characteristic by a distinctly angular, sharply trapezoidal head (Fig. 7D), *E. (C.) magnus* and *E. (C.) shargi* by more oval trapezoidal head with more broadly rounded corners (Fig. 10D, E and Fig. 43D respectively), and *E. (C.) longimaculatus* by more or less rounded, ellipsoid shape of head (Fig. 28D). Interspecific differences in the shape of head are most distinct in fully grown or late instars of males (and females in *E. (C.) magnus*).

- ix) postero-lateral projection on tergum X:** presence/absence (Fig. 11K–M, arrows and 17L respectively) and its size.

We also figure a shape of medial emargination of female sternum IX and spatulate setae on dorsal surface of femora (figures in the guide include the variability from proximal to distal margin of femora of all leg pairs). Despite a relatively wide range of variability in these characters, it may be helpful in identification of some species.

Identification guide to larvae of Caucasian species of *Caucasiron*

How to use the guide?

The dichotomous key divides *Caucasiron* species into two morphological groups, further divided into subgroups. They do not correspond with the phylogeny and merely represent groupings defined for the practical purpose of species identification.

Some characters within the key are subject to variation in some species. For instance, *E. (C.) caucasicus* usually has a median hypodermal femur spot, but in rare instances it is absent. We deal with this ambiguity by placing such species both in Group A (femur spot present) and B (femur spot absent). Thus, there are sometimes multiple paths leading to the same species in the key.

Most *Caucasiron* species are defined on the basis of a particular combination of several morphological characters. Following species identification using the dichotomous key, it is recommended to compare all the remaining diagnostic characters for a given species, provided in detail in the “Main morphological diagnostic characters of larvae” for each species. Variability of morphological diagnostic characters is described in the remarks section.

The “Main morphological diagnostic characters of larvae” were described based on late-instar larvae (fully-grown larvae). The order of characters is not concise in relation to all species; it always starts with the most prominent character for a given species after which the value of subsequent characters for species identification diminishes. For each species included in the guide, geographical and altitudinal distribution with frequency of sampling sites is provided. The construction of distribution maps was based on published records (Table 1) and our unpublished data. Brief information on distribution

Table 1. Records of *Caucasiron* species from the Caucasus and adjacent areas. Abbreviations used: A-Armenia; N-Nakhchivan; Te-eastern Turkey; T-Turkey*; G-Georgia; AZ-Azerbaijan; I-Iran; Iq-Iraq; Is-Israel; S-Syria; Rw-Russia (western Caucasus); Rc-Russia (central Caucasus); Sa-Samos Island; C-Cyprus Island.

Species	Records and references
<i>E. (C.) caucasicus</i> (Tshernova, 1938)	N-Tshernova (1938); Rw,A,G,N -Sinitshenkova (1976), Palatov and Sokolova (2018); AZ -Sinitshenkova (1976); Rc -Cherchesova (2004); Te -Braasch (1981), Koch (1988), Kazancı (2001); T -Kazancı and Türkmen (2012)*, Türkmen and Kazancı (2015)
<i>E. (C.) znojki</i> (Tshernova, 1938)	N,AZ -Tshernova (1938); G,Rc -Sinitshenkova (1976), Braasch (1980), Cherchesova (2004), Khazeeva (2010); A -Sinitshenkova (1976); Te -Braasch (1981), Türkmen and Kazancı (2015), Aydınli (2017); T -Kazancı and Türkmen (2012)*; I -Bojková et al. (2018)
<i>E. (C.) nigripilosus</i> (Sinitshenkova, 1976)	G -Sinitshenkova (1976); Rc -Sinitshenkova (1976), Braasch (1979), Khazeeva (2010); Rw -Braasch (1979); Iq -Al-Zubaidi et al. (1987); Te -Kazancı (2001); T -Kazancı and Türkmen (2012)*; I -Hrivniak et al. (2020a, b); C -Hrivniak et al. (2020a, b)
<i>E. (C.) magnus</i> (Braasch, 1978)	Rw -Braasch (1978,1980), Palatov and Sokolova (2018); G -Braasch (1980); A -Braasch (1980); T -Kazancı and Türkmen (2012)*, Rc -Cherchesova (2004)
<i>E. (C.) alpestris</i> (Braasch, 1979)	Rw ; Rc -Braasch (1979), Palatov and Sokolova (2018); Te -Kazancı (1986, 2001)**; T -Kazancı and Türkmen (2012)*, Aydınli (2017)**,
<i>E. (C.) soldani</i> (Braasch, 1979)	Rw ; Rc -Braasch (1979)
<i>E. (C.) sinitshenkovae</i> (Braasch & Zimmerman, 1979)	Rc ; Rw ; G -Braasch and Zimmermann (1979)
<i>E. (C.) longimaculatus</i> (Braasch, 1980)	G -Braasch (1980), Martynov et al. (2016)**; Te -Kazancı and Braasch (1988)**; Kazancı (2001)**; T -Kazancı and Türkmen (2012)**
<i>E. (C.) inanicus</i> (Braasch & Soldán, 1979)	I -Braasch and Soldán (1979), Mousavi and Hakobyan (2017), Bojková et al. (2018), Hrivniak et al. (2020a, b)
<i>E. (C.) insularis</i> (Braasch, 1983)	Sa -Braasch (1983), Hrivniak et al. (2020a, b)
<i>E. (C.) bicolliculatus</i> Hrivniak 2017	G -Martynov et al. (2016), Hrivniak et al. (2017); Te -Türkmen and Kazancı (2015), Hrivniak et al. (2017); A -Švihla (1975)***
<i>E. (C.) turcicus</i> Hrivniak, Türkmen & Kazancı, 2019	Te -Hrivniak et al. (2019)
<i>E. (C.) alborzicus</i> Hrivniak & Sroka, 2020	I -Hrivniak et al. (2020a)
<i>E. (C.) shargi</i> Hrivniak & Sroka, 2020	I -Hrivniak et al. (2020a)
<i>E. (C.) zagrosicus</i> Hrivniak & Sroka, 2020	I -Hrivniak et al. (2020a)

* without exact localisation, not included in distribution maps.

** doubtful record not included in distribution maps.

*** unpublished record included in the distribution map.

is also given directly in the key. Abbreviations correspond with points of the compass; central Greater Caucasus refers to area from Mount Elbrus to Mount Kazbek. In the description of habitat, altitudinal distribution is divided into three categories: low (up to 500 m a.s.l.), middle (500–1500 m), and high (above 1500 m). This serves only for the purpose of rough orientation, since actual environmental conditions on a given altitude may vary significantly because of high climatic heterogeneity within the region. The list of synonyms given for each species includes all generic/subgeneric combinations under which the species is mentioned in the literature, always with the reference to the first study using a given combination.

Key to species

- 1 Medial hypodermal femur spots present (e.g., Fig. 13F, G) **group A**
- Coloration pattern on abdominal sterna present (Figs 4B; 13B; 46B)
..... **subgroup A1**, p. 9
- Coloration pattern on abdominal sterna absent (Figs 28B; 37B; 43B)
..... **subgroup A2**, p. 9
- 2 Medial hypodermal femur spots absent (e.g., Fig. 16F) **group B**
- Coloration pattern on abdominal sterna present (Figs 4B; 7B; 16B; 31B;
34B; 40B; 19J–K) **subgroup B1**, p. 10
- Coloration pattern on abdominal sterna absent (e.g., Figs 10B; 25B)
..... **subgroup B2**, p. 11

subgroup A1

- 1 Abdominal sterna II–VI with a pair of oblique stripes (Figs 4J; 22I, J; 46I) ... **2**
- Abdominal sterna II–V (VI) with a pair of triangular spots (Fig. 13J) and abdominal terga with lateral stripes extended dorso-posteriorly (Fig. 13H, I, arrows) ***E. (C.) nigripilosus* (W and Central Greater Caucasus, Turkey, Iraq, N Iran)**, p. 19
- 2 Stripes on abdominal sterna II–VI widened anteriorly (Fig. 46I, arrows) and abdominal terga with lateral stripes extended dorso-posteriorly (Fig. 46H, arrows) ***E. (C.) zagrosicus* (S and SW Iran)**, p. 45
- Stripes on abdominal sterna II–VI not widened anteriorly (Figs 4J; 22I, J) ... **3**
- 3 Abdominal terga V–VII with crown-like medial macula (Fig. 4I)
..... ***E. (C.) caucasicus* (widespread in the Caucasus)**, p. 11
- Abdominal terga V–VII with stripe-like medial macula and a pair of distinct antero-lateral stripes (Fig. 22G, arrows) ***E. (C.) iranicus* (N Iran)**, p. 29

subgroup A2

- 1 Medial hypodermal femur spots distinctly elongated (Fig. 28F–H); setae on abdominal terga wide at base and denticles along posterior margin of tergum VII narrow (Fig. 29E); gill plates III without distinct projection (Fig. 29G);

- gill plates VII narrow (Figs 28L, M; 29H–L)
 ***E. (C.) longimaculatus* (central Greater Caucasus)**, p. 32
- Medial hypodermal femur spots rounded, not distinctly elongated, gill plates III with well-developed projection; setae on abdominal terga hair-like (e.g., Fig. 38E)..... **2**
- 2 Abdominal terga V–VII with stripe-like medial macula and lateral stripes extended dorso-posteriorly (Fig. 37G, arrows); gill plates VII narrow (Figs 37I; 38H–K).....***E. (C.) turcicus* (NE Turkey, Georgia)**, p. 40
- Abdominal terga V–VII with more or less triangular or T-shaped medial macula, lateral stripes not extended dorso-posteriorly (Fig. 43I–K); gill plates VII wide (Figs 43M; 44H, I).....***E. (C.) shargi* (N Iran)**, p. 45

subgroup B1

- 1 Setae on abdominal terga wide at base **2**
- Setae on abdominal terga hair-like **3**
- 2 Abdominal terga II–IX with a pair of postero-medial protuberances (Fig. 34H, arrows; protuberances are most developed on terga VI–VIII and best visible from dorso-lateral view); abdominal terga V–VII with stripe-like medial macula, which is often anteriorly and posteriorly widened (Fig. 34G, H); abdominal sterna II–VI as on Fig. 34J–L.....
 ***E. (C.) bicolliculatus* (NE Turkey, W Caucasus, Armenia)**, p. 36
- Abdominal terga without postero-medial protuberances; terga V–VII with well-defined triangular maculae (Fig. 19H, I); sterna not intensively pigmented, pattern of sterna II–VI as on Fig. 19J, K.....
 ***E. (C.) soldani* (W and central Greater Caucasus)**, p. 24
- 3 Postero-lateral projections on tergum X distinct (Fig. 41K, arrow); abdominal sterna II–VI with circular medial macula (Fig. 40L–N); gill plates VII wide (Figs 40J, K; 41H–J)..... ***E. (C.) alborzicus* (N Iran)**, p. 41
- Postero-lateral projections on tergum X absent or indistinct, coloration pattern of abdominal sterna different..... **4**
- 4 Abdominal sterna II–VI yellowish, with a pair of black oblique stripes or brownish rounded medial macula **5**
- All or at least abdominal sterna VIII–IX intensively red (Fig. 7L), with reddish to brownish maculation (Fig. 7M) including a longitudinal stripe (Figs 7N–P; 31J) and a pair of reddish oblique (Fig. 7K, a) and/or medio-lateral stripes (Fig. 7K, b) **6**
- 5 Abdominal sterna II–VI with a pair of black oblique stripes (Fig. 4J); abdominal terga V–VII with crown-like medial macula (Fig. 4I)
 ***E. (C.) caucasicus* (widespread in the Caucasus, E Turkey)**, p. 11
- Abdominal sterna II–VI with brownish rounded medial macula (Fig. 16I); abdominal terga V–VII with narrow stripe-like medial macula (widened on terga VIII–IX, Fig. 16G, H, arrows)
 ***E. (C.) alpestris* (W and central Greater Caucasus)**, p. 23

- 6 Gill plate VII wide (Figs 7J, L–P; 8H–L); denticles along posterior margin of tergum VII relatively long, strongly sclerotized and dense (Fig. 8E); postero-lateral projections on tergum X present or absent (Fig. 8M, N)
 ***E. (C.) znojkoï s. l. (widespread in the Caucasus, Turkey)***, p. 13
- Gill plates VII narrow (Figs 31K; 32H, I); denticles along posterior margin of tergum VII relatively short and poorly sclerotized (Fig. 32E); postero-lateral projections on tergum X absent (Fig. 32J).....
 ***E. (C.) insularis (Samos Island, Greece)***, p. 35

subgroup B2

- 1 Postero-lateral projections on tergum X present (Fig. 11K–M); dorsal surface of labrum with dense bristle-like setae (Fig. 11A); gill plates VII wide or slightly narrowed (Figs 10K; 11H–J)
 ***E. (C.) magnus (widespread in the Caucasus, Turkey)***, p. 18
- Postero-lateral projections on tergum X absent (Figs 20L, 26L); gill plates VII distinctly narrowed (Figs 19L; 20H–K; 25J; 26H–K); dorsal surface of labrum with sparse hair-like setae (Figs 20A; 26A)..... **2**
- 2 Abdominal terga V–VII with narrowed triangular medial macula and a pair of anterolateral spots (Fig. 25H; arrows); gill plates III without distinct projection (Fig. 26G); setae on terga not distinctly widened at base, often elongated (Fig. 26E) ***E. (C.) sinitschenkovaë (W and central Greater Caucasus)***, p. 30
- Abdominal terga V–VII with well-defined triangular medial maculae, without a pair of anterolateral spots (Fig. 19H, I); setae on terga wide at base (Fig. 20E); gill plates III with well-developed projection (Fig. 20G).....
 ***E. (C.) soldani (W and central Greater Caucasus)***, p. 24

Morphological diagnostics, distribution, and habitat of individual species

Epeorus (Caucasiron) caucasicus (Tshernova, 1938)

Figs 3–5

Cynigma caucasica Tshernova, 1938

Epeorus (Iron) (Tshernova, 1938); in Tshernova (1974)

Iron fuscus Sinitschenkova, 1976; jun. syn.; in Braasch (1979)

Epeorus (Caucasiron) caucasicus (Tshernova, 1938); in Kluge (1997b)

Type locality. Azerbaijan, The Nakhchivan Autonomous Republic, a stream in the vicinity of the upper Sakarsu River (3000 m a.s.l.).

Distribution. Georgia, south-western Russia, Azerbaijan, Armenia, eastern Turkey (Fig. 3). One of the most widespread species in the Caucasus.

Habitat. Larvae inhabit small streams and rivers at middle and high altitude, most frequently found above 1000 m a.s.l. Altitudinal range of sampling sites 496–2474 m a.s.l. (Fig. 3).

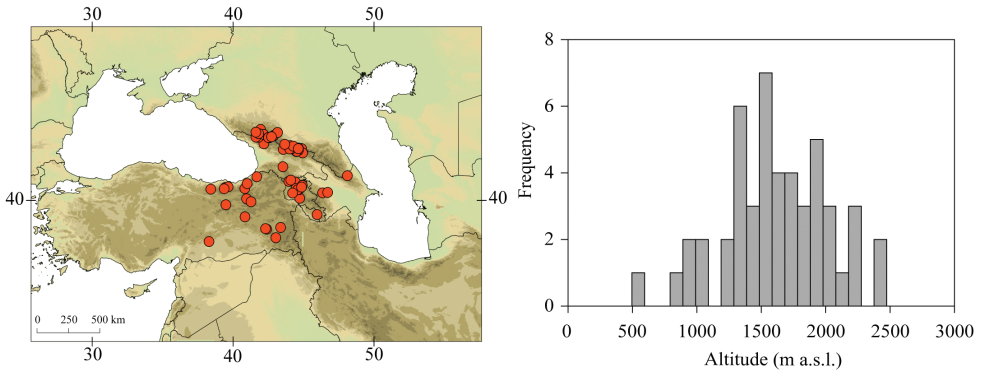


Figure 3. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) caucasicus*.

Main morphological diagnostics of larvae. (i) abdominal sterna II–VI with a pair of oblique stripes; nerve ganglia often with stripes or spots (Fig. 4B, J); (ii) abdominal terga V–VII with crown-like medial macula (Fig. 4A, I, arrow); (iii) femora with medial hypodermal spot (Fig. 4G, H), sporadically absent or poorly visible (Fig. 4F); (iv) setae on abdominal terga hair-like (Fig. 5E); (v) gill plates III with well-developed projection (Fig. 5G); (vi) tergum X with poorly developed postero-lateral projections (Fig. 5M, arrow) or without postero-lateral projections (Fig. 5L).

Remarks. Morphology. Coloration pattern of abdominal sterna as in *E. (C.) iranica* (Figs 22I, J), similar pattern in *E. (C.) zagrosicus* (Fig. 46I). Lateral stripes on abdominal terga sporadically dorso-posteriorly extended as in *E. (C.) nigripilosus* (Fig. 13H, I, arrows). A projection on gill plates III usually well-developed, a slight reduction observed in specimens collected from central Armenia.

Taxonomy. This species was described based on male imagines from the Nakhchivan Autonomous Republic (upper Sakarsu River) (Tshernova 1938). The type series is deposited in the Institute of Zoology, Russian Academy of Sciences, Saint Petersburg (IZ) (Kluge 1995). Female imago not described; the larva described by Sinitshenkova (1976) from several localities in Russia, Armenia, and Azerbaijan. Larvae and imagines were associated based on the same sampling sites (a part of the larval material originated from the vicinity of the type locality) and a similarity in the coloration of abdomen of the larva and imagines. The description and validity of larval diagnostic characters were discussed by Braasch (1979, 1980). According to him, Sinitshenkova (1976) described the larva of *E. (C.) znojtkoi* under the name *E. (C.) caucasicus* by mistake. This opinion was supported by the investigation of imagines reared from larvae corresponding to *E. (C.) caucasicus* described by Sinitshenkova (1976). Imagines corresponded to *E. (C.) znojtkoi* as were described by Tshernova (1938). The larva belonging to *E. (C.) caucasicus* was also described in Sinitshenkova (1976), but under erroneous attribution to newly proposed species *E. (C.) fuscus*. Later, *E. (C.) fuscus* was considered as a synonym of *E. (C.) caucasicus* (Braasch 1979; Braasch and Soldán 1979).

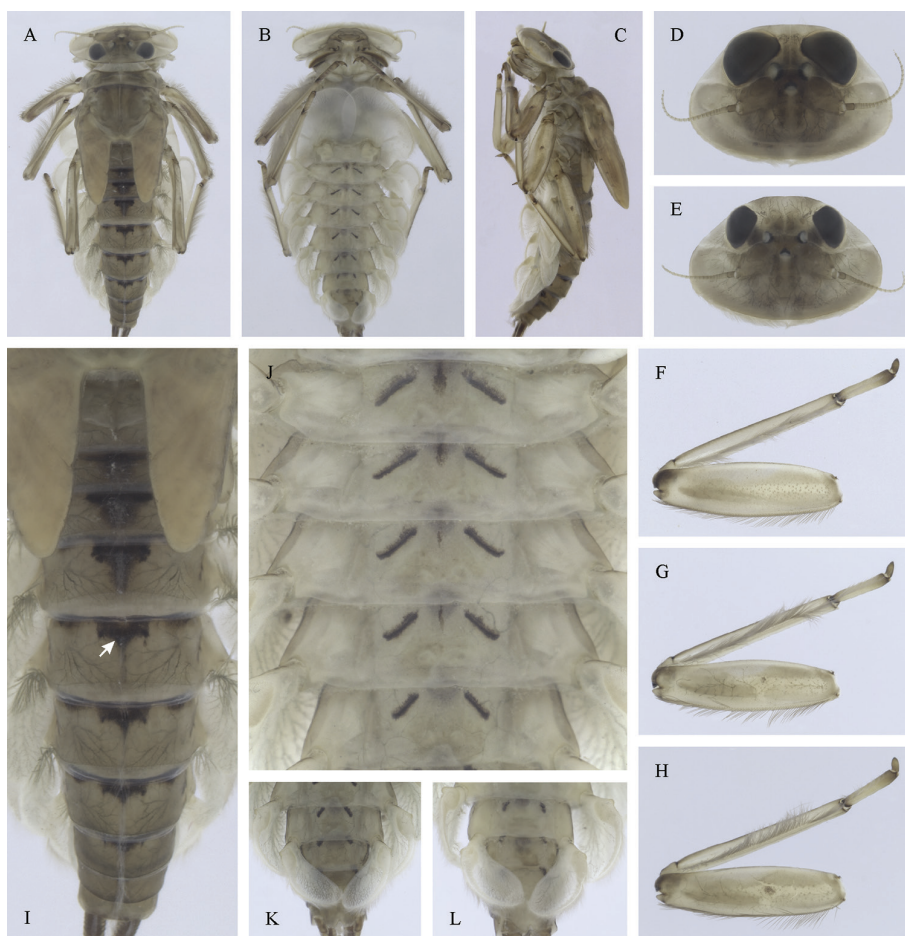


Figure 4. *Epeorus* (*Caucasiron*) *caucasicus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F–H** middle leg in dorsal view **I** abdominal terga (arrow points on medial macula) **J** abdominal sterna II–VI **K, L** gills VII (in natural position from ventral view).

***Epeorus* (*Caucasiron*) *znojko* (Tshernova, 1938), sensu lato**

Figs 6–8

Iron znojko Tshernova, 1938

Epeorus (*Iron*) *znojko* (Tshernova, 1938); in Tshernova (1974)

Iron caucasicus (Tshernova, 1938); in Sinitshenkova (1976) partim

Iron znojko Tshernova, 1938; in Sinitshenkova (1976) partim

Epeorus (*Caucasiron*) *znojko* (Tshernova, 1938); in Kluge (1997b)

Type locality. Azerbaijan, Nakchivan Autonomous Republic, Giljan-tshaj (Gilljak) (2000–2100 m a.s.l.).



Figure 5. *Epeorus (Caucasiron) caucasicus*, larva: **A** labrum (left half in dorsal view, right half in ventral view) with detail of hair-like seta **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII with detail of hair-like seta **F** gill I **G** gill III (arrow points on distinct projection on costal margin) **H** gill VII (flattened on slide) **I–K** gill VII (in natural position from ventral view), variability in shape **L, M** abdominal segments VIII–X in lateral view (arrow points on postero-lateral projection) **N** sternum IX of female with observed variability.

Distribution. Georgia, south-western Russia, Azerbaijan, Armenia, Turkey, northern Iran (Fig. 6). The most widespread species in the Caucasus.

Habitat. Larvae inhabit streams and rivers of various sizes, from larger braided low altitude rivers to small streams at high altitude. Altitudinal range of sampling sites

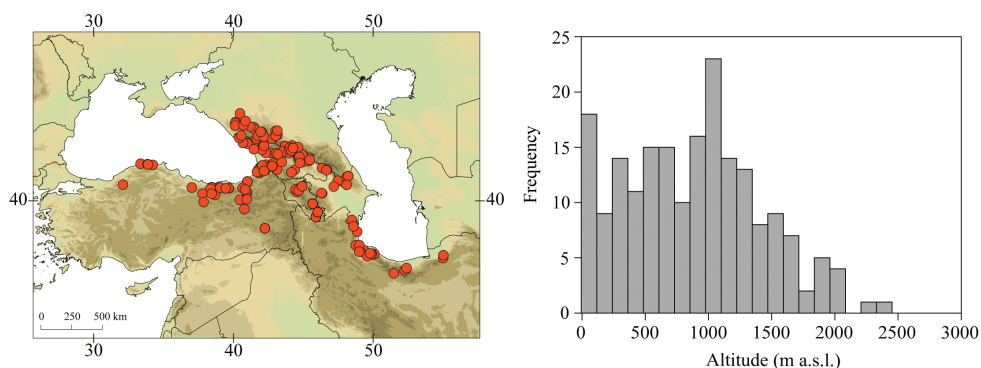


Figure 6. Geographical (left) and vertical (right) distribution of *Epeorus* (*Caucasiron*) *znojtkoi*.

–6–2453 m a.s.l. (Fig. 6). Most frequently found in low and middle altitudes. Often syntopic with *E. (C.) magnus*.

Main morphological diagnostics of larvae. (i) abdominal terga II–IV with triangular medial macula and terga V–VII with T shaped medial macula (Fig. 7A, G–I); (ii) abdominal sterna intensively red or reddish (Fig. 7B, L, M), with a pair of reddish oblique stripes (Fig. 7K, a) and/or reddish medio-lateral stripes (Fig. 7K, b), or with reddish to brownish longitudinal stripe on all sterna or at least on sterna VIII and IX (Fig. 7N–P) (iii); tergum X with short postero-lateral projections (Fig. 8M, arrow) or without postero-lateral projections (Fig. 8N); (iv) femora without medial hypodermal spot (Fig. 7F); (v) gill plates VII (in natural position from ventral view) wide (Figs 7J, L–P, 8H–L); (vi) denticles along posterior margin of tergum VII strongly sclerotized and dense (Fig. 8E); (vii) gill plates III with well-developed projection (Fig. 8G); (viii) shape of head sharply trapezoidal in males (Fig. 7D).

Remarks. Morphology. The reduction of reddish coloration of abdominal sterna observed particularly in specimens collected from Turkey (Fig. 7N) and northern Iran (Fig. 7O, P). Similar coloration pattern of sterna as present in *E. (C.) insularis* (Fig. 31J).

Taxonomy. This species was described based on male and female subimagines and imagines from the Nakchivan Autonomous Republic (Tshernova 1938). The type series is deposited in IZ (Kluge 1995). The larva was described by Sinitshenkova (1976) based on material collected in Georgia, Russia (the central Greater Caucasus), Armenia and the type locality. Larvae were identified as species *znojtkoi*, based on the proximity of its type locality and the similarity of markings on abdominal terga. However, the description of larva is confusing, because the larva of *E. (C.) znojtkoi* was erroneously described under the name *E. (C.) caucasicus* by Sinitshenkova (1976) (Braasch, 1980). Therefore, the larva described by Sinitshenkova (1976) as *E. (C.) znojtkoi* should belong to a different species. Its diagnostic characters correspond to those of *E. (C.) magnus* that was later described by Braasch (1978). These characters include: (i) body length: Tshernova (1938) noted 9.5–12 mm for imagines of species *E. (C.) znojtkoi*; contrary to Sinitshenkova (1976) who noted 14–19 mm for the larvae. Larvae of species *magnus* exhibit 20–24 mm as described by Braasch (1978); (ii) shape of head: trapezoidal head with rounded



Figure 7. *Epeorus (Caucasiron) znojkoii*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F** middle leg in dorsal view **G–I** abdominal terga **J** gills VII (in natural position from ventral view) **K** abdominal sterna II–VI (a, position of oblique stripes b, position of medio-lateral stripes) **L–P** abdominal sterna, variability in coloration pattern (**L** Georgia **M, O, P** Iran **N** Turkey).

edges as figured by Sinitshenkova (1976) is typical for *E. (C.) magnus* (Fig. 10D, E), not to *E. (C.) znojkoii* with more angular edges of head (Fig. 7D); (iii) setation of labrum: the shape of labrum and dense setae on its dorsal surface as figured by Sinitshenkova (1976) is characteristic for *E. (C.) magnus* (Fig. 11A); (iv) coloration of abdominal sterna: an absence of coloration on abdominal sterna as described by Sinitshenkova (1976) is typical for *E. (C.) magnus* (Fig. 10J); *E. (C.) znojkoii* possess reddish sterna and gills.

Distribution. *E. (C.) znojkoii* is considered as a species complex containing several lineages (Hrivniak et al. 2020b). They are distributed in the Pontic Mts. in Turkey (*Caucasiron* sp. 5 in Hrivniak et al. 2020b), the Alborz Mts. in Iran (*Caucasiron* sp. 4 in Hrivniak et al. 2020b), and the Lesser Caucasus in Georgian Adjara (*Caucasiron* sp. 6 in Hrivniak et al. 2020b). The lineages are not formally described now and fall into the group *E. (C.) znojkoii* s. l. in this identification guide.



Figure 8. *Epeorus (Caucasiron) znojkoii*, larva: **A** labrum (left half in dorsal view, right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII and its variability **F** gill I **G** gill III **H** gill VII (flattened on slide) **I-L** gill VII (in natural position from ventral view), variability in shape **M, N** abdominal segments VIII-X in lateral view (arrow points on postero-lateral projection) **O** sternum IX of female with observed variability.

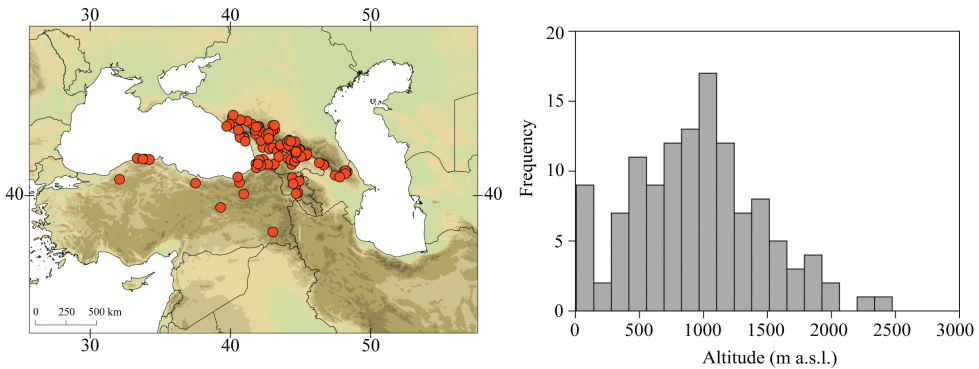


Figure 9. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) magnus*.

Epeorus (Caucasiron) magnus (Braasch, 1978)

Figs 9–11

*Iron znojko*i Tshernova, 1938; in Sinitshenkova (1976), partim

Iron magnus Braasch, 1978

Epeorus (Iron) magnus (Braasch, 1978); in Kluge (1988)

Epeorus (Caucasiron) magnus (Braasch, 1978); in Kluge (1997b)

Type locality. Russia, Krasnodar krai, western Caucasus, Sochi River (20 km above Sochi; 800 m a.s.l.).

Distribution. Georgia, south-western Russia, Azerbaijan, Armenia, Turkey (Fig. 9). One of the most widespread species in the Caucasus.

Habitat. Larvae inhabit streams and rivers of various sizes, from larger braided low-altitude rivers to small streams at high altitude. Altitudinal range of sampling sites 6–2474 m a.s.l. (Fig. 9). Most frequently found at low and middle altitude. Often syntopic with *E. (C.) znojko*i.

Main morphological diagnostics of larvae. (i) shape of head in male and female oval, trapezoidal (Fig. 10D, E); (ii) tergum X with well-developed postero-lateral projections (Fig. 11K–M, arrows), sporadically poorly developed; (iii) abdominal sterna without coloration pattern (Fig. 10B, J); (iv) abdominal terga V–VII with triangular medial macula (Fig. 10H), sporadically poorly visible (Fig. 10I); (v) femora without medial hypodermal spot (Fig. 10F, G); (vi) dorsal surface of labrum densely covered by bristle-like setae (Fig. 11A); (v) setae on abdominal terga hair-like (Fig. 11E); (vi) gill plates III with well-developed projection (Fig. 11G); (vii) denticles along posterior margin of tergum VII strongly sclerotized, dense and curved (Fig. 11E).

Remarks. Morphology. The largest species occurring in the Caucasus. The body size of larvae 20–24 mm, cerci 20–22 mm (Braasch 1978).

Taxonomy. Original description based on the larvae from Russia (western Caucasus) (Braasch 1978). The type series is currently deposited in the collection of Stuttgart State Museum of Natural History, Stuttgart, Germany (SMNS). Imagines (male and

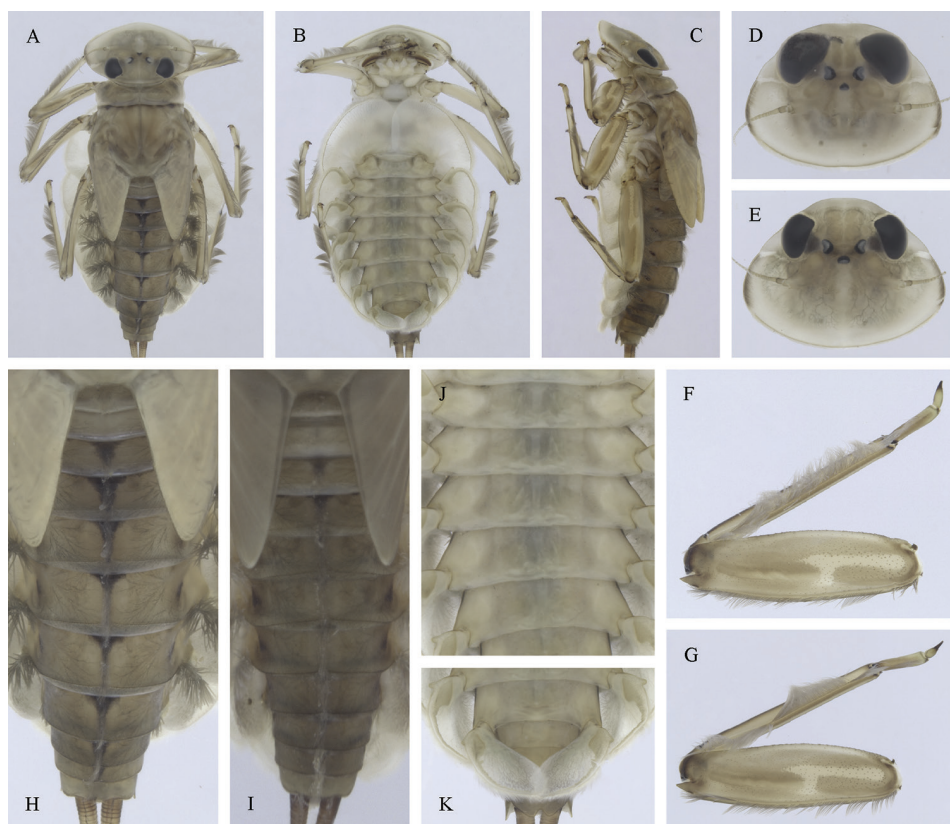


Figure 10. *Epeorus* (*Caucasiron*) *magnus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F, G** middle leg in dorsal view **H, I** abdominal terga **J** abdominal sterna II–VI **K** gills VII (in natural position from ventral view).

female) and female subimago described by Braasch (1980) based on material from Russia, Armenia and Georgia. We assume the larva of *E. (C.) magnus* was erroneously described under the name *znojko*i by Sinitshenkova (1976) (see remarks to *E. (C.) znojko*i s. l. for details).

***Epeorus* (*Caucasiron*) *nigripilosus* (Sinitshenkova, 1976)**

Figs 12–14

Iron nigripilosus Sinitshenkova, 1976

Epeorus (*Iron*) *nigripilosus* (Sinitshenkova, 1976); in Kluge (1995)

Epeorus (*Caucasiron*) *nigripilosus* (Sinitshenkova, 1976); in Kluge (2004)

Type locality. Georgia, Mtskheta-Mtianeti Region, Kistinka (= Khde, Khdistkhali) River (along the Georgian Military Road, 1300 m a.s.l.).

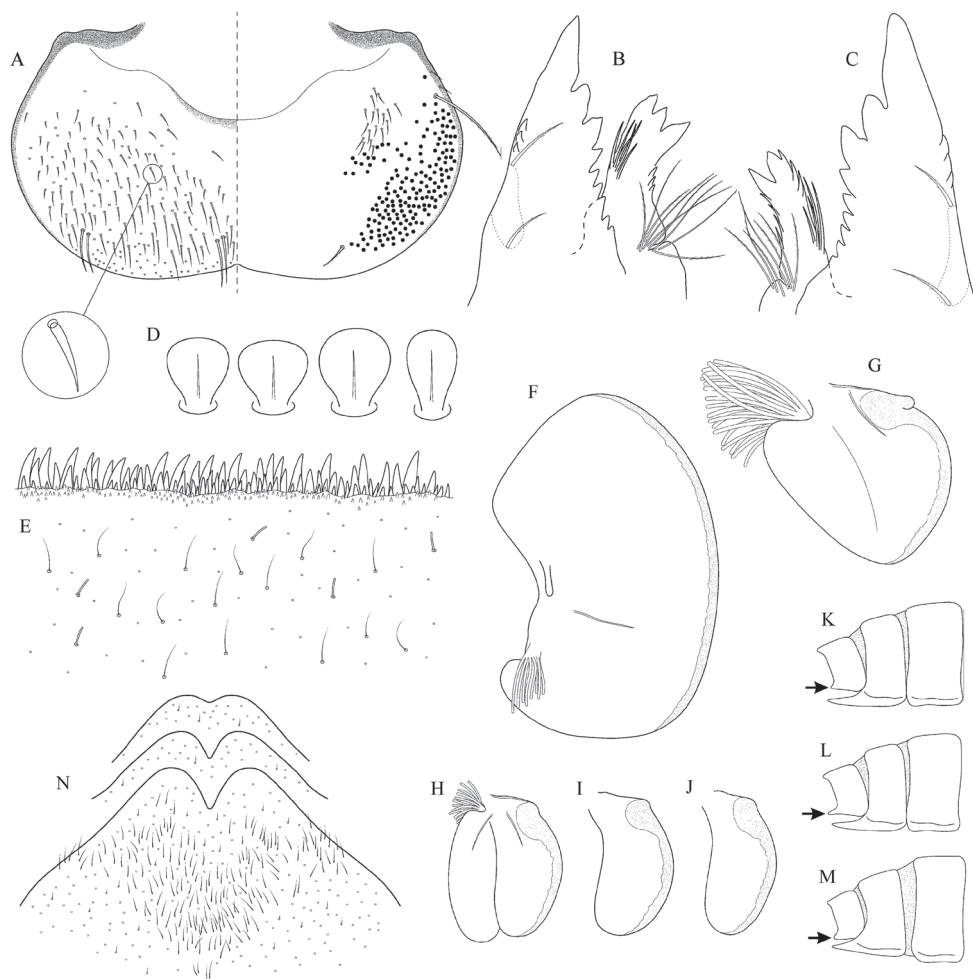


Figure 11. *Epeorus (Caucasiron) magnus*, larva: **A** labrum (left half in dorsal view right half in ventral view) with detail of bristle-like seta **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I, J** gill VII (in natural position from ventral view) variability in shape **K–M** abdominal segments VIII–X in lateral view (arrow points on postero-lateral projection) **N** sternum IX of female with observed variability.

Distribution. Georgia, south-western Russia, Turkey, Cyprus Island, northern Iraq, northern Iran (Fig. 12).

Habitat. Larvae inhabit small streams and rivers at low to high altitude. Altitudinal range of sampling sites 280–2100 m a.s.l. (Fig. 12). Most frequently found above 1000 m a.s.l.

Main morphological diagnostics of larvae. (i) abdominal sterna II–VI with a pair of triangular spots; nerve ganglia often with spots (Fig. 13B, J); (ii) abdominal

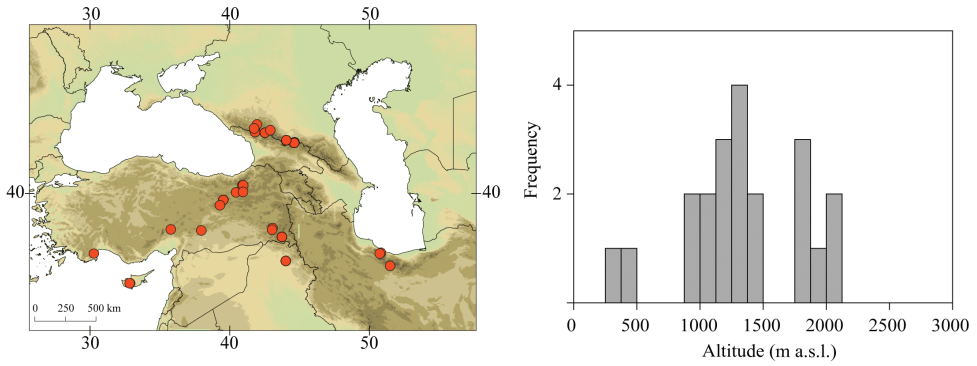


Figure 12. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) nigripilosus*.

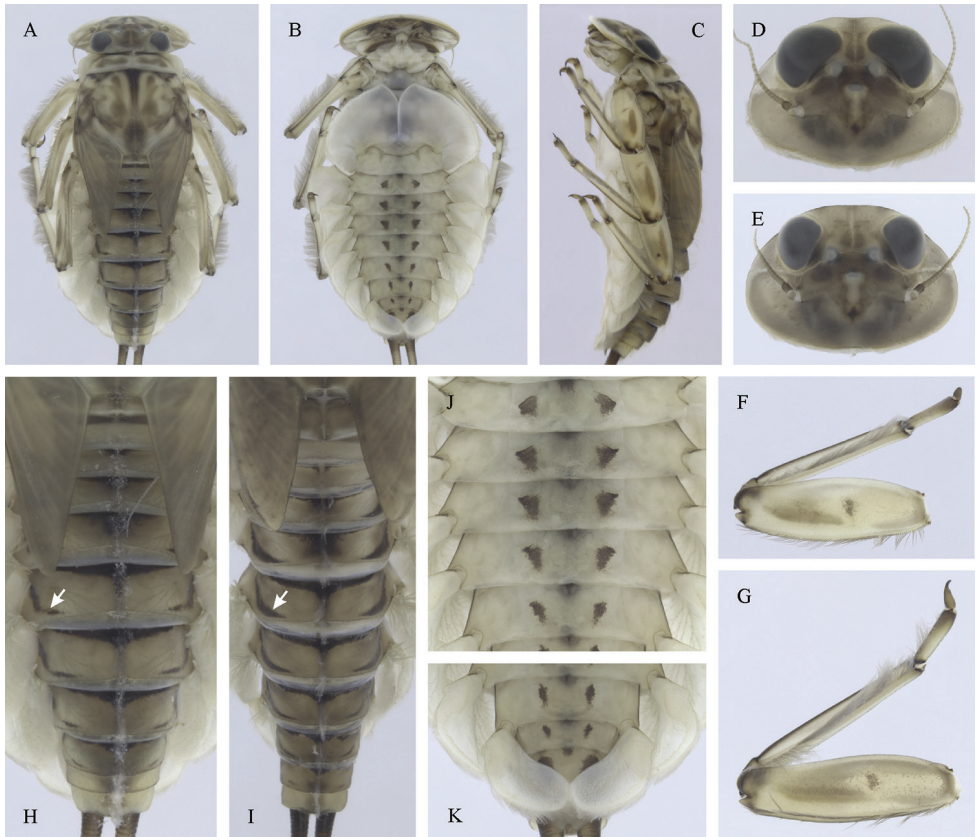


Figure 13. *Epeorus (Caucasiron) nigripilosus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F, -G** middle leg in dorsal view **H, -I** abdominal terga (arrows point on dorso-posteriorly extended lateral stripes) **J** abdominal sterna II-VI **K** gills VII (in natural position from ventral view).

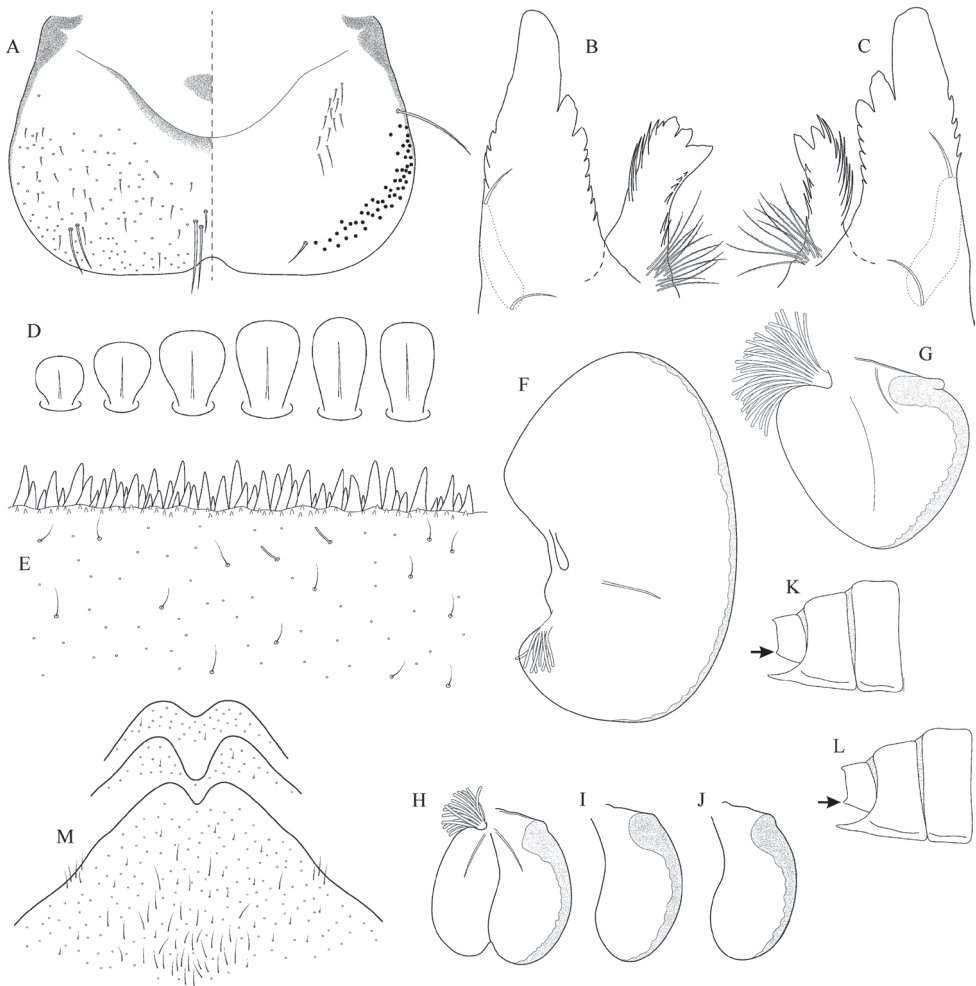


Figure 14. *Epeorus (Caucasiron) nigripilosus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of right mandible **C** incisors of left mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I, J** gill VII (in natural position from ventral view) variability in shape **K, L** abdominal segments VIII–X in lateral view (arrows point on postero-lateral projection) **M**, sternum IX of female with observed variability.

terga V–VII with lateral stripes extended dorso-posteriorly (Fig. 13H, I, arrows); (iii) tergum X with postero-lateral projections (Fig. 14K, L, arrows); (iv) femora with rounded medial hypodermal spot (Fig. 13F, G); (v) setae on abdominal terga hair-like (Fig. 14E); (vi) denticles along posterior margin of tergum VII strongly sclerotized and dense (Fig. 14E); (vii) gill plates VII (in natural position from ventral view) wide (Figs 13K, 14H–J); (viii) gill plates III with developed projection (Fig. 14G).

Remarks. Taxonomy. This species was described based on larvae from Georgia (Kistinka River) (Sinitshenkova 1976). Type series is deposited in IZ (Kluge 1995). Male imago was described by Braasch (1979) based on the material from the western

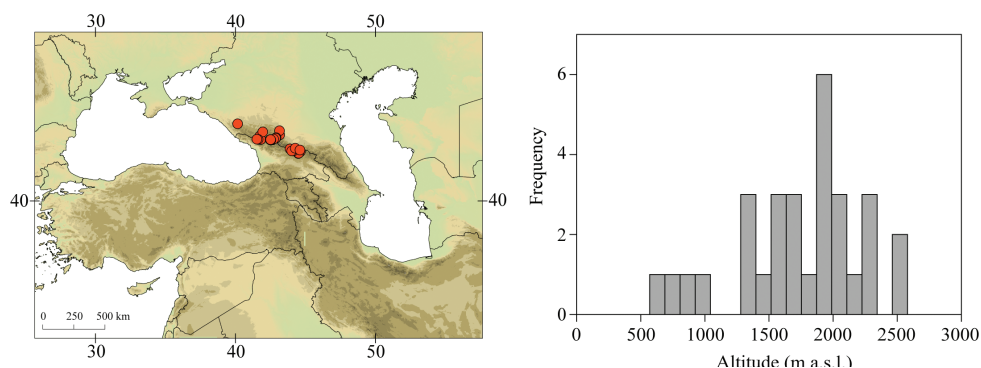


Figure 15. Geographical (left) and vertical (right) distribution of *Epeorus* (*Caucasiron*) *alpestris*.

Caucasus (Teberda River) associated with larvae according to similar coloration of abdominal terga and sterna. Female imago not described. Male genitalia similar to *E. (C.) caucasicus* according to Braasch (1979).

Epeorus (*Caucasiron*) *alpestris* (Braasch, 1979)

Figs 15–17

Iron alpestris Braasch, 1979

Epeorus (*Iron*) *alpestris* (Braasch, 1979); in Kluge (1988)

Epeorus (*Caucasiron*) *alpestris* (Braasch, 1979); in Kluge (1997b)

Type locality. Russia, The Karachay-Cherkess Republic, western Greater Caucasus, Teberda (Glacier Alibek – stream, 1800–1900 m a.s.l.).

Distribution. Georgia, south-western Russia. Species endemic to the Greater Caucasus (Fig. 15).

Habitat. Larvae inhabit small streams and rivers at middle and high altitude in the western and central Greater Caucasus. Altitudinal range of sampling sites 570–2580 m a.s.l (Fig. 15). Most frequently found at altitudes above 1200 m a.s.l. Often syntopic with *E. (C.) soldani* and at higher altitude with *E. (C.) sinitschenkova*.

Main morphological diagnostics of larvae. (i) abdominal terga V–VII with narrow stripe-like medial macula; widened on terga VIII–IX (Fig. 16G, H, arrows); (ii) abdominal sterna II–VI with rounded medial macula (Fig. 16B, I); (iii) femora without medial hypodermal spot (Fig. 16F); (iv) tergum X without postero-lateral projections (Fig. 17L); (v) gill plates III with well-developed projection (Fig. 17G); (vi) setae on abdominal terga hair-like (Fig. 17E); (vii) dorsal surface of labrum with sparse hair-like setae (Fig. 17A); (viii) gill plates VII (in natural position of ventral view) wide (Figs 16J, K, 17H–K).

Remarks. Taxonomy. This species was described based on the male imago and larva from western Greater Caucasus (Braasch 1979). The type series is currently deposited in SMNS. Imagines and larvae were associated based on the coloration of abdomen. Female imago not described.

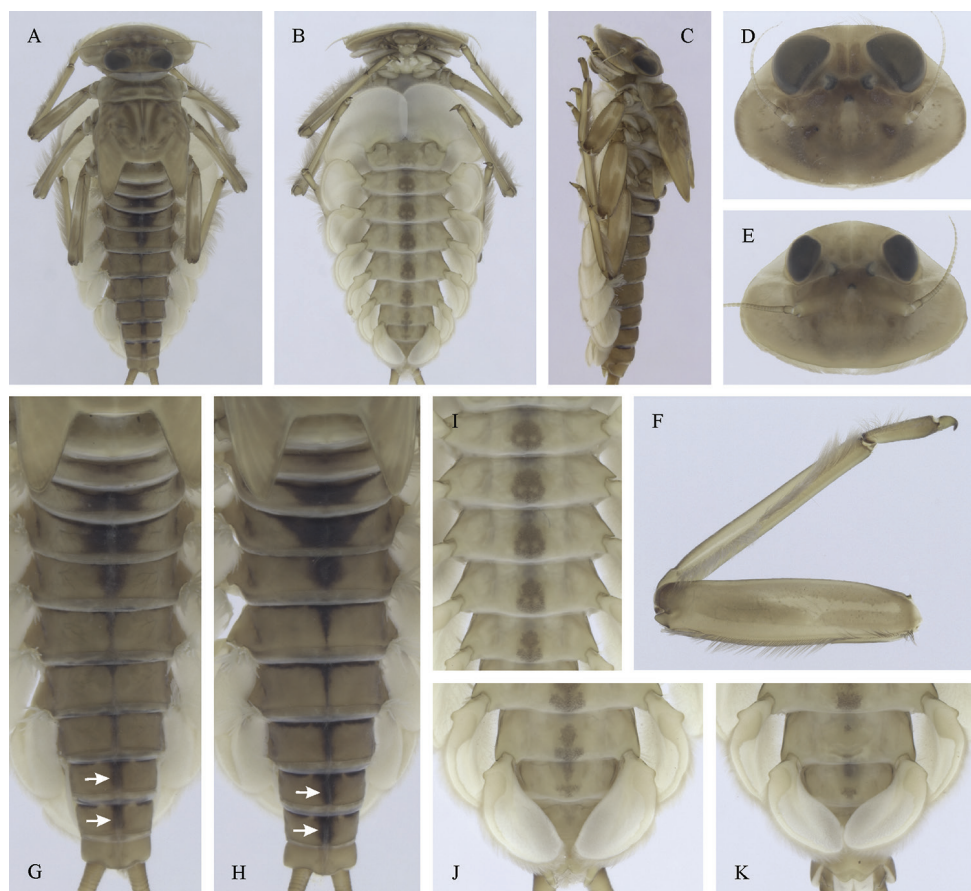


Figure 16. *Epeorus (Caucasiron) alpestris*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F** middle leg in dorsal view **G, H** abdominal terga (arrows point on widened medial maculae) **I** abdominal sterna II–VI **J, K** gills VII (in natural position from ventral view).

***Epeorus (Caucasiron) soldani* (Braasch, 1979)**

Figs 18–20

Iron soldani Braasch, 1979

Epeorus (Iron) soldani (Braasch, 1979); in Kluge (1988)

Epeorus (Caucasiron) soldani (Braasch, 1979); in Kluge (1997b)

Type locality. Russia, The Karachay-Cherkess Republic, western Greater Caucasus, Teberda (Glacier Alibek – stream, 1800–1900 m a.s.l.).

Distribution. Georgia, south-western Russia. Species endemic to the Greater Caucasus (Fig. 18).

Habitat. Larvae inhabit small streams and rivers at middle and high altitudes in the western and central Greater Caucasus. Frequently found above 1000 m a.s.l. Altitudinal

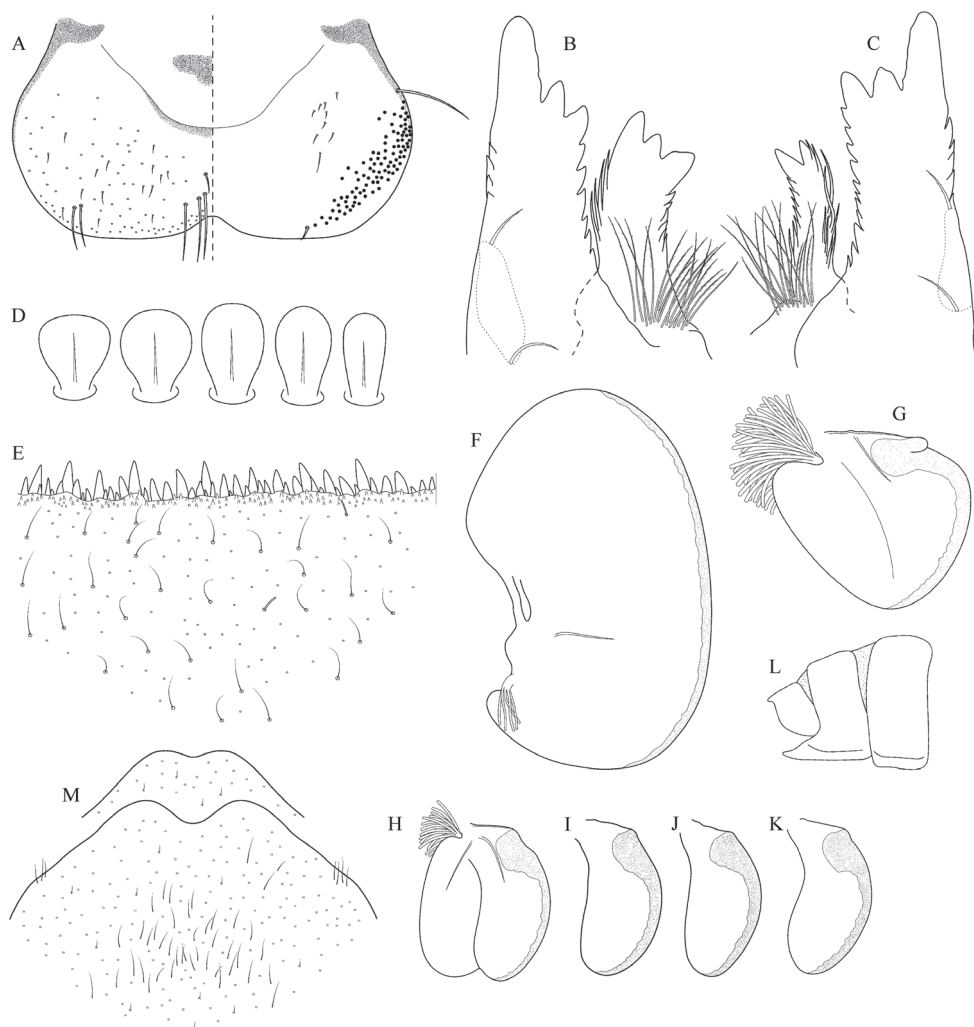


Figure 17. *Epeorus* (*Caucasiron*) *alpestris*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I–K** gill VII (in natural position from ventral view) variability in shape **L** abdominal segments VIII–X in lateral view **M** sternum IX of female with observed variability.

range of sampling sites 426–1900 m a.s.l. (Fig. 18). Often syntopic with *E. (C.) alpestris* and *E. (C.) sinitschenkova*.

Main morphological diagnostics of larvae. (i) abdominal terga V–VII with well-defined triangular medial maculae (Fig. 19H, I); (ii) abdominal sterna II–VI either without pattern or with indistinct pattern as on Fig. 19J, K; (iii) setae on abdominal terga wide at base (Fig. 20E); (iv) femora without medial hypodermal spot (Fig. 19F, G); (v) tergum X without postero-lateral projections (Fig. 20L); (vi) gill plates III with

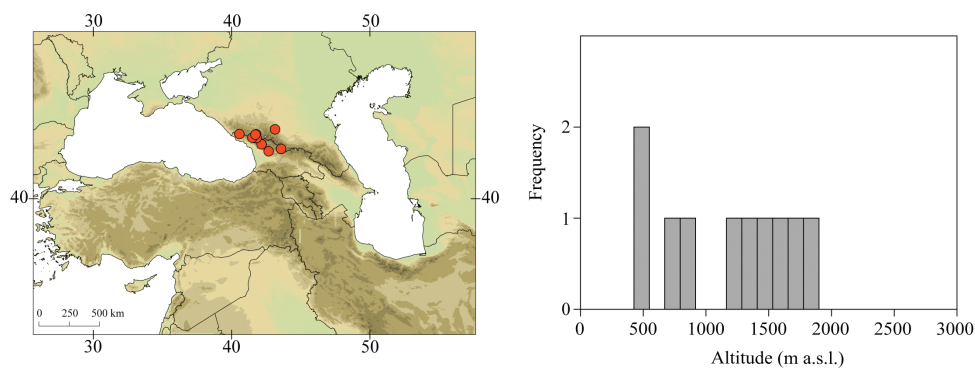


Figure 18. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) soldani*.



Figure 19. *Epeorus (Caucasiron) soldani*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F, G** middle leg in dorsal view **H, I** abdominal terga **J, K** abdominal sterna II–VI **L** gills VII (in natural position from ventral view).

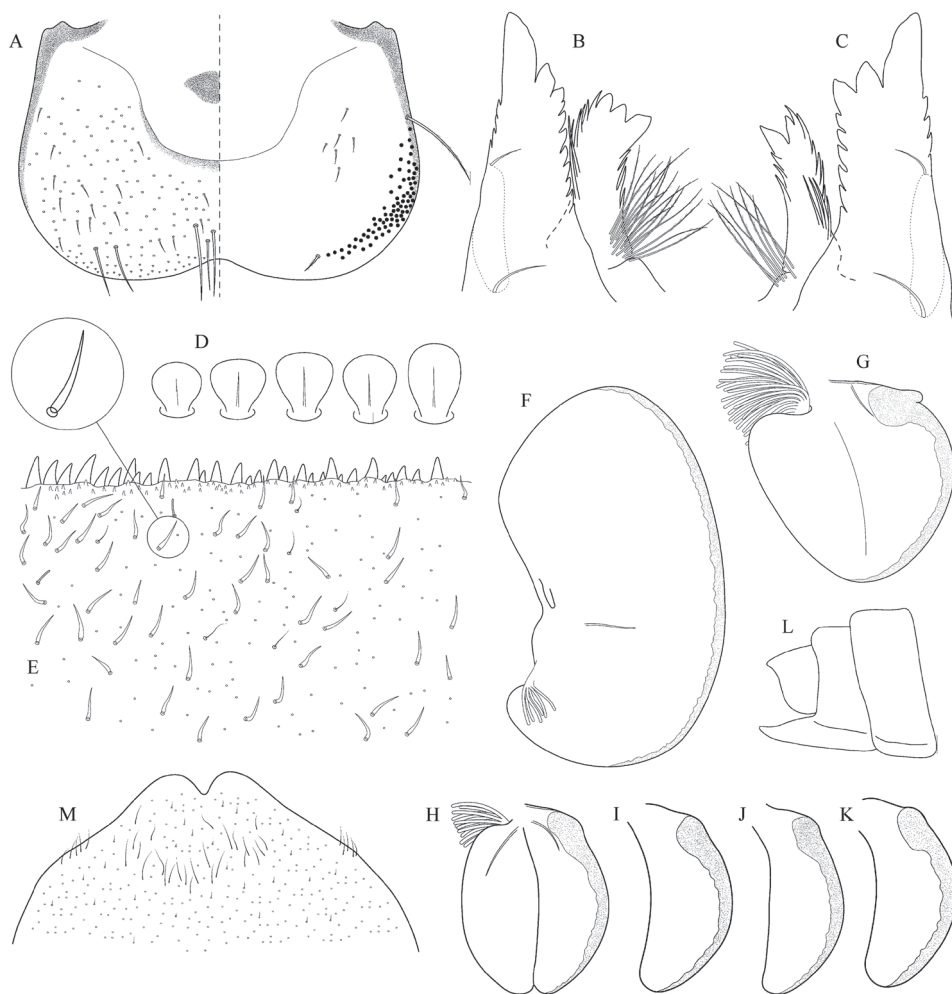


Figure 20. *Epeorus* (*Caucasiron*) *soldani*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII with detail of basally wide seta **F** gill I **G** gill III **H** gill VII (flattened on slide) **I–K** gill VII (in natural position from ventral view) variability in shape **L** abdominal segments VIII–X in lateral view **M** sternum IX of female.

well-developed projection (Fig. 20G); (vii) gill plates VII (in natural position of ventral view) narrow (Figs 19L; 20H–K); (viii) denticles along posterior margin of tergum VII relatively sparse and triangular (Fig. 20E).

Remarks. Taxonomy. This species was described based on male imago and larva from the western Greater Caucasus (Braasch 1979). The type series is currently deposited in SMNS. Larva associated with imago based on the coloration of abdomen. Female imago not described. The lineage *Caucasiron* sp. 7 detected by Hrivniak et al. (2020b) is distributed in Georgia and morphologically corresponds to *E. (C.) soldani*. Therefore, *E. (C.) soldani* may represent a species complex.

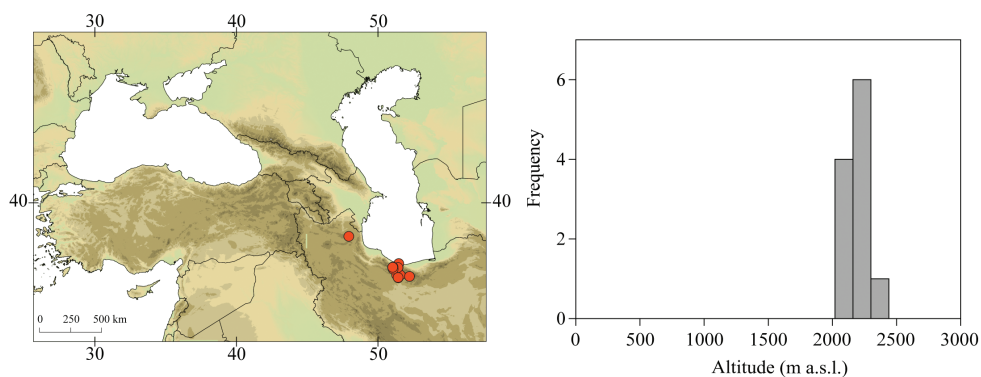


Figure 21. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) iranicus*.

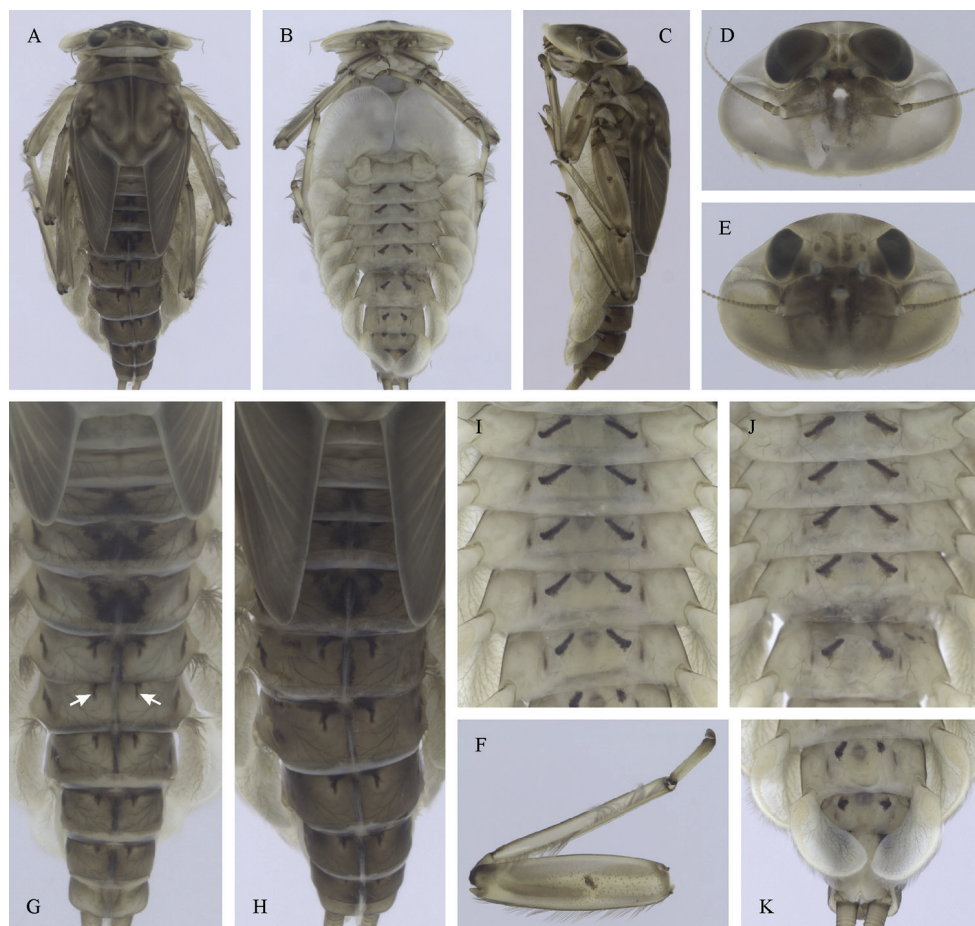


Figure 22. *Epeorus (Caucasiron) iranicus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F** middle leg in dorsal view **G, H** abdominal terga (arrows point antero-lateral stripes of medial macula) **I, J** abdominal sterna II–VI **K** gills VII (in natural position from ventral view).



Figure 23. *Epeorus* (*Caucasiron*) *iranicus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I** gill VII (in natural position from ventral view) **J** sternum IX of female **K, L** abdominal segments VIII–X in lateral view (arrow points on postero-lateral projection).

***Epeorus* (*Caucasiron*) *iranicus* (Braasch & Soldán, 1979)**

Figs 21–23

Iron caucasicus iranicus Braasch & Soldán, 1979

Epeorus (*Caucasiron*) *caucasicus iranicus* (Braasch & Soldán, 1979); in Bojková et al. (2018)

Epeorus (*Caucasiron*) *iranicus* (Braasch & Soldán, 1979); in Hrivniak et al. (2020b)

Type locality. Iran, Tehran Province, river in the Darban-Tal (Darban Valley), 2100 m a.s.l.

Distribution. Northern Iran. Species endemic to the Alborz Mountains (Fig. 21).

Habitat. Larvae inhabit streams at altitudes above 2000 m a.s.l. in the western and central Alborz. Altitudinal range of sampling sites 2020–2440 m a.s.l. (Fig. 21). Often syntopic with *E. (C.) alborzicus*.

Main morphological diagnostics of larvae. (i) abdominal sterna II–VI with a pair of oblique stripes; nerve ganglia often with stripes or spots (Fig. 22B, I, J); (ii) abdominal terga V–VII with stripe-like medial macula and a pair of distinct antero-lateral stripes (Fig. 22G, arrows); (iii) femora with rounded medial hypodermal spot (Fig. 22F); (iv) gill III with well-developed projection (Fig. 23G); (v) setae on abdominal terga hair-like (Fig. 23E); (vi) tergum X with poorly developed postero-lateral projections (Fig. 23K, arrow) or without postero-lateral projections (Fig. 23L).

Remarks. Morphology. Coloration pattern on abdominal sterna as in *E. (C.) caucasicus* (Fig. 4J), similar pattern in *E. (C.) zagrosicus* (Fig. 46I).

Taxonomy. This species was described as a subspecies of *E. (C.) caucasicus* based on larvae collected in the Alborz Mts. (Braasch and Soldán 1979). Elevated to species level by Hrivniak et al. (2020b) based on a phylogenetic analysis of all Caucasian *Epeorus* (*Caucasiron*) species. The holotype probably lost. Paratypes are currently deposited in SMNS and Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic (IECA). Imagines and subimagines not described.

Epeorus (Caucasiron) sinitschenkova (Braasch & Zimmerman, 1979)

Figs 24–26

Iron sinitschenkova Braasch & Zimmermann, 1979

Epeorus (Iron) sinitschenkova (Braasch & Zimmermann, 1979); in Kluge (1995)

Epeorus (Caucasiron) sinitschenkova (Braasch & Zimmermann, 1979); in Kluge (2004)

Type locality. Russia, the Kabardino-Balkarian Republic, central Greater Caucasus, right tributary of Dongoserun (Donguz-Orun-Baksan) River (2100 m a.s.l.).

Distribution. Georgia, south-western Russia. Species endemic to the Greater Caucasus (Fig. 24).

Habitat. Larvae inhabit small streams and rivers at middle and high altitude in the western and central Greater Caucasus. Altitudinal range of sampling sites 760–2580 m a.s.l. (Fig. 24). Most frequently found above 1800 m a.s.l. Often syntopic with *E. (C.) alpestris* and at lower altitude with *E. (C.) soldani*.

Main morphological diagnostics of larvae. (i) abdominal terga V–VII with narrowed triangular medial macula and a pair of anterolateral spots (Fig. 25H; arrows); (ii) abdominal sterna without coloration pattern (Fig. 25B, I); (iii) femora without medial hypodermal spot (Fig. 25F, G); (iv) gill plates VII (in natural position from ventral view) narrow (Figs 25J, 26H–K); (v) gill plates III with poorly developed projection (Fig. 26G); (vi) setae on abdominal terga not distinctly wide at base, often elongated (Fig. 26E); (vii) tergum X without postero-lateral projections (Fig. 26L).

Remarks. Taxonomy. Original description based on male imago and larva from the Greater Caucasus (Braasch and Zimmermann 1979). The type series is currently

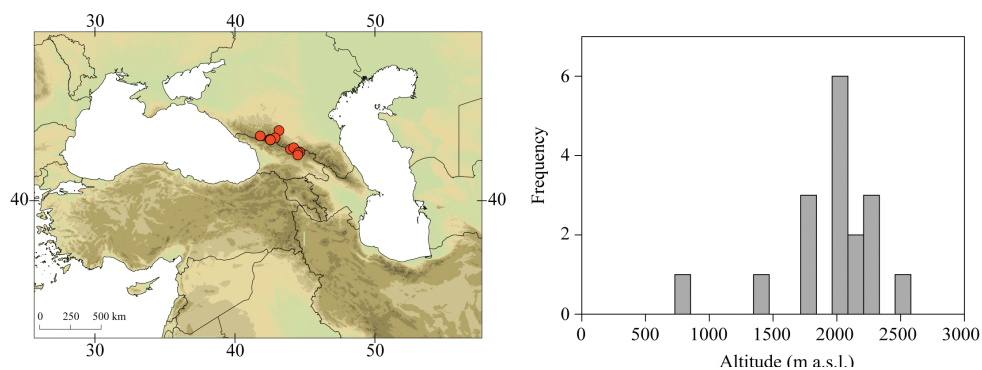


Figure 24. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) sinitschenkova*.

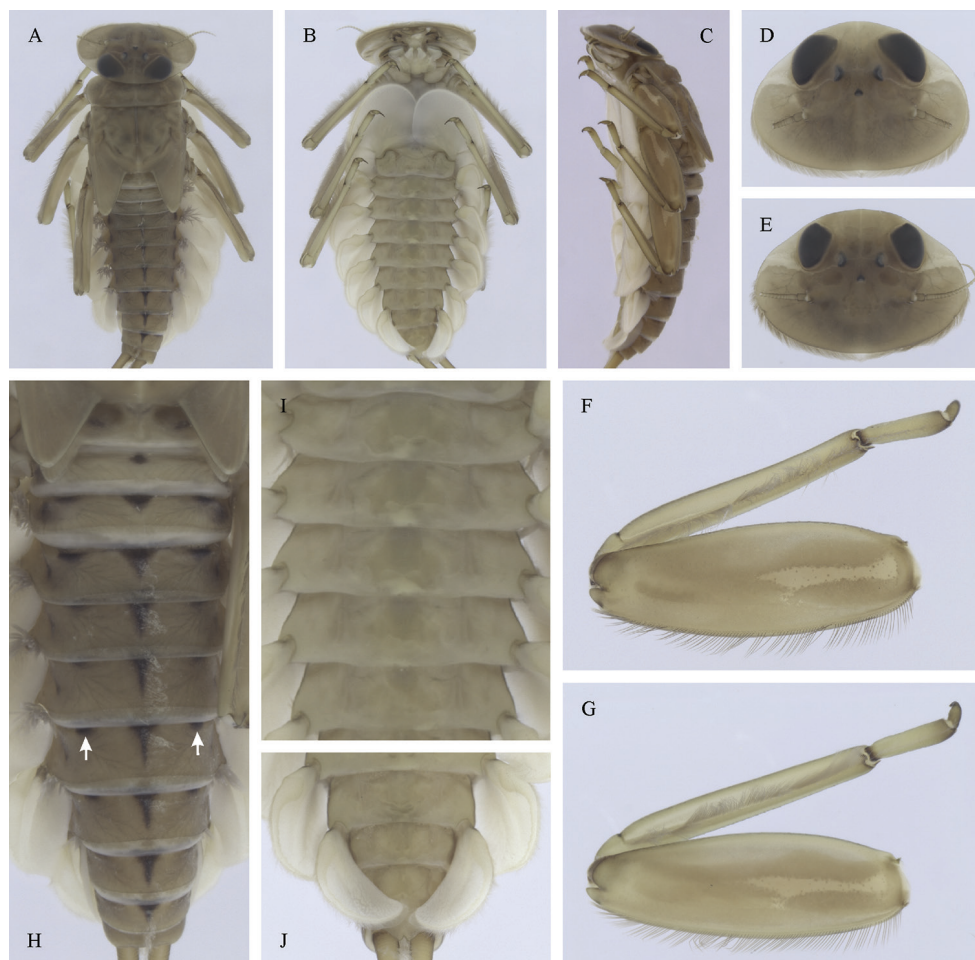


Figure 25. *Epeorus (Caucasiron) sinitschenkova*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F, G** middle leg in dorsal view **H** abdominal terga (arrows point on anterolateral spots) **I** abdominal sterna II–VI **J** gills VII (in natural position from ventral view).

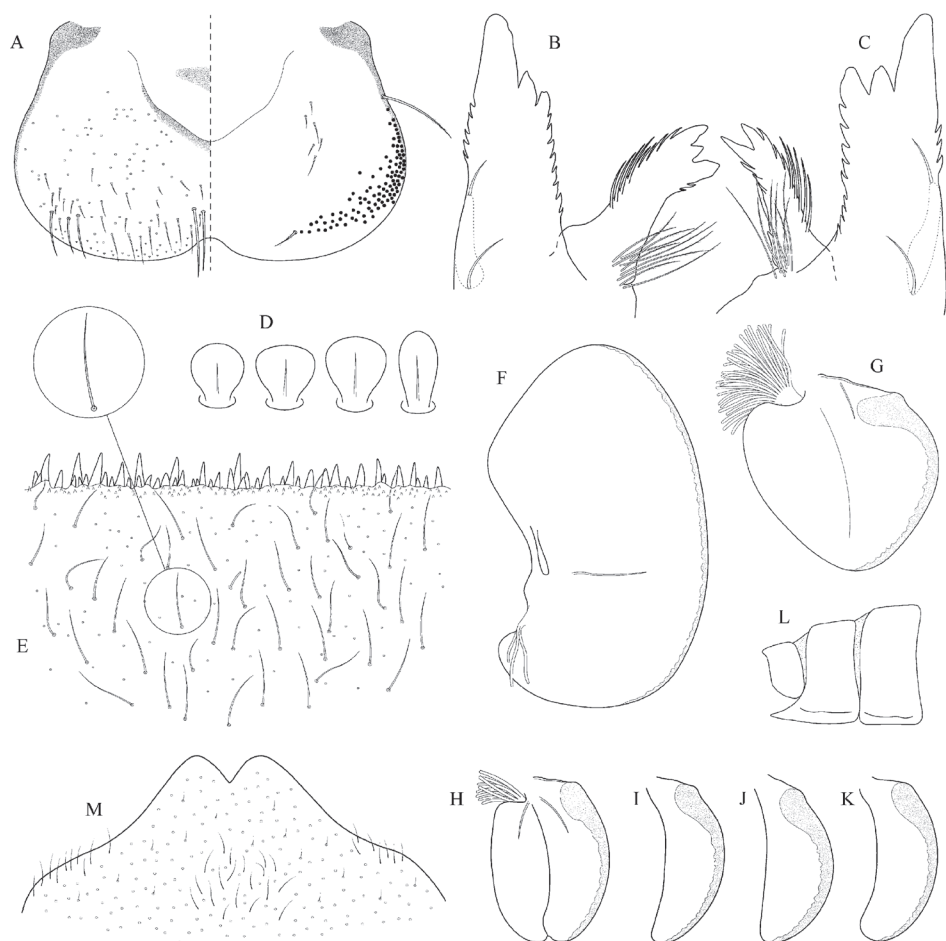


Figure 26. *Epeorus (Caucasiron) sinitschenkova*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII with detail of slightly widened elongated seta **F** gill I **G** gill III **H** gill VII (flattened on slide) **I–K** gill VII (in natural position from ventral view) variability in shape **L** abdominal segments VIII–X in lateral view **M** sternum IX of female.

deposited in SMNS. Female imago not described in detail. The association of imagines and larvae based on the colour pattern of abdominal terga and sterna in material from the same locality.

***Epeorus (Caucasiron) longimaculatus* (Braasch, 1980)**

Figs 27–29

Iron longimaculatus Braasch, 1980

Epeorus (Caucasiron) longimaculatus (Braasch, 1980); in Kluge (2004)

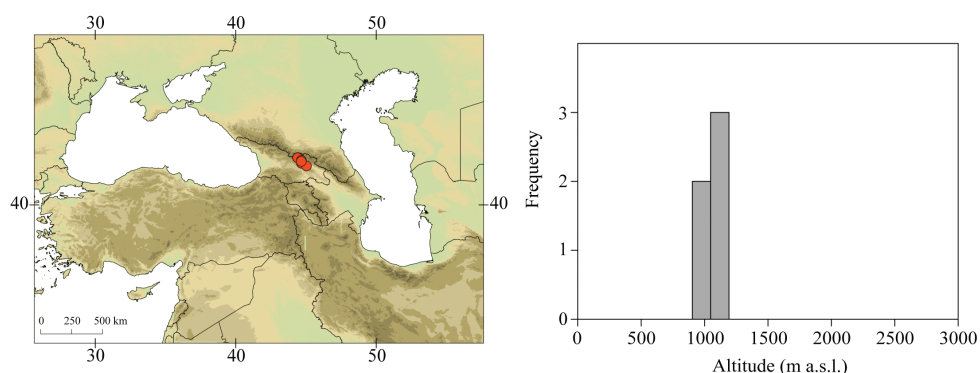


Figure 27. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) longimaculatus*.



Figure 28. *Epeorus (Caucasiron) longimaculatus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F-H** middle leg in dorsal view **I-K** abdominal terga **L, M** gills VII (in natural position from ventral view) **N-P** abdominal sterna II-VI.

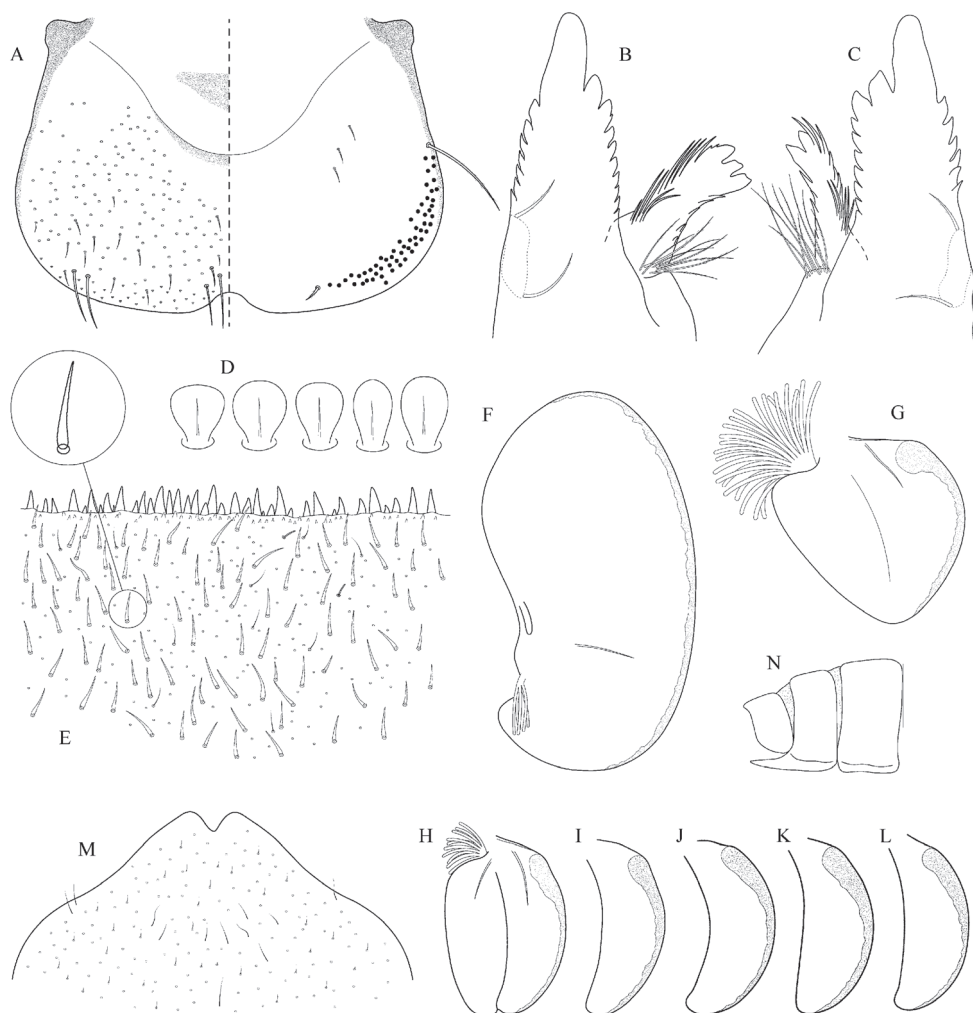


Figure 29. *Epeorus (Caucasiron) longimaculatus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII with detail of basally wide seta **F** gill I **G** gill III **H** gill VII (flattened on slide) **I–L** gill VII (in natural position from ventral view) variability in shape **M** sternum IX of female **N** abdominal segments VIII–X in lateral view.

Type locality. Georgia, Mtskheta-Mtianeti Region, central Greater Caucasus, tributary of Aragvi River, 3 km above Pasanauri (1400–1500 m a.s.l.).

Distribution. Georgia. Species endemic to the Greater Caucasus (Fig. 27).

Habitat. Larvae inhabit small streams and rivers at middle altitude in the central Greater Caucasus. Altitudinal range of sampling sites 903–1193 m a.s.l. (Fig. 27).

Main morphological diagnostics of larvae. (i) femora with elongated medial hypodermal spot (Fig. 28F–H); (ii) setae on abdominal terga wide at base (Fig. 29E);

(iii) gill plates III without distinct projection (Fig. 29G); (iv) gill plates VII (in natural position from ventral view) narrow (Figs 28L, M, 29H–L); (v) denticles along posterior margin of tergum VII narrowed (Fig. 29E); (vi) abdominal terga V–VII with narrowed triangular medial macula (Fig. 28I–K); (vii) abdominal sterna without coloration pattern (Fig. 28B, N–P); (viii) tergum X without postero-lateral projections (Fig. 29N); (ix) shape of head of male ellipsoid (Fig. 28D).

Remarks. Taxonomy. This species described based on male subimago and larva collected in central Greater Caucasus (Braasch 1980). The type series is currently deposited in SMNS. Larva associated with the subimago according to the coloration of abdomen. Male and female imagines not described.

Epeorus (*Caucasiron*) *insularis* (Braasch, 1983)

Figs 30–32

Iron znojtkoi insularis Braasch, 1983

Epeorus (*Caucasiron*) *insularis* (Braasch, 1983); in Hrivniak et al. (2020b)

Type locality. Greece, Samos Island, stream east of Pírgos, 37°3'N/26°49'E; 300 m a.s.l.

Distribution. Known only from few sites in Samos Island (Fig. 30).

Habitat. Larvae inhabit small forested streams at 128–440 m a.s.l. (Fig. 30).

Main morphological diagnostics of larvae. (i) abdominal terga V–VII with T-shaped medial macula (Fig. 31I); (ii) abdominal sterna V–VII with reddish to brownish longitudinal stripe (Fig. 31B, J); (iii) tergum X without postero-lateral projections (Fig. 32J); (iv) gill plates VII (in natural position from ventral view) narrow (Figs 31K, 32H, I); (v) gill plates III with well-developed projection (Fig. 32G); (vi) setae on abdominal terga hair-like (Fig. 32E); (vii) denticles along posterior margin of tergum VII relatively short and poorly sclerotized (Fig. 32E).

Remarks. Morphology. Coloration of abdominal terga and sterna as in *E. (C.) znojtkoi* s.l. (Fig. 7N–P).

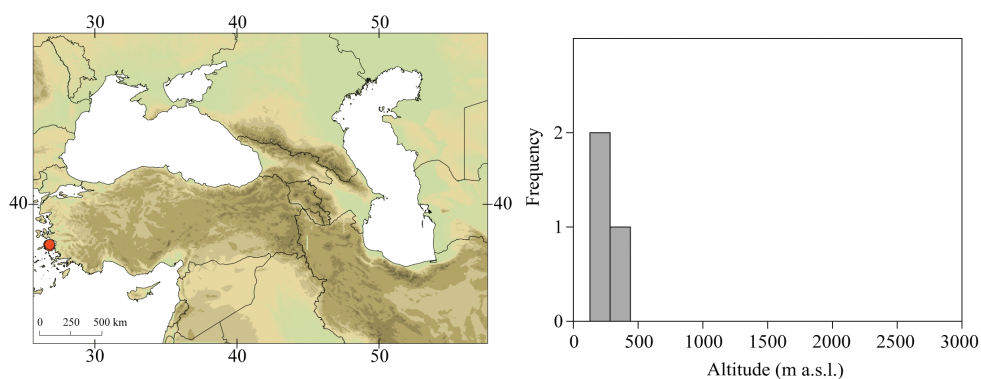


Figure 30. Geographical (left) and vertical (right) distribution of *Epeorus* (*Caucasiron*) *insularis*.



Figure 31. *Epeorus (Caucasiron) insularis*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F–H** middle leg in dorsal view **I** abdominal terga **J** abdominal sterna II–VI **K** gills VII (in natural position from ventral view).

Taxonomy. This species was described by Braasch (1983) based on imagines as a subspecies of *E. (C.) znojkoii*. Elevated to species level in Hrivniak et al. (2020b) based on a phylogenetic analysis of all Caucasian *Epeorus (Caucasiron)* species. The type series is currently deposited in SMNS.

***Epeorus (Caucasiron) bicolliculatus* Hrivniak, 2017**

Figs 33–35

Epeorus alpicola (Eaton, 1871); in Türkmen and Kazancı (2015), partim
Epeorus sylvicola (Pictet, 1865); in Türkmen and Kazancı (2015), partim
Epeorus (Caucasiron) sp.; in Martynov et al. (2016)

Type locality. Georgia, Autonomous Republic of Adjara, vicinity of Chakhati village, Kintrishi River; 41°45'43"N/41°58'34"E; 325 m a.s.l.

Distribution. Georgia, north-eastern Turkey, Armenia, south-western Russia (Fig. 33).

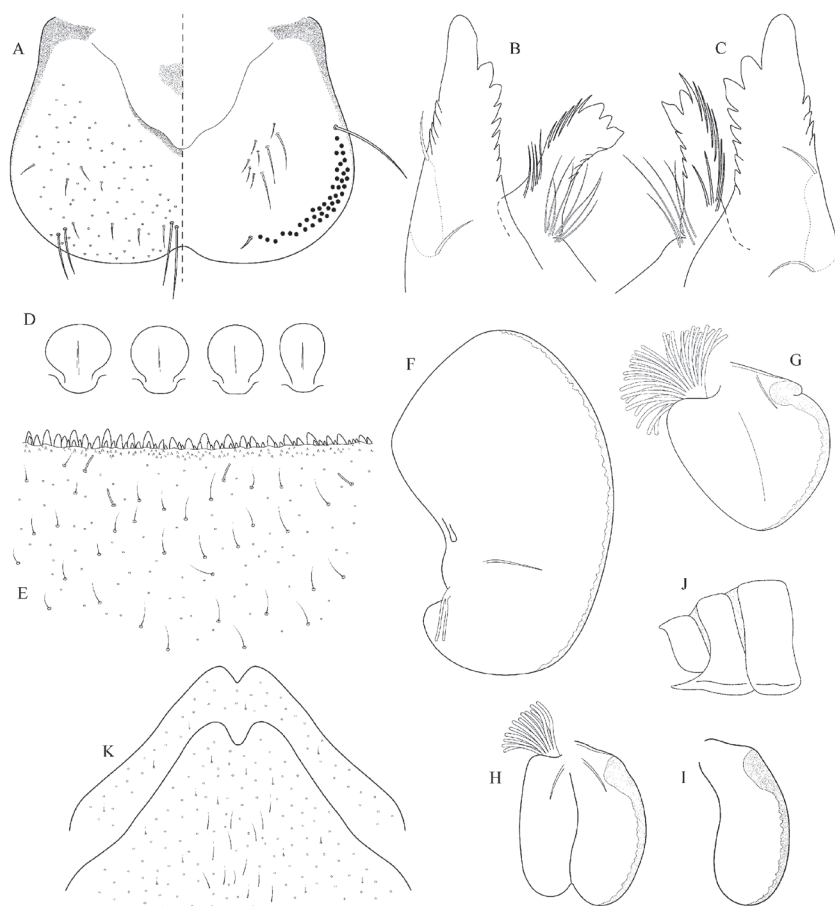


Figure 32. *Epeorus* (*Caucasiron*) *insularis*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I** gill VII (in natural position from ventral view) **J** abdominal segments VIII–X in lateral view **K** sternum IX of female with observed variability.

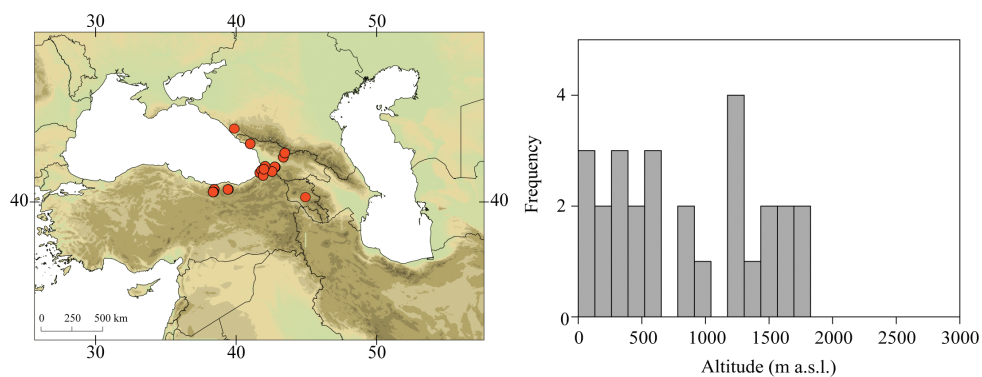


Figure 33. Geographical (left) and vertical (right) distribution of *Epeorus* (*Caucasiron*) *bicolliculatus*.



Figure 34. *Epeorus* (*Caucasiron*) *bicolliculatus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F** middle leg in dorsal view **G** abdominal terga **H** abdominal terga VI–X (arrows point on postero-medial protuberances) **I** gills VII (in natural position from ventral view) **J–L** abdominal sterna II–VI.

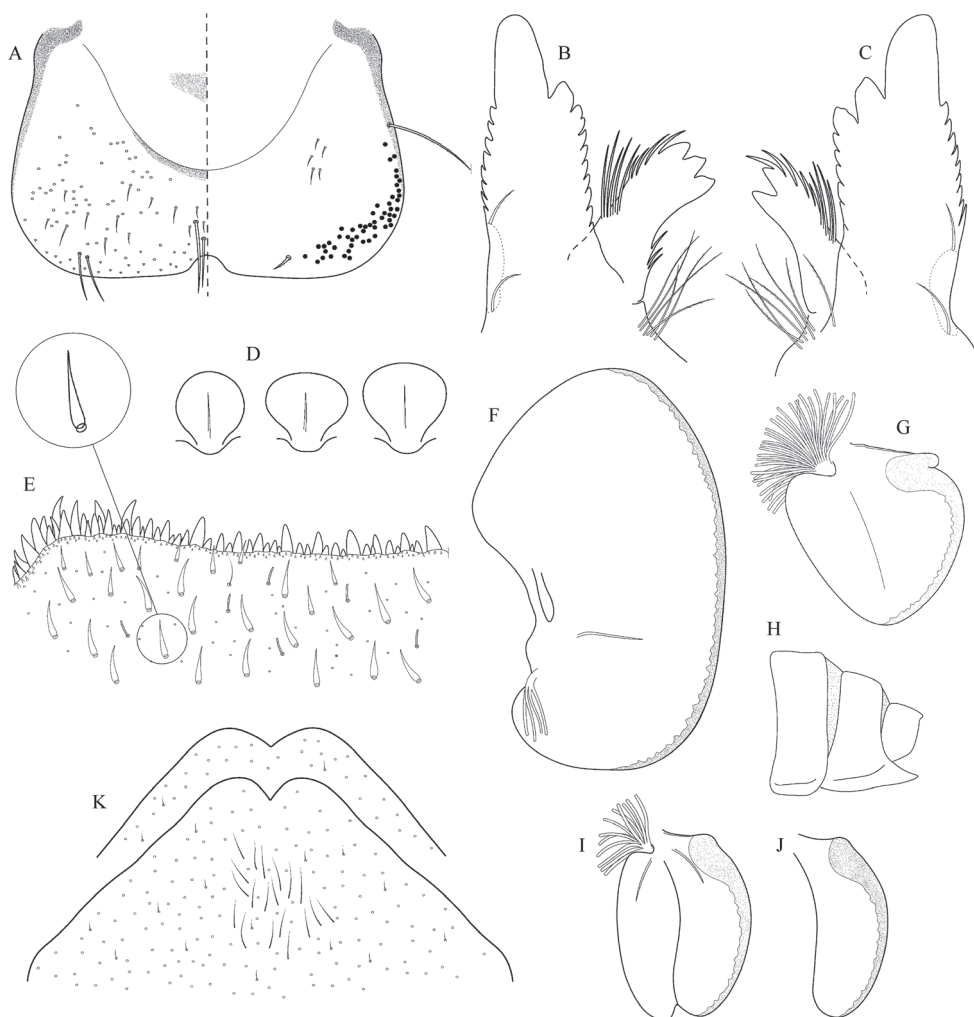


Figure 35. *Epeorus* (*Caucasiron*) *bicolliculatus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII with detail of basally wide setae **F** gill I **G** gill III **H** abdominal segments VIII–X in lateral view **I** gill VII (flattened on slide) **J** gill VII (in natural position from ventral view) **K** sternum IX of female with observed variability.

Habitat. Larvae inhabit streams and rivers of different sizes, from to middle-sized rivers at low altitude to small streams at high altitudes. Altitudinal range of sampling sites 40–1804 m a.s.l. (Fig. 33).

Main morphological diagnostics of larvae. (i) abdominal terga II–IX with paired postero-medial protuberances (Fig. 34H, arrows); (ii) abdominal terga V–VII with stripe-like medial macula, often anteriorly and posteriorly widened, and with antero-lateral stripes (Fig. 34G, H); (iii) abdominal sterna as on Fig. 34B, J–L; (iv) setae on abdominal terga wide at base (Fig. 35E); (v) gill plates VII (in natural position from ventral view)

narrow (Figs 34I, 35I, J); (vi) femora without medial hypodermal spot (Fig. 34F, blurred macula may be present in darker specimens); (vii) tergum X without postero-lateral projections (Fig. 35H); (viii) gill plates III with well-developed projection (Fig. 35G).

Remarks. Taxonomy. This species was described based on the larva, male subimago and imago (associated by rearing), female imago (associated by DNA analysis) and eggs. Material was collected from the western Lesser Caucasus (Hrivniak et al. 2017). The type series is currently deposited in IECA.

***Epeorus (Caucasiron) turcicus* Hrivniak, Türkmen & Kazancı, 2019**

Figs 36–38

Type locality. Turkey, Artvin Province, Camili Village, Merata Plateau, unnamed mountain stream; 41°26'30"N/42°04'41"E; 2190 m a.s.l.

Distribution. North-eastern Turkey, Georgia (Fig. 36). Known only from few sites in the Camili (Machakheli) District in Turkey and central Georgia.

Habitat. Larvae inhabit small streams at middle and high altitudes. Altitudinal range of sampling sites 928–2388 m a.s.l. (Fig. 36).

Main morphological diagnostics of larvae. (i) femora with medial hypodermal spot (Fig. 37F); (ii) abdominal terga V–VII with stripe-like medial macula with lateral stripes extended dorso-posteriorly (Fig. 37G, arrows); (iii) abdominal sterna without coloration pattern, nerve ganglia often coloured (Fig. 37B, H); (iv) gill plates VII (in natural position from ventral view) narrow (Figs 37I, 38H–K); (v) setae on abdominal terga hair-like like (Fig. 38E); (vi) tergum X without postero-lateral projections (Fig. 38L); (vii) gill plates III with well-developed projection (Fig. 38G).

Remarks. Taxonomy. This species described based on larvae collected from Pontic Mts. (Hrivniak et al. 2019). Imagines not described. The type series is currently deposited in IECA and collection of N. Kazancı and G. Türkmen (Hacettepe University, Department of Biology, Biomonitoring Laboratory, Turkey).

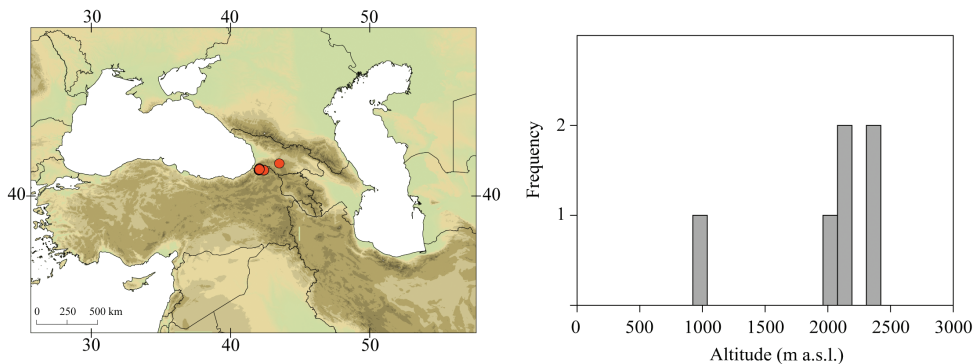


Figure 36. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) turcicus*.



Figure 37. *Epeorus* (*Caucasiron*) *turcicus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F** middle leg in dorsal view **G** abdominal terga (arrows point on dorso-posteriorly extended lateral stripes) **H** abdominal sterna II–VI **I** gill VII (in natural position from ventral view).

***Epeorus* (*Caucasiron*) *alborzicus* Hrivniak & Sroka, 2020**

Figs 39–41

Type locality. Iran, Mazandaran Province, Panjab village, unnamed brook (left tributary of Haraz River); 36°05'52.818"N/52°15'15.987"E (locality no. 152); 955 m a.s.l.

Distribution. Northern Iran. Species endemic to the Alborz Mountains (Fig. 39).

Habitat. Larvae inhabit small rivers at middle and high altitude in the central Alborz. Altitudinal range of sampling sites 750–2438 m a.s.l. (Fig. 39). Most frequently found at altitudes above 1000 m a.s.l. At high altitudes often syntopic with *E. (C.) iranicus*.

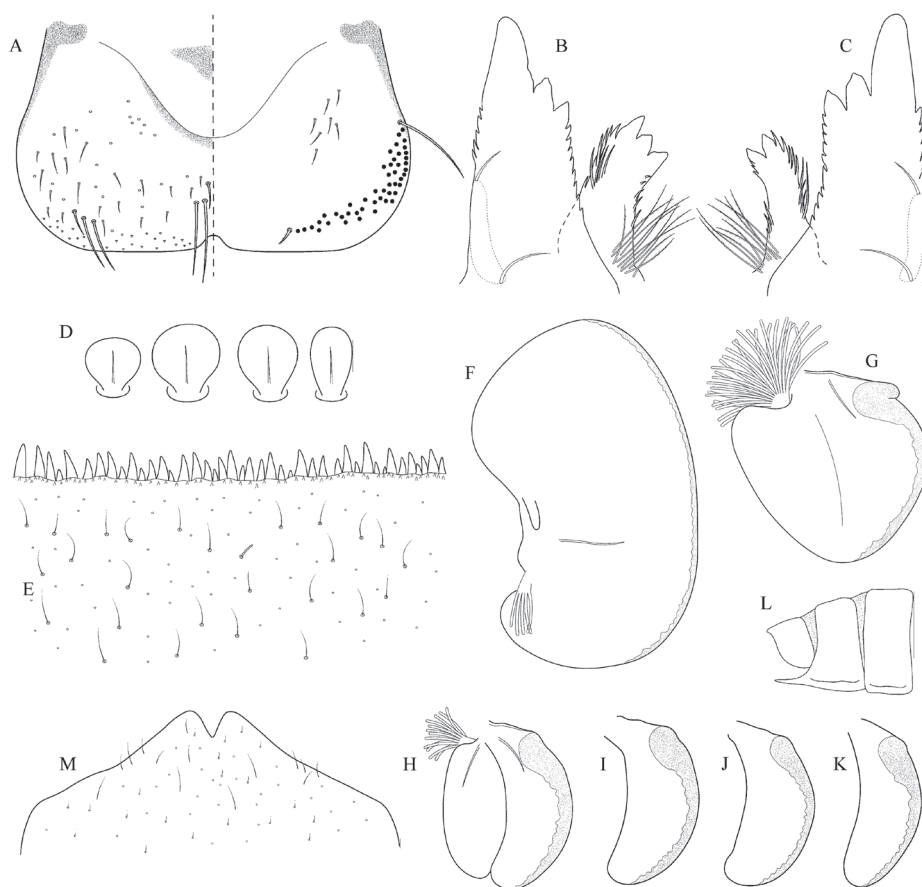


Figure 38. *Epeorus (Caucasiron) turcicus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I–K** gill VII (in natural position from ventral view) variability in shape **L** abdominal segments VIII–X in lateral view **M** sternum IX of female.

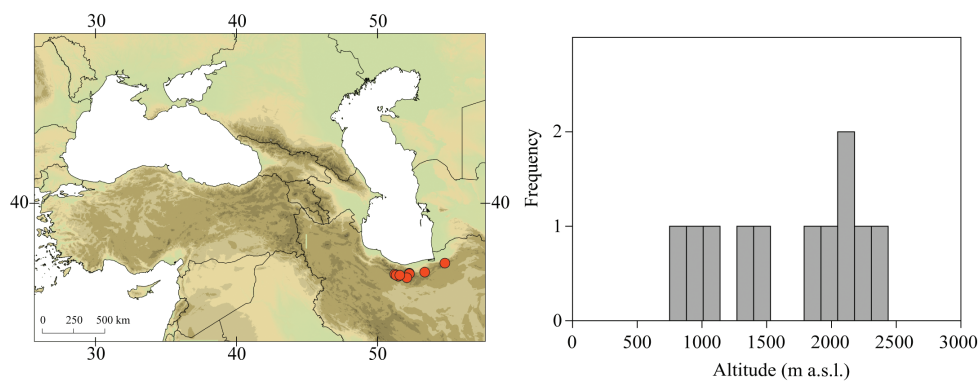


Figure 39. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) alborzicus*.



Figure 40. *Epeorus* (*Caucasiron*) *alborzicus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F, G** middle leg in dorsal view **H, I** abdominal terga **J, K** gills VII (in natural position from ventral view) **L–N** abdominal sterna II–VI.

Main morphological diagnostics of larvae. (i) abdominal terga as on Fig. 40H, I; (ii) abdominal sterna II–VI with circular central medial macula of various intensity (Fig. 40B, L–N); (iii) tergum X with postero-lateral projections (Fig. 41K, arrow), (iv) femora without medial hypodermal spot (Fig. 40F, G); (v) gill plates VII (in natural position from ventral view) wide (Figs 40J, K, 41H–J);

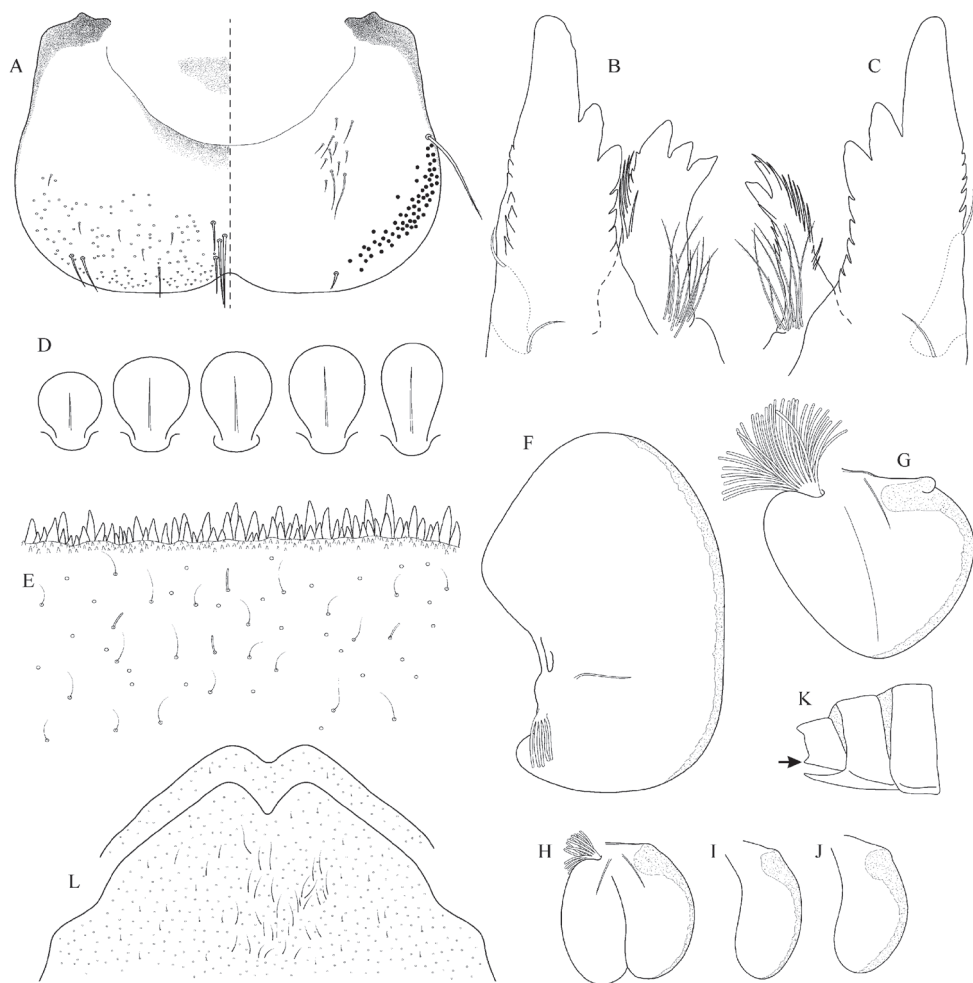


Figure 41. *Epeorus (Caucasiron) alborzicus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I, J** gill VII (in natural position from ventral view) variability in shape **K** abdominal segments VIII–X in lateral view (arrow points on postero-lateral projection) **L** sternum IX of female with observed variability.

(vi) setae on abdominal terga hair-like (Fig. 41E); (vii) gill plates III with well-developed projection (Fig. 41G).

Remarks. Taxonomy. This species was described based on larvae collected from Alborz Mts. (Hrivniak et al. 2020a). Imagines not described.

The type series is currently deposited in SMNS, IECA, and Natural History Museum and Genetic Resources, Department of Environment, Tehran, Iran (MMTT_DOE).

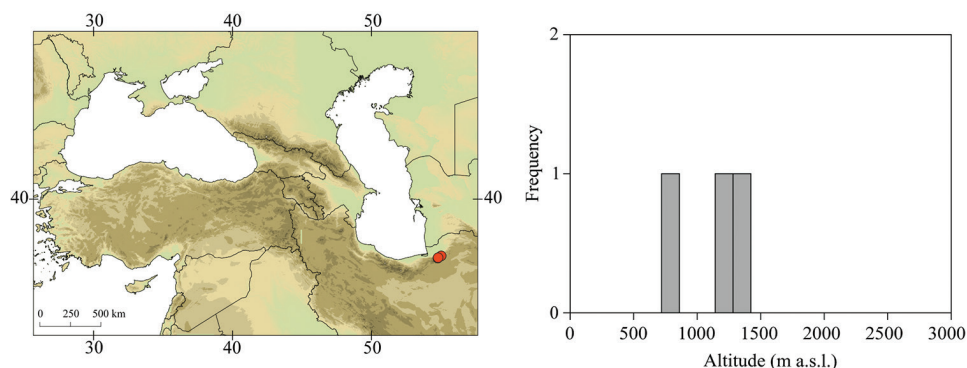


Figure 42. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) shargi*.

***Epeorus (Caucasiron) shargi* Hrivniak & Sroka, 2020**

Figs 42–44

Type locality. Iran, Golestan Province, Shirinabad village, unnamed river; 36°48'01.44"N/ 55°01'05.78"E (locality no. 108); 740 m a.s.l.

Distribution. Northern Iran. Known only from three sites in the eastern Alborz (Fig. 42).

Habitat. Larvae inhabit streams at middle altitude, 740–1450 m a.s.l. (Fig. 42).

Main morphological diagnostics of larvae. (i) abdominal terga V–VII with triangular or T-shaped medial macula (Fig. 43I–K); (ii) abdominal sterna without coloration pattern (Fig. 43B, L); (iii) tergum X without postero-lateral projections (Fig. 44J); (iv) femora with medial hypodermal spot (Fig. 43F–H); (v) gill plates VII (in natural position from ventral view) wide (Figs 43M, 44H, I); (vi) setae on abdominal terga hair-like (Fig. 44E); (vii) gill plates III with well-developed projection (Fig. 44G); (viii) shape of head of male oval trapezoidal (Fig. 43D).

Remarks. Taxonomy. This species was described based on larvae collected from Alborz Mts. (Hrivniak et al. 2020a). Imagines not described. The type series is currently deposited in SMNS, IECA, and MMTT_DOE.

***Epeorus (Caucasiron) zagrosicus* Hrivniak & Sroka, 2020**

Figs 45–47

Type locality. Iran, Chaharmahal and Bakhtiari Province, Dimeh village, Chehme-Dimeh River, 32°30'11.62"N, 50°13'04.45"E; 2220 m a.s.l.

Distribution. South-western Iran. Known only from few sites in the central Zagros (Fig. 45).

Habitat. Larvae inhabit streams and rivers at high altitude, 1721–2402 m a.s.l. (Fig. 45).



Figure 43. *Epeorus (Caucasiron) shargi*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F–H** middle leg in dorsal view **I–K** abdominal terga **L** abdominal sterna II–VI **M** gills VII (in natural position from ventral view).

Main morphological diagnostics of larvae. (i) abdominal sterna II–VI with a pair of anteriorly widened oblique stripes (Fig. 46B, I, arrows); (ii) abdominal terga V–VII with triangular, stripe-like or crown-like medial macula (Fig. 46G, H), often with lateral stripes extended dorso-posteriorly (Fig. 46H, arrows); (iii) tergum X with postero-lateral projections (Fig. 47L, M, arrows); (iv) femora with medial hypodermal spot (Fig. 46F); (v) setae on abdominal terga hair-like (Fig. 47E); (vi) gill plates III with well-developed projection (Fig. 47G); (vii) gill plates VII (in natural position from ventral view) relatively wide (Figs 46J, K, 47H–K).

Remarks. Taxonomy. This species was described based on larvae collected from Zagros Mts. (Hrivniak et al. 2020a). Imagines not described. The type series is currently deposited in SMNS, IECA, and MMTT_DOE. The lineage *Caucasiron* sp. 2 detected by Hrivniak et al. (2020b) is distributed in Turkey (Taurus Mts.) and morphologically corresponds to *E. (C.) zagrosicus*. Therefore, *E. (C.) zagrosicus* may represent a species complex.

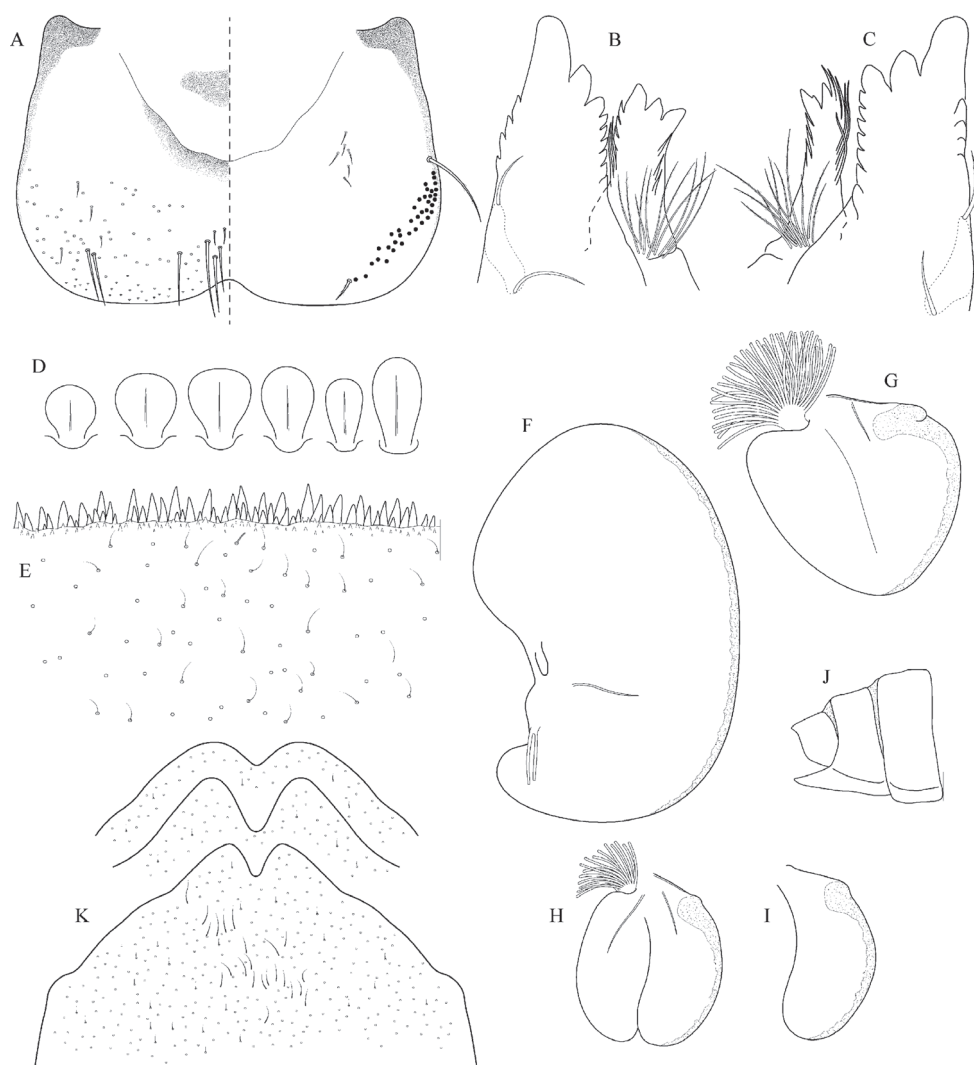


Figure 44. *Epeorus* (*Caucasiron*) *shargi*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I** gill VII (in natural position from ventral view) **J** abdominal segments VIII–X in lateral view **K** sternum IX of female with observed variability.

Concluding remarks

This contribution represents the first complete source of information for the routine identification of the larvae of all fifteen *Caucasiron* species occurring in the Caucasus and adjacent areas. It is possible that additional new *Caucasiron* species will be de-

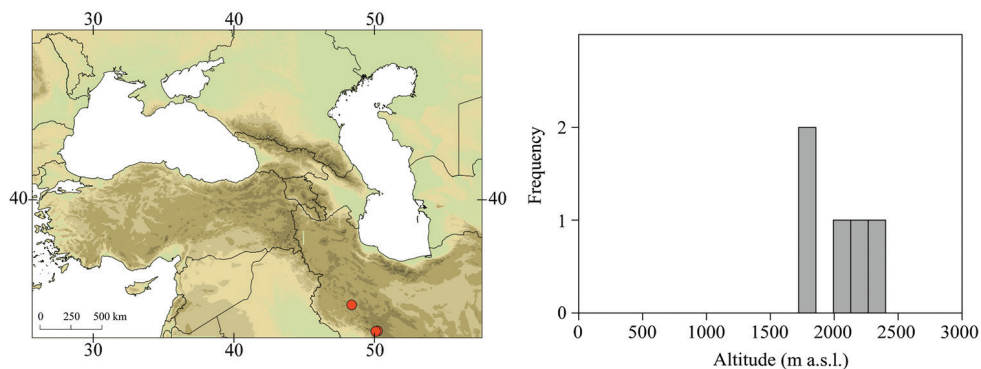


Figure 45. Geographical (left) and vertical (right) distribution of *Epeorus (Caucasiron) zagrosicus*.

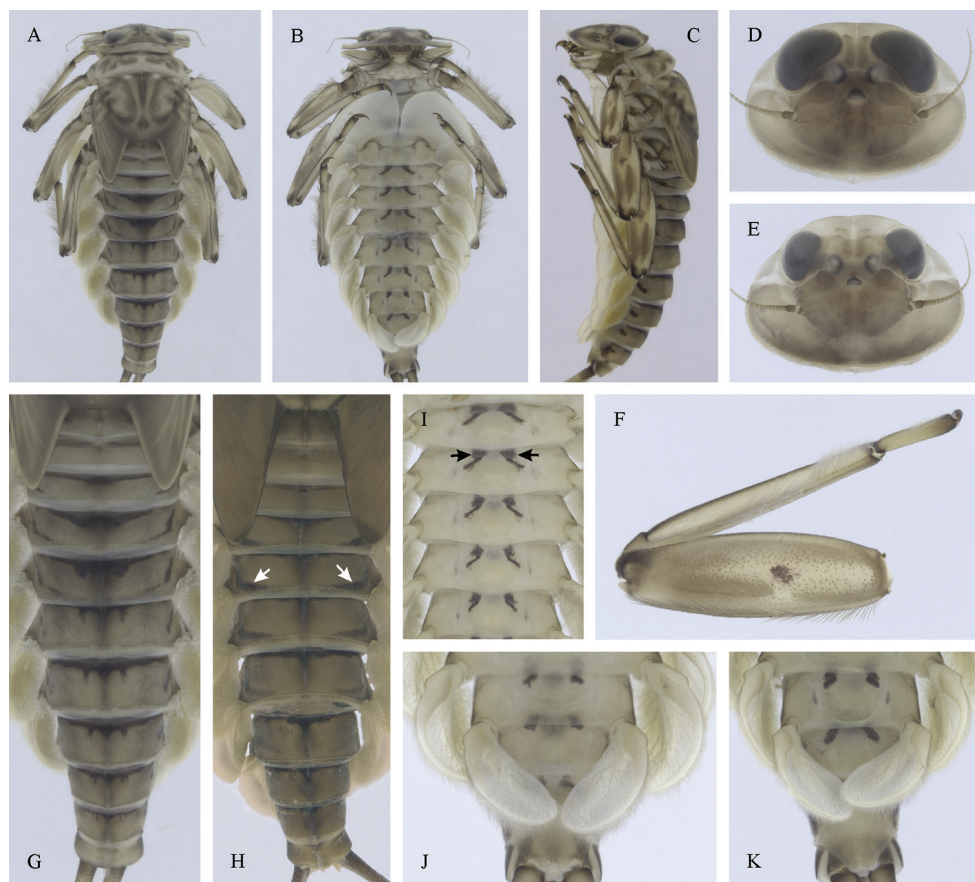


Figure 46. *Epeorus (Caucasiron) zagrosicus*, larva: **A** habitus in dorsal view **B** habitus in ventral view **C** habitus in lateral view **D** head of male in dorsal view **E** head of female in dorsal view **F** middle leg in dorsal view **G, H** abdominal terga (arrows point on dorso-posteriorly extended lateral stripes) **I** abdominal sterna II–VI **J, K** gills VII (in natural position from ventral view).

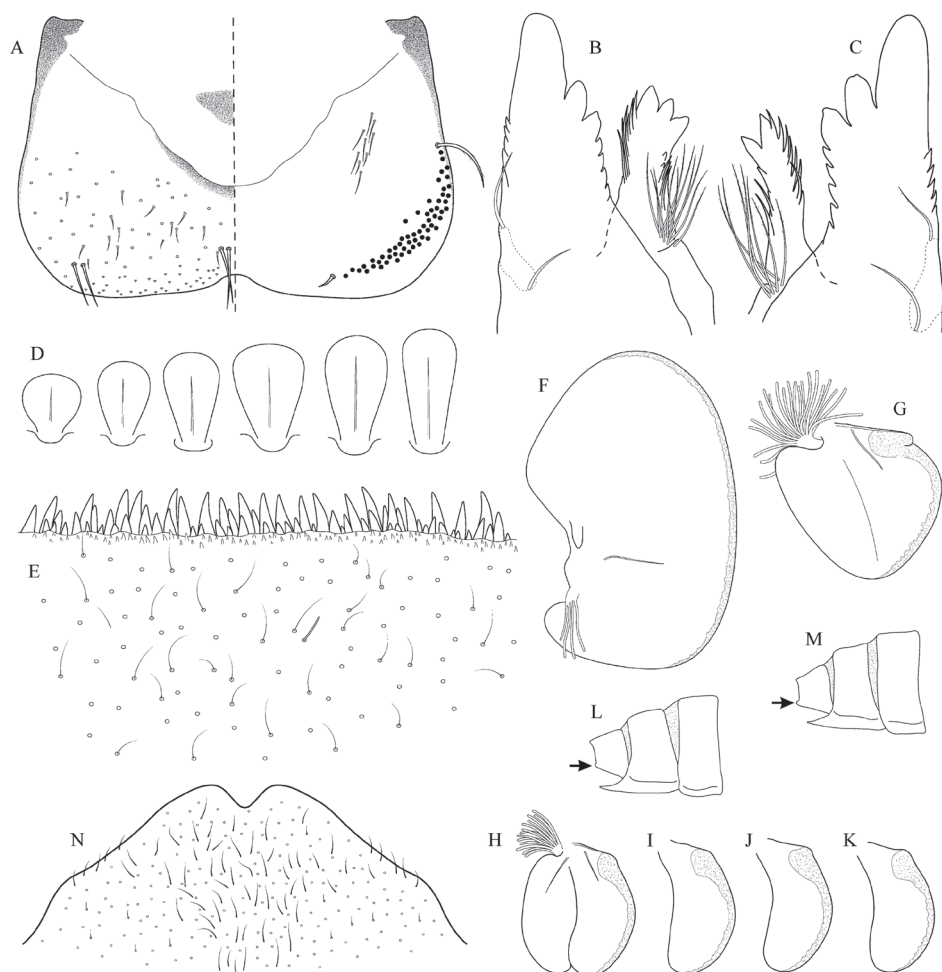


Figure 47. *Epeorus* (*Caucasiron*) *zagrosicus*, larva: **A** labrum (left half in dorsal view right half in ventral view) **B** incisors of left mandible **C** incisors of right mandible **D** setae on dorsal surface of femora **E** surface and posterior margin of abdominal tergum VII **F** gill I **G** gill III **H** gill VII (flattened on slide) **I–K** gill VII (in natural position from ventral view) variability in shape **L, M** abdominal segments VIII–X in lateral view (arrow point on postero-lateral projections) **N** sternum IX of female.

scribed from the region and some morphologically and genetically variable taxa, such as *E. (C.) znojkoii*, will be split into several species. This identification guide describes the state of the art at the time of publication.

All species of *Caucasiron* mayflies are charismatic animals, unique to the region. Some of them are endemic in a relatively limited area (especially for the Greater Caucasus and the Alborz Mts.) and may have considerable conservation value. We hope that this work will contribute to an increase in the knowledge of *Caucasiron* mayflies among hydrobiologists and ecologists. We would also like to encourage regional researchers to incorporate *Caucasiron* species as indicators in their biomonitoring surveys and water quality assessments.

Acknowledgements

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The species of *Timarcha* Samouelle, 1819 described by Linnaeus (Coleoptera, Chrysomelidae)

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Abstract

Linnaeus described five species presently included in the genus *Timarcha*: *Chrysomela goettingensis*, *Tenebrio caeruleus*, *Tenebrio laevigatus*, *Tenebrio latipes*, and *Tenebrio rugosus*. After a study of the relevant material, the identity of these species has been established. The following synonyms are proposed or confirmed: *Timarcha goettingensis* (Linnaeus, 1758) = *T. latipes* (Linnaeus, 1767), **syn. nov.**; *Timarcha caerulea* (Linnaeus, 1758), **comb. nov.** = *T. balearica* Gory, 1833, **syn. nov.** = *T. balearica* Pérez Arcas, 1865, **syn. nov.**; *Timarcha rugosa* (Linnaeus, 1767) = *T. scabra* (Olivier, 1807), **syn. conf.** = *T. generosa* Erichson, 1841, **syn. conf.**; *Timarcha laevigata* (Linnaeus, 1767) = *T. tenebricosa* (Fabricius, 1775), **syn. conf.**. The type of *Tenebrio caeruleus* is a Chrysomelidae currently belonging to genus *Timarcha* and therefore can no longer be considered a Tenebrionidae (*Helops caeruleus*) nor the type species of genus *Helops*. For the sake of nomenclatural stability, an application to the International Commission on Zoological Nomenclature to change the relative precedence of *Timarcha caerulea* and retain usage of *T. balearica* will be made. An application to change the relative precedence of *Timarcha laevigata* has been submitted, which would lead to the conservation of usage of *T. tenebricosa* as valid. Lectotypes are designated for *Chrysomela goettingensis*, *Tenebrio latipes*, *Tenebrio caeruleus*, *Timarcha balearica* Gory, *T. balearica* Pérez Arcas, *Tenebrio rugosus*, *Chrysomela scabra*, *Timarcha generosa*, *Tenebrio laevigatus*, and *Chrysomela tenebricosa*. For each of the valid species the diagnosis, distribution, and host-plant data are reported.

Keywords

Chrysomelinae, Europe, leaf beetles, nomenclature, North Africa, synonyms, taxonomy, Tenebrionidae

Introduction

The taxonomy of the genus *Timarcha* Samouelle, 1819 (Coleoptera, Chrysomelidae) is among the most challenging of all Palaearctic chrysomelids because: i) the types were rarely consulted by authors, and ii) there exists a high variability in traits as size, sculpture and form of the pronotum (Petitpierre 1970; Tiberghien 1971; Gómez-Zurita 2008; Kippenberg 2010). Therefore, a revisionary work on this genus is required (Daccordi et al. 2020).

As a starting point for the revision of the genus *Timarcha*, we have studied the species authored by Carl Linnaeus. He described five species presently belonging to this genus. They are *Chrysomela goettingensis* Linnaeus, 1758, *Tenebrio caeruleus* Linnaeus, 1758, *Tenebrio laevigatus* Linnaeus, 1767, *Tenebrio latipes* Linnaeus, 1767, and *Tenebrio rugosus* Linnaeus, 1767. One of us (MAAZ) studied and photographed the types of *T. latipes* and *T. laevigatus* in the collection of the Linnean Society of London. As well, consultation of photographs of Linnean types on the website of the Linnean Collections (<http://linnean-online.org/>) of the Linnean Society of London, together with a study of type specimens of other nominal species in other museums, led us to reconsider the availability and nomenclatural status of the five species of *Timarcha* described by Linnaeus.

Material and methods

Measurements of body length were made using the ocular grid of a Lomo MBS-10 binocular microscope at 10× magnification. Body size was considered the total length of the specimen from the anterior region of head to the apex of elytron. Photographs of type specimens of *Tenebrio laevigatus* and *T. latipes* were taken with a Canon EOS 7D camera attached to a MP-E 65 mm f/2.8 1–5× macro lens. Photographs of type specimens of *Chrysomela goettingensis*, *Tenebrio caeruleus*, and *T. rugosus* were kindly provided by Linnean collections staff (The Linnean Society of London) and of *Timarcha balearica* Gory, 1833 by Antoine Mantilleri (MNHN). Types, and their parts, of *Timarcha balearica* Pérez-Arcas, 1865, *T. scabra* (Olivier, 1807), and *T. generosa* Erichson, 1841 were photographed with an Olympus Stylus TG-3 digital compact camera. Photographs of other specimens or their parts were done with a Canon EOS 550D attached to a bellows with a Schneider Componon-S 50mm f/2.8 objective. Combine ZM was used for resolving the stack of photos.

The methodology to name the vestiture under of the tarsi I–III, to dissect the sclerites of endophallus, and to inflate the endophallus is explained by Daccordi et al. (2020).

In the treatment of type material from the collection of Carl Linnaeus, we have followed Recommendation 73F (International Commission on Zoological Nomenclature 1999) and have designated “a lectotype rather than assume a holotype”. The designation of lectotypes in this paper has been made by the three authors jointly, unless otherwise indicated in the labels of the types.

Host plants are given using their valid names. If a name, now a synonym, was originally mentioned, this follows the valid name between round brackets. Plant nomenclature follows APG IV (2016) for families and The Plant List (<http://www.th-epantlist.org>) for genera and species names.

The material examined is housed in the following collections (curators mentioned between round brackets):

- LSUK** The Linnean Collections of the Linnean Society, London (Isabelle Charmentier, Suzanne Ryder)
MNCN Museo Nacional de Ciencias Naturales, Madrid (Mercedes París)
MNHN Muséum National d'Histoire Naturelle, Paris (Antoine Mantilleri)
ZMHB Museum für Naturkunde der Humboldt-Universität, Berlin (Johannes Frisch, Bernd Jäger)
ZMUK Zoologisches Museum, Universität Kiel, Kiel (Michael Kuhlmann).

The label data for all type specimens is cited as follows: a double slash (//) divides the texts on different labels, a single slash (/) divides the text in different rows. Type localities are cited with their original spellings. Comments and notes are cited in square brackets: [p] preceding data are printed, [h] preceding data are handwritten, [w] white label, [r] red label.

The webpage of the Linnean Collections (<http://linnean-online.org/>), of the Linnean Society of London, has been a critical source of information.

Results

Timarcha goettingensis (Linnaeus, 1758)

Figures 1–7

Chrysomela goettingensis Linnaeus 1758: 368 (original description).

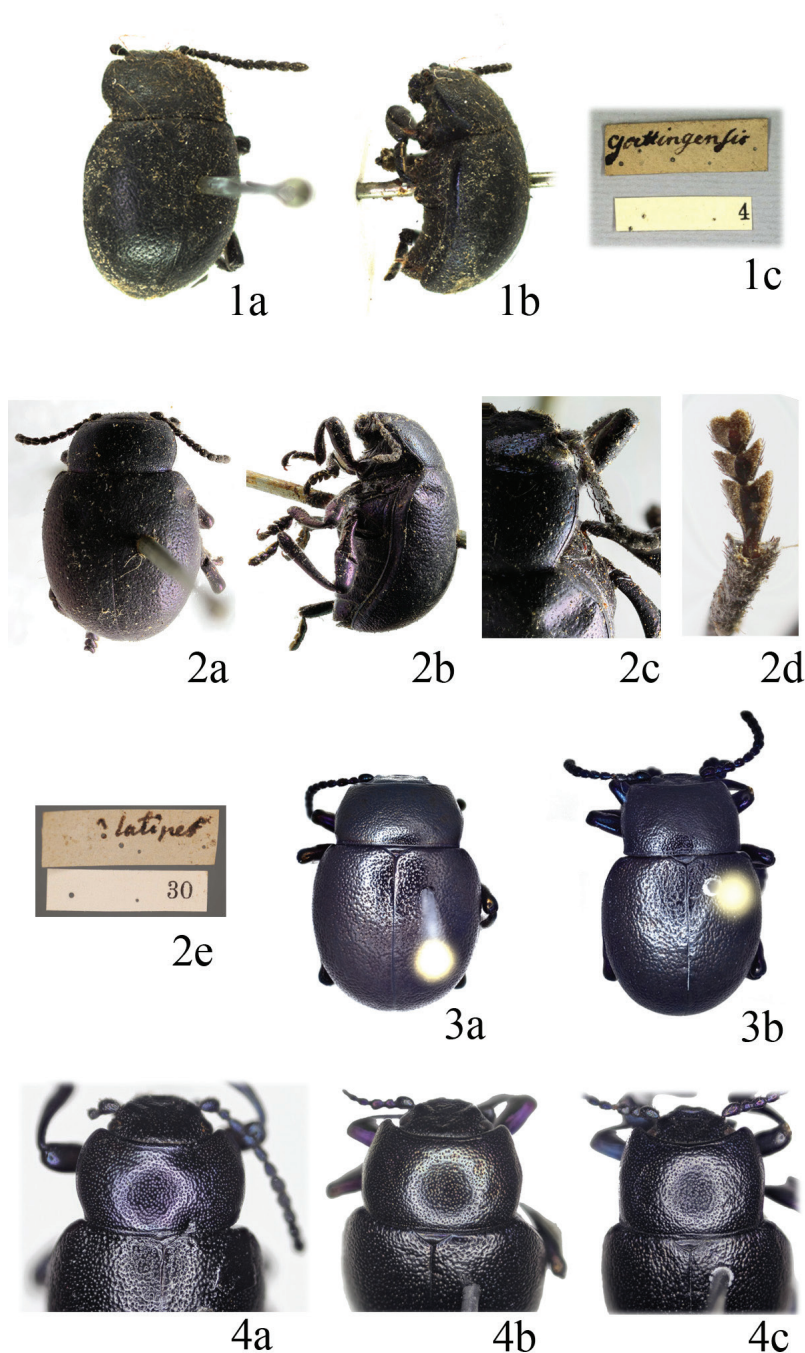
Tenebrio latipes Linnaeus 1767: 678 (original description), syn. nov.

Type localities. *Chrysomela goettingensis*: “Germania”. *Tenebrio latipes*: “Africa” [type locality wrong].

Type material. *Chrysomela goettingensis*: not examined. The images of the **lectotype** (♂, presently designated, Fig. 1), labelled “goettingensis [w, h, Linnaeus’ handwriting] // 4 [w, p]” (LSUK, code LINN 5537), are available at <http://linnean-online.org/22922/>.

Tenebrio latipes: **Lectotype** (♀, presently designated, Fig. 2): “LSL INS 6579 [p] // latipes [h, Linnaeus’ handwriting] // 30” (LSUK, code LINN 6579). Examined by one of us (MAAZ), images are also available at <http://linnean-online.org/23904/>.

Comments. In the Linnean collections there are two different species under *Chrysomela goettingensis*. One of them, specimen LINN 5537 labelled “goettingensis” [w, h, Lin-



Figures 1–4. *Timarcha goettingensis* 1 lectotypus of *Chrysomela goettingensis* in dorsal (a) and side (b) view, and label (c) (photos provided by The Linnean Society, with permission to reproduce 2 lectotypus of *Tenebrio latipes* in dorsal (a) and side (b) view, lateral side of pronotum (c), underside of third metatarsomere (d), and label (e) (photos by Alonso Zarazaga and Ren Li, with permission to reproduce from The Linnean Society) 3 male habitus from Germany, Frankenhausen (a) and Germany, Erfurt (b) 4 pronota of males from France, Lozère (a), Germany, Bad Frankenhausen (b) and Germany, Erfurt (c).

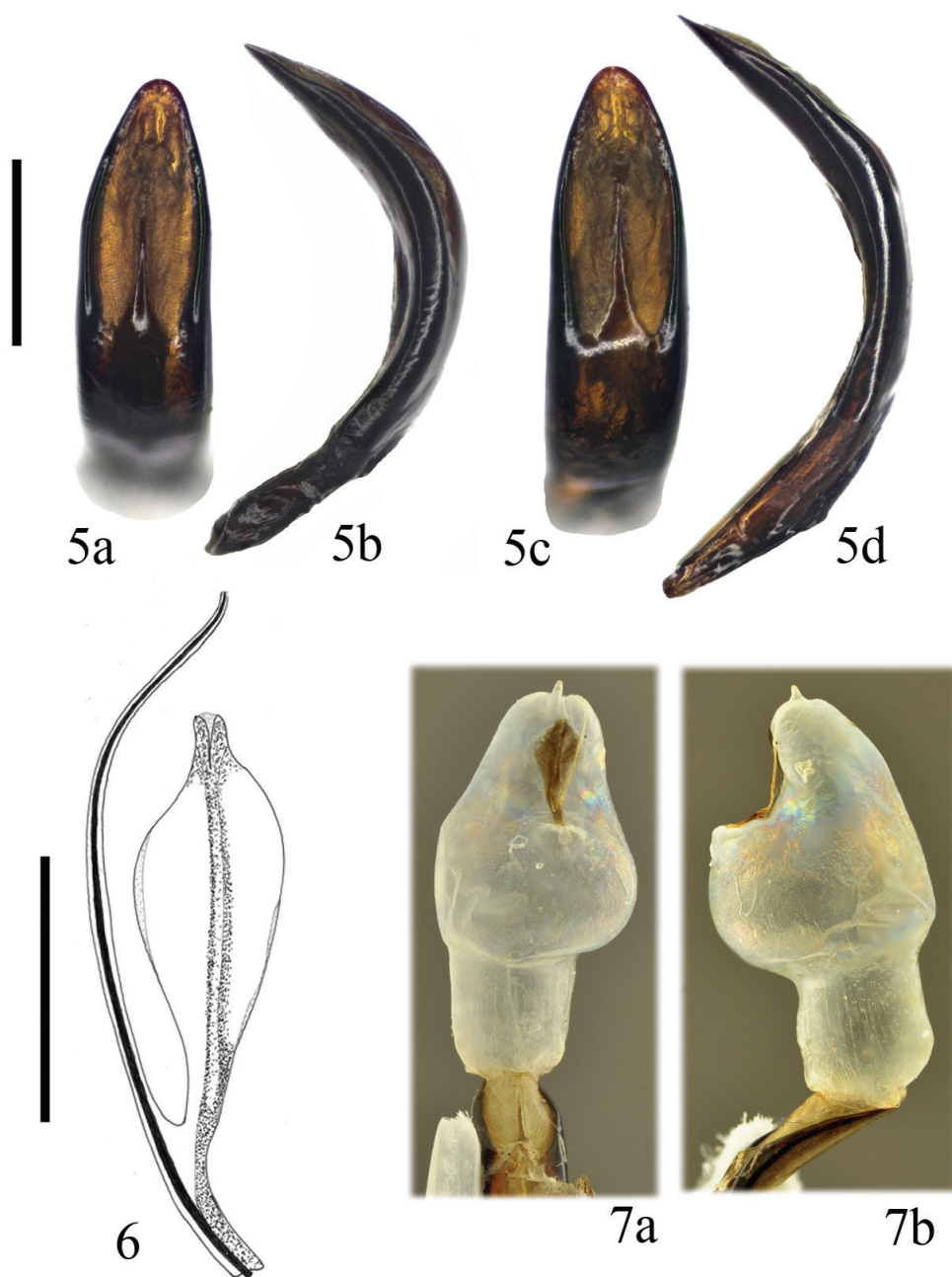
naeus' handwriting] (<http://linnean-online.org/22922/>), is the lectotype of *Chrysomela goettingensis* Linnaeus, 1758: 368 (presently in *Timarcha*, Fig. 1). The other species have the codes LINN 5536 (labelled "goettingensis" [w, h, Linnaeus' handwriting]) (<http://linnean-online.org/22921/>), LINN 5538 (no labelled) (<http://linnean-online.org/22923/>), LINN 5539 (no labelled) (<http://linnean-online.org/22924/>), and are to be considered syntypes of *Chrysomela goettingensis* Linnaeus, 1760: 160, although this species is currently known as *Chrysolina sturmi* (Westhoff 1882: 268) (see Waterhouse 1864: 18; Weise 1916: 96; Bieńkowski 2001: 162). An additional specimen, LINN 5540 (<http://linnean-online.org/22925/>), is also the latter species but cannot be a syntype because it comes from Fenwick Skrimshire, who was born after the publication of Linnaeus's work.

Authors such as Weise (1916: 207), Winkler (1930: 1298), Bechyně (1945a: 103, 1947a: 59, 1948: 50), Jolivet (1967a: 225); Warchałowski (2010: 629), and Gómez-Zurita and Kippenberg (2010: 440 (pars)) have identified as "*Timarcha latipes* (Linnaeus)" specimens belonging to *T. punctella* Marseul, 1871 species group (Daccordi and Vela unpubl. data). However, the original description of *Tenebrio latipes* Linnaeus, 1767 clearly says that it is half the size of *T. laevigata*. In fact, the lectotype of *T. latipes* measures 8.4 mm (Fig. 2), obviously much smaller than *T. punctella* or species similar to it from North Africa. The type locality "Africa" given by Linnaeus (1867) for *T. latipes* is incorrect.

Diagnosis. Males: 7.4–12.6 mm (lectotype of *T. goettingensis*: 8.7 mm; Fig. 1); females: 8.4–14.5 mm (lectotype of *T. latipes* is an unextended specimen measuring 8.4 mm; Fig. 2). Black or black with bluish luster (Fig. 3). Highly variable species in brightness, puncturation, form of the pronotum and elytra, and size. Sides of the pronotum regularly, slightly curved or almost straight, with the widest point in the basal third or at base, never cordiform, completely margined or with lateral margins obliterated at different extent (Fig. 4). Puncturation on the pronotum and elytra dense, regular, heavily or weakly marked, usually stronger on the elytra, not or conspicuously vermiculated (Figs 3, 4). Mesoventrite variable with apophysis a bit prominent, slightly forked, or more or less emarginated or almost straight, never clearly bituberculated. Vestiture of the female tarsi: (1, 1, 1/3–3/4; 1, 1, 1/3–3/4; 1, 4/5–1, 1/2–3/4). The aedeagus is slender and progressively narrowed towards the apex in dorsal view and regularly curved in side view (Fig. 5). Sclerites of the internal sac of the aedeagus (Fig. 6) with a paired phanera in romboïd form, which is an important diagnostic character to separate this from other closely related species. The inflated endophallus of an approximate locotype (coming near Göttinga in central Germany) (Fig. 7) is shown. A number of species and subspecies, whose taxonomical rank has yet to be studied, have been described in association with this species (Winkelman and Debreuil 2008; Warchałowski 2010; Gómez-Zurita and Kippenberg 2010).

Distribution. Most of Europe, from northern Spain to European Russia, and reaching Great Britain and Sweden (Gómez-Zurita and Kippenberg 2010)

Host plants. *Scabiosa atropurpurea* L. (= *S. maritima* L.) (Caprifoliaceae); *Plantago lanceolata* L., *P. coronopus* L. (Plantaginaceae), *Cruciata laevipes* Opiz, *Galium aparine* L., *G. arenarium* Loisel., *G. mollugo* L., *G. odoratum* (L.) Scop. (= *Asperula odorata* L.), *G. saxatile* L., *G. uliginosum* L., *G. verum* L., *Rubia peregrina* L. (Rubiaceae) (Jolivet and Petitpierre 1973; Winkelman and Debreuil 2008; Tiberghien 2016).



Figures 5–7. *Timarcha goettingensis* **5** aedeagi of Germany, Erfurt in dorsal (**a**) and side view (**b**) and Germany, Bad Frankenhausen in dorsal (**c**) and side (**d**) view **6** sclerite of the endophallus from Germany, Bad Frankenhausen, in dorsolateral view (taken from Daccordi et al. 2020) **7** everted endophallus from Germany, Frankenhausen in dorsal (**a**) and side (**b**) view (taken from Daccordi et al. 2020). Scale bars: 1 mm (**5**), 0.5 mm (**6**).

***Timarcha balearica* Gory, 1833**

Figures 8–14

Tenebrio caeruleus Linnaeus 1758: 418 (original description), syn. nov. Application for reversal of precedence will be submitted to the International Commission on Zoological Nomenclature (see comments below).

Tenebrio caeruleus Linnaeus 1767: 677 (repeated description).

Timarcha caerulea (Linnaeus 1758), nov. comb.

Timarcha balearica Gory 1833: pl. 49 (original description).

Timarcha balearica Gory 1844: 300 (text description).

Timarcha balearica Pérez Arcas 1865: 180 (original description). Synonymized with *T. balearica* Gory by Fairmaire and Allard (1873: 152). Synonymy confirmed.

Timarcha balearica var. *violaceus* Pic 1919: 20 (unavailable infrasubspecific name).

Timarcha balearica var. *martini* Pic 1919: 20 (unavailable infrasubspecific name).

Timarcha balearica ab. *viridipennis* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *coerulescens* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *longicornis* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *nigriventris* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *olivacea* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *semicoerulea* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *discolor* Bechyně 1946: 30 (unavailable infrasubspecific name).

Timarcha balearica ab. *tricolor* Bechyně 1946: 30 (unavailable infrasubspecific name).

Type localities. *Tenebrio caeruleus*: “Hispania”. *Timarcha balearica* G.: “Les Iles Baléares”. *Timarcha balearica* P. A.: “Mahón (Menorca), Alcudia de Mallorca”.

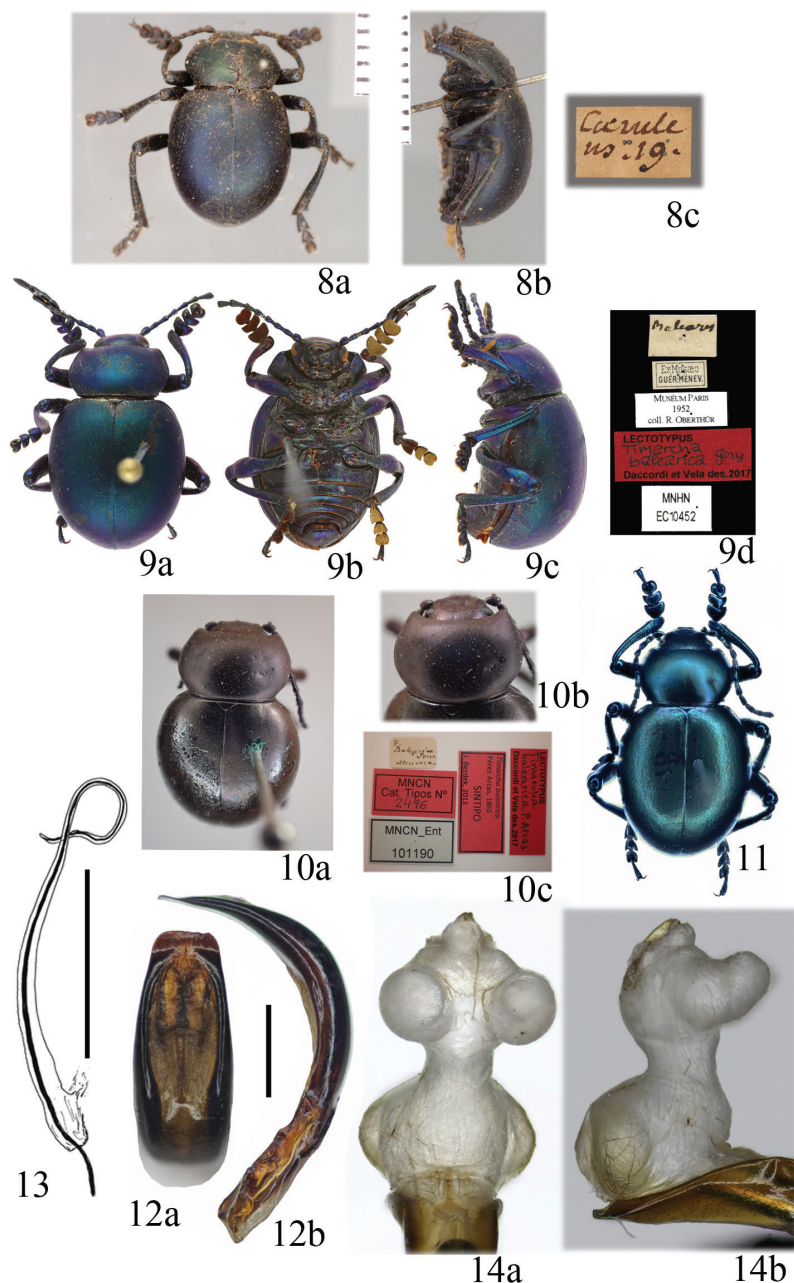
Type material. *Tenebrio caeruleus*: not examined. The images of the **lectotype** (♂, presently designated, Fig. 8), labelled “coerule / us 19’ [w, h, Linnaeus’s handwriting]” (LSUK, code LINN 6569), are available at <http://linnean-online.org/23894/>.

Timarcha balearica G.: **Lectotype** (♂, presently designated, Fig. 9): “Balears [h. by Blanchard] // Ex-Musaeo / GUÉR.-MÉNEV. [p, w] // **lectotypus** [p] / Timarcha / balearica Gory [h] / Daccordi et Vela des. 2017 [p, r]”. **Paralectotypes**: 2 ♂♂: same label text as lectotype, but **paralectotypus** instead of **lectotypus** (MNH, Col. Oberthür).

Timarcha balearica P.-A.: **Lectotype** (♂, presently designated, Fig. 10): “T. / Balearica / Perez / Menorca [h. by Pérez Arcas, w] // MNCN / Cat. Tipos N° / 2496 [p, r] // MNCN_Ent / 101190 [p, grey] // Timarcha balearica / Pérez Arcas, 1865 / SINTIPO / J. Bezdek, 2013 [p, r] // **lectotypus** [p] / Timarcha / balearica P. Arcas [h] / Daccordi et Vela des. 2017 [p, r]” (MNCN).

Comments. *Timarcha balearica* was described for the first time as figure 8 in planche 49 (Gory 1833). Later, Gory (1844: 300) published a text description (see Bousquet 2016 for exact publication dates).

Linnaeus (1758: 418) described *Tenebrio caeruleus* (Fig. 8; here considered a synonym of *Timarcha balearica*), with these words: “T. apterus caeruleus, thorace sub-



Figures 8–14. *Timarcha balearica* 8 lectotypus of *Tenebrio caeruleus* in dorsal (a) and side (b) view, and label (c) (photos provided by The Linnean Society, with permission to reproduce) 9 lectotypus of *Timarcha balearica* Gory in dorsal (a), ventral (b) and side (c) view, and label (d) (photos provided by Antoine Mantilleri (MNHN), with permission to reproduce) 10 lectotypus of *Timarcha balearica* Pérez Arcas, habitus (a), pronotum (b) and labels (c) 11 male habitus from Spain, Balears, Palma de Mallorca 12 aedeagus from Spain, Balears, Palma de Mallorca, in dorsal (a) and side (b) view 13 sclerite of the endophallus from Spain, Palma de Mallorca, in side view (taken from Daccordi et al. 2020) 14 Everted endophallus from Spain, Balears, Menorca, Mahón, in dorsal (a) and side (b) view. Scale bars: 1 mm (12), 0.5 mm (13).

orbiculato, coleoptris obtusis. Habitat in Hispania”. Some years later, Linnaeus (1764: 98) made an extended description expanding the locality to “Europa australiore” and provided more characters: “Corpus magnitudine, colore, statura & facie *T. mortisagi*, sed. Antennae caeruleae, apice nigrae, nec totae nigrae. Thorax brevior, postice parum rotundatus, nec postice truncatus. Elytra marginibus lateralibus atro-caerulescentibus, apice obtuso nec acuminato. Femora atro-caerulescentia, nitida, nec nigra opaca”. Later, Linnaeus (1767: 677) turned back repeating exactly the description of 1758, but not that of 1764.

Fabricius (1775: 257) proposed the combination *Helops caeruleus* (Coleoptera, Tenebrionidae) for a beetle from “Europa australi”, making a reference to the Linnaean descriptions of 1758 and 1764, but adding “elytris striatis” and “antennae pedesque nigrae” to the description of Linnaeus (1758: 418); these characters are clearly not found in the type of *Tenebrio caeruleus* Linnaeus, 1758, where the elytra are smooth and legs are bluish. To date, the type species of the genus *Helops* (Coleoptera, Tenebrionidae) is *Tenebrio caeruleus* Linnaeus, 1758 (Nabozhenko et al. 2008; International Commission on Zoological Nomenclature 2009), but this statement should be changed as most probably *Helops caeruleus* was described by Fabricius, not by Linnaeus (in *Tenebrio*). Interestingly, Illiger (1802: 410) rightly stated that *Tenebrio caeruleus* should be considered as belonging to genus *Chrysomela* (genus *Timarcha* was not described until 1819 by Samouelle). However, since 1802 no one has mentioned *Tenebrio caeruleus* as a Chrysomelidae.

For the sake of stability (Art. 23.2, International Commission on Zoological Nomenclature 1999), it would be convenient to apply the reversal of precedence and declare *Timarcha balearica* Gory, 1833 a *nomen protectum*. The requirements of Art. 23.9.1.2 are met by quoting the following references: Jolivet (1967b, 1995), Petitpierre (1970, 1973, 1985, 2011), Jolivet and Petitpierre (1973, 1981), Petitpierre et al. (1993), Chevin (1994), Petitpierre and Juan (1994), Santiago-Blay and Fain (1994), Steinhausen (1994), Jolivet and Hawkeswood (1995), Jolivet (1998), Teunissen (2002), Warchałowski (2003, 2010), Gómez-Zurita (2004, 2008), Gómez-Zurita et al. (2000, 2004), Gómez-Zurita and Galián (2005), Davison and Blaxter (2005), Jolivet and Poinar (2007), Gómez-Zurita and Kippenberg (2010), Mravinac et al. (2011), Jolivet et al. (2014), Petitpierre and Anichtchenko (2018), Petitpierre (2019), Daccordi et al. (2020). However, the name *Tenebrio caeruleus* Linnaeus, 1758 does not meet the requirements of Art. 23.9.1.1, because, until the present, it has been used in its misinterpreted concept of a Tenebrionidae of genus *Helops*. Therefore, an application is to be submitted to the International Commission of Zoological Nomenclature in order to maintain usage of *T. balearica* as a valid species, under Art. 23.9.3. Nomenclatural stability would be negatively affected by using *Tenebrio caerulea* Linnaeus (presently combined in *Timarcha*) as a valid name owing to its current ambiguity.

Diagnosis. Males: 12.0–14.5 mm (lectotype of *Tenebrio caeruleus*: 12.2 mm, Fig. 8; lectotype of *T. balearica* Gory: 12.7 mm, Fig. 9; lectotype of *T. balearica* P. Arcas: 12.0 mm, Fig. 10); females: 14.9–17.3 mm. Coloration variable from black, greenish, bluish, or copper-violet, or a combination; 11 color variations have been described (Pic 1919; Bechyň 1946; Compte 1956). Lateral sides of the pronotum

curved, narrower at base; margin conspicuous on all four sides except in lateral sides near the base, where it is obliterated. Pronotum and elytra smooth, puncturation absent (Fig. 11). Mesoventrite divergently bituberculated. Vestiture tarsal formulae: ♂♂ (0, 0, 0; 0, 0, 0; 1/3, 0, 0), ♀♀ (1/4, 0, 0; 1/4–1/3, 0, 0; 3/4, 0, 0), very distinctive in females. Aedeagus very characteristic in its truncate apex in dorsal view; in side view it is curved in its second half (Fig. 12). Sclerite of internal sac of aedeagus with a much reduced phanera and a looped flagellum (Fig. 13; see also Petitpierre 1970: fig. 8 and Petitpierre 2019: fig. 19). The inflated endophallus is as illustrated (Fig. 14; see also Petitpierre and Anichtchenko 2018: fig. 11).

Distribution. Balearic Islands: Mallorca and Menorca (Tenenbaum 1915; Jolivet 1953; Compte 1956).

Host plants. *Rubia peregrina* L. (= *R. angustifolia* L.), *Galium* spp., *Asperula* spp. (Rubiaceae) (Jolivet 1953; Jolivet and Petitpierre 1973; Jolivet and Poinar 2004), *Plantago lanceolata* L. (Plantaginaceae) (Petitpierre 1985).

Timarcha rugosa (Linnaeus, 1767)

Figures 15–22

Tenebrio rugosus Linnaeus 1767: 678 (original description).

Chrysomela scabra Olivier 1807: 507 (original description). Synonymized by Fairmaire (1884: 89). Synonymy confirmed.

Timarcha generosa Erichson 1841: 189 (original description). Synonymized by Fairmaire and Allard (1873: 161). Synonymy confirmed.

Type localities. *Tenebrio rugosus*: “Africa” [other localities mentioned in the original description as “Hispania” and “Gallia” are erroneous and should not be taken into consideration following Recommendation 76A.2 of the Code (International Commission on Zoological Nomenclature 1999)]. *Chrysomela scabra*: “côte de Barbarie”. *Timarcha generosa*: “Bona”.

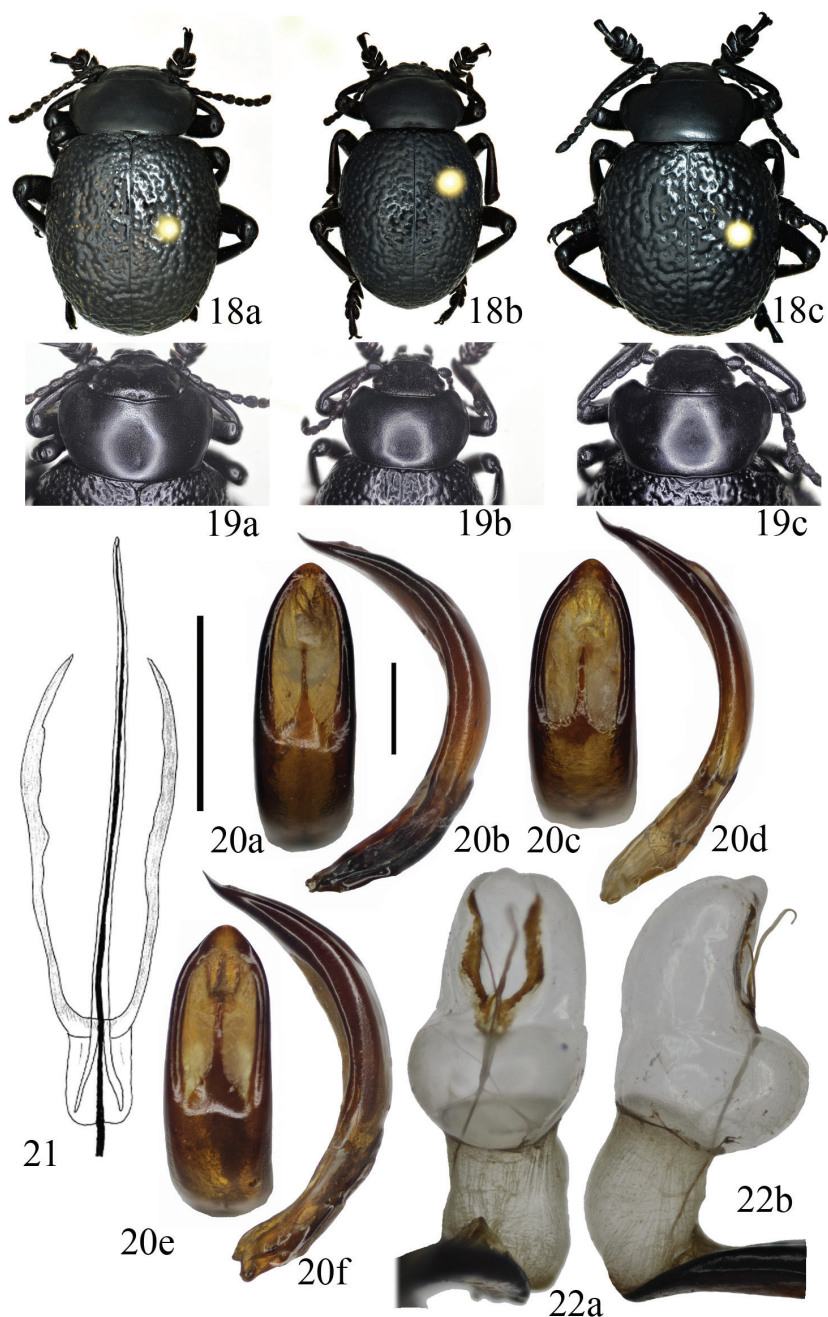
Type material. *Tenebrio rugosus*: not examined. The photographs of the **lectotype** (♀, presently designated, Fig. 15), labelled “rugosus / chalybeata [reversal, w, h, Linnaeus’ handwriting] // 27 [w, p]” (LSUK, code LINN 6576), are available at <http://linnean-online.org/23901/>.

Chrysomela scabra: **Lectotype** (♀, presently designated, Fig. 16): “COLLECTION / OLIVIER / TYPE [round green label, p] // **lectotypus** [p] / Timarcha / scabra Olivier [h] / Daccordi et Vela des. 2017 [p, r] // Timarcha [p] / rugosa L. [h] / Daccordi et Vela det. 2017 [p, w]” (MNHN).

Timarcha generosa: **Lectotype** (♂, presently designated, Fig. 17): “generosa / Er. / chalconota Dej. / Bona Wagner [h, w] // v. generosa Er. [h, bluish label] // 19114 [p, w] // Type [p, r] // **syntype** / Timarcha generosa / Erichson, 1841 / labelled by MFNB



Figures 15–17. *Timarcha rugosa* **15** lectotypus of *Tenebrio rugosus* in dorsal (**a**) and side (**b**) view, and label in upper (**c**) and lower view (**d**) (photos provided by The Linnean Society, with permission to reproduce) **16** lectotypus of *Timarcha scabra* in dorsal (**a**), and ventral (**b**) view, pronotum (**c**) and label (**d**) **17** Lectotypus of *Timarcha generosa* in dorsal view (**a**), pronotum (**b**) and label (**c**).



Figures 18–22. *Timarcha rugosa* **18** male habitus from Algeria, Mandoura (**a**), Morocco, Debdou (**b**) and Morocco, Aguelmame Sidi Ali (**c**) **19** pronota of males from Algeria, Mandoura (**a**), Morocco, Debdou (**b**) and Morocco, Aguelmam Sidi Ali (**c**) **20** aedeagi from Morocco, Oujda in dorsal (**a**) and side (**b**) view, Morocco, Aguelmame Sidi Ali in dorsal (**c**) and side (**d**) view and Morocco, Annual in dorsal (**e**) and side (**f**) view **21** sclerite of the endophallus from Algeria, Batna in dorsal view (taken from Daccordi et al. 2020) **22** Everted endophallus from Morocco, Ain Benimathar, in dorsal (**a**) and side view (**b**). Scale bars: 1 mm (**20**), 0.5 mm (**21**).

2016 [p, r] // **lectotypus** [p] / *Timarcha* / *generosa* Erichson [h] / Daccordi et Vela des. 2017 [p, r]" (ZMHB). **Paralectotype**: 1 ♂ "Hist.-Coll. (Coleoptera) / Nr. 19114 / *Timarcha generosa* Erichs. / Bona, Wagner / Zool. Mus. Berlin [p, w] // 19114 [p, w] // Type [p, r] // **syntype** / *Timarcha generosa* / Erichson, 1841 / labelled by MFNB 2016 [p, r] // **paralectotypus** [p] / *Timarcha* / *generosa* Erichs. [h] / Daccordi et Vela des. 2017 [p, r]" (ZMHB). **Paralectotype**: 1 ♀ "Hist.-Coll. (Coleoptera) / Nr. 19114 / *Timarcha generosa* Erichs. / Bona, Wagner / Zool. Mus. Berlin [p, w] // 19114 [p, w] // Type [p, r] // **syntype** / *Timarcha generosa* / Erichson, 1841 / labelled by MFNB 2016 [p, r] // **paralectotypus** [p] / *Timarcha* / *generosa* Erichs. [h] / Daccordi et Vela des. 2017 [p, r]" (ZMHB). All the specimens carry a label: "TIMARCHA [p] / *rugosa* L. [h] / Daccordi et Vela det. 2017 [p, w].

Comments. Fairmaire (1884: 89) and Fairmaire and Allard (1873: 161), respectively, considered *Chrysomela scabra* and *Timarcha generosa* as junior synonyms of *T. rugosa*, and we can confirm these decisions. However, since Bechyně (1947a: 56) to present, *T. generosa* and *T. scabra* were regarded as separate species (Gómez-Zurita and Kippenberg 2010: 439, 441; Warchałowski 2010: 625). The lectotype of *T. generosa* designated herein has blackish legs, but it is interesting that the two paralectotypes have reddish legs, showing this color variation which is not uncommon in several *Timarcha* species.

Diagnosis. Males: 11.8–19.0 mm (lectotype of *T. generosa*: 15.8 mm, Fig. 17); females: 13.7–21.2 mm (lectotype of *Tenebrio rugosus* is an extended specimen measuring 23.5 mm, Fig. 15; lectotype of *Timarcha scabra* is an unextended specimen measuring 15.6 mm, Fig. 16). Species variable in size, form of pronotum, and elytral sculpture. Black, shining or matte, sometimes with bronze tan. Legs black or femora and tibiae reddish, also antennomeres I–V can be reddish at base in populations of northern Algeria. Pronotum cordiform or subcordiform, with maximum width at distal 1/3, reborded even at posterior angles, without or with weak punctures which are not very dense (Fig. 19). Elytra not or weakly punctured, always conspicuously vermiculate, giving a rugose aspect (Fig. 18). Mesoventrite straight or weakly emarginate, not or weakly prominent. In ventral view, meso- and metatarsomere III slightly emarginated at apex in males; in females, this emargination is well marked, which is a differential feature relative to other species. Vestiture tarsal formulae: ♂♂ (0,0,0; 0–1/3,0,0; 0–4/5 (very finely),0,0), ♀♀ (1, 1, 1; 1, 1, 1; 1, 1, 1). Aedeagus somewhat variable, generally broad (Fig. 20c, e) but sometimes narrower (Fig. 20a) in dorsal view, and also more or less curved in side view (Fig. 20b, d, f). Sclerites of internal sac of aedeagus, in dorsoventral view, with wide or fine, slightly curved, and paired phanera, and a straight flagellum (Figs. 21, 22). The inflated endophallus is shown in Figure 22.

Distribution. Tunisia, Algeria, and Morocco. Spain and France, as in the original description of *T. rugosus*, are wrong.

Host plants. *Plantago albicans* L. (Plantaginaceae), *Asperula* sp. (Rubiaceae) (Jolivet 1966).

***Timarcha tenebricosa* (Fabricius, 1775)**

Figures 23–29

Tenebrio laevigatus Linnaeus 1767: 678 (original description), syn nov. Application for reversal of precedence submitted to the International Commission on Zoological Nomenclature (see comments below).

Tenebrio coeruleus Berkenhout 1769: 111 (non *T. caeruleus* Linnaeus 1758: 418). Synonymized with *T. tenebricosa* by Stephens (1829: 224).

Chrysomela tenebricosa Fabricius 1775: 94 (unjustified replacement name). Synonymized explicitly with *T. laevigatus* by Duftschmid (1825: 161) and Stephens (1831: 348).

Chrysomela tenebriosa: Fabricius 1781: 116 (incorrect spelling).

Chrysomela tenebrioides: Gmelin 1790: 1667 (incorrect spelling).

Chrysomela tenebricosa: Olivier 1791: 689; Rossi 1790: 74; Herbst 1794: 104; Fabricius 1801: 423; Panzer 1797: 44, 1; Illiger 1802: 410; Latreille 1804: 376; Olivier 1807: 508. Schönherr 1808: 239.

Tenebrio coeruleus: Berkenhout 1795: 109.

Timarcha tenebricosa: Samouelle 1819: 213 (combination); Kirby 1826: 99; Stephens 1829: 224; Herrich-Schäffer 1838: 156, 21b; Gemminger and Harold 1871: 3462; Fairmaire and Allard 1873: 169; Weise 1882: 321, 1916: 211; Marseul 1883: 49; Heyden et al. 1883: 197; Fairmaire 1884: 93; Reitter 1913: 108; Bechyně 1945b: 7; Bechyně 1947b: 8; Müller 1952: 450; Jeanne 1967: 8; Mohr 1966: 191; Petitpierre 1970: 5, 1973: 10; Tiberghien 1971: 190, 2014: 2; Minelli and Vittorelli 1976: 20; Kippenberg 1994: 86; Lopatin et al. 2004: 83; Winkelman and Debreuil 2008: 42; Warchałowski 2003: 223, 2010: 628; Gómez-Zurita and Kippenberg 2010: 442; Petitpierre and Anichtchenko 2018: 364; Petitpierre 2019: 109.

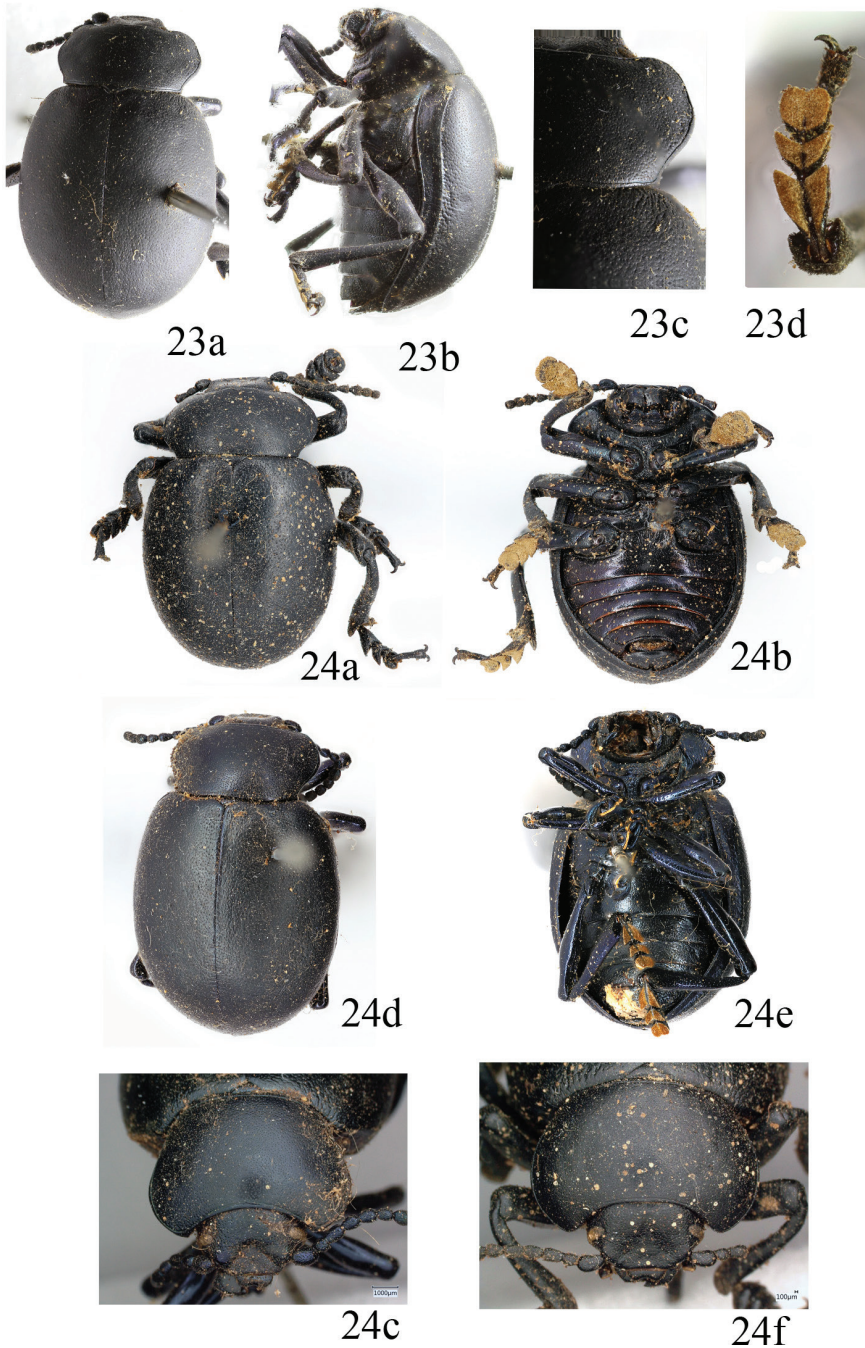
Chrysomela laevigata: Duftschmid 1825: 161 (combination).

Timarcha laevigata: Latreille 1829: 150 (virtual combination); Stephens 1831: 348; 1839: 308; Dufour 1843: 106; Küster 1847: 91; Little 1838: 237; Shuckard and Spry 1861: 70; Steiner 1864: 208; Waterhouse 1864: 26; Brunetti 1880: 235; Cuní-Martorell and Martorell-Peña 1876: 321; Cuní-Martorell 1885: 62, 1888: 159; Apfelbeck 1907: 506.

Type localities. *Tenebrio laevigatus*: “Africa” [wrong type locality]. *Chrysomela tenebricosa*: “Europa australiori”.

Type material. *Tenebrio laevigatus*: **Lectotype** (♀, designated herein, Fig. 23): “laevigatus [h, probably by Linnaeus] // 29 [p]” (LSUK, code LINN 6578). Examined by one of us (MAAZ), images are also available at <http://linnean-online.org/23903/>.

Chrysomela tenebricosa: not examined. Syntypes (1 ♂, 1 ♀, Fig. 24) (ZMUK) were examined from photographs. Minelli and Vittorelli (1976) designated *in litteris* (1974) the male as the “lectoholotypus”, the female as the “lectoallotypus”. Here we formally designate the male as **lectotype** (Fig. 24a–c), and the female as **paralectotype** (Fig. 24d–f).



Figures 23, 24. *Timarcha tenebricosa* 23 lectotypus of *Tenebrio laevigatus* in dorsal (a) and side view (b), pronotum (c) and metatarsus in lower view (d) (photos by Alonso Zarazaga and Ren Li, with permission to reproduce from The Linnean Society) 24 typi of *Chrysomela tenebricosa* Fabricius, lectotypus male in dorsal (a) and ventral (b) view, and pronotum (c), paralectotypus female in dorsal (d) and ventral (e) view, and pronotum (f) (photographed by Michael Kuhlmann, Zoologischen Museum Kiel, reproduced with permission).

Comments. The lectotype of *T. laevigata* (L.), a female (Fig. 23), has the pronotum and elytra finely and regularly punctured, the mesoventrite protruding and slightly bituberculated, and the metatarsus setose with the vestiture formula (1, 1/2, 1/3) (Fig. 23d); this perfectly fits specimens of the well-known European *T. tenebricosa*. On the other hand, females of *Timarcha laevigata* auct. nec Linnaeus, 1767 from North Africa, i.e. *T. turbida* Erichson, 1841: 189, or even *T. punctella* Marseul, 1871: 387, are different in that they have a pronotum with finer punctures, the mesoventrite very scarcely protruding and slightly emarginated but not bituberculate, and the female metatarsus with a large glabrous strip underside and a vestiture formula (1, 1, 1/2–3/4) (*T. turbida*) or (1,1,1) (*T. punctella*).

As the name *Timarcha laevigata*, in the sense here fixed as a synonym of *T. tenebricosa*, has at least one usage since 1899 (in Apfelbeck 1907: 500, 502, 506), one of the two conditions required by International Code of Zoological Nomenclature (1999, Art. 23.9.1.1) for reversal of precedence is not accomplished. Besides, the name *T. laevigata*, although wrongly applied to a North African species, has been profusely used until now (see e.g. Gómez-Zurita and Kippenberg 2010; Warchałowski 2010). An application has been submitted to the International Commission of Zoological Nomenclature (Vela et al. 2018) to maintain usage of *T. tenebricosa* as a valid species. The type species of *Timarcha* Samouelle is *Chrysomela tenebricosa* Fabricius 1775, by subsequent designation by Chevrolat (1843: 655 in Löbl and Smetana 2011: 50).

Diagnosis. Males: 14.6–17.2 mm; females: 16.1–18.2 mm (lectotype of *Tenebrio laevigata* = 17.5 mm; Fig. 23). Black or with bluish luster. Surface microreticulate, with a dull aspect (Fig. 25). Pronotum subcordiform, or cordiform, usually widest at the anterior 1/3, completely rebordered by a fine furrow, sides regularly curved. However, there is a much variation in the form of pronotum (Fig. 26), and the lateral sides near the base may be straight (Fig. 26a, d), sinuate (Fig. 26b), or both straight and sinuate (e.g. left side straight, right side sinuate; Fig. 26c). Puncturation on pronotum (Fig. 26) and elytra dense, regular, moderately marked, on a smooth surface never vermiculate. Mesoventrite with apophysis somewhat protruding, more or less emarginate, sometimes slightly bituberculate. Vestiture tarsal formulae: ♂♂ (0,0,0; 0,0,0; 1/3–1/2,0,0), ♀♀ (1/2,0,0; 1/2–3/4,0–1/2,0–1/2; 3/4–1,1/3–1/2,1/3–1/2). Aedeagus variable but always with paddle-shaped at the apex in dorsal view and strongly curved with sinuate apex in lateral view (Fig. 27). Sclerites of the internal sac of aedeagus with a long, curved flagellum that is somewhat widened before the apex in dorso-lateral view (Fig. 28); the phanera consist in two paired wings elongated and curved. The inflated endophallus is shown in Figure 29 (see also Petitpierre and Anichtchenko 2018: fig. 3). Thirteen subspecies have been described (Bechyně 1945b, 1948; Müller 1952), whose taxonomic status is very doubtful (Minelli and Vitorelli 1976; Warchałowski 2010).

Distribution. Most of Europe, from northern Spain to Great Britain and Ireland, eastwards to Georgia, Azerbaijan, and Asiatic Turkey; not recorded in Scandinavia (Gómez-Zurita and Kippenberg 2010).



Figures 25–29. *Timarcha tenebricosa* **25** male habitus from France, Poitiers **26** pronota of males of Crimea (a), Austria, Vienna (b), France, Paimport (c) and France, Poitiers (d) **27** aedeagi from France, Alps Maritimes, Caussols in dorsal (a) and side (b) view, France, Normandie, Bréal in dorsal (c) and side (d) view, Crimea in dorsal (e) and side (f) view **28** sclerite of the endophallus from England, Launceston in dorsolateral view (taken from Daccordi et al. 2020) **29** Everted endophallus from France, Paimport in dorsal (a) and side view (b). Scale bars: 1 mm (27), 0.5 mm (28).

Host plants. On Rubiaceae: *Asperula cynanchica* L., *Cruciata laevipes* Opiz, *Galium aparine* L., *G. mollugo* L., *G. parisiense* (L.), *G. verum* L., *Rubia peregrina* L. (Jolivet and Petitpierre 1973; Winkelman and Debreuil 2008).

Discussion

The difficult task of studying the types of old species has been greatly facilitated with the quick access to high-quality images. As a result, it is now easier for taxonomists to verify the status of old synonyms or interpretations which were based upon very short, generalized descriptions. The high-quality images of the types in the Linnaean collections have proven extremely useful for the zoological community. In addition, most museums and their curators are willing to help with search for and loan of types, which can facilitate taxonomic work. Incorrect species concepts, not based on the examination of name-bearing types, have sometimes been maintained despite the identity of extant type material, which has been carefully cared for and maintained for many years for the benefit of science.

In the case of genus *Timarcha*, whose revision is very necessary, the slow and sometimes difficult work of consulting types has become absolutely necessary, as various authors have made different interpretations for a long time. Although historical misinterpretations of *T. laevigata* and *T. latipes* have ascribed these to different North African species or even to both sexes of the same species, these two species are actually two very different European species. Also, *T. caerulea* is revealing, as it was considered a Tenebrionidae, when in actuality the type is clearly a male of the genus *Timarcha*, identical to *T. balearica* (Chrysomelidae).

The main synonyms presented here can be summarized as follows:

***Timarcha goettingensis* (Linnaeus, 1758)**

= *T. latipes* (Linnaeus, 1767), syn. nov.

***Timarcha balearica* Gory, 1833 (to be proposed to ICZN as a *nomen protectum*)**

= *Timarcha caerulea* (Linnaeus, 1758), syn. nov., comb. nov. (to be proposed to ICZN as a *nomen oblitum*)

***Timarcha rugosa* (Linnaeus, 1767)**

= *T. scabra* (Olivier, 1807), syn. conf.

= *T. generosa* Erichson, 1841, syn. conf.

***Timarcha tenebricosa* (Fabricius, 1775) (proposed to ICZN as a *nomen protectum*)**

= *T. laevigata* (Linnaeus, 1767), syn. conf. (proposed to ICZN as a *nomen oblitum*)

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First detection of an ocellate octopus in the Revillagigedos ecoregion, a biodiversity hotspot located in the Tropical East Pacific Province

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Abstract

The biodiversity of mollusks, particularly cephalopods, has not been exhaustively determined in the Revillagigedos ecoregion, which is a biodiversity hotspot for several marine groups located in the Tropical East Pacific Province. In our study, we detected and examined ocellate octopuses from Socorro and Clarion Islands, and determined their identity using morphological criteria and molecular data from two mitochondrial genes (COIII and COI). The taxon identified was *Octopus oculifer*, a species considered endemic to the Galapagos Archipelago. In addition, according to our analyses, *O. mimus*, *O. hubbsorum* and *O. oculifer* are very closely related and may represent a species complex comprised of three morphotypes. We found that the evolutionary relationships among octopuses are not determined by the presence of ocelli. This study is the first to report a clade represented by ocellate and non-ocellate species, in addition, the identity of cephalopods in the Revillagigedos was determined with analytical support.

Keywords

Benthic octopus, Cephalopoda, Octopod, synonym, Tropical Pacific

Introduction

Octopuses are soft-bodied cephalopods of the order Octopoda Leach, 1818, which comprises 13 families with around 300 pelagic or benthic species (Jereb et al. 2016). Benthic octopuses are either holobenthic, inhabiting the sea floor during the whole life cycle, or merobenthic, with a planktonic distribution during early stages (Villanueva and Norman 2008; Sauer et al. 2019). The family Octopodidae d'Orbigny 1940 includes 13 ocellate species catalogued in two genera, *Octopus* Cuvier, 1797 and *Amphioctopus* Fischer, 1882 (Jereb et al. 2016). Ocellate species (*Octopus cyanea* (Gray, 1849), *Amphioctopus exannulatus* (Norman, 1993), *A. fangsiao* (d'Orbigny, 1839–1841), *A. kagoshimensis* (Ortmann, 1888), *A. mototi* (Norman, 1993), *A. neglectus* (Nateewathana & Norman, 1999), *A. rex* (Nateewathana & Norman, 1999), *A. siamensis* (Nateewathana & Norman, 1999), *A. ovulum* (Sasaki, 1917), *O. bimaculatus* Verrill, 1883 and *O. bimaculoides* Pickford & McConnaughey, 1949) inhabit the Indian, Indo-Pacific and northwestern Pacific Oceans, except for *O. maya* Voss & Solis, 1966 and *O. oculifer* (Hoyle, 1904), which are considered endemic to the Yucatan Peninsula and the Galapagos Archipelago, respectively (Jereb et al. 2016).

Ocelli are considered an important diagnostic trait within octopodids, and are defined as false eye-spots in the form of round or ovoid conglomerations of chromatophores that may possess an outer concentric dark or light ring and an iridescent blue, purple, gold, or green inner ring (Packard and Hochberg 1977; Jereb et al. 2016). For octopodids, diagnostic features are highly valuable and needed, mainly due to the increased number of taxonomic confusions that derive from overlapped morphological characters among species (Norman and Hochberg 2005). Perhaps the most outstanding example of this problematic aspect is *O. vulgaris* Cuvier, 1797, which is considered a complex species that is being disentangled into different species (e.g., *Octopus insularis* Leite & Haimovici, 2008) and morphotypes by using morphological and molecular approaches (see Gleadall (2016), González-Gómez et al. (2018) and Amor et al. (2019)). Octopodids from the northeastern Pacific are no exception, for instance, Pliego-Cardenas et al. (2014) suggested that *O. mimus* Gould, 1852 and *O. hubbsorum* Berry, 1983 could be conspecific, and Díaz-Santana-Iturrios et al. (2019) confirmed that *O. californicus* Berry, 1911 and *O. alecto* Berry, 1953 should be reassigned into new genera.

Determining the biodiversity of octopodids is relevant given that several species constitute fishery resources (4.8 million tons extracted during 2005–2014) (Sauer et al. 2019) or present aquaculture potential (Baltazar et al. 2000; Iglesias et al. 2000; Chapela et al. 2006), thus, it is important to implement species-specific conservation and management measurements, especially in poorly known areas such as islands. Many insular systems are biodiversity hotspots (Hazen et al. 2013), often difficult to access, which hinders the characterization of biodiversity. The Revillagigedo Archipelago is an insular system declared Biosphere Reserve since 1994, and later, in 2016, the World Heritage Committee included it in the World Heritage List of the United Nations Educational, Scientific and Cultural Organization; more recently, in 2017, it

was declared National Park (CONANP 2004; SEMARNAT 2017; UNESCO 2019). The Archipelago is located in the Tropical East Pacific Province and the Revillagigedos ecoregion, according to Spalding et al. (2007). Revillagigedos is considered a biodiversity hotspot, at least for sea turtles, sharks, whales and giant manta ray (Dutton et al. 2014; Muntaner 2016; SEMARNAT 2017). Moreover, in Mexican waters, Clarion represents one of the five islands with the greatest marine species richness, and Socorro is one of the nine islands with the highest number of strict endemism (Koleff et al. 2009). Determining the identity of taxa in biodiversity hotspots can be informative to understand and monitor global biodiversity patterns, especially in groups that respond markedly to the current climate change, as is the case for cephalopods (Renema et al. 2008; Robinson et al. 2009; Rodhouse et al. 2014). We visited Revillagigedos during 2018 with the purpose of characterizing the coastal malacofauna of the Archipelago and found ocellated octopuses; thus, in this study, the objective was to identify these octopodids collected in Clarion and Socorro Islands through morphological comparisons and partial COIII and COI gene sequence analyses.

Methods

The Revillagigedo Archipelago is located approximately 390 km southwest of the southern tip of the Baja California peninsula and 890 km west from Manzanillo harbor, between 17°39'19" and 20°00'31"N, and 110°04'41" and 115°28'17"W. The Archipelago is comprised of four volcanic islands: Roca Partida, San Benedicto, Clarion and Socorro (CONANP 2004; SEMARNAT 2017). The octopuses evaluated in this study were fished for self-consumption by crew of the Mexican Navy in the military bases of Clarion and Socorro in accordance with the regulations stated by SEMARNAT (2017) for the Revillagigedo National Park and the permit to develop scientific research on the malacofauna from the Archipelago (official document number: F00.1.DRPBCPN.DIR.PNR.-001/2018) during June, 2018. In this convenience sampling, a total of 49 individuals were collected in Socorro ($n = 8$) and Clarion ($n = 41$) islands (Table 1) (Fig. 1). Due to the sampling particularities (convenience sampling), we were not able to transfer whole specimens from the Archipelago to the laboratory, instead, the morphological identification and morphometric measurements were conducted *in situ* and a small piece of tissue of each specimen was preserved in vials with 96% ethanol. However, in order to account for a type specimen of our sampling, a whole specimen was frozen and transferred to the laboratory facilities, preserved in 96% ethanol and deposited as ICML-EMU-12678 in the Regional Collection of Marine Invertebrates (ICML-EMU), Instituto de Ciencias de Mar y Limnología, Unidad Mazatlán, UNAM, in Mazatlán, México.

In Socorro Island, specimens were captured in each location by free and scuba diving using a hook; and in Clarion Island, octopuses were collected with a hook in the rocky intertidal during the lowest tide of each sampling site and day. Octopuses were sacrificed right after fishing. Individuals were sexed according to the presence (male) or absence (female) of a hectocotylized arm. Maturity stages for males and females were

Table 1. Data of octopuses from the Revillagigedo Archipelago.

Specimen number	DML (cm)	Sex	Maturity stage	Sampling site
1	6.5	M	I	Clarion Island
2	10	F	I	Clarion Island
3	7.5	F	I	Clarion Island
4	7	M	I	Clarion Island
5	8	M	I	Clarion Island
6	10	F	N/A	Clarion Island
7	8	F	N/A	Clarion Island
8	7.8	F	N/A	Clarion Island
9	7.5	F	N/A	Clarion Island
10	7	F	N/A	Clarion Island
11	8	F	N/A	Clarion Island
12	8	M	N/A	Clarion Island
13	10.5	M	III	Clarion Island
14	8.8	F	N/A	Clarion Island
15	7.3	F	N/A	Clarion Island
16	6.6	F	N/A	Clarion Island
17	3.1	F	N/A	Clarion Island
18	7.5	F	N/A	Clarion Island
19	6	F	N/A	Clarion Island
20	6.5	F	N/A	Clarion Island
21	10.5	M	III	Clarion Island
22	10	F	II	Clarion Island
23	7.5	M	III	Clarion Island
24	6.5	F	I	Clarion Island
25	6.5	F	I	Clarion Island
26	8	F	II	Clarion Island
27	9	F	II	Clarion Island
28	5.5	F	I	Clarion Island
29	7	F	I	Clarion Island
30	5.6	F	I	Clarion Island
31	5.7	F	I	Clarion Island
32	6.5	F	I	Clarion Island
33	6	F	I	Clarion Island
34	6.8	M	II	Clarion Island
35	6.8	F	II	Clarion Island
36	7.3	F	II	Clarion Island
37	8.3	F	II	Clarion Island
38	7.5	M	II	Clarion Island
39	6.9	M	II	Clarion Island
40	6.5	F	I	Clarion Island
41	6.5	F	I	Clarion Island
42	9	F	II	Socorro Island
43	6.3	F	II	Socorro Island
44	9	F	III	Socorro Island
45	8	F	II	Socorro Island
46	11.5	M	III	Socorro Island
47	11	M	III	Socorro Island
48	11	M	III	Socorro Island
49	12.8	F	III	Socorro Island

determined with the same criterion considered by Alejo-Plata et al. (2009). Octopuses were identified at the genus level using the morphological characteristics described in Jereb et al. (2016). For identification at the species level, the diagnoses of taxa of the genus *Octopus* that were reported for the Revillagigedo Islands by CONANP (2018)

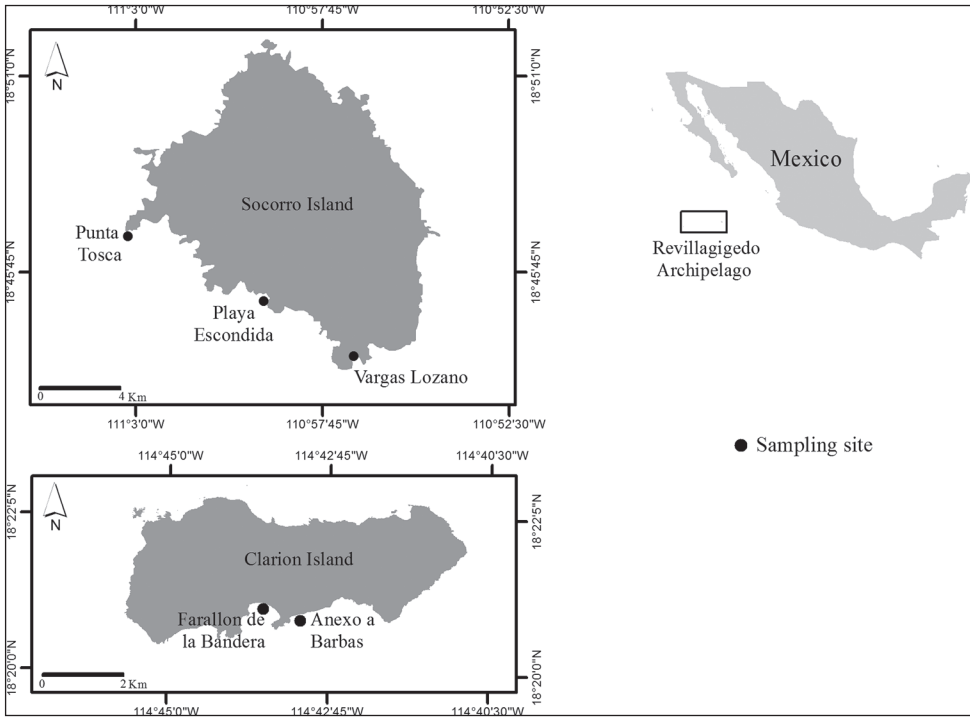


Figure 1. Study area. Sampling sites of octopuses from the Revillagigedo Archipelago.

(*O. bimaculatus* and *O. hubbsorum*) and ocellate octopuses from the Eastern Pacific (*O. oculifer* and *O. bimaculoides*) were considered (Verrill 1883; Pickford and McConaughy 1949; Berry 1953). The coloration patterns observed in the individuals were photographed with a Canon PowerShot D30 subaquatic camera. Images of morphological features of the octopus were recorded using a Canon EOS rebel T5 coupled to a stereoscopic microscope (Iroscope ES-24).

The dorsal mantle length (DML) and total weight (TW) were recorded to determine the length-weight relationship (LWR). Class intervals were determined following the Sturge's rule. The following potential equation was employed to evaluate LWR:

$$TW = a * DML^b$$

where: TW = dependent variable (total weight), a = coefficient of proportionality, DML = independent variable (dorsal mantle length), b = allometry coefficient (weight per unit of length).

The type of growth was estimated based on Student's t-test for the "b" values obtained from the model and compared to a theoretical value of $b=3$ which represents isometric growth.

Table 2. Accession numbers of sequences (COIII and COI) obtained from GenBank of the octopodid species and specimens evaluated in this study.

Species	Accession number COIII	Accession number COI
<i>Octopus maya</i>	GU362546.1	MH293049.1
	–	KX611862.1
<i>Octopus cyanea</i>	AB573224.1	AB191280.1
	AJ628220.1	MK593394.1
<i>Octopus oculifer</i>	AJ628235.1	–
<i>Octopus hubbsorum</i>	KF225011.1	KY985096.1
	KF225010.1	KF225005.1
<i>Octopus bimaculoides</i>	KF225012.1	KY985076.1
	X83104.1	KF225006.1
<i>Octopus bimaculatus</i>	KT335840.1	KY985047.1
	NC_028547.1	KT335828.1
<i>Enteroctopus dofleini</i>	X83103.1	AB191272.1
	FJ603531.1	AB477017.1
<i>Octopus insularis</i>	KX219649.1	KY492362.1
	KX219648.1	KX611859.1
<i>Octopus vulgaris</i>	JQ085601.1	AB052253.1
	FN424384.1	KU525767.1
<i>Octopus tetricus</i>	AJ628240.1	MH289829.1
	JX680530.1	AF000056.1
<i>Octopus fitchi</i>	MK450541.1	MK450541.1
	KT335844.1	KT335832.1
<i>Octopus mimus</i>	KT335842.1	KT335830.1
	KT314263.1	GU355923.1
<i>Amphioctopus exannulatus</i>	AJ628223.1	–
<i>Amphioctopus fangsiao</i>	AB573188.1	HQ846155.1
	AB573186.1	AB430517.1
<i>Amphioctopus kagoshimensis</i>	AB573193.1	HQ846125.1
	AJ628226.1	HQ846123.1
<i>Amphioctopus mototi</i>	AJ628233.1	–
<i>Amphioctopus neglectus</i>	MH899749.1	MH899749.1
<i>Amphioctopus ovulum</i>	AB573198.1	HQ846159.1
	AB573197.1	AB430524.1
<i>Robsonella fontaniana</i>	KT314259.1	KF774313.1
	KC792301.1	–
<i>Hapalochlaena fasciata</i>	AJ628210.1	MF440346.1
	AB573212.1	JN790685.1
<i>Abdopus aculeatus</i>	AB573185.1	GQ900726.1
	AJ628213.1	LT604981.1
<i>Ameloctopus litonalis</i>	AJ628207.1	HM104255.1
<i>Eledone cirrhosa</i>	HM104251.1	KM517898.1
	–	MH293107.1
<i>Bathypolypus sponsalis</i>	FJ603530.1	KX078469.1
<i>Mussoctopus longibrachus</i>	KM459494.1	KM459478.1
	KM459486.1	KM459478.1
<i>Vampyroteuthis infernalis</i>	NC_009689.1	NC_009689.1
Specimen No.38	–	MN259102.1
Specimen No.44	MN259103.1	MN259099.1
Specimen No.45	MN259104.1	MN259100.1
Specimen No.48	MN259105.1	MN259101.1

For the molecular approach, sequences deposited in GenBank (Table 2) of the octopus species reported for the Revillagigedo Islands, ocellate octopuses of the world and species of various octopod genera were compared to our sequences. Separate analyses of partial COIII and COI sequences were performed to support the morphological

identification. For this research, four individuals (Specimens No. 44, 45 and 48 from Socorro Island and Specimen No. 38 from Clarion Island) were selected as representatives of the whole sample. DNA was extracted using the salt-extraction method. For COIII gene, a partial fragment was PCR amplified using the primers developed by Simon et al. (1994) as follows: amplifications were conducted in 12 μ l reactions consisting of 2.4 μ l of Colorless GoTaq Flexi Reaction Buffer (5 $\times$ -Mg), 1.2 μ l of MgCl₂ (25 mM), 0.6 μ l of dNTPmix (10 mM), 0.6 μ l of each primer (10 μ M), 0.1 μ l of *Taq* polymerase (5 U/ μ l), 2.4 μ l of combinatorial PCR enhancer solution (5 $\times$) (Ralser et al. 2006), 2.1 μ l of Milli-Q H₂O, and 2 μ l of extracted DNA (32 ng/ μ l). The thermal cyclers conditions were the following: 2 min at 94 °C for denaturation, followed by 35 cycles of 40 sec at 94 °C, 40 sec at 50 °C, and 1:30 min at 72 °C, and a final extension of 10 min at 72 °C. For COI gene, a partial fragment was PCR amplified using the primers developed by Folmer et al. (1994) as follows: amplifications were conducted in 15 μ l reactions consisting of 2.1 μ l of Buffer *Taq* (5 $\times$ -Mg), 1.5 μ l of MgCl₂ (25 mM), 0.25 μ l of each dNTP (10 mM), 0.9 μ l of each primer (10 μ M), 0.1 μ l of *Taq* polymerase (5 U/ μ l), 3 μ l of combinatorial PCR enhancer solution (5 $\times$) (Ralser et al. 2006), 1.5 μ l of Milli-Q H₂O, and 4 μ l of extracted DNA (32 ng/ μ l). The thermal cyclers conditions were the following: 4 min at 94 °C for denaturation, followed by 30 cycles of 30 sec at 94 °C, 30 sec at 52 °C, and 1 min at 72 °C, and a final extension of 10 min at 72 °C. PCR products (COIII and COI) were analyzed with agarose-gel (1%) electrophoresis and stained with GelRed. All products amplified successfully, except for the COIII-gene sequence of Specimen No. 38, thus, it was not included in the respective analysis. Amplified products were sequenced in both directions with the same primers used for PCR (MACROGEN INC., South Korea).

All partial COIII and COI sequences were assembled and edited using BIOEDIT 7.2.6 software (Hall 1999). Edited sequences were deposited in the GenBank (Accession Numbers: MN259099–MN259105) (Table 2). All partial sequences of each gene were aligned using MUSCLE (Edgar 2004) in MEGA 7 software (Kumar et al. 2016). The phylogenetic relationships among octopodids were reconstructed using Bayesian inference in MR. BAYES v3 (Huelsenbeck and Ronquist 2001) with the GTR+G+I model (Tavaré 1986) (selected by BIC in MEGA 7 software). The analysis was conducted with four default heated chains, running 1 million MCMC iterations and saving at every 1000th generation. The first 1000 trees were discarded as burn-in. Inter-specific genetic distances were estimated by Kimura-2-parameter model (Kimura 1980) in MEGA 7 software.

Results

The individuals analyzed belonged to the genus *Octopus* Cuvier, 1797; these presented an ink sac and suckers in a two-row arrangement. The specimens presented ocelli (Fig. 2) and were identified as *Octopus oculifer* according to the morphological characteristics specified in its original description (Table 3).



Figure 2. Ocellus of specimen No. 48 **A** defrosted **B** preserved in 96% ethanol.

Diagnosis of the specimens collected in the Revillagigedo Archipelago

The morphological features observed in the octopuses evaluated in this study are shown in Fig. 3A–G. Arm length ranged 3.5 to 4.4 times mantle length. Arm formula $3>4>2>1$. Each arm with 230 to 280 suckers. Enlarged suckers on arms II and III. Gills with 10 lamellae per demibranch. Funnel organ W-shaped. Radula with 9 ele-

Table 3. Diagnostic features of species of the genus *Octopus* reported for the Revillagigedo islands and ocellate species of the world. Species identified in bold.

Species	Arm index	Arm formula	Sucker counts	Ocelli	Lamellae per demibranch	Funnel organ shape
<i>O. bimaculatus</i>	4 to 5	3>2>4>1	200 to 320	Yes	8 to 10	W
<i>O. oculifer</i>	3.5 to 4.5	3>2>4>1	230 to 280	Yes	8 to 10	W
<i>O. hubbsorum</i>	3 to 4	3>2>4>1	240	No	9 to 11	W
<i>O. bimaculoides</i>	3 to 3.5	3>2>4>1	140 to 190	Yes	8 to 10	W
<i>O. maya</i>	3 to 4.5	3>4>2>1	N/A	Yes	9 to 10	W
<i>O. cyanea</i>	4 to 6	4=3=2>1	450 to 500	Yes	9 to 11	W
<i>A. exannulatus</i>	2 to 3	3>4>2>1	120 to 190	Yes	8	W
<i>A. kagoshimensis</i>	2 to 3	4=3=2>1	150 to 170	Yes	8 to 9	W
<i>A. mototi</i>	2.5 to 3	3=4>2>1	140 to 170	Yes	9 to 11	W
<i>A. neglectus</i>	2 to 3	4=3>2>1	110 to 125	Yes	7 to 8	W
<i>A. rex</i>	2 to 3	4>3>2>1	134 to 184	Yes	8 to 9	W
<i>A. siamensis</i>	2 to 3	4=3>2>1	100 to 140	Yes	7 to 8	W
<i>A. ovulum</i>	N/A	4=3=2>1	59 to 70	Yes	15 to 17	W

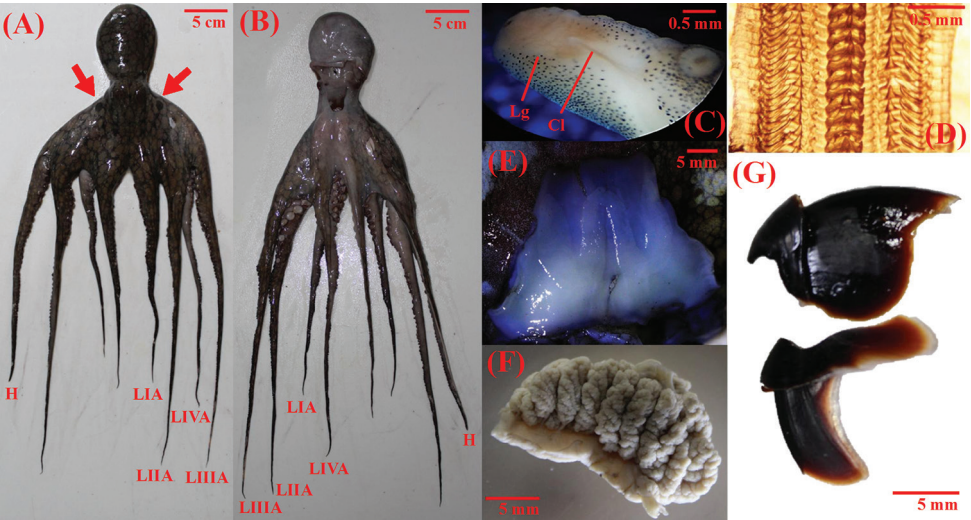


Figure 3. Morphological features. Morphological features of *Octopus oculifer* from the Revillagigedo Archipelago **A** dorsal view; H: hectocotylus, LI-IVA: left arms I-IV **B** ventral view; LI-IVA: left arms I-IV **C** ligula Lg and calamus Cl **D** H: hectocotylus, radulae **E** funnel organ shape **F** demibranch **G** upper and lower beaks.

ments, 7 rows of teeth plus marginal plates. Right third arm of males hectocotylized (with 180 suckers). Ligula tiny, 0.3% of hectocotylized arm. Calamus small, 0.25% of hectocotylized arm. Upper beak: rostral tip blunt and thick; rostral curvature well-defined and extended anteroventrally; the hood extends in posterodorsal direction; crest curved dorsoposteriorly; dorsal portion of the lateral wall sharply angled towards the tip of crest; wing and shoulder compressed posteriorly into an almost vertical position; jaw angle and edge concave ventrally. Lower beak: pointed rostral tip; jaw edge extends in moderate slope posteroventrally; wing fold slightly angled; wing extended in dorsoposterior direction; the dorsal edge raised in the central por-



Figure 4. Coloration patterns. Coloration patterns observed in live individuals of *Octopus oculifer* from the Revillagigedo Archipelago **A** pale body with few reddish/brown spots randomly placed throughout the mantle and arms, entire body with a rugose aspect **B** brown and smooth body with large well-defined white ovals throughout mantle and arms **C** rugose and reddish body with large cream ovals of different size **D** red and smoother (still rugose) body with lesser number of cream ovals of different size **E** dark red body without ovals and a smooth skin.

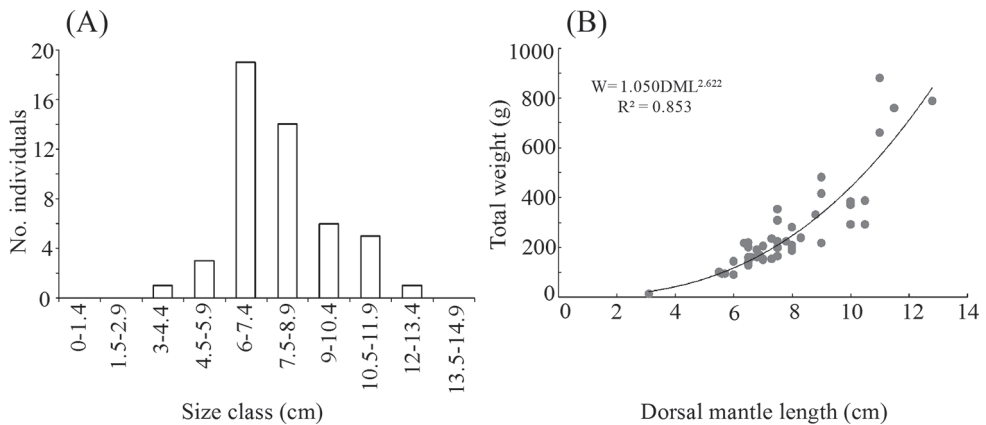


Figure 5. Length-weight relationship of *Octopus oculifer* from the Revillagigedo Archipelago **A** number of individuals per size class **B** length-weight plot.

tion; wall moderately curved posteroventrally towards the tip; free corner of the wall blunt; ventral edge of the wall curved. Color: five coloration patterns were observed in live individuals (Fig. 4), from pale and rugose body with few reddish-brown spots to completely dark-red smooth body without ovals. False-eye spots (ocelli) present as purplish black spot with a small pale central spot; ocelli are bound in an outer pale

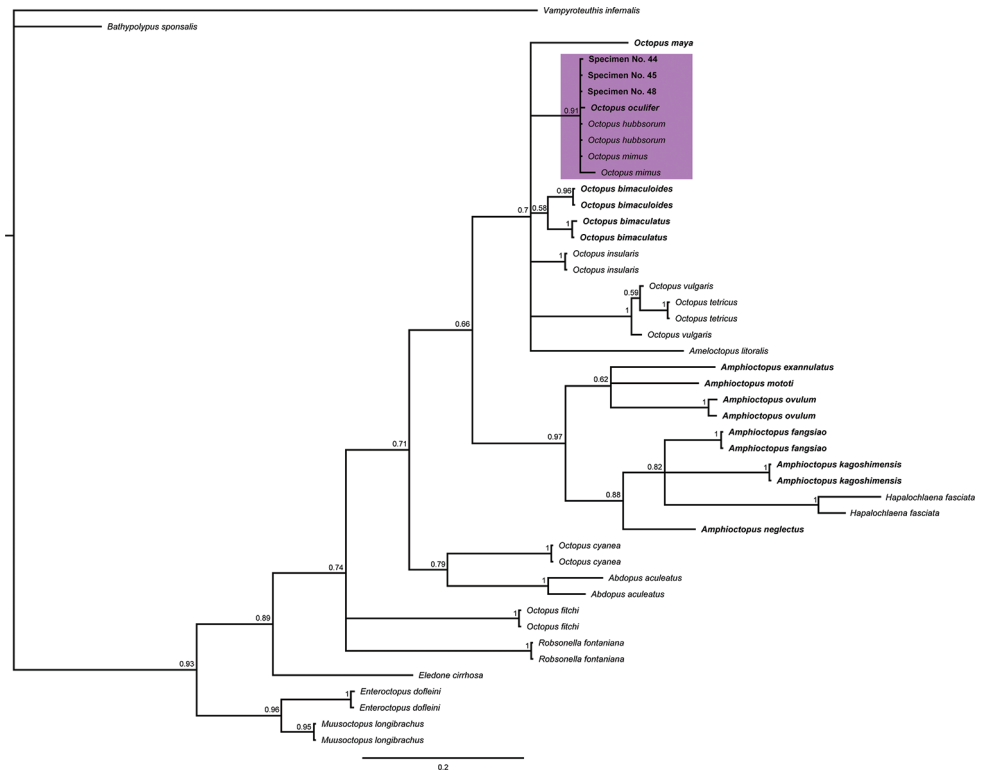


Figure 6. COIII Molecular phylogeny. Molecular phylogeny of COIII-gene sequences (273 bp: 136 variable and 137 conserved) of ocellate and non- ocellate octopus species. Ocellated octopuses are bold-faced. Purple rectangle indicates the clade containing the specimens evaluated in this study.

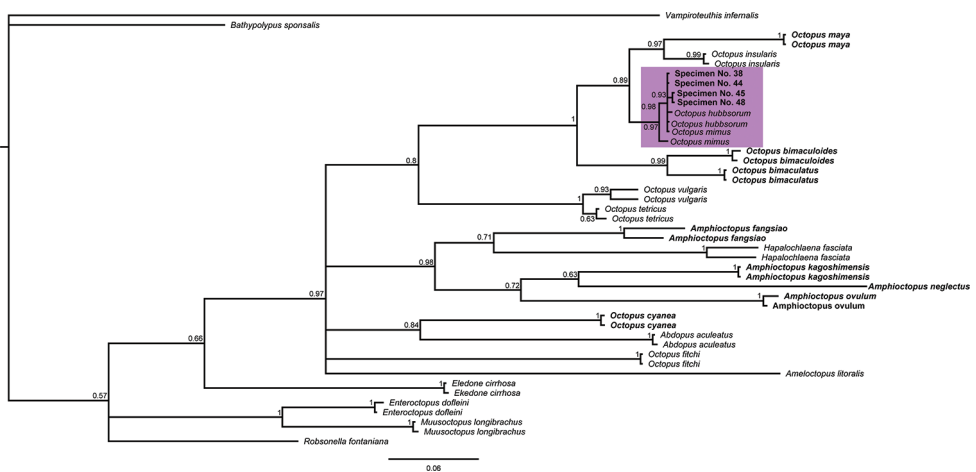


Figure 7. COI Molecular phylogeny. Molecular phylogeny of COI-gene sequences (474 bp: 193 variable and 281 conserved) of ocellate and non- ocellate octopus species. Ocellated octopuses are bold-faced. Purple rectangle indicates the clade containing the specimens evaluated in this study.

Table 4. Genetic distances among octopuses collected and reported in the Revillagigedo Archipelago (RA) and ocellate and non-ocellate octopuses of the world estimated for a fragment of COIII gene sequences. *Octopus maya* (Oct may), *Amphioctopus exalatus* (Amp exa), *Amphioctopus fangsiao* (Amp fan), *Amphioctopus kagoshimensis* (Amp kag), *Amphioctopus mototi* (Amp mot), *Amphioctopus neglectus* (Amp neg), *Amphioctopus oculum* (Amp ovi), *Octopus cyanea* (Oct cyd), *Our spei-* *mens* (Our spe), *Octopus oculifer* (Oct ovi), *Octopus hubbsorum* (Oct hub), *Octopus bimaculoides* (Oct bdes), *Octopus bimaculatus* (Oct bms), *Enteroctopus dofleini* (Ent dof), *Octopus insularis* (Oct ins), *Octopus vulgaris* (Oct vul), *Octopus tetricus* (Oct tet), *Octopus fitchi* (Oct fit), *Robsonella fontianiana* (Rob fon), *Hapalochlaena fasciata* (Hap fas), *Abdopus aculeatus* (Abd acu), *Ameloctopus littoralis* (Ame lit), *Eledone cirrhosa* (Ele cir), *Bathypolypus sponsalis* (Bat spo), *Musoctopus longibrachis* (Muu lon) and *Octopus mimus* (Oct mim).

Sp	Oct may	Amp exa	Amp fan	Amp mot	Amp neg	Amp ovi	Oct cya	Our spe	Oct ovi	Oct hub	Oct bdes	Oct bus	Ent dof	Oct ins	Oct vul	Oct tet	Oct fit	Rob fon	Hap fas	Abd acu	Arne lit	Ele cir	Bat spo	Muu lon	Oct mim
Oct may	0%																								
Amp exa	17.9%																								
Amp fan	15.5%	14.1%																							
Amp kag	19.9%	15.2%	12.0%																						
Amp mot	17.9%	13.5%	12.9%	17.0%																					
Amp neg	16.4%	15.5%	11.4%	14.7%	14.1%																				
Amp ovi	18.5%	15.0%	15.4%	14.7%	14.4%	14.2%																			
Oct cya	18.2%	17.0%	15.8%	18.2%	18.5%	19.9%	17.6%																		
Our spe	13.3%	15.7%	15.4%	18.7%	16.2%	15.1%	14.6%																		
Oct ovi	13.5%	16.1%	15.8%	18.5%	16.4%	15.8%	14.7%	15.0%	0.4%																
Oct hub	13.2%	15.8%	15.5%	18.8%	16.1%	16.1%	15.0%	14.7%	0.3%	8.8%															
Oct bdes	10.3%	17.0%	15.2%	19.1%	15.5%	14.7%	15.1%	15.2%	8.9%	9.1%	8.8%														
Oct bus	10.7%	15.5%	15.8%	19.1%	16.7%	15.0%	14.7%	16.1%	8.5%	8.7%	8.4%	4.8%													
Ent dof	20.8%	20.2%	17.9%	17.6%	19.6%	17.0%	19.9%	19.2%	17.7%	17.3%	17.6%	16.9%	17.0%												
Oct ins	11.1%	15.2%	14.4%	18.5%	16.1%	15.0%	16.4%	8.3%	8.5%	8.2%	9.7%	8.4%	18.8%												
Oct vul	14.1%	16.7%	15.2%	19.4%	16.7%	16.7%	17.4%	17.0%	12.1%	12.3%	12.0%	11.6%	11.4%	21.0%	11.7%										
Oct tet	15.0%	17.3%	15.5%	20.2%	18.5%	15.8%	18.8%	13.3%	13.5%	13.2%	12.9%	12.2%	21.4%	12.3%	3.4%										
Oct fit	21.4%	18.5%	17.9%	17.9%	16.1%	18.2%	19.1%	19.9%	18.3%	18.5%	18.2%	18.2%	19.1%	20.5%	18.2%	20.1%	19.9%								
Rob fon	20.5%	19.4%	18.8%	19.4%	19.4%	21.4%	20.5%	18.8%	17.2%	17.6%	17.3%	18.5%	19.2%	19.8%	19.1%	19.1%	19.6%	20.2%							
Hap fas	19.5%	18.5%	13.2%	15.5%	15.7%	13.9%	15.4%	17.7%	17.5%	17.4%	17.4%	16.7%	16.7%	19.1%	16.7%	15.8%	16.3%	17.6%	20.5%						
Abd acu	18.9%	17.2%	17.0%	18.5%	18.9%	18.0%	17.7%	16.6%	15.3%	15.5%	15.2%	16.4%	17.6%	21.7%	16.0%	16.4%	17.7%	19.1%	19.9%	18.5%					
Arne lit	17.9%	17.9%	18.2%	19.6%	17.6%	17.0%	18.0%	17.9%	14.8%	15.0%	14.7%	13.5%	13.9%	18.6%	13.5%	14.2%	14.1%	19.4%	19.6%	18.9%	18.3%				
Ele cir	22.3%	22.3%	20.5%	22.0%	20.8%	19.9%	21.6%	19.6%	19.5%	19.6%	19.4%	21.4%	21.4%	18.8%	20.7%	20.5%	22.0%	22.6%	18.5%	20.7%	22.9%				
Bat spo	19.4%	20.8%	16.1%	18.2%	20.8%	19.9%	18.6%	18.2%	18.1%	18.5%	18.2%	17.6%	19.5%	17.4%	18.5%	22.1%	22.6%	19.4%	18.5%	21.8%	22.0%	19.1%	20.5%		
Muu lon	21.1%	19.1%	16.4%	17.0%	17.6%	16.4%	18.5%	19.6%	18.1%	18.5%	18.2%	16.4%	17.3%	7.8%	19.6%	21.6%	22.3%	18.8%	19.4%	18.6%	21.4%	18.8%	19.1%	15.5%	
Oct mim	13.5%	15.4%	15.2%	18.8%	16.0%	16.1%	14.8%	14.5%	0.7%	1.0%	0.7%	9.1%	8.8%	18.0%	8.2%	12.2%	13.2%	18.2%	17.7%	17.7%	15.5%	15.1%	19.4%	18.2%	0%

Table 5. Genetic distances among octopuses collected and reported in the Revillagigedo Archipelago (RA) and ocellate and non-ocellate octopuses of the world estimated for a fragment of COI gene sequences. *Octopus maya* (Oct may), *Amphioctopus fangsiao* (Amp fan), *Amphioctopus kugoshimensis* (Amp kag), *Amphioctopus neglectus* (Amp neg), *Amphioctopus oululum* (Amp ou), *Octopus cyanea* (Oct cya), *Our specimens* (Our spe), *Octopus hubbsorum* (Oct hub), *Octopus bimaculoides* (Oct bles), *Octopus bimaculatus* (Oct bms), *Enteroctopus dofleini* (Ent dof), *Octopus insularis* (Oct ins), *Octopus vulgaris* (Oct vul), *Octopus tetricus* (Oct tet), *Octopus fitchi* (Oct fit), *Robsonella fontianiana* (Rob fon), *Hapalochlaena fasciata* (Hap fas), *Abdopus aculeatus* (Abd acu), *Ameloctopus littoralis* (Ame lit), *Bathypolypus sponsalis* (Bat spo), *Eledone cirrhosa* (Ele cir), *Muusoctopus longibrachis* (Muu lon), *Octopus mimus* (Oct mim).

Species	Oct may	Amp fan	Amp lag	Amp neg	Amp ou	Oct cya	Our spe	Oct hub	Oct bles	Oct bms	Ent dof	Oct ins	Oct vul	Oct tet	Oct fit	Rob Jon	Hap fas	Abd acu	Ame lit	Bat spo	Ele cir	Muu lon	Oct mim
Oct may	0%																						
Amp fan	15.0%																						
Amp kag	17.5%	13.9%																					
Amp neg	17.7%	15.8%	14.1%																				
Amp ou	16.7%	13.6%	13.8%	14.5%																			
Oct cya	13.2%	15.8%	14.8%	16.2%	14.0%																		
Our spe	7.3%	14.0%	18.0%	17.2%	17.5%	14.8%																	
Oct hub	7.3%	14.2%	18.0%	17.4%	17.5%	14.6%	0.2%																
Oct bles	10.9%	14.1%	15.5%	17.9%	16.9%	15.4%	9.6%																
Oct bms	10.5%	14.0%	16.5%	18.1%	17.4%	14.2%	9.8%	6.2%															
Ent dof	18.0%	18.9%	19.8%	17.5%	16.4%	16.2%	18.6%	18.6%	19.1%	19.1%													
Oct ins	7.7%	14.8%	16.5%	16.6%	16.1%	13.4%	5.9%	5.9%	9.7%	9.7%	17.6%												
Oct vul	14.3%	16.4%	16.0%	15.8%	17.6%	16.4%	12.8%	13.0%	14.3%	13.6%	18.6%	11.8%											
Oct tet	14.3%	15.7%	16.6%	15.1%	16.7%	15.1%	12.1%	12.3%	14.7%	14.5%	17.1%	11.8%	3.4%										
Oct fit	17.1%	15.6%	16.9%	17.3%	15.9%	15.9%	15.9%	15.9%	18.7%	18.8%	18.1%	16.7%	17.1%	15.8%									
Rob Jon	16.0%	17.5%	16.7%	15.6%	15.9%	16.6%	16.4%	16.1%	16.1%	17.3%	14.1%	15.8%	17.8%	16.8%	15.2%								
Hap fas	16.1%	14.1%	14.8%	17.1%	17.0%	16.1%	16.4%	16.4%	15.7%	16.0%	19.4%	15.5%	16.1%	15.3%	17.6%	17.4%							
Abd acu	16.8%	17.0%	17.3%	16.5%	18.2%	13.5%	16.5%	16.5%	18.2%	16.1%	20.9%	16.1%	16.9%	17.0%	16.4%	16.8%	16.8%						
Ame lit	18.6%	18.9%	19.0%	18.6%	16.8%	18.2%	18.9%	18.7%	18.0%	18.8%	17.3%	17.3%	17.7%	17.3%	16.2%	17.1%	18.2%	18.5%					
Bat spo	18.1%	17.3%	16.7%	16.0%	15.8%	15.8%	18.2%	18.2%	17.5%	18.4%	14.9%	17.5%	17.9%	16.8%	18.6%	14.1%	18.1%	17.3%	19.4%				
Ele cir	16.4%	16.9%	16.8%	19.7%	18.7%	15.1%	16.9%	16.9%	16.9%	17.2%	17.6%	17.0%	16.7%	14.9%	16.8%	15.3%	18.1%	16.0%	17.8%	16.4%			
Muu lon	19.3%	20.3%	18.6%	17.4%	16.9%	17.4%	20.5%	21.0%	20.5%	19.7%	9.4%	19.5%	18.5%	17.4%	20.0%	14.8%	19.1%	19.5%	19.8%	15.6%	17.4%		
Oct mim	7.4%	14.1%	17.9%	17.5%	17.4%	14.7%	0.5%	0.5%	9.5%	9.5%	18.7%	5.8%	12.9%	15.8%	15.8%	16.2%	16.2%	16.6%	18.8%	18.1%	17.0%	20.1%	0%

ring. The individuals analyzed in this study presented isometric growth ($b = 2.62$; $\tau = 2.38$; $p = 0.07$) (Fig. 5).

The phylogenetic trees of COIII and COI-gene sequences showed that the specimens from the Revillagigedo Archipelago belong to a clade associated with the ocellated octopus *O. oculifer* and the non-ocellate octopuses *O. hubbsorum* and *O. mimus* (Figs 6, 7). Similarly, the octopuses from the Revillagigedo Archipelago and sequences regarded as *O. oculifer*, *O. hubbsorum* and *O. mimus* presented low genetic distance ($<1\%$) (Tables 4, 5). In addition, lower genetic distance was observed between ocellated and non-ocellate octopuses than between non-ocellate octopuses of the same genus (e.g., *O. insularis* and *O. bimaculatus* COIII=8.4% and COI=9.7% vs *O. insularis* and *O. vulgaris* COIII=11.7% and COI=11.8%) (Tables 3, 4). Closer relationships were also observed between ocellated and non-ocellate octopuses than between non-ocellate octopuses in the phylogenetic trees (e.g., clade comprising the individuals collected in the Revillagigedo Archipelago and clade containing *Hapalochlaena fasciata*) (Figs 6, 7).

Discussion

In this study we analyzed octopuses from the Revillagigedo Archipelago in an attempt to increase knowledge concerning cephalopods in this geographic area. We identified the octopuses to the species level primarily, according to their morphological attributes, and secondarily, using partial sequences of COIII and COI genes following Vecchione et al. (2017). The individuals were identified as *Octopus oculifer* (Hoyle, 1904) based on morphological and molecular examinations; however, the overlap of characters among the species reviewed in literature, especially between *O. oculifer* and *O. hubbsorum*, and the slight variation of arm formula in regard to original description (i.e., $3>4>2>1$ instead of $3>2>4>1$), explained why Jereb et al. (2016) pointed out that these species are a confusing complex that needs to be carefully re-evaluated (Table 3). Unfortunately, there is no holotype designated for *O. oculifer*, however, the original description is well-delineated and it is the only official conduit that endorses the identity of the species. In our study, there was no reason to suspect that the octopuses evaluated belonged to a new (undescribed) ocellated species or to any ocellated octopus other than *O. oculifer*.

For octopodids, particularly for the species evaluated in this study, a great deal of the taxonomic confusion is related to the fact that the morphological attributes are not standardized among species and that the diagnoses of octopodids from the northeastern Pacific had not been updated since the species descriptions, except for the validation of *O. bimaculatus* and *O. bimaculoides* within the genus *Octopus* performed by Norman and Hochberg (2005), and the recent evaluation performed by Díaz-Santana-Iturrios et al. (2019) for eight species of the genus *Octopus*. Thus, in this study, we provided a detailed characterization of the specimens collected in the Revillagigedo Archipelago and described attributes that were not included in the diagnosis of *O. oculifer* such as

images of anatomic parts and coloration patterns, description of beaks, and length-weight relationship. We suggest that such procedures should be addressed in further research concerning octopods from the Eastern Pacific in general.

The endemism and geographic distribution of *O. oculifer* restricted to the Galapagos Islands was well documented (Hoyle 1904; Edgar et al. 2004; Molina et al. 2004; Jereb et al. 2016); in fact, this area is the known distribution of the species to date. Contrastingly, in our research we found *O. oculifer* in the Revillagigedo Archipelago, Mexico, which is located approximately 3242 km northwest from the Galapagos Islands, Ecuador. Hence, with this information, in this study we report an increase of the distribution range of *O. oculifer*, which could be related to ocean-current patterns putatively used as dispersal mechanism during the paralarval stage, as was detected for larvae of the lobe coral *Porites lobate* Dana, 1846 in that same area (Galapagos and Revillagigedo Archipelago) (Reyes-Bonilla et al. 1999). Another explanation for this distribution expansion could be associated with changes in climatic conditions and similarities between original and new environments (Arkhipkin and Laptikhovski 2008; Stewart et al. 2014; Ramos et al. 2015), given that the Revillagigedos and the Galapagos Islands belong to the same realm (Tropical Eastern Pacific) according to Spalding et al. (2007). Thus, it is likely that the presence of *O. oculifer* in the Revillagigedo islands is related to the incipient climate change, which is beneficial for the abundance and distribution of cephalopods (Doubleday et al. 2016). It is worth noting, however, that the Revillagigedo Archipelago is a remote protected area, which limits human access, and therefore, the cephalopod diversity has been determined incidentally and with unreliable observations. As a result, octopods are only included as part of the mollusks and macroinvertebrate fauna of the Revillagigedo Archipelago in generalized taxonomic lists (i.e., González-Nakawaga and Sánchez-Nava 1986; Holguín-Quinones et al. 1992; Ortega and Castellanos 1994; Ortega et al. 1995; Cabrera-Mancilla and Bautista-Moreno 2002; CONANP 2004; Friscione-Carrascosa 2005; Bedolla-Guzmán 2007; CONANP 2018) that do not specify the identification criteria or that employ taxonomic keys (i.e., Keen 1971; Abbott and Dance 1998) that were not developed by specialized cephalopod taxonomists. These lists include identifications at the genus level (*Octopus* sp.), tentative determinations (*Octopus* cf. *bimaculatus*) and/or erroneous identifications, such as *Callistoctopus macropus* (Risso, 1826) (formerly *O. macropus*), from the Mediterranean and northwestern Africa (Jereb et al. 2016). Thus, it is more probable that the presence *O. oculifer* in the Revillagigedo Archipelago occurred much earlier in geological time due to different ocean current patterns putatively used as dispersal mechanism during the paralarval stage, as explained earlier, than during the current climate change, and that its presence was not noticed until this detailed revision.

In addition, the molecular analyses of partial COIII and COI sequences strongly evidenced that *O. oculifer*, *O. hubbsorum*, and *O. mimus* are very closely related (interspecific distance lower than 1%) and it is highly likely that these taxa are conspecific and represent a species complex comprised by three morphotypes. However, our finding should be further confirmed with type material (when available) and complete re-

descriptions must be performed to support that these taxa are the same species. Moreover, the closer evolutionary relationships found between ocellated and non-ocellated octopuses compared to the relationships among non-ocellated octopuses indicate that the presence of ocelli is not a determinant character in octopodid classification and therefore, it should not be considered a diagnostic attribute.

Conclusions

In this research, we conclude that according to our integrative species identification, the specimens collected in the Revillagigedo Archipelago are *Octopus oculifer*. According to our molecular analyses the non-ocellate *O. hubbsorum* and *O. mimus* and the ocellate *O. oculifer* are very closely related and might constitute a single species comprised of three morphotypes. In addition, ocelli should not be considered a diagnostic attribute for octopodids but rather a supplemental character.

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Description of a new *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from Guizhou Province, China

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Abstract

A new species of the genus *Megophrys* is described from Guizhou Province, China. Molecular phylogenetic analyses based on mitochondrial DNA indicated the new species as a clade clustered into the *Megophrys* clade. The new species can be distinguished from its congeners by a combination of the following characters: body size moderate (SVL 40.0–45.5 mm in males and 48.9–51.2 mm in females); vomerine teeth absent; tongue not notched behind; tympanum distinctly visible, oval; a small horn-like tubercle at the edge of each upper eyelid; two metacarpal tubercles in hand; toes with rudimentary webbing; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level of mid-eye when leg stretched forward; in breeding males, an internal single subgular vocal sac present and brownish nuptial pads, made up of black nuptial spines, present on the dorsal base of the first two fingers.

Keywords

Molecular phylogenetic analysis, morphology, new species, taxonomy

* These authors have contributed equally to this work.

Introduction

The Asian horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura: Megophryidae Bonaparte, 1850) is widely distributed in eastern and central China, throughout southeastern Asia, and extending to the islands of the Sunda Shelf and the Philippines (Frost 2020). This group was indicated to be a monophyletic group by most molecular phylogenetic studies (e.g., Chen et al. 2017; Mahony et al. 2017; Liu et al. 2018; Li et al. 2018a; Liu et al. 2020; Wang et al. 2020) though the taxonomic profiles especially on generic assignments of species in the group are still on debate (e.g., Tian and Hu 1983; Dubois 1987; Rao and Yang 1997; Lathrop 1997; Jiang et al. 2003; Delorme et al. 2006; Fei et al. 2009; Fei and Ye 2016; Chen et al. 2017; Deuti et al. 2017; Mahony et al. 2017; Liu et al. 2018; Frost 2020). Currently, the genus *Megophrys* contains 106 species, of which, 49 species were described in the last ten years (Frost 2020). Molecular phylogenetic frameworks even still proposed many cryptic species in the genus (e.g., Chen et al. 2017; Liu et al. 2018). In Guizhou Province, China, in recent five years, four *Megophrys* species have been described, and they are, *M. liboensis* Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017, *M. leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018, *M. jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020, and *M. chishuiensis* Xu, Li, Liu, Wei & Wang, 2020.

During field surveys in Anlong County, Guizhou Province, China, we collected eight *Megophrys* specimens. Molecular phylogenetic analyses and morphological comparisons supported it as an undescribed species and it is described herein as a new species.

Materials and methods

Sampling

Three adult females and five adult males of the undescribed species were collected from Anlong County, Guizhou Province, China (Fig. 1; Table 1). The toads were firstly euthanised using isoflurane, and then the specimens were fixed in 75% ethanol for preservation. Tissue samples were taken and preserved separately in 95% ethanol prior to fixation. The specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).

Phylogenetic analyses

Six specimens of the undescribed species were included in the molecular analyses (Table 2). Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Two fragments of the mitochondrial 16S rRNA (16S) and cytochrome oxidase subunit I (COI) genes were amplified. For 16S gene, the primers P7 (5'-CGCCTGTTTACCAAAAACAT-3') and P8 (5'-CCGGTCTGAACTCAGATCACGT-3') were used following Simon et al. (1994), and for COI gene, Chmf4

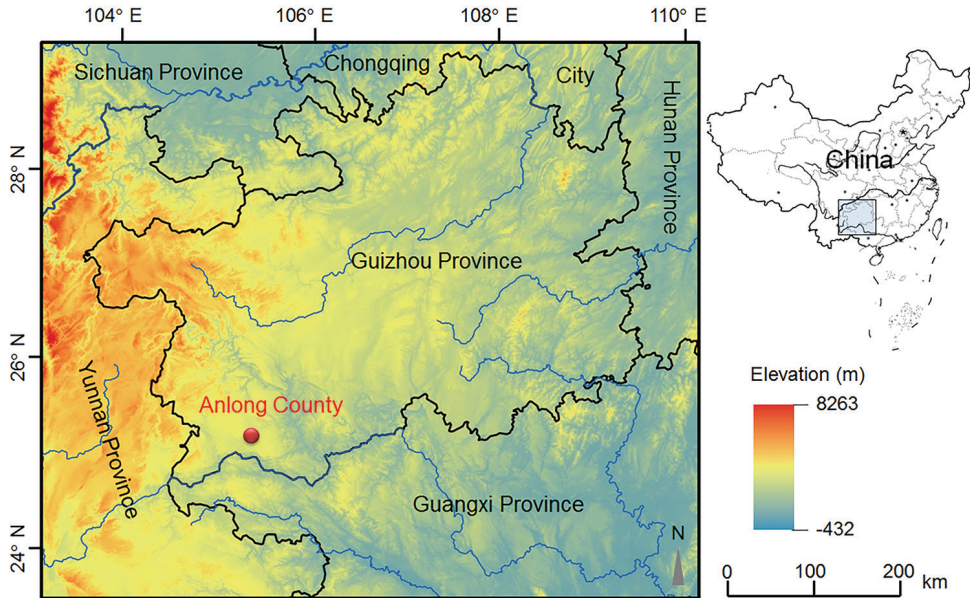


Figure 1. Geographical location of the type locality of *Megophrys anlongensis* sp. nov., Anlong County, Guizhou Province, China.

(5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRT-GRCCRAARAATCA-3') were used following Che et al. (2012). The fragments were amplified under the following conditions: an initial denaturing step at 95 °C for 4 min; 36 cycles of denaturing at 95 °C for 30 s, annealing at 52 °C (for 16S)/47 °C (for COI) for 40 s and extending at 72 °C for 70 s. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. New sequences were deposited in GenBank (for GenBank accession numbers see Table 2).

For molecular analyses, the available sequence data for congeners of *Megophrys* were downloaded from GenBank (Table 2), primarily from previous studies (Chen et al. 2017; Liu et al. 2018). For phylogenetic analyses, corresponding sequences of one *Leptobranchella oshanensis* (Liu, 1950) and one *Leptobranchium boringii* (Liu, 1945) were also downloaded from GenBank (Table 2), and used as outgroups according to Mahony et al. (2017). Sequences were assembled and aligned using the Clustalw module in BioEdit v.7.0.9.0 (Hall 1999) with default settings. Alignments were checked by eye and revised manually if necessary. For phylogenetic analyses of mitochondrial DNA, the dataset concatenated with 16S and COI gene sequences. To avoid under- or over-parameterisation (Lemmon and Moriarty 2004; McGuire et al. 2007), the best partition scheme and the best evolutionary model for each partition were chosen for the phylogenetic analyses using PARTITIONFINDER v. 1.1.1 (Robert et al. 2012). In this analysis, 16S gene and each codon position of COI gene were defined, and Bayesian Inference Criteria was used. As a result, the analysis suggested that the best partition scheme is 16S gene/each codon position of COI gene,

Table 1. Measurements of the adult specimens of *Megophrys anlongensis* sp. nov. Units given in mm. See abbreviations for the morphological characters in Materials and methods section.

Voucher	Sex	SVL	HDL	HDW	SL	IND	IOD	UEW	ED	TYD	LAL	IW	HLL	THL	TL	TW	TFL	FL
CIBAL20190531021	♂	45.5	12.3	15.9	6.0	5.6	4.1	4.0	4.7	3.1	19.9	5.0	70.8	18.8	22.5	5.2	33.1	22.7
CIBAL20190531019	♂	41.1	12.4	14.0	5.0	4.5	3.2	4.3	4.8	2.7	18.8	4.3	65.4	17.7	22.3	5.1	29.4	19.9
CIBAL20190531017	♂	42.5	11.5	14.4	5.1	4.7	4.1	3.7	4.4	3.1	19.7	4.2	67.7	20.2	22.1	5.5	31.1	21.5
CIBAL20190531020	♂	42.5	11.6	14.5	6.0	5.2	3.4	4.3	5.1	3.5	19.4	4.5	63.9	19.9	20.9	5.2	29.2	19.0
CIBAL20190531018	♂	40.0	13.0	14.5	5.6	4.7	4.0	3.8	4.6	2.7	19.1	3.8	65.2	19.7	21.2	5.0	29.6	20.0
Range of males		40.0–45.5	11.5–13.0	14.0–15.9	5.0–6.0	4.5–5.6	3.2–4.1	3.7–4.3	4.4–5.1	2.7–3.5	18.8–19.9	3.8–5.0	63.9–70.8	17.7–20.2	20.9–22.5	5.0–5.5	29.2–33.1	19.0–22.7
Mean ± SD of males		42.3 ± 2.04	12.1 ± 0.62	14.6 ± 0.71	5.5 ± 0.48	4.9 ± 0.45	3.8 ± 0.41	4.0 ± 0.28	4.7 ± 0.25	3.0 ± 0.36	19.4 ± 0.44	4.34 ± 0.44	66.6 ± 2.70	19.3 ± 1.03	21.8 ± 0.73	5.2 ± 0.18	30.3 ± 1.64	20.6 ± 1.47
CIBAL20190531022	♀	51.2	12.9	17.4	6.5	5.7	4.2	4.7	4.9	3.3	23.6	4.0	83.7	26.0	27.5	6.0	38.6	25.6
CIBAL20190811015	♀	48.9	13.1	16.1	5.5	5.1	3.1	4.1	4.9	2.9	23.8	3.7	83.4	24.7	26.2	5.3	38.0	26.0
CIBAL20190811014	♀	49.4	13.2	16.5	6.0	5.3	4.0	4.5	5.1	3.1	24.5	3.3	88.2	25.4	28.0	5.0	40.4	20.5
Range of females		48.9–51.2	12.9–13.2	16.0–17.0	5.5–6.5	5.1–5.7	3.1–4.2	4.1–4.7	4.9–5.1	2.9–3.3	23.6–24.5	3.3–4.0	83.4–88.2	24.7–26.0	26.2–28.0	5.0–6.0	38.0–40.4	20.5–26.0
Mean ± SD of females		49.8 ± 1.21	13.1 ± 0.17	16.7 ± 0.66	6.0 ± 0.50	5.4 ± 0.31	3.8 ± 0.59	4.4 ± 0.31	5.0 ± 0.12	3.1 ± 0.21	24.0 ± 0.48	3.7 ± 0.35	85.1 ± 2.68	25.4 ± 0.64	27.2 ± 0.92	5.4 ± 0.51	39.0 ± 1.24	24.0 ± 3.06

Table 2. Information for samples used in molecular phylogenetic analyses in this study.

ID	Species	Voucher number	Locality	GenBank accession number	
				16S	COI
1	<i>Megophrys anlongensis</i>	CIBAL20190531018	Anlong County, Guizhou, China	MT823184	MT823261
2	sp. nov.	CIBAL20190531017	Anlong County, Guizhou, China	MT823185	MT823262
3		CIBAL20190531022	Anlong County, Guizhou, China	MT823186	MT823263
4		CIBAL20190811014	Anlong County, Guizhou, China	MT823187	MT823264
5		CIBAL20190811015	Anlong County, Guizhou, China	MT823188	MT823265
6		CIBAL20190531019	Anlong County, Guizhou, China	MT823189	MT823266
7	<i>Megophrys nankunensis</i>	SYS a004498	Nankun Shan, Guangdong, China	MK524108	MK524139
8	<i>Megophrys dongguanensis</i>	SYS a001972	Yinping Shan, Guangdong, China	MK524098	MK524129
9	<i>Megophrys cheni</i>	SYS a001427	Jinggang Shan, Jiangxi, China	KJ560391	–
10	<i>Megophrys obesa</i>	SYS a002272	Heishiding Nature Reserve, Guangdong, China	KJ579122	–
11	<i>Megophrys ombrophila</i>	KRM18	Wuyishan, Fujian, China	KX856404	–
12	<i>Megophrys wugongensis</i>	SYS a002610	Wugongshan Scenic Area, Jiangxi, China	MK524114	MK524145
13	<i>Megophrys lini</i>	SYS a002370	Suichuan, Jiangxi, China	KJ560412	–
14	<i>Megophrys xiangnanensis</i>	SYS a002874	Yangming Shan, Hunan, China	MH406713	MH406165
15	<i>Megophrys nanlingensis</i>	SYS a001959	Nanling Nature Reserve, Guangdong, China	MK524111	MK524142
16	<i>Megophrys kuatunensis</i>	SYS a001579	Wuyi Shan, Fujian, China	KJ560376	–
17	<i>Megophrys jinggangensis</i>	KIZ07132	Chashan Forest Farm, Jiangxi, China	KX811840	KX812108
18	<i>Megophrys xianjuensis</i>	CIBXJ190505	Xianju, Zhejiang, China	MN563753	MN563769
19	<i>Megophrys lishuiensis</i>	WYF00169	Lishui, Zhejiang, China	KY021418	–
20	<i>Megophrys huangshanensis</i>	KIZ022004	Huang Shan, Anhui, China	KX811821	KX812107
21	<i>Megophrys boettgeri</i>	Tissue ID: YPXJK033	Wuyi Shan, Fujian, China	KX811814	KX812104
22	<i>Megophrys liboensis</i>	GNUG:20160408003	Libo, Guizhou, China	MF285262	–
23	<i>Megophrys mufuimontana</i>	SYS a006391	Mufu Shan, Hunan, China	MK524105	MK524136
24	<i>Megophrys mirabilis</i>	SYS a002192	Huaping Nature Reserve, Guangxi, China	MH406669	MH406109
25	<i>Megophrys shunhuangensis</i>	HNNU16SH02	Shunhuang Mountains, Hunan, China	MK836037	–
26	<i>Megophrys acuta</i>	SYS a001957	Heishiding Nature Reserve, Guangdong, China	KJ579118	–
27	<i>Megophrys leishanensis</i>	CIBLS20171101001	Leigong Shan, Guizhou, China	MK005310	MK005306
28	<i>Megophrys shimentaina</i>	SYS a002077	Shimentai Nature Reserve Guangdong, China	MH406655	MH406092
29	<i>Megophrys yangmingensis</i>	SYS a002877	Yangming Shan, Hunan, China	MH406716	MH406168
30	<i>Megophrys jiulianensis</i>	SYS a002107	Jiulian Shan, Jiangxi, China	MK524099	MK524130
31	<i>Megophrys wushanensis</i>	KIZ045469	Guangwu Shan, Sichuan, China	KX811838	KX812094
32	<i>Megophrys baolongensis</i>	KIZ019216	Baolong, Chongqing, China	KX811813	KX812093
33	<i>Megophrys tuberogranulata</i>	Tissue ID: YPX10987	Badagongshan Nature Reserve, Hunan, China	KX811823	KX812095
34	<i>Megophrys binchuanensis</i>	KIZ019441	Jizu Shan, Yunnan, China	KX811849	KX812112
35	<i>Megophrys sangzhiensis</i>	SYSa004307	Zhangjiajie, Hunan, China	MH406798	MH406260
36	<i>Megophrys spinata</i>	SYSa002227	Leigong Shan, Guizhou, China	MH406676	MH406116
37	<i>Megophrys binlingensis</i>	SYSa005313	Wawu Shan, Sichuan, China	MH406892	MH406354
38	<i>Megophrys angka</i>	KIZ040591	Kiew Mae Pan nature trail, Chiang Mai, Thailand	MN508052	–
39	<i>Megophrys omeimontis</i>	KIZ025765	Emei Shan, Sichuan, China	KX811884	KX812136
40	<i>Megophrys palpebralespinosa</i>	KIZ011603	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KX811888	KX812137
41	<i>Megophrys jingdongensis</i>	KIZ-LC0805067	Huanglianshan National Nature Reserve, Yunnan, China	KX811872	KX812131
42	<i>Megophrys daweimontis</i>	KIZ048997	Dawei Shan, Yunnan, China	KX811867	KX812125
43	<i>Megophrys wuliangshanensis</i>	KIZ046812	Huangcaoling, Yunnan, China	KX811881	KX812129
44	<i>Megophrys fansipanensis</i>	VNMN 2018.01	Lao Cai, Sa Pa, Vietnam	MH514886	–
45	<i>Megophrys boanglienensis</i>	VNMN 2018.02	Lao Cai, Sa Pa, Vietnam	MH514889	–
46	<i>Megophrys minor</i>	KIZ01939	Qingcheng Shan, Sichuan, China	KX811896	KX812145
47	<i>Megophrys jiangi</i>	CIBKKS20180722006	Kuankuosui Nature Reserve, Guizhou, China	MN107743	MN107748
48	<i>Megophrys chishuiensis</i>	CIBCS20190518031	Chishui Nature Reserve, Guizhou, China	MN954707	MN928958
49	<i>Megophrys brachykolos</i>	ROM 16634	Hong Kong, China	KX811897	KX812150
50	<i>Megophrys elfina</i>	ZMMU ABV-00454	Bidoup Mountain, Lam Dong, Vietnam	KY425379	–
51	<i>Megophrys gerti</i>	ITBCZ 1108	Nui Chua National Park, Ninh Thuan, Vietnam	KX811917	KX812161
52	<i>Megophrys synoria</i>	FMNH 262778	O'Reang, Mondolkiri, Cambodia	KY022198	–
53	<i>Megophrys microstoma</i>	KIZ048799	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811914	KX812156
54	<i>Megophrys hansi</i>	KIZ010360	Phong Dien Nature Reserve, Thua Thien Hue, Vietnam	KX811913	KX812155

ID	Species	Voucher number	Locality	GenBank accession number	
				16S	COI
55	<i>Megophrys pachyproctus</i>	KIZ010978	Beibeng, Xizang, China	KX811908	KX812153
56	<i>Megophrys baluensis</i>	ZMH A13125	Gunung Kinabalu National Park, Kogopan Trail, Malaysia	KJ831310	–
57	<i>Megophrys stejnegeri</i>	KU 314303	Pasonanca Natural Park, Zamboanga, Philippines	KX811922	KX812052
58	<i>Megophrys ligayae</i>	ZMMU NAP-05015	Palawan, Philippines	KX811919	KX812051
59	<i>Megophrys nasuta</i>	KIZ019419	Malaysia	KX811921	KX812054
60	<i>Megophrys kobayashii</i>	UNIMAS 8148	Gunung Kinabalu National Park, Sabah, Malaysia	KJ831313	–
61	<i>Megophrys edwardinae</i>	FMNH 273694	Bintulu, Sarawak, Malaysia	KX811918	KX812050
62	<i>Megophrys aceras</i>	KIZ025467	Khao Nan National Park, Nakhon Si Thammarat, Thailand	KX811925	KX812159
63	<i>Megophrys zhangii</i>	KIZ014278	Zhangmu, Xizang, China	KX811765	KX812084
64	<i>Megophrys sanu</i>	K5198/ZS111393	–	KX894679	–
65	<i>Megophrys katabhako</i>	ZSIA11799	–	KX894669	–
66	<i>Megophrys periosa</i>	BNHS 6061	West Kameng dist., Arunachal Pradesh, India	KY022309	MH647528
67	<i>Megophrys himalayana</i>	SDBDU2009.75	East Siang dist., Arunachal Pradesh, India	KY022311	–
68	<i>Megophrys glandulosa</i>	KIZ048439	Husa, Yunnan, China	KX811762	KX812075
69	<i>Megophrys medogensis</i>	KIZ06621	Beibeng, Xizang, China	KX811767	KX812082
70	<i>Megophrys flavipunctata</i>	SDBDU2009.297	East Khasi Hills dist., Meghalaya, India	KY022307	MH647536
71	<i>Megophrys maosonensis</i>	KIZ016045	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811780	KX812080
72	<i>Megophrys mangshanensis</i>	KIZ021786	Nanling National Forest Park, Guangdong, China	KX811790	KX812079
73	<i>Megophrys oreocrypta</i>	BNHS 6046	West Garo Hills dist., Meghalaya, India	KY022306	–
74	<i>Megophrys major</i>	SYSa002961	Zhushihe, Yunnan, China	MH406728	MH406180
75	<i>Megophrys parva</i>	SYSa003042	Zhushihe, Yunnan, China	MH406737	MH406189
76	<i>Megophrys auralensis</i>	NCSM 79599	Aural, Kampong Speu, Cambodia	KX811807	–
77	<i>Megophrys dringi</i>	UNIMAS 8943	Gunung Mulu National Park, Sarawak, Malaysia	KJ831317	–
78	<i>Megophrys gigantica</i>	SYSa003933	Wuliang shan, Yunnan, China	MH406775	MH406235
79	<i>Megophrys shapingsensis</i>	KIZ014512	Liziping Nature Reserve, Sichuan, China	KX811904	KX812060
80	<i>Megophrys wawuensis</i>	KIZ025799	Wawu Shan, Sichuan, China	KX811902	KX812062
81	<i>Megophrys nankiangensis</i>	CIB ZYC517	Nanjiang, Sichuan, China	KX811900	–
82	<i>Megophrys lancip</i>	MZB:Amp:22233	–	KY679891	–
83	<i>Megophrys montana</i>	LSUMZ 81916	Sukabumi, Java, Indonesia	KX811927	KX812163
84	<i>Megophrys popei</i>	SYS a000589	Naling Nature Reserve, Guangdong, China	KM504251	–
85	<i>Megophrys carinense</i>	Tissue ID: YPX20455	Dayao Shan, Guangxi, China	KX811811	KX812057
86	<i>Megophrys feae</i>	KIZ046706	Huangcaoling, Yunnan, China	KX811810	KX812056
87	<i>Megophrys chuannanensis</i>	CIB20050081	Hejiang, Sichuan, China	KM504261	–
88	<i>Megophrys intermedia</i>	ZFMK 87596	U Bo, Phong Nha-Ke Bang NP, Vietnam	HQ588950	–
89	<i>Leptobranchium boringii</i>	Tissue ID: YPX37539	Emei Shan, Sichuan, China	KX811930	KX812164
90	<i>Leptobranchella oshanensis</i>	KIZ025778	Emei Shan, Sichuan, China	KX811928	KX812166

and selected GTR + G + I model as the best model for each partition. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian Inference (BI) methods, implemented in PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.12 (Ronquist and Huelsenbeck 2003), respectively. For the ML tree, branch supports were drawn from 10,000 nonparametric bootstrap replicates. In BI, two runs each with four Markov chains were simultaneously run for 50 million generations with sampling every 1,000 generations. The first 25% trees were removed as the “burn-in” stage followed by calculations of Bayesian posterior probabilities and the 50% majority-rule consensus of the post burn-in trees sampled at stationarity. Finally, genetic distance between species based on uncorrected *p*-distance model was estimated on each gene using MEGA v. 6.06 (Tamura et al. 2013).

Morphological comparisons

All eight adult specimens of the undescribed species were measured (Table 1). The terminology and methods followed Fei et al. (2009). Measurements were taken with a dial caliper to 0.1 mm. Seventeen morphometric characters of adult specimens were measured:

ED	eye diameter (distance from the anterior corner to the posterior corner of the eye);	LW	lower arm width (maximum width of the lower arm);
FL	foot length (distance from tarsus to the tip of fourth toe);	SVL	snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
HDL	head length (distance from the tip of the snout to the articulation of jaw);	SL	snout length (distance from the tip of the snout to the anterior corner of the eye);
HDW	maximum head width (greatest width between the left and right articulations of jaw);	TFL	length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
HLL	hindlimb length (maximum length from the vent to the distal tip of the Toe IV);	THL	thigh length (distance from vent to knee);
IND	internasal distance (minimum distance between the inner margins of the external nares);	TL	tibia length (distance from knee to tarsus);
IOD	interorbital distance (minimum distance between the inner edges of the upper eyelids);	TW	maximal tibia width;
LAL	length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);	TYD	maximal tympanum diameter;
		UEW	upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

The undescribed species was also compared with all other congeners on morphology. Comparative data were obtained from related species as described in literature (Table 3).

Bioacoustics data

The advertisement calls of the undescribed species were recorded from the holotype specimen CIBAL20190531018 in the field on 31 May 2019 in Anlong County, Guizhou Province, China. The advertisement call of the undescribed species was recorded in the stream at ambient air temperature of 18.5 °C and air humidity of 83%. SONY PCM-D50 digital sound recorder was used to record within 30 cm of the calling individual. The sound files in wave format were resampled at 48 kHz with sampling depth 24 bits. The sonograms and waveforms were generated by WaveSurfer software (Sjöander and Beskow 2000) from which all parameters and characters were measured. Ambient temperature was taken by a digital hygrothermograph.

Table 3. Bibliographic references for morphological characters for congeners of the genus *Megophrys*.

Species	Literature obtained
<i>M. aceras</i> Boulenger, 1903	Boulenger 1903
<i>M. acuta</i> Wang, Li & Jin, 2014	Li et al. 2014
<i>M. ancræ</i> Mahony, Teeling & Biju, 2013	Mahony et al. 2013
<i>M. angka</i> Wu, Suwannapoom, Poyarkov, Chen, Pawangkhanant, Xu, Jin, Murphy & Che, 2019	Wu et al. 2019
<i>M. auralensis</i> Ohler, Swan & Daltry, 2002	Ohler et al. 2002
<i>M. baluensis</i> (Boulenger, 1899)	Boulenger 1899a
<i>M. baolongensis</i> Ye, Fei & Xie, 2007	Ye et al. 2007
<i>M. binchuanensis</i> Ye & Fei, 1995	Ye and Fei 1995
<i>M. binlingensis</i> Jiang, Fei & Ye, 2009	Fei et al. 2009
<i>M. boettgeri</i> (Boulenger, 1899)	Boulenger 1899b
<i>M. brachykolos</i> Inger & Romer, 1961	Inger and Romer 1961
<i>M. carinense</i> (Boulenger, 1889)	Boulenger 1889
<i>M. caobangensis</i> Nguyen, Pham, Nguyen, Luong, and Ziegler, 2020	Nguyen et al. 2020
<i>M. caudoprocta</i> Shen, 1994	Shen 1994
<i>M. cheni</i> (Wang & Liu, 2014)	Wang et al. 2014
<i>M. chishuiensis</i> Xu, Li, Liu, Wei & Wang, 2020	Xu et al. 2020
<i>M. chuannanensis</i> (Fei, Ye & Huang, 2001)	Fei et al. 2001
<i>M. damrei</i> Mahony, 2011	Mahony 2011
<i>M. daweimontis</i> Rao & Yang, 1997	Rao and Yang 1997
<i>M. dongguanensis</i> Wang & Wang, 2019	Wang et al. 2019b
<i>M. dringi</i> Inger, Stuebing & Tan, 1995	Inger et al. 1995
<i>M. eduardinae</i> Inger, 1989	Inger 1989
<i>M. elfina</i> Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony, 2017	Poyarkov et al. 2017
<i>M. fansipanensis</i> Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
<i>M. feae</i> Boulenger, 1887	Boulenger 1887
<i>M. feii</i> Yang, Wang & Wang, 2018	Yang et al. 2018
<i>M. flavipunctata</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. gerti</i> (Ohler, 2003)	Ohler 2003
<i>M. gigantica</i> Liu, Hu & Yang, 1960	Liu et al. 1960
<i>M. glandulosa</i> Fei, Ye & Huang, 1990	Fei et al. 1990
<i>M. hansi</i> (Ohler, 2003)	Ohler 2003
<i>M. himalayana</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. hoanglienensis</i> Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
<i>M. huangshanensis</i> Fei & Ye, 2005	Fei and Ye 2005
<i>M. insularis</i> (Wang, Liu, Lyu, Zeng & Wang, 2017)	Wang et al. 2017a
<i>M. intermedia</i> Smith, 1921	Smith 1921
<i>M. jiangi</i> Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020	Liu et al. 2020
<i>M. jingdongensis</i> Fei & Ye, 1983	Fei et al. 1983
<i>M. jinggangensis</i> (Wang, 2012)	Wang et al. 2012
<i>M. julianensis</i> Wang, Zeng, Lyu & Wang, 2019	Wang et al. 2019b
<i>M. kalimantanensis</i> Munir, Hamidy, Matsui, Iskandar, Sidik & Shimada, 2019	Munir et al. 2019
<i>M. kobayashii</i> Malkmus & Matsui, 1997	Malkmus and Matsui 1997
<i>M. koui</i> Mahony, Foley, Biju & Teeling, 2017	Mahony et al. 2017
<i>M. kuatunensis</i> Pope, 1929	Pope 1929
<i>M. lancip</i> Munir, Hamidy, Farajallah & Smith, 2018	Munir et al. 2018
<i>M. leishanensis</i> Li, Xu, Liu, Jiang, Wei & Wang, 2018	Li et al. 2018a
<i>M. lekaguli</i> Stuart, Chuaynkern, Chan-ard & Inger, 2006	Stuart et al. 2006
<i>M. liboensis</i> (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017)	Zhang et al. 2017
<i>M. ligayae</i> Taylor, 1920	Taylor 1920
<i>M. lini</i> (Wang & Yang, 2014)	Wang et al. 2014
<i>M. lishuiensis</i> (Wang, Liu & Jiang, 2017)	Wang et al. 2017b
<i>M. longipes</i> Boulenger, 1886	Boulenger 1886
<i>M. major</i> Boulenger, 1908	Boulenger 1908
<i>M. mangshanensis</i> Fei & Ye, 1990	Fei et al. 2012
<i>M. maosonensis</i> Bourret, 1937	Bourret 1937
<i>M. medogensis</i> Fei, Ye & Huang, 1983	Fei et al. 1983
<i>M. megacephala</i> Mahony, Sengupta, Kamei & Biju, 2011	Mahony et al. 2011
<i>M. microstoma</i> (Boulenger, 1903)	Boulenger 1903
<i>M. minor</i> Stejneger, 1926	Stejneger 1926
<i>M. mirabilis</i> Lyu, Wang & Zhao	Lyu et al. 2020

Species	Literature obtained
<i>M. montana</i> Kuhl & Van Hasselt, 1822	Kuhl and Van Hasselt 1822
<i>M. monticola</i> (Günther, 1864)	Günther 1864
<i>M. mufumontana</i> Wang, Lyu & Wang, 2019	Wang et al. 2019b
<i>M. nankiangensis</i> Liu & Hu, 1966	Hu and Liu 1966
<i>M. nankunensis</i> Wang, Zeng & Wang, 2019	Wang et al. 2019b
<i>M. nanlingensis</i> Lyu, Wang, Liu & Wang, 2019	Wang et al. 2019b
<i>M. nasuta</i> (Schlegel, 1858)	Schlegel 1858
<i>M. obesa</i> Wang, Li & Zhao, 2014	Wang et al. 2014
<i>M. ombrophila</i> Messenger & Dahn, 2019	Messenger et al. 2019
<i>M. omeimontis</i> Liu, 1950	Liu 1950
<i>M. oreocrypta</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. oropedion</i> Mahony, Teeling & Biju, 2013	Mahony et al. 2013
<i>M. orientalis</i> Li, Lyu, Wang & Wang, 2020	Li et al. 2020
<i>M. pachyproctus</i> Huang, 1981	Huang and Fei 1981
<i>M. palpebralespinosa</i> Bourret, 1937	Bourret 1937
<i>M. parallela</i> Inger & Iskandar, 2005	Inger and Iskandar 2005
<i>M. parva</i> (Boulenger, 1893)	Boulenger 1893
<i>M. periosa</i> Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
<i>M. popei</i> (Zhao, Yang, Chen, Chen & Wang, 2014)	Zhao et al. 2014
<i>M. robusta</i> Boulenger, 1908	Boulenger 1908
<i>M. rubrimeria</i> Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017	Tapley et al. 2017
<i>M. sangzhiensis</i> Jiang, Ye & Fei, 2008	Jiang et al. 2008
<i>M. serchhipii</i> (Mathew & Sen, 2007)	Mathew and Sen 2007
<i>M. shapingensis</i> Liu, 1950	Liu 1950
<i>M. shimentaina</i> Lyu, Liu & Wang	Lyu et al. 2020
<i>M. shuichengensis</i> Tian & Sun, 1995	Tian and Sun 1995
<i>M. shunhuangensis</i> Wang, Deng, Liu, Wu & Liu, 2019	Wang et al. 2019a
<i>M. spinata</i> Liu & Hu, 1973	Hu et al. 1973
<i>M. stejnegeri</i> Taylor, 1920	Taylor 1920
<i>M. synoria</i> (Stuart, Sok & Neang, 2006)	Stuart et al. 2006
<i>M. takensis</i> Mahony, 2011	Mahony 2011
<i>M. tuberogranulata</i> Shen, Mo & Li, 2010	Mo et al. 2012
<i>M. vegrandis</i> Mahony, Teeling, Biju, 2013	Mahony et al. 2013
<i>M. wawuensis</i> Fei, Jiang & Zheng, 2001	Fei et al. 2012
<i>M. wugongensis</i> Wang, Lyu & Wang, 2019	Wang et al. 2019b
<i>M. wuliangshanensis</i> Ye & Fei, 1995	Ye and Fei 1995
<i>M. wushanensis</i> Ye & Fei, 1995	Ye and Fei 1995
<i>M. xianjuensis</i> Wang, Wu, Peng, Shi, Lu & Wu, 2020	Wang et al. 2020
<i>M. xiangnanensis</i> Lyu, Zeng & Wang	Lyu et al. 2020
<i>M. yangningensis</i> Lyu, Zeng & Wang	Lyu et al. 2020
<i>M. zhangii</i> Ye & Fei, 1992	Ye and Fei 1992
<i>M. zunheboensis</i> (Mathew & Sen, 2007)	Mathew and Sen 2007

Results

Phylogenetic analyses

Aligned sequence matrix of 16S+COI contains 1104 bp. ML and BI trees of the mitochondrial DNA dataset presented almost consistent topology (Fig. 2). In mitochondrial DNA trees, all samples of the undescribed species were clustered into one clade which was nested into the *Megophrys* clade. However, the relationships between the undescribed species and its related species were not resolved though it was likely sister to *M. binchuanensis* in topology.

Genetic distances between samples of the undescribed species either on 16S or on COI genes were below 0.2% much lower than the interspecific genetic distance between recognised *Megophrys* species (Suppl. materials 1, 2). The genetic distance between the undescribed species and its closest related species *M. binchuanensis* were

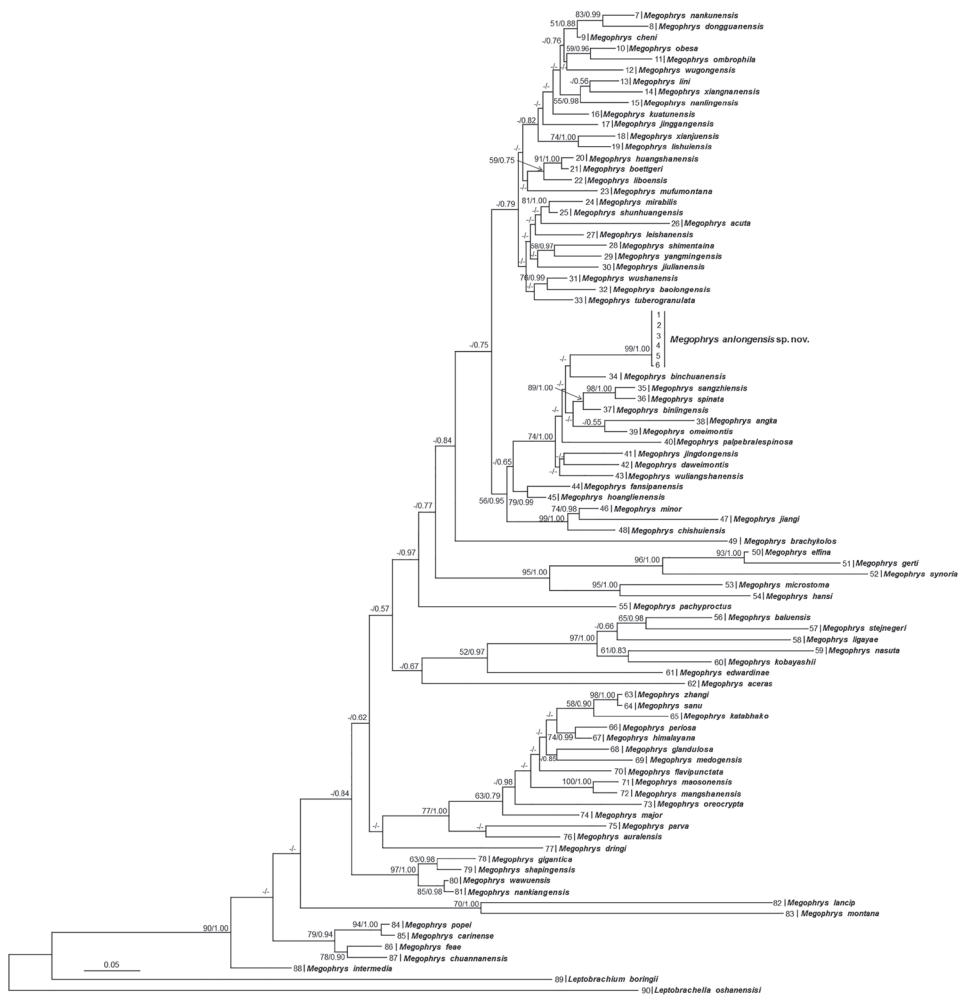


Figure 2. Maximum likelihood (ML) tree of the genus *Megophrys* reconstructed based on the 16S rRNA and COI gene sequences. Bayesian posterior probability/ML bootstrap supports were denoted beside each node. Samples 1–90 refer to Table 2.

2.3% and 10.0% on 16S and COI, respectively, which was higher than or at the same level with those among many pairs of sister species, such as, 2.1% and 6.3% on 16S and COI respectively between *M. wushanensis* and *M. baolongensis*, 1.7% and 3.8% on 16S and COI respectively between *M. spinata* and *M. sangzhiensis* (Suppl. materials 1, 2).

Morphological comparisons

The new species could be identified from its congeners in a series of morphological characters (Suppl. material 3). The detailed demonstration based on morphological comparisons see the following section on describing the new species.

Taxonomic account

Megophrys anlongensis sp. nov.

<http://zoobank.org/9D151886-5AD4-43A9-A32C-A2FCB16DA74F>

Holotype. CIBAL20190531018 (Figs 3, 4), adult male, from Anlong County, Guizhou Province, China (24.9899277°N, 105.5990611°E, ca. 1290 m a. s. l.), collected by Jing Liu on 31 May 2019.

Paratype. Four adult males and three females from the same place as holotype collected by Shi-Ze Li and Jing Liu. CIBAL20190531017, CIBAL20190531019, CIBAL20190531021 and CIBAL20190531022 collected on 31 May 2019 by Jing Liu, and CIBAL20190811014 and CIBAL20190811015 collected by Shi-Ze Li on 11 August 2019.

Diagnosis. *Megophrys anlongensis* sp. nov. is assigned to the genus *Megophrys* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like, projecting beyond the lower jaw; canthus rostralis distinct; chest glands small and round, closer to the axilla than to midventral line; femoral glands on rear part of thigh; vertical pupils.

Megophrys anlongensis sp. nov. could be distinguished from its congeners by a combination of the following morphological characters: (1) body size moderate (SVL 40.0–45.5 mm in males and 48.9–51.2 mm in females); (2) vomerine teeth absent; (3) tongue not notched behind; (4) a small horn-like tubercle at the edge of each upper eyelid; (5) tympanum distinctly visible, oval; (6) two metacarpal tubercles on hand; (7) toes with rudimentary webbing; (8) heels overlapping when thighs are positioned at right angles to the body; (9) tibiotarsal articulation reaching the level of mid-eye when leg stretched forward; (10) an internal single subgular vocal sac in male; (11) in breeding males, brownish nuptial pads, made up of black nuptial spines, present on the dorsal base of the first two fingers.

Description of holotype. (Figs 3, 4). SVL 40.0 mm; head width larger than head length slightly (HDW/HDL ratio about 1.1); snout obtusely pointed, protruding well beyond the margin of the lower jaw in ventral view; loreal region vertical and concave; canthus rostralis well-developed; top of head flat in dorsal view; eye large, eye diameter 35.4% of head length; pupils vertical; nostril orientated laterally, closer to snout than eye; tympanum distinct, 60% of eye diameter; vomerine ridges present and vomerine teeth absent; margin of tongue smooth, not notched behind.

Forelimbs slender, the length of lower arm and hand 47.9% of SVL; fingers slender, relative finger lengths: I < II < V < III; tips of digits globular, without lateral fringes; subarticular tubercle distinct at the base of each finger; two metacarpal tubercles, prominent, oval-shaped, the inner one bigger than the outer one.

Hindlimbs slender; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the middle eye when leg stretched forward; tibia length longer than thigh length; relative toe lengths I < II < V < III < IV; tips of toes round, slightly dilated; subarticular tubercles present on each toes; toes with

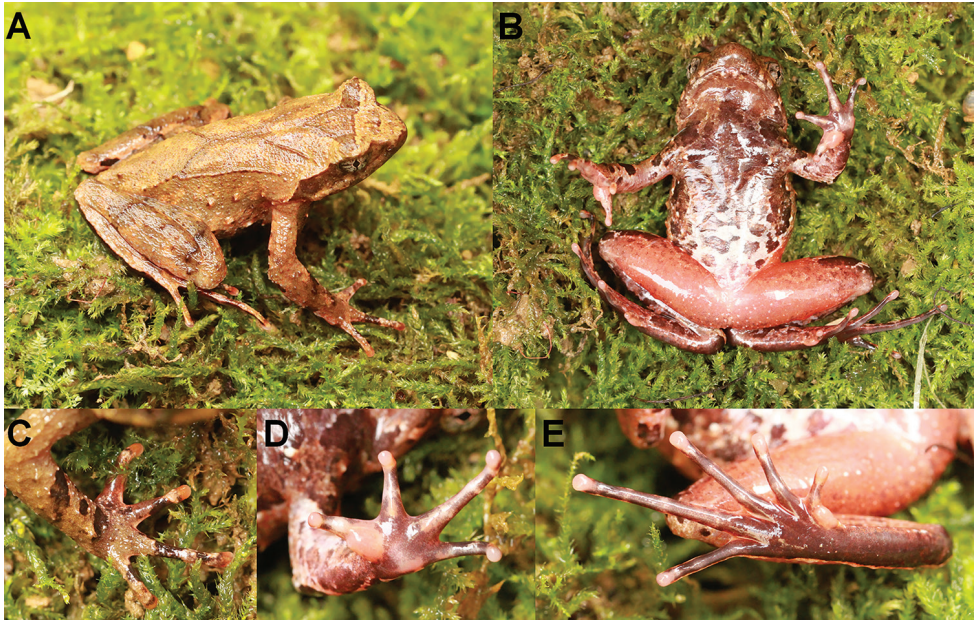


Figure 3. Photographs of the holotype CIBAL20190531018 of *Megophrys anlongensis* sp. nov. in life **A** dorsal view **B** ventral view **C** dorsal view of hand **D** ventral view of hand. **E** ventral view of foot.

rudimentary webbing and narrow lateral fringe; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent.

Dorsal skin rough, several large warts scattered on flanks; a small horn-like tubercle at the edge of each upper eyelid; tubercles on the dorsum forming a weak X-shaped ridge, two dorsolateral parallel ridges on either side of the X-shaped ridges; an inverted triangular brown speckle between two upper eyelids; several tubercles on the flanks and dorsal surface of thighs and tibiae; supratympanic fold distinct.

Ventral surface smooth; numerous granules scattered on flanks; glands on chest indistinct; numerous white granules on outer thighs and posterior end of the body distinctly protruding and forming an arc-shaped swelling above the anal region.

Colouration of holotype in life. (Fig. 3). Dorsal brown, an inverted triangular brown speckle between the eyes; X-shaped ridges on the dorsum, four dark transverse bands on the dorsal surface of the thigh and shank; ventral surface of body brown with white spots; several dark brown and white vertical bars on the lower and upper lip; ventral surface of anterior limb orange, with some brown spots and posterior limb orange with numerous white granules; tip of digits pale grey; inner metatarsal tubercle and two metacarpal tubercles pinkish; soles uniform black; pectoral glands white.

Colouration of holotype in preservation. (Fig. 4). Colour of dorsal surface fades to taupe; the inverted triangular brown speckle between the eyes and X-shaped ridges on dorsum are more distinct; ventral surface greyish white; creamy white substitutes

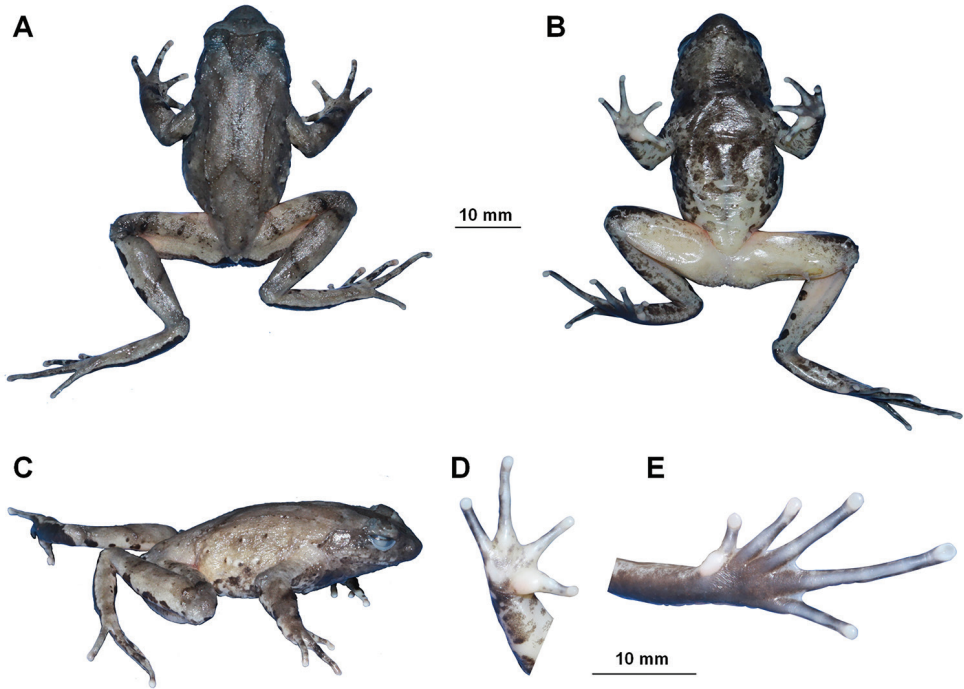


Figure 4. Photographs of the holotype specimen CIBAL20190531018 of *Megophrys anlongensis* sp. nov. **A** dorsal view **B** ventral view **C** lateral view **D** ventral view of hand **E** ventral view of foot.

the purple-grey on tip of digits; the posterior of ventral surface of body, inner of thigh and upper of tibia fades to creamy white.

Variation. In CIBAL20190531017 the inverted triangular brown speckle is connected to the X-shape ridge (Fig. 5A), and the ventral surface is reddish brown with creamy white in the posterior of belly (Fig. 5B); in CIBAL20190531022 an X-shaped marking on the dorsum (Fig. 5C), and anterior of ventral surface is brownish (Fig. 5D); in CIBAL20190811014 dorsal skin more rough, some black warts scattered on dorsal (Fig. 5E), and the white spots on ventral surface are less numerous and some black spots are mixed with the white spots or brown spots on ventral surface (Fig. 5F).

Advertisement call. The call description is based on recordings of the holotype CIBAL20190531018 (Fig. 6) calling from a shrub leaf near a streamlet, and the ambient air temperature was 18.5 °C. Each call consists of 14–26 (mean 22.5 ± 4.4 , $N = 6$) notes. Call duration was 2832–5621 ms (mean 4413 ± 972 , $N = 6$). Call interval was 6812–14387 ms (mean 10878 ± 2701 , $N = 5$). Each note had a duration of 129–211 ms (mean 167 ± 0.02 , $N = 135$) and the intervals between notes 34–94 ms (mean 57 ± 0.01 , $N = 128$). Amplitude modulation within note was apparent, beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note. The average dominant frequency was 2469 ± 197.47 (2250–3000 Hz, $N = 6$).



Figure 5. Colour variation in *Megophrys anlongensis* sp. nov. **A** dorsolateral view of the specimen CIBAL20190531017 **B** ventral view of the male specimen CIBAL20190531017 **C** dorsolateral view of the specimen CIBAL20190531022 **D** ventral view of the specimen CIBAL20190531022 **E** dorsolateral view of the specimen CIBAL20190811014 **F** ventral view of the specimen CIBAL20190811014.

Secondary sexual characters. Adult males have a single subgular vocal sac. In breeding males, brownish nuptial pads, made up of black nuptial spines, present on the dorsal bases of the first two fingers (Fig. 3C).

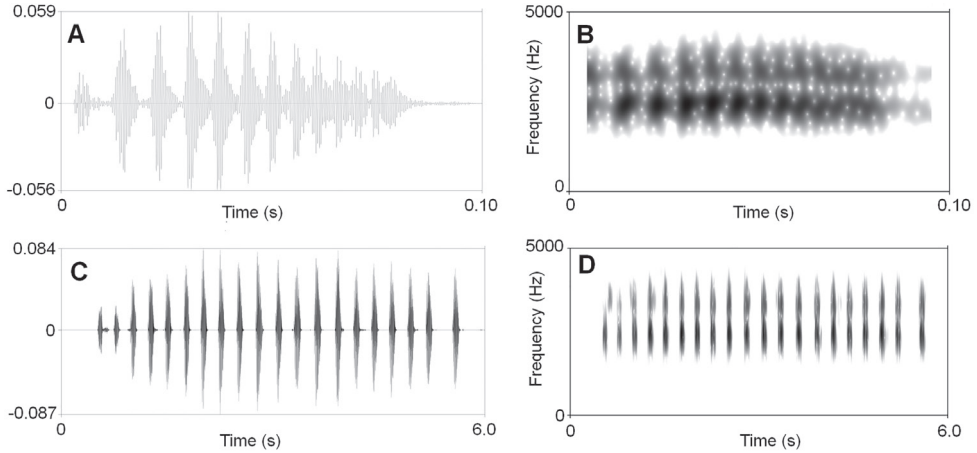


Figure 6. Visualisation of advertisement calls of *Megophrys anlongensis* sp. nov. **A** waveform showing one note **B** sonogram showing one note **C** waveform showing 20 notes of one call **D** sonogram showing 20 notes of one call.

Comparisons. By body size medium, *Megophrys anlongensis* sp. nov. differs from *M. aceras*, *M. acuta*, *M. angka*, *M. auralensis*, *M. binchuanensis*, *M. boettgeri*, *M. caobangensis*, *M. cheni*, *M. daweimontis*, *M. dringi*, *M. elfina*, *M. feii*, *M. gerti*, *M. jinggangensis*, *M. jiulianensis*, *M. kuatunensis*, *M. leishanensis*, *M. lishuiensis*, *M. microstoma*, *M. mufumontana*, *M. nankunensis*, *M. nanlingensis*, *M. obesa*, *M. ombrophila*, *M. oropedion*, *M. pachyproctus*, *M. palpebralespinosa*, *M. rubrimera*, *M. serchhipii*, *M. shimentaina*, *M. shunhuangensis*, *M. vegrandis*, *M. wugongensis*, *M. wuliangshanensis*, *M. wushanensis*, *M. xianjuensis*, *M. yangmingensis*, *M. zhang*i, and *M. zunhebotoensis* (SVL > 40.0 mm in the new species vs. maximum SVL < 39.0 mm in the latter), and differs from *M. carinense*, *M. caudoprocta*, *M. chuannanensis*, *M. damrei*, *M. feae*, *M. flavipunctata*, *M. gigantea*, *M. glandulosa*, *M. himalayana*, *M. kalimantanensis*, *M. kobayashii*, *M. lekaguli*, *M. ligayae*, *M. mangshanensis*, *M. medogensis*, *M. mirabilis*, *M. nasuta*, *M. omeimontis*, *M. orientalis*, *M. periosa*, *M. platyparietus*, *M. popei*, *M. sangzhiensis*, *M. shapingensis*, and *M. shuichengensis* (maximum SVL < 52.0 mm in the new species vs. minimum SVL > 54.0 mm in the latter), and differs from *M. edwardinae* and *M. monticola* (SVL 48.9–51.2 mm in female in the new species vs. 69–82 mm in *M. edwardinae* and 40.5 mm in *M. monticola*).

By vomerine teeth absent, *Megophrys anlongensis* sp. nov. differs from *M. ancræ*, *M. baluensis*, *M. carinense*, *M. caudoprocta*, *M. chuannanensis*, *M. damrei*, *M. daweimontis*, *M. dongguanensis*, *M. fansipanensis*, *M. feae*, *M. flavipunctata*, *M. glandulosa*, *M. himalayana*, *M. hoanglienensis*, *M. insularis*, *M. intermedia*, *M. jingdongensis*, *M. jinggangensis*, *M. jiulianensis*, *M. kalimantanensis*, *M. kobayashii*, *M. lancip*, *M. lekaguli*, *M. liboensis*, *M. ligayae*, *M. longipes*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. megacephala*, *M. montana*, *M. nankunensis*, *M. nanlingensis*, *M. nasuta*, *M. omeimontis*, *M. oreocrypta*, *M. orientalis*, *M. oropedion*, *M. pachyproctus*, *M. palpebralespinosa*,

M. parallela, *M. parva*, *M. periosa*, *M. platyparietus*, *M. popei*, *M. robusta*, *M. rubrimera*, *M. serchhipii*, *M. shimentaina*, *M. stejnegeri*, *M. takensis*, *M. zhang*i, and *M. zunhebot-oensis* (vs. present in the latter).

By a small horn-like tubercle at the edge of each upper eyelid, *Megophrys anlongensis* sp. nov. differs from *M. aceras*, *M. acuta*, *M. carinense*, *M. caudoprocta*, *M. chuannanensis*, *M. feae*, *M. gerti*, *M. hans*i, *M. intermedia*, *M. intermedia*, *M. jinggangensis*, *M. kalimantanensis*, *M. kou*i, *M. lancip*, *M. liboensis*, *M. microstoma*, *M. montana*, *M. nasuta*, *M. orientalis*, *M. palpebralespinosa*, *M. platyparietus*, *M. popei*, *M. shuichengensis*, *M. stejnegeri*, and *M. synoria* (vs. having a prominent and elongated tubercle in the latter).

By tongue not notched behind, *Megophrys anlongensis* sp. nov. differs from *M. ancr*ae, *M. baolongensis*, *M. binlingensis*, *M. boettgeri*, *M. carinense*, *M. chen*i, *M. chuannanensis*, *M. damrei*, *M. dringi*, *M. fansipanensis*, *M. feae*, *M. fei*i, *M. flavipunctata*, *M. gerti*, *M. glandulosa*, *M. hoanglienensis*, *M. huangshanensis*, *M. insularis*, *M. jiulianensis*, *M. jingdongensis*, *M. kalimantanensis*, *M. kuatunensis*, *M. liboensis*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. minor*, *M. nankiangensis*, *M. nanlingensis*, *M. omeimontis*, *M. oropedion*, *M. pachyproctus*, *M. parallela*, *M. popei*, *M. robusta*, *M. sangzhiensis*, *M. shapingensis*, *M. shuichengensis*, *M. spinata*, *M. vegrandis*, *M. wawuensis*, *M. zhang*i, and *M. zunhebotoensis* (vs. tongue notched behind in the latter).

By toes with narrow lateral fringes, *Megophrys anlongensis* sp. nov. differs from *M. angka*, *M. baolongensis*, *M. brachykolos*, *M. caobangensis*, *M. chishuiensis*, *M. damrei*, *M. daweimontis*, *M. dongguanensis*, *M. fansipanensis*, *M. feae*, *M. himalayana*, *M. hoanglienensis*, *M. huangshanensis*, *M. insularis*, *M. jiang*i, *M. jiulianensis*, *M. kalimantanensis*, *M. kou*i, *M. lekaguli*, *M. lishuiensis*, *M. major*, *M. mangshanensis*, *M. medogensis*, *M. megacephala*, *M. microstoma*, *M. minor*, *M. nankunensis*, *M. obesa*, *M. ombrophila*, *M. oreocrypta*, *M. oropedion*, *M. pachyproctus*, *M. parva*, *M. periosa*, *M. shunhuangensis*, *M. takensis*, *M. tuberogranulata*, *M. wawuensis*, *M. wugongensis*, *M. wuliangshanensis*, and *M. xianjuensis* (vs. lacking lateral fringes on toes in the latter), and differs from *M. binchuanensis*, *M. boettgeri*, *M. carinense*, *M. chen*i, *M. chuannanensis*, *M. dringi*, *M. fei*i, *M. gigantica*, *M. glandulosa*, *M. intermedia*, *M. jingdongensis*, *M. liboensis*, *M. lini*, *M. orientalis*, *M. palpebralespinosa*, *M. platyparietus*, *M. shapingensis*, *M. shuichengensis*, *M. spinata*, and *M. xiangnanensis* (vs. with wide lateral fringes in the latter).

By toes with rudimentary webbing, *Megophrys anlongensis* sp. nov. differs from *M. brachykolos*, *M. carinense*, *M. flavipunctata*, *M. jingdongensis*, *M. jinggangensis*, *M. lini*, *M. major*, *M. palpebralespinosa*, *M. popei*, *M. shuichengensis*, and *M. spinata* (vs. at least one-fourth webbed in the latter).

By heels overlapping when thighs are positioned at right angles to the body, *Megophrys anlongensis* sp. nov. differs from *M. acuta*, *M. brachykolos*, *M. dongguanensis*, *M. huangshanensis*, *M. kuatunensis*, *M. nankunensis*, *M. obesa*, *M. ombrophila*, and *M. wugongensis* (vs. not meeting in the latter).

By tibiotarsal articulation reaching to the level of mid-eye when leg stretched forward, *Megophrys anlongensis* sp. nov. differs from *M. daweimontis*, *M. glandulosa*, *M. lini*, *M. major*, *M. medogensis*, *M. obesa*, and *M. sangzhiensis* (vs. reaching the anterior corner of the eye or beyond eye or nostril or tip of snout in the latter), differs from

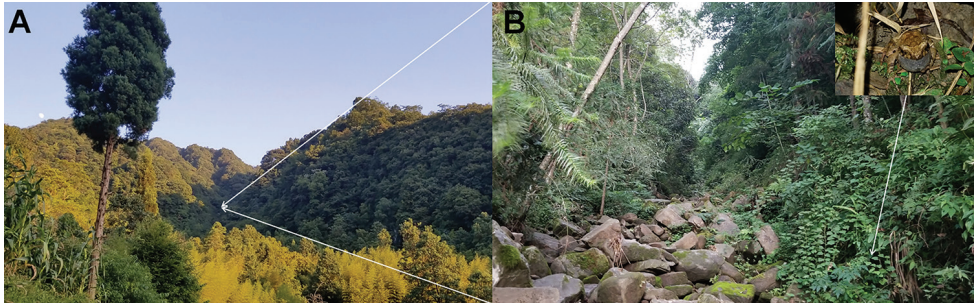


Figure 7. Habitats of *Megophrys anlongensis* sp. nov. in the type locality, Anlong County, Guizhou Province, China **A** landscape of montane forests in the type locality **B** a mountain stream where toads of the new species live (insert the holotype standing on the leaf beside the stream).

M. mufumontana (vs. reaching tympanum in males and to the eye in females in the latter), and differs from *M. chishuiensis* (vs. reaching the level between tympanum and eye in the latter).

By having an internal single subgular vocal sac in male, *Megophrys anlongensis* sp. nov. differs from *M. caudoprocta*, *M. shapingensis*, and *M. shuichengensis* (vs. vocal sac absent in the latter).

Megophrys anlongensis sp. nov. is genetically closest to *M. binchuanensis*. The new species could be identified from *M. binchuanensis* distinctly by having a bigger body size (SVL 40.0–45.5 mm in males and 48.9–51.2 in females in the new species vs. SVL 32.0–36.0 mm in males and 40.2–42.5 mm in females in the latter), having narrow lateral fringes on toes (vs. wide in the latter), and heels overlapping when thighs are positioned at right angles to the body (vs. just meeting in the latter).

Distribution and habitats. *Megophrys anlongensis* sp. nov. is known only from the type locality, Anlong County, Guizhou Province, China at elevations between 1400–1600 m. The individuals were frequently found near the streams surrounded by evergreen broadleaved forests (Fig. 7).

Etymology. The specific name *anlongensis* refers to the known distribution of this species, Anlong County, Guizhou Province, China. We propose the common English name “Anlong horned toad”, and Chinese name “An Long Jiao Chan” (安龙角蟾).

Discussion

Southwestern China was proposed as biodiversity hotspot (Myers et al. 2000). Guizhou Province, China is an important part of southwestern China, especially concerning the particular environments of karst rocky desertification, and knowledge of biodiversity levels and/or patterns are still seriously lacking in this region. Recently, a series of new amphibian species were described from Guizhou Province (Zhang et al. 2017; Li et al. 2018a, b, 2019a, b; Lyu et al. 2019; Wang et al. 2019c; Luo et al. 2020; Liu et al. 2020; Wei et al. 2020; Xu et al. 2020, Li et al. 2020), highlighting the underestimation of

the species diversity of this province. For the genus *Megophrys*, molecular phylogenetic differences still suggested some cryptic species in or near this region (Liu et al. 2018), but *Megophrys anlongensis* sp. nov. was not found before. This indicates that more work should focus on detailed information for describing such species, and additionally, comprehensive and in-depth surveys should be led to discover more cryptic species of the genus in this province. According to our surveys, habitat degradation due to construction and human activities are impacting the population of *Megophrys anlongensis* sp. nov. Hence, it is urgent for us to understand its population status and suggest strategies for supplying conservation needs of the species.

Acknowledgements

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Supplementary material 1

Table S1. Uncorrected p -distances between the *Megophrys* species based on the 16S gene sequences.

Authors: Shi-Ze Li, Ning-Ning Lu, Jing Liu, Bin Wang

Data type: statistical data

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Link: <https://doi.org/10.3897/zookeys.986.57119.suppl1>

Supplementary material 2

Table S2. Uncorrected p -distances between the *Megophrys* species based on the COI gene sequences

Authors: Shi-Ze Li, Ning-Ning Lu, Jing Liu, Bin Wang

Data type: statistical data

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Link: <https://doi.org/10.3897/zookeys.986.57119.suppl2>

Supplementary material 3

Table S3. Diagnostic characters separating the new species described in this study from other species of *Megophrys*

Authors: Shi-Ze Li, Ning-Ning Lu, Jing Liu, Bin Wang

Data type: species data

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A definition of the *Goniurosaurus yingdeensis* group (Squamata, Eublepharidae) with the description of a new species

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Abstract

A definition of the *Goniurosaurus yingdeensis* group is presented in this study, on the basis of morphological and phylogenetic analyses based on a series of additional specimens. Moreover, a new species of this group, *Goniurosaurus varius* **sp. nov.**, is proposed for northern Guangdong Province, China. The new species can be distinguished from the other two congeners of this group by the following unique characters: one or two internasals; enlarged supraorbital tubercles absent; paravertebral tubercles between limb insertions 27–29; dorsal tubercle rows at midbody 21–24; ten precloacal pores in males and absent in females; body bands with black spots; iris orange-red.

Keywords

Goniurosaurus varius sp. nov., *Goniurosaurus zhelongi*, morphology, phylogeny, taxonomy

Introduction

The eublepharid genus *Goniurosaurus* Barbour, 1908, currently contains 21 species that are distributed in east and Southeast Asia (Uetz et al. 2020). Nine of those species were described in last decade (Wang et al. 2014; Yang and Chan 2015; Zhou et al. 2018; Zhou et al. 2019; Zhu et al. 2020). In previous studies based on morphological analysis, the genus *Goniurosaurus* was suggested to be divided into three species groups (Grismer et al. 1999, 2002; Wang et al. 2013, 2014). The *G. kuroiwa* group is composed of five endemic species in the Ryukyu Archipelago, Japan: *G. kuroiwa* (Namiye, 1912), *G. orientalis* (Maki, 1931), *G. splendens* (Nakamura & Uéno, 1959), *G. toyamai* Grismer, Ota & Tanaka, 1994, and *G. yamashinae* (Okada, 1936). The *G. lichtenfelderi* group is composed of four species in the Gulf of Tonkin, Hainan island and Guangdong Province, China: *G. hainanensis* Barbour, 1908, *G. lichtenfelderi* (Mocquard, 1897), *G. yingdeensis* Wang, Yang & Cui, 2010, and *G. zhelongi* Wang, Jin, Li & Grismer, 2014. The *G. luii* group is composed of five species from northern Vietnam, through the China-Vietnam border, to southern Guangxi Zhuang Autonomous Region and Guizhou Province, China, include *G. araneus* Grismer, Viets & Boyle, 1999, *G. bawanglingensis* Grismer, Shi, Orlov, & Ananjeva, 2002, *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008, *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008, *G. liboensis* Wang, Yang & Grismer, 2013, and *G. luii* Grismer, Viets & Boyle, 1999. However, a recent molecular phylogenetic study suggested that the genus *Goniurosaurus* could be divided into four species groups (Liang et al. 2018). According to their proposition, *G. yingdeensis* and *G. zhelongi* formed an independent clade, the *G. yingdeensis* group, which only occurs in northern Guangdong Province, China. However, the morphological definition of this newly proposed species group has not been given so far, which may bring chaos to subsequent research.

During the herpetological surveys conducted from 2015 to 2019, a number of *Goniurosaurus* specimens were collected from northern Guangdong Province, China that should be placed in the *G. yingdeensis* group on the basis of both morphological and molecular analyses. Furthermore, these specimens can be distinguished from congeners by discrete morphological differences and genetic divergences, and represent an unidentified taxon within the *G. yingdeensis* group. In the present study, this taxon is described as a new species and the *Goniurosaurus yingdeensis* group is revised and defined.

Materials and methods

Sampling

Sixteen specimens of *Goniurosaurus yingdeensis* were collected from the Shimentai National Nature Reserve, Yingde City, Guangdong Province (including six type specimens) for morphological comparison, and four specimens (SYS r001271, 1272, 1493, 2115) were used in the phylogenetic analysis. Nine specimens of *G. zhelongi* were collected

from the Shimentai National Nature Reserve, Yingde City, Guangdong Province (including five type specimens) for morphological comparison, and four specimens (SYS r000816, 1491, 1492, 2108) were used in the phylogenetic analysis. Five specimens of the undescribed species were collected from the Nanling National Nature Reserve, Chengjia Yao Ethnic Township, Yangshan County, Guangdong Province, China, and all of them were used in phylogenetic analysis. Following euthanasia, all specimens were fixed in 10% formalin and transferred to 75% alcohol; they are deposited in the Museum of Biology, Sun Yat-sen University (SYS), Guangdong Province, China.

Due to the cryptic diversity in genus *Goniurosaurus*, we choose sequences from type series or topotype specimen for molecular analysis if available, to ensure the taxonomic identity of the species being studied. A total of 10 samples from four known species (one sample of *Goniurosaurus bawanglingensis*, four samples of *G. yingdeensis*, three samples of *G. zhelongi*, and two samples of *G. zhoui*) and five samples of the unidentified species were used. Tissues samples were taken before the specimens were fixed in 10% formalin, preserved in 99% alcohol, and stored at -40°C . Sequences of other species of *Goniurosaurus* follow Liang et al. (2018); for details see Table 1.

Species delimitation

The general lineage concept (GLC; de Queiroz 2007) adopted herein proposes that a species constitutes a population of organisms evolving independently from other such populations owing to a lack of gene flow. By “independently”, it is meant that new mutations arising in one species cannot spread readily into another species (Barracough et al. 2003; de Queiroz 2007). Integrative studies on the nature and origins of species are using an increasingly wide range of empirical data to delimit species boundaries (Coyne and Orr 1998; Fontaneto et al. 2007; Knowles and Carstens 2007; Leaché et al. 2009), rather than relying solely on morphology and traditional taxonomic methods. Under the GLC implemented herein, molecular phylogenies were used to recover monophyletic mitochondrial lineages of individuals (populations) in order to develop initial species-level hypotheses – the grouping stage of Hillis (2019). Discrete color pattern data and univariate and multivariate analyses of morphological data were then used to search for characters and morphospacial patterns bearing statistically significant differences that were consistent with the previous designations of the species-level hypotheses, the construction of boundaries representing the hypothesis-testing step of Hillis (2019), thus providing independent diagnoses to complement the molecular analyses.

Morphological characters

Measurements were taken following Ziegler et al. (2008) using digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. Abbreviations of morphological characters are as follows: **SVL** snout-vent length (from tip of snout to vent); **TaL** tail length (from vent to tip of tail); **HL** head length (from tip of snout to posterior margin of ear opening); **HW** maximum head width; **SE** snout-to-eye

Table 1. Localities, voucher information, and GenBank accession numbers for all specimens used in this study.

Species name	Locality	Specimen voucher	16S	Cytb	References
Ingroup: <i>Goniurosaurus</i>					
(1) <i>G. varius</i> sp. nov.	Yangshan, Guangdong, China	SYS r002330	MT995753	MT995768	This study
(2) <i>G. varius</i> sp. nov.		SYS r002331	MT995754	MT995769	This study
(3) <i>G. varius</i> sp. nov.		SYS r002333	MT995755	MT995770	This study
(4) <i>G. varius</i> sp. nov.		SYS r002362	MT995756	MT995771	This study
(5) <i>G. varius</i> sp. nov.		SYS r002363	MT995757	MT995772	This study
(6) <i>G. bawanglingensis</i>	Bawangling, Hainan, China	SYS r002162	MT995758	MT995773	This study
(7) <i>G. bawanglingensis</i>		BL-RBZ-021	MH247190	MH247201	Liang et al. 2018
(8) <i>G. hainanensis</i>	Jianfengling, Hainan, China	SYS r000349	KC765080	N/A	Wang et al., 2013
(9) <i>G. huiliensis</i>	Vietnam	N/A	AB853453	AB853479	Honda et al. 2014
(10) <i>G. kuroiwaie</i>	Northern Okinawajima Island, Japan	N/A	AB853448	AB853473	Honda et al. 2014
(11) <i>G. liboensis</i>	Libo, Guizhou, China	SYS r000217	KC900230	N/A	Wang et al. 2013
(12) <i>G. luii</i>	Jingxi, Guangxi, China	SYS r000255	KC765083	N/A	Wang et al. 2013
(13) <i>G. luii</i>		SYS r000256	KC765084	N/A	Wang et al. 2013
(14) <i>G. luii</i>	Cao Bang, Vietnam	ZFMK 87057	EU499391	N/A	Ziegler et al. 2008
(15) <i>G. orientalis</i>	Iejima Island, Japan	N/A	AB853446	AB853467	Honda et al. 2014
(16) <i>G. splendens</i>	Tokunoshima Island, Japan	N/A	AB853451	AB853477	Honda et al. 2014
(17) <i>G. yamashinae</i>	Kumejima Island, Japan	N/A	AB853442	AB853460	Honda et al. 2014
(18) <i>G. yingdeensis</i>	Yingde, Guangdong, China	SYS r001271	MT995759	MT995774	This study
(19) <i>G. yingdeensis</i>		SYS r001272	MT995760	MT995775	This study
(20) <i>G. yingdeensis</i>		SYS r001493	MT995761	MT995776	This study
(21) <i>G. yingdeensis</i>		SYS r002115	MT995762	MT995777	This study
(22) <i>G. zhelongi</i>	Yingde, Guangdong, China	SYS r000816	KJ423105	MT995778	Wang et al. 2014, this study
(23) <i>G. zhelongi</i>		SYS r001491	MT995763	MT995779	This study
(24) <i>G. zhelongi</i>	Yingde, Guangdong, China	SYS r001492	MT995764	MT995780	This study
(25) <i>G. zhelongi</i>		SYS r002108	MT995765	MT995781	This study
(26) <i>G. zhoui</i>	Central area, Hainan, China	SYS r002213	MT995766	MT995782	This study
(27) <i>G. zhoui</i>		SYS r002214	MT995767	MT995783	This study
(28) <i>G. zhoui</i>		BL-RBZ-001	MH247196	MH247207	Liang et al. 2018
Outgroup					
(29) <i>Hemitheconyx taylori</i>	East Africa	N/A	AB308457	N/A	Jonniaux and Kumazawa 2008

distance (measured from tip of snout to the boney anterior margin of the orbit); **EE** eye-to-ear distance (from the boney posterior margin of the orbit to posterior margin of ear opening); **SPL** supralabials; **IFL** infralabials; **N** nasal scales surrounding nare; **IN** internasals; **PostIN** granular scales bordering the internasals; **PM** postmentals; **GP** gular scales bordering postmentals; **CIL** eyelid fringe scales or ciliaria; **PO** preorbital scales (number of scales in a line from posterior margin of external naris to anterior margin of the orbit); **GST** granular scales surrounding dorsal tubercles; **PTL** paravertebral tubercles between limb insertions; **DTR** dorsal tubercle rows at midbody; **MB** scales around midbody; **PP** precloacal pores; **PAT** postcloacal tubercles. Bilateral scale counts are given as left/right.

Data of characters of known congeners were taken from the literature (Grismer et al. 1999, 2002; Orlov et al. 2008; Ziegler et al. 2008; Blair et al. 2009; Wang et al. 2010, 2013, 2014; Yang and Chan 2015; Zhou et al. 2018) and 30 museum specimens of the seven species listed in the Appendix 1 were examined.

DNA Extraction, Polymerase Chain Reaction (PCR), and sequencing

Genomic DNA was extracted from muscle tissue samples, using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Partial segments of the mitochondrion genes 16S ribosomal RNA gene (16S) and Cytochrome b gene (Cytb) were amplified. Primers used for 16S were r16S-5L (5'-GGTMMYGCCTGCCCAGTG-3') and 16sbr-H (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991) and for Cytb the primers were L14731 (5'-TGGTCTGAAAAACCATTGTTG-3') (Honda et al. 2014) and H15149m (5'-GCMCCTCAGAAKGATATTTGYCCTCA-3') (Chambers and MacAvoy 1999). The PCR procedure was performed with an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min (Liang et al. 2018). PCR products were purified with spin columns and then sequenced with forward primers using BigDye Terminator Cycle Sequencing Kit as per the guidelines on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd.

Phylogenetic analyses

Twenty sequences from eleven known *Goniurosaurus* species and one out-group sequence from *Hemitheconyx taylori* in the Eublepharidae used to root the tree, were obtained from GenBank and incorporated into our dataset (Table 1). DNA sequences were aligned by the Clustal W with default parameters (Thompson et al. 1997) and trimmed with gaps partially deleted in MEGA 6 (Tamura et al. 2013). Two gene segments, with 482 base pairs (bp) of 16S and 396 bp of Cytb, were concatenated serially into an 878 bp sequence, and further divided into two partitions based upon each gene. The partitions were tested in jmodeltest v2.1.2 with Akaike and Bayesian information criteria, all resulting the best-fitting nucleotide substitution models of GTR+I+G. Sequence data were analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012), and maximum likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in the BI analysis with 10,000,000 generations each and sampled every 1000 generations with the first 25% of samples discarded as burn-in, resulting in a potential scale reduction factor (PSRF) of < 0.005. In the ML analysis, a bootstrap consensus tree inferred from 1000 replicates was generated. Uncorrected pairwise sequence divergences utilizing the 16s gene were calculated using MEGA 6 (provide ref for MEGA 6).

Statistical analyses of morphology

An analysis of variance (ANOVA) was conducted on characters with statistically similar variances (i.e., p values ≤ 0.05 in a Levene's test) to search for the presence of statistically significant mean differences ($p < 0.05$) across the data set. Characters bearing statistical differences were subjected to a TukeyHSD test to ascertain which population

pairs differed significantly from each other for those particular characters. The mensural characters were scaled to SVL in order to remove any potential effects of allometry using the following equation: $X_{adj} = \log(X) - \beta[\log(SVL) - \log(SVL_{mean})]$, where X_{adj} = adjusted value; X = measured value; β = unstandardized regression coefficient for each population; and SVL_{mean} = overall average SVL of all populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000). Boxplots were generated in order to visualize the range, mean, and degree of differences between pairs of species bearing statistically different mean values for sets of characters.

Results

The ML and BI analyses resulted in essentially identical topologies (Fig. 1). Uncorrected pairwise sequence divergences are reported in Table 2. The phylogenetic analyses showed that *Goniurosaurus* can be divided into four strongly supported clades consistent with the recognition of the four species groups proposed by Liang et al. (2018), i.e., the *G. kuroiwaie* group, *G. lichtenfelderi* group, *G. luii* group, and *G. yingdeensis* group.

The *Goniurosaurus yingdeensis* group is divided into three subclades with moderate genetic differences among them (3.3–4.7%), two of which represent *G. yingdeensis* and *G. zhelongji*, respectively; the third subclade is composed of the new population

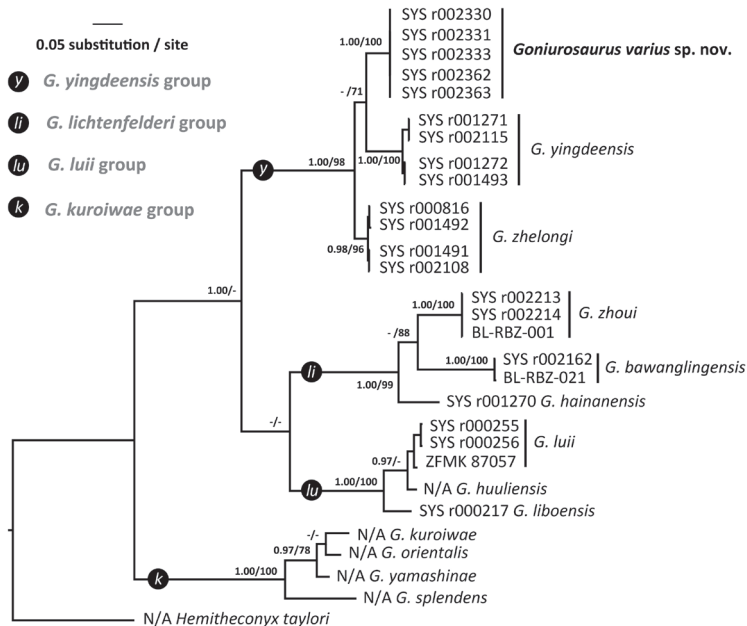


Figure 1. Bayesian inference tree of 13 species of *Goniurosaurus*, based on the partial DNA sequences of the mitochondrial 16S rRNA and Cytb genes. *Hemitheconyx taylora* is the outgroup. Numbers before slash indicate Bayesian posterior probabilities (> 0.94 retained) and numbers after slash are bootstrap support for ML (1000 replicates) analyses (> 70 retained). The hyphen represents bootstrap values ≤ 0.94 or ≤ 70.

from northern Guangdong Province with a high nodal support value (1.00 in BI and 100% in ML) and low intrapopulational genetic differentiation (0–0.3%) and represents an unnamed species of *Goniurosaurus* (Table 2). Additionally, this population has a combination of characteristics (see below) distinguishing it from other species in the *G. yingdeensis* group while showing significant differences from all known congeners. ANOVAs and subsequent TukeyHSD tests recovered significantly different mean values among various combinations of species across various combinations of characters (Tables 3, 4; Figs 2, 3).

Based on phylogeny and corroborating statistically significant differences in morphology (Figs 1–3), we propose that the northern Guangdong Province population is a new species of the *Goniurosaurus yingdeensis* group. The discovery of this new species has provided valuable new morphological and genetic information on this species group. The previous designation of the *Goniurosaurus yingdeensis* species group was on the basis solely on molecular data and lacked a morphological definition. Therefore, along with the description of a new species, we provide the first morphological definition of the *Goniurosaurus yingdeensis* group.

Systematics

Class Reptilia Laurenti, 1768

Order Squamata Oppel, 1811

Family Eublepharidae Boulenger, 1883

Genus *Goniurosaurus* Barbour, 1908

Goniurosaurus yingdeensis group

Morphological definition. This species group can be differentiated from the other species groups by the combination of the following characters: (1) base of claws sheathed by four scales, two lateral scales of claw short and shell-shaped; (2) precloacal pores fewer than 15 in males and absent in most females; precloacal pores form a continuous transverse series not extending onto the femora; (3) enlarged row of supraorbital tubercles indistinct or absent; (4) nuchal loop rounded posteriorly; and (5) four body bands between the nuchal loop and the caudal constriction.

Comparison. The *Goniurosaurus yingdeensis* group can be distinguished from the three other known species groups by the base of claws being sheathed by four scales, two lateral scales of claw short and shell-shaped vs. claws sheathed by four scales, two lateral scales of claw long, curved in *G. lichtenfelderi* group and *G. luii* group, and not sheathed by four scales in *G. kuroiwa* group; precloacal pores less than 15 in males vs. 17–46 in *G. lichtenfelderi* group (37–46 in *G. bawanglingensis*, 24–32 in *G. hainanensis*, 17–32 in *G. lichtenfelderi*, 36–38 in *G. zhoui*), 16–33 in *G. luii* group (18–22 in *G. araneus*, 16–21 in *G. catbaensis*, 25–28 in *G. huuliensis*, 26–28 in *G. kadoorieorum*, 31–33 in *G. kwangsiensis*, 23–28 in *G. liboensis*, 23–29 in *G. luii*) and absent in *G. kuroiwa* group.

Table 2. Uncorrected *P*-distance of 16S gene among 13 *Goniurosaurus* species and the outgroup species used in this study.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>G. nairui</i> sp. nov.	0–0.3													
2 <i>G. zhelongi</i>	3.3–3.9	0–0.3												
3 <i>G. yingdeensis</i>	4.1–4.7	4.7	0–0.5											
4 <i>G. huiliensis</i>	11.1	13.3	13.6–13.9	/										
5 <i>G. lui</i>	12.3–12.6	13.6–13.9	12.6–13.3	1.6–1.9	0–0.8									
6 <i>G. liboensis</i>	12.7	12.6–12.7	13.0–13.3	3.9	3.6–4.2	/								
7 <i>G. zhoui</i>	14.8–15.1	16.7–16.8	17.1	14.1	14.7–15.3	14.6	0–0							
8 <i>G. hainanensis</i>	15.1–15.4	17.0	16.4	13.4	13.7–14.1	13.7	5.6	/						
9 <i>G. huanwanglingensis</i>	16.2–16.8	15.8–16.5	17.9–18.2	15.4–15.7	14.7–15.7	15.0–15.3	5.6–5.8	74–7.7	0–0.3					
10 <i>G. orientalis</i>	15.7–16.0	17.1–17.4	15.4–15.7	19.0	19.3–19.7	19.3	18.0	18.8	19.1–19.5	/				
11 <i>G. yamashinae</i>	16.0–16.3	17.7–18.0	16.0–16.3	18.6	19.3–19.6	19.3	17.1	18.4	19.7–20.1	1.1	/			
12 <i>G. karoiuiae</i>	17.4–17.7	18.8–19.1	16.8–17.1	18.7	18.7–19.1	19.0	17.0	18.1	18.4–18.7	1.4	1.9	/		
13 <i>G. splendens</i>	17.8–18.1	19.5–19.9	16.8–17.2	20.4	19.7–20.1	20.0	18.8	18.8	21.0–21.4	4.5	4.2	5.0	/	
14 <i>Hemibaconyx taylori</i>	17.4–17.7	18.0–18.4	19.3–19.4	23.1	24.5–24.6	23.1	22.2	24.0	25.8–26.2	18.5	18.1	19.2	20.4	/

Table 3. ANOVA *F* values and TukeyHSD adjusted *p* values for pairs of species bearing statistically significant mean values in the listed characters.

	ANOVA <i>F</i>	TukeyHSD <i>p</i> adjusted
Eye to ear distance (EE)	169.5	
<i>yingdeensis-varius</i>		2.24E-14
<i>zhelongi-varius</i>		7.50E-12
<i>zhelongi-yingdeensis</i>		0.0004
Snout to eye distance (SE)	5.717	
<i>zhelongi-yingdeensis</i>		0.0098
Head length (HL)	5.087	
<i>zhelongi-yingdeensis</i>		0.0126
Maximum head width (HW)	4.292	
<i>zhelongi-yingdeensis</i>		0.0244
Infralabials (IFL)	6.493	
<i>zhelongi-varius</i>		0.0168
<i>zhelongi-yingdeensis</i>		0.0106
Nasal scales surrounding nares (N)	5.773	
<i>zhelongi-yingdeensis</i>		0.0086
Internasals (IN)	13.75	
<i>yingdeensis-varius</i>		0.0022
<i>zhelongi-yingdeensis</i>		0.0003
Granular scales bordering internasals (PostIN)	3.548	
<i>zhelongi-yingdeensis</i>		0.0449
Postmentals (PM)	21.43	
<i>zhelongi-varius</i>		0.0007
<i>zhelongi-yingdeensis</i>		4.58E-06
Gular scales bordering postmentals (GP)	9.196	
<i>zhelongi-yingdeensis</i>		0.0008
Eyelid fringe scales or cilia (CIL)	4.898	
<i>zhelongi-yingdeensis</i>		0.0146
Preorbital scales (PO)	15.52	
<i>yingdeensis-varius</i>		0.0012
<i>zhelongi-yingdeensis</i>		0.0001
Dorsal tubercle rows at midbody (DTR)	12.2	
<i>zhelongi-yingdeensis</i>		0.0001

Summary statistics of the species of the *Goniurosaurus yingdeensis* group are listed in Table 4. Additional comparisons of morphological characteristics are provided in Table 5 and Fig. 4.

Goniurosaurus yingdeensis Wang, Yang & Cui, 2010

Figs 4A, 5A, 6, 8B, 9B

[English name: Yingde Leopard Gecko]

[Chinese formal name: 英德睑虎]

Type material. Holotype. SYS r000504, adult male, collected from Guoshanyao Village, Yingde City, Guangdong Province, China. **Paratypes.** Five specimens from the same locality as holotype. Three adult males SYS r000501–0503, an adult female SYS r000535 and a juvenile female SYS r000536.

Additional specimens. Four adult males (SYS r000788, SYS r000815, SYS r001493, SYS r002115); two adult females (SYS r001271–1272), a subadult female

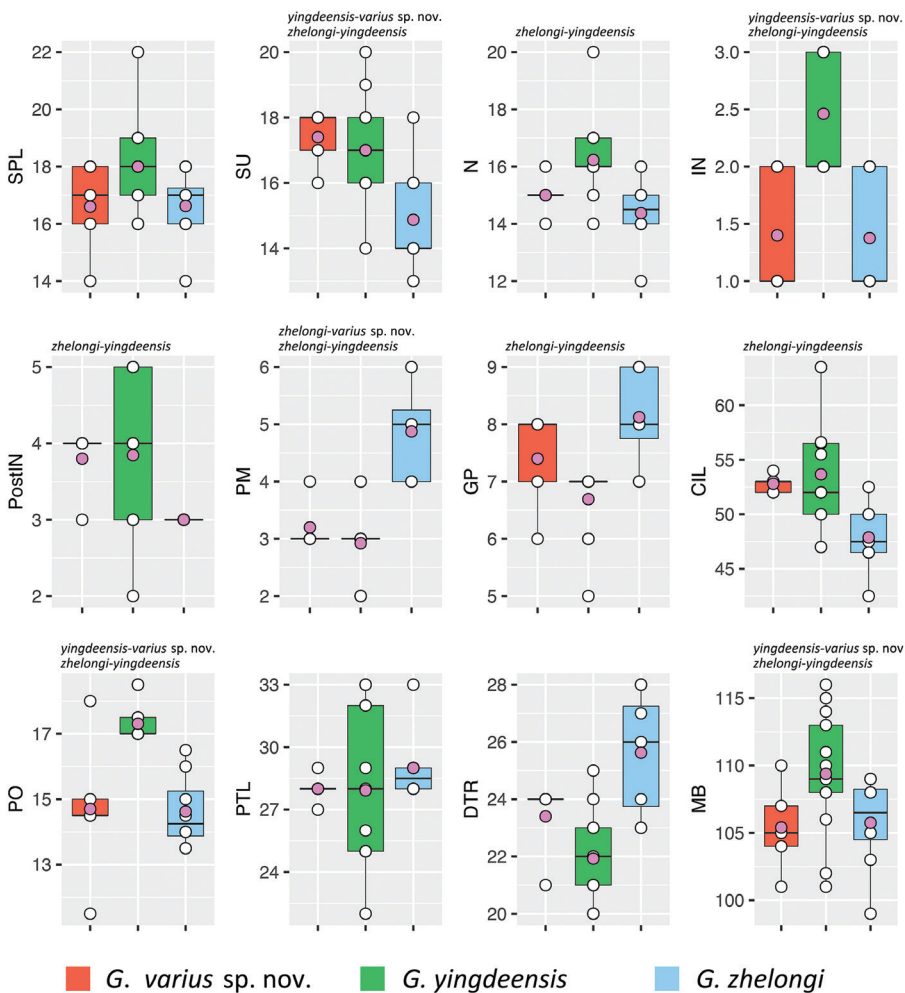


Figure 2. Boxplots showing characters bearing significantly different mean values. Species pairs bearing significantly different mean values between them are listed above each plot. Abbreviations are in the materials and methods.

(SYS r000536) and a juvenile female (SYS r000552). All specimens collected from the Shimentai National Nature Reserve.

Variation. Overall morphology, coloration, and scalation data of the newly discovered populations of *G. yingdeensis* are in general agreement with the description of the holotype by Wang et al. (2010). Males have 10–13 distinct preloacal pores, whereas preloacal pores are present but indistinct in two adult females (SYS r000535, SYS r001652) and a subadult female (SYS r000536), absent in another two adult females (SYS r001271, SYS r001272); internasal usually numbering two or three, but single in the two females (SYS r001271, SYS r001652). Additional variation in scale counts and measurements are shown in Table 6. For female preloacal pores see Fig. 6.

Table 4. Summary statistics for meristic and adjusted mensural characters among the species of the *Goniurosaurus yingdeensis* group. SD = standard deviation and N = sample size.

Scaled mensural characters	<i>varius</i> sp. nov. (N = 5)	<i>yingdeensis</i> (N = 13)	<i>zbelongi</i> (N = 8)
HL			
mean ($\pm$ SD)	3.1 ($\pm$ 0.02)	3.1 ($\pm$ 0.05)	3.2 ($\pm$ 0.04)
Range	3.08–3.14	3.02–3.21	3.12–3.23
HW			
Mean ($\pm$ SD)	2.7 ($\pm$ 0.04)	2.7 ($\pm$ 0.04)	2.8 ($\pm$ 0.03)
Range	2.67–2.77	2.66–2.77	2.71–2.83
SE			
Mean ($\pm$ SD)	2.2 ($\pm$ 0.06)	2.2 ($\pm$ 0.04)	2.2 ($\pm$ 0.03)
Range	2.14–2.29	2.10–2.24	2.16–2.24
EE			
Mean ($\pm$ SD)	2.6 ($\pm$ 0.05)	2.2 ($\pm$ 0.02)	2.2 ($\pm$ 0.07)
Range	2.52–2.66	2.01–2.15	2.11–2.31
Meristic characters			
SPL			
Mean ($\pm$ SD)	16.6 ($\pm$ 1.67)	18.0 ($\pm$ 1.58)	16.6 ($\pm$ 1.30)
Range	14–18	16–22	14–18
IFL			
Mean ($\pm$ SD)	17.4 ($\pm$ 0.89)	17.0 ($\pm$ 1.53)	14.9 ($\pm$ 1.64)
Range	16–18	14–20	13–18
N			
Mean ($\pm$ SD)	15 ($\pm$ 0.71)	16.2 ($\pm$ 1.42)	14.4 ($\pm$ 1.19)
Range	14–16	14–20	12–16
IN			
Mean ($\pm$ SD)	1.4 ($\pm$ 0.54)	2.5 ($\pm$ 0.52)	1.4 ($\pm$ 0.52)
Range	1 or 2	2 or 3	1 or 2
PostIN			
Mean ($\pm$ SD)	3.8 ($\pm$ 0.44)	3.8 ($\pm$ 0.99)	3 ($\pm$ 0.00)
Range	3 or 4	2–5	3
PM			
Mean ($\pm$ SD)	3.2 ($\pm$ 0.44)	2.9 ($\pm$ 0.64)	4.9 ($\pm$ 0.83)
Range	3 or 4	2–4	4–6
GP			
Mean ($\pm$ SD)	7.4 ($\pm$ 0.89)	6.7 ($\pm$ 0.63)	8.1 ($\pm$ 0.83)
Range	6–8	5–7	7–9
CIL			
Mean ($\pm$ SD)	52.8 ($\pm$ 0.83)	53.7 ($\pm$ 5.33)	47.9 ($\pm$ 3.01)
Range	52–54	47–63.5	42.5–52.5
PO			
Mean ($\pm$ SD)	14.7 ($\pm$ 2.31)	17.3 ($\pm$ 0.56)	14.6 ($\pm$ 1.13)
Range	11.5–18	17–18.5	13.5–16.5
PTL			
Mean ($\pm$ SD)	28.0 ($\pm$ 0.71)	27.9 ($\pm$ 3.64)	29.0 ($\pm$ 1.69)
Range	27–29	22–33	28–33
DTR			
Mean ($\pm$ SD)	23.4 ($\pm$ 1.34)	21.9 ($\pm$ 1.50)	25.6 ($\pm$ 2.07)
Range	21–24	20–25	23–28
MB			
Mean ($\pm$ SD)	105.4 ($\pm$ 3.36)	109.4 ($\pm$ 4.59)	105.8 ($\pm$ 3.49)
Range	101–110	101–116	99–109

Diagnosis. (1) medium size, measuring 82.0–96.3 mm in SVL in adults; (2) TaL and SVL almost equal in adult with original tail; (3) nasal scales surrounding nares 7–11; (4) internasals 1–3; (5) eyelid fringe scales 46–64; (6) granular scales of the upper eye-

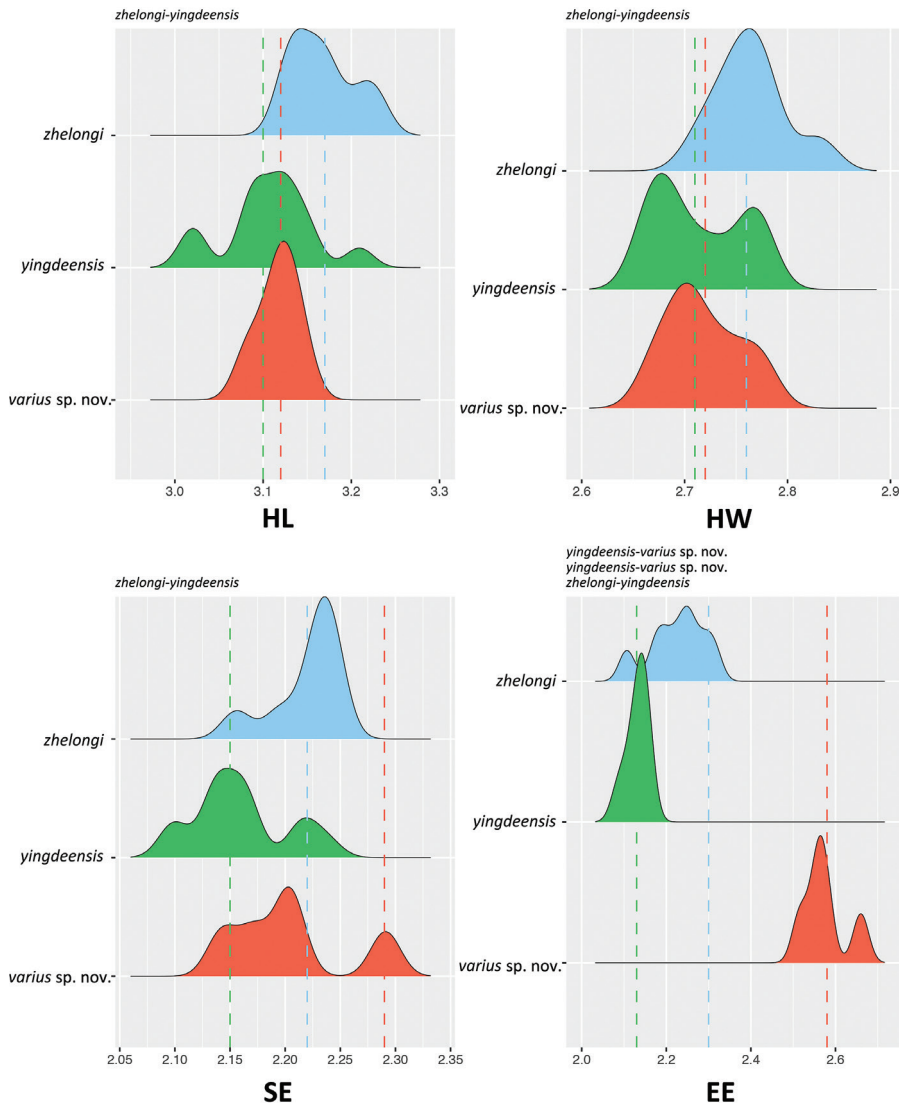


Figure 3. Ridge plots showing characters bearing significantly different mean values. Species pairs bearing significantly different mean values between them are listed above each plot. Abbreviations are in the Materials and methods.

lids similar in size to those on the top of the head; (7) scales around midbody 101–116; (8) dorsal tubercle rows at midbody 20–25; (9) paravertebral tubercles between limb insertions 22–33; (10) claws sheathed by four scales, two lateral scales short and shell-shaped; (11) axillary pockets deep; (12) precloacal pores 10–13, distinct in males, barely visible or not visible in females; (13) dorsal ground color of head, body, and limbs of adults brown; (14) presence of a thin, cream colored nuchal loop, posteriorly rounded; (15) presence of four thin, cream colored, and immaculate body bands between the nuchal loop and the

Table 5. Diagnostic characters distinguishing *Goniurosaurus varius* sp. nov. from all other known species of *Goniurosaurus*. Data come from Grismer et al. 1999, 2002; Orlov et al. 2008; Ziegler et al. 2008; Blair et al. 2009; Wang et al. 2010, 2013, 2014; Yang and Chan 2015; Zhou et al. 2018.

Character	<i>G. kuroiwae</i> group (5 spp.)	<i>G. lichtenfelderi</i> group (4 spp.)	<i>G. luii</i> group (7 spp.)	<i>G. yingdeensis</i> group (3 species)		
				<i>G. varius</i> sp. nov.	<i>G.</i> <i>yingdeensis</i>	<i>G. zhelongi</i>
Scales of upper eyelid one-half the size of scales on the top of head or equal in size	Equal	Equal	Equal or 1/2	Equal	Equal	Equal
Enlarged row of supraorbital tubercles	Absent	Absent or present	Absent or present	Absent	Absent or indistinct	Absent or indistinct
Eyelid fringe scales	<52	43–77	41–67	50–56	46–64	42–53
No. of paravertebral tubercles	Unknown	23–36	27–38	27–29	22–33	28–33
Dorsal tubercle rows at midbody	Unknown	19–22	20–25	21–24	20–25	23–28
Scales around midbody	Unknown	95–140	112–147	101–110	101–116	99–109
Nasal scales surrounding nares	Unknown	8–9	5–9	7–9	7–11	6–8
Internasals	Unknown	1	0–3	1–2	1–3	1–2
Tubercles between orbits	Present or absent	Present or absent	Present or absent	Present	Present	Absent
Claws sheathed by scales	Absent	Present	Present	Present	Present	Present
Lateral scales of claw sheaths	Absent	Long, curved	Long, curved	Short, shell-shaped	Short, shell-shaped	Short, shell-shaped
No. of precloacal pores in males	0	17–46	16–33	10	10–13	9–12
Posterior shape of nuchal loop	Rounded	Protracted or rounded	Protracted	Rounded	Rounded	Rounded
No. of body bands between nuchal loop and the caudal constriction	3 or 4	3 or 4	3, 4 or 5	4	4	4
Dark spotting in body bands	Present or absent	Present or absent	Present or absent	Present or absent	Absent	Absent
Lateral spotting on belly present or absent	Absent	Absent	Present or absent	Present	Present	Present

caudal constriction, edged in black anteriorly and posteriorly; (16) body bands without dark spots; (17) chin, throat, thorax, and ventral surfaces of limbs white, dark brown spots present, ventral surfaces of body dull white, interspersed with dark brown scales, dark brown lateral spots on belly; (18) iris gray, becoming orange near pupil.

Distribution. *Goniurosaurus yingdeensis* is currently only known from the Yingde City, Guangdong Province, China.

Goniurosaurus zhelongi Wang, Jin, Li & Grismer, 2014

Figs 5B, 8C, 9C

[English name: Zhe-Long's Leopard Gecko]

[Chinese formal name: 蒲氏睑虎]

Type material. Holotype. SYS r000770, adult male, collected from the Shimentai National Nature Reserve, Yingde City, Guangdong Province, China. **Paratypes.** Four specimens, bearing the same data as the holotype. Three adult females SYS r000551, SYS r000765–0766 and one adult male SYS r000816.

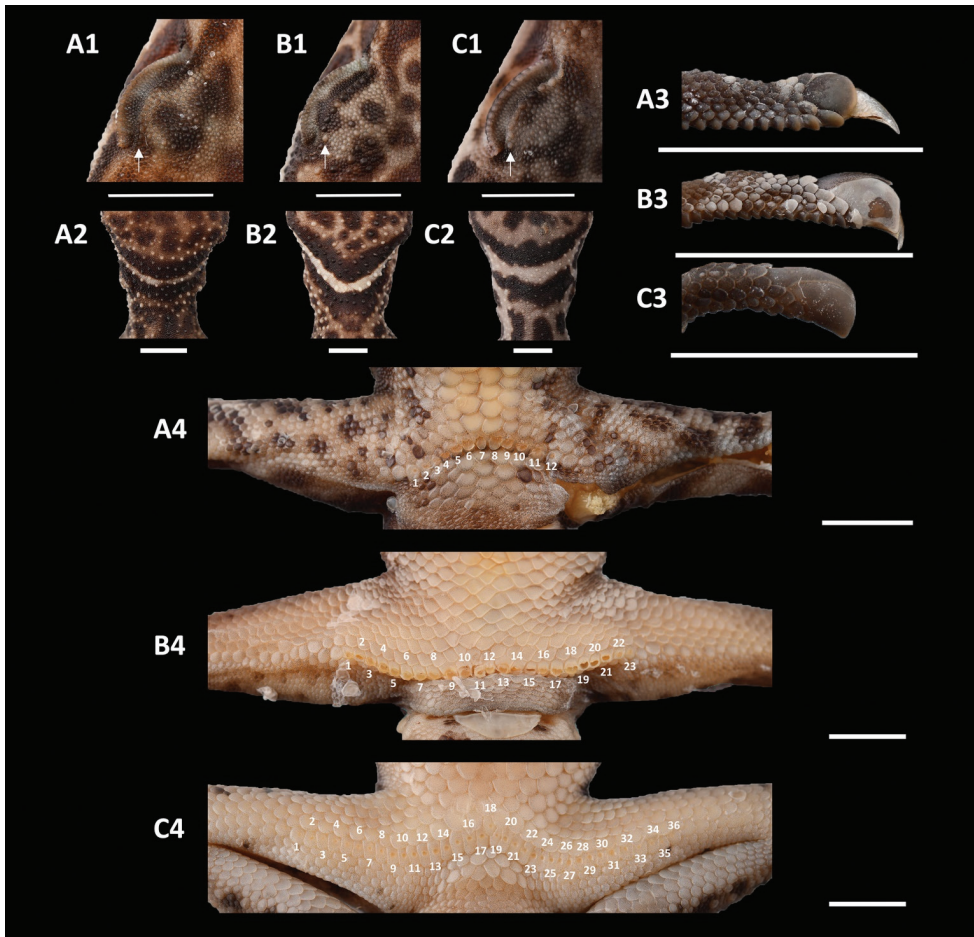


Figure 4. Comparisons of morphological characteristics of three species group in the genus *Goniurosaurus* **A** *Goniurosaurus yingdeensis* group (*G. yingdeensis*) **B** *Goniurosaurus luii* group (*G. liboensis*) **C** *Goniurosaurus lichtenfelderi* group (*G. zhoui*) **1** enlarged row of supraorbital tubercles **2** shape of nuchal loop **3** sheathed claws **4** number and position of preloacal pores. Scale bars: 5 mm. Photographs by Shuo Qi.

Additional specimens. Two adult males (SYS r001491, SYS r001492) and an adult female (SYS r002108). All specimens collected from the Shimentai National Nature Reserve.

Variation. Overall morphology, coloration, and scalation data of the newly discovered populations of *G. zhelongi* are in general agreement with the description of the holotype by Wang et al. (2014). Preloacal pores usually nine in males, 12 in an adult male (SYS r001491) and absent in female; internasal single or two. Additional variation in scale counts and measurements are shown in Table 7.

Diagnosis. (1) medium size, measuring 86.0–93.4 mm in SVL in adults; (2) TaL 0.85 times as long as SVL; (3) nasal scales surrounding nares 6–8; (4) internasal one or two; (5) eyelid fringe scales 42–53; (6) granular scales of the upper eyelids similar in size to those on the top of the head; (7) scales around midbody 99–109; (8) dorsal

Table 6. Mensural (mm) and meristic diagnostic characters (minimum/maximum) of specimens of *Goniurosaurus jingdeensis*. See Materials and methods for abbreviations. * holotype, # paratype.

[illegible]



Figure 5. The general aspects of *Goniurosaurus yingdeensis* and *Goniurosaurus zhelongzi* **A** *Goniurosaurus yingdeensis* **B** *Goniurosaurus zhelongzi*; (1) adult; (2) juvenile. Photographs by Jian Wang and Shuo Qi.

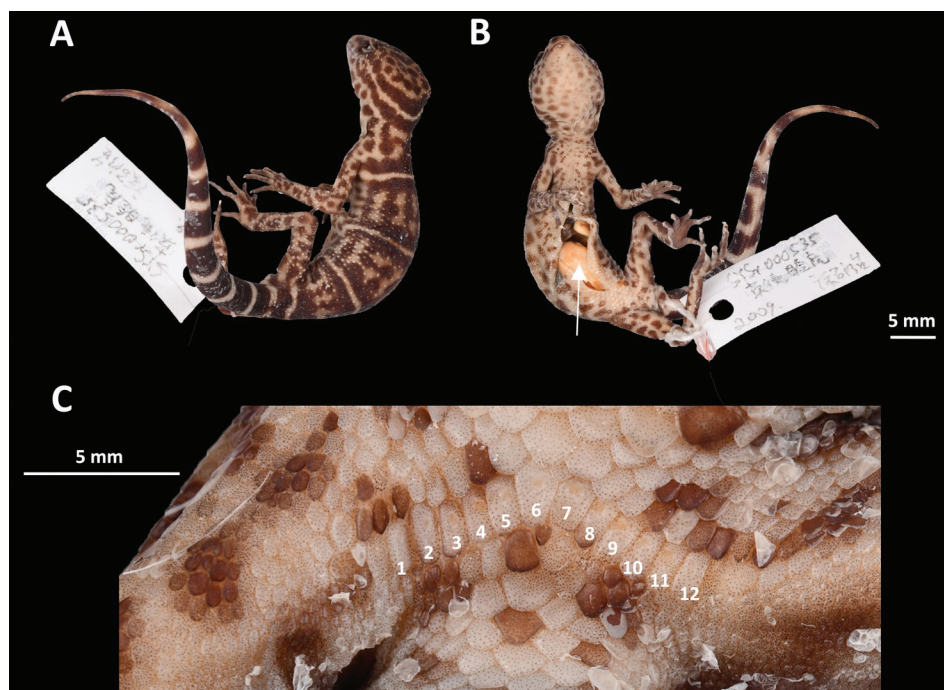


Figure 6. Adult female paratype (SYS r000535) of *Goniurosaurus yingdeensis* **A** dorsal view **B** ventral view, the white arrow denotes an egg in the fallopian tube **C** close-up of the preloacal region. Photographs by Shuo Qi.

Table 7. Mensural (mm) and meristic diagnostic characters (minimum/maximum) of specimens of *Goniurosaurus zhejiangi*. See Materials and methods for abbreviations. * holotype, # paratype.

Morphological character	SYS r000551 #	SYS r000765 #	SYS r000766 #	SYS r000770 *	SYS r000816 #	SYS r001491	SYS r001492	SYS r002108
Sex	Female	Female	Female	Male	Male	Male	Male	Female
SVL	91.5	93.4	91.6	86.0	88.1	90.8	87.1	87.9
TaL	Regenerated	79.6	Regenerated	Regenerated	Regenerated	Regenerated	77.8	80.0
HL	24.4	23.4	23.9	22.4	22.8	25.5	23.3	24.5
HW	15.8	15.9	16.1	15.6	15.7	17.1	14.8	16.0
SE	9.7	9.5	9.5	8.9	9.1	9.5	8.3	9.2
EE	9.8	9.9	9.9	9.5	9.6	9.2	7.8	8.5
SPL	8/8	7/7	8/8	9/8	9/8	9/8	9/9	10/8
IFL	7/7	6/7	7/7	7/7	7/7	9/9	8/8	8/8
N	7/7	7/8	6/6	8/8	7/7	8/7	7/8	7/7
IN	1	1	1	2	1	2	2	1
PostIN	3	3	3	3	3	3	3	3
PM	6	4	6	4	5	5	5	4
GP	9	7	8	8	8	9	7	9
CIL	47/48	47/48	48/45	52/48	49/51	47/46	43/42	52/53
PO	14/14	13/15	15/15	14/15	13/14	16/16	14/13	16/17
GST	10–12	9–11	11–12	9–12	10–12	9–11	10–12	9–11
PTL	33	28	29	29	28	28	28	29
DTR	26	26	27	28	28	23	24	23
MB	108	105	109	108	109	99	105	103
PP	0	0	0	9	9	12	9	0
PAT	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2

tubercle rows at midbody 23–28; (9) paravertebral tubercles between limb insertions 28–33; (10) claws sheathed by four scales, two lateral scales short and shell-shaped; (11) axillary pockets deep; (12) 9–12 precloacal pores in males and absent in females; (13) dorsal ground color of head, body, and limbs of adults brownish-black; (14) a thin, cream colored, posteriorly rounded nuchal loop; (15) four thin, cream colored, and immaculate body bands between the nuchal loop and the caudal constriction, edged in black anteriorly and posteriorly; (16) body bands without dark spots; (17) chin, throat, thorax, and ventral surfaces of body white, tinged brownish, with dark brown lateral spots; (18) iris gray-white, tinged with orange.

Distribution. *Goniurosaurus zhelongi* is currently only known from the Shimentai National Nature Reserve, Yingde City, Guangdong Province, China.

***Goniurosaurus varius* sp. nov.**

<http://zoobank.org/E28DD1FB-9EDD-4E6A-ACAE-5F831BC310BD>

Figs 7, 8A, 9A, 10

[English name: Nanling Leopard Gecko]

[Chinese formal name: 南岭睑虎]

Type material. *Holotype*. SYS r002333, adult male (Fig. 7), collected by Liang Zhang on 20 September 2019 from Nanling National Nature Reserve (ca 560 m a.s.l.), Chengjia Yao Ethnic Township, Yangshan County, Guangdong Province, China. **Paratypes.** One adult male (SYS r002331) and three adult females (SYS r002330, SYS r002362–2363), collected by Zhi-Ren Zhang, Yu Zhang, and Peng Cen on 6 August 2018, from Nanling National Nature Reserve, Chengjia Yao Ethnic Township at elevations between 180 and 560 m.

Additional specimens. Five individuals from the Nanling National Nature Reserve, Yangshan County, including a road-killed adult (SYS r002357), and four captured/released individuals (two juveniles, one adult male, and one adult female). All released individuals were photographed and measured for morphological examination. The tips of the tails were removed for future molecular analyses (SYS r002355, 2358, 2359, 2360), but not used these in current phylogenetic analysis.

Etymology. The specific name *varius* means varied or diverse in Latin and refers to its variable dorsal color pattern. As the type locality locates within the Nanling National Nature Reserve, we suggest the common name as “Nanling Leopard Gecko”.

Diagnosis. *Goniurosaurus varius* sp. nov. can be distinguished from all other congeners by the combination of the following characters: (1) adult body size moderate, measuring 81.5–86.3 mm in SVL; (2) nasal scales surrounding nares 7–9; (4) inter-nasal usually single, rarely two; (5) eyelid fringe scales 50–56; (6) granular scales of the upper eyelids similar in size to those on the top of the head; (7) scales around midbody 101–110; (8) dorsal tubercle rows at midbody 21–24; (9) paravertebral tubercles between limb insertions 27–29; (10) claws sheathed by four scales, dorsal scale small, two lateral scales short and shell-shaped; (11) axillary pockets deep; (12) pres-

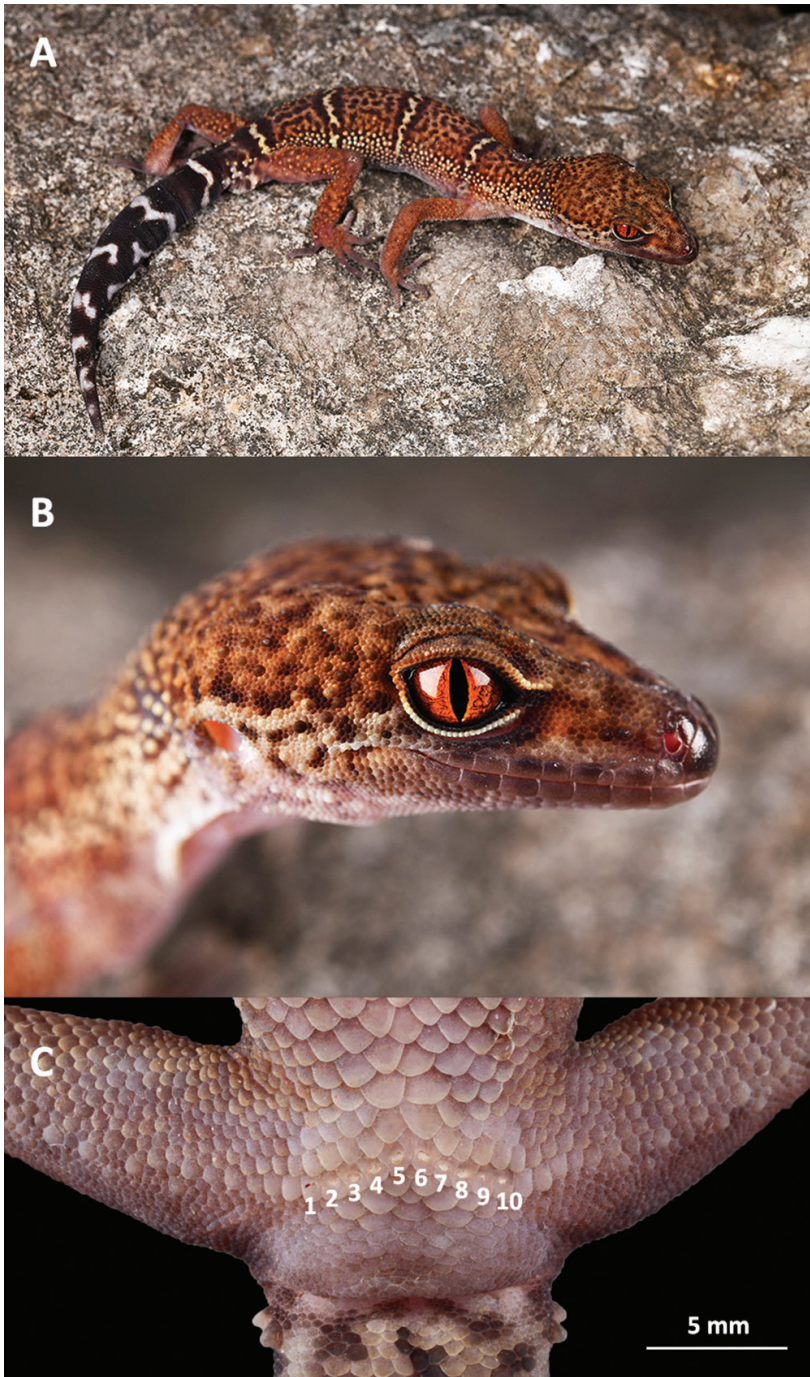


Figure 7. **A** Dorsolateral view of the adult male holotype SYS r002333 of *Goniurosaurus varius* sp. nov. in life **B** sculation and coloration characters of the head of the adult male holotype SYS r002333 of *Goniurosaurus varius* sp. nov. **C** ten precloacal pores in adult male holotype SYS r002333. Photographs by Shuo Qi.

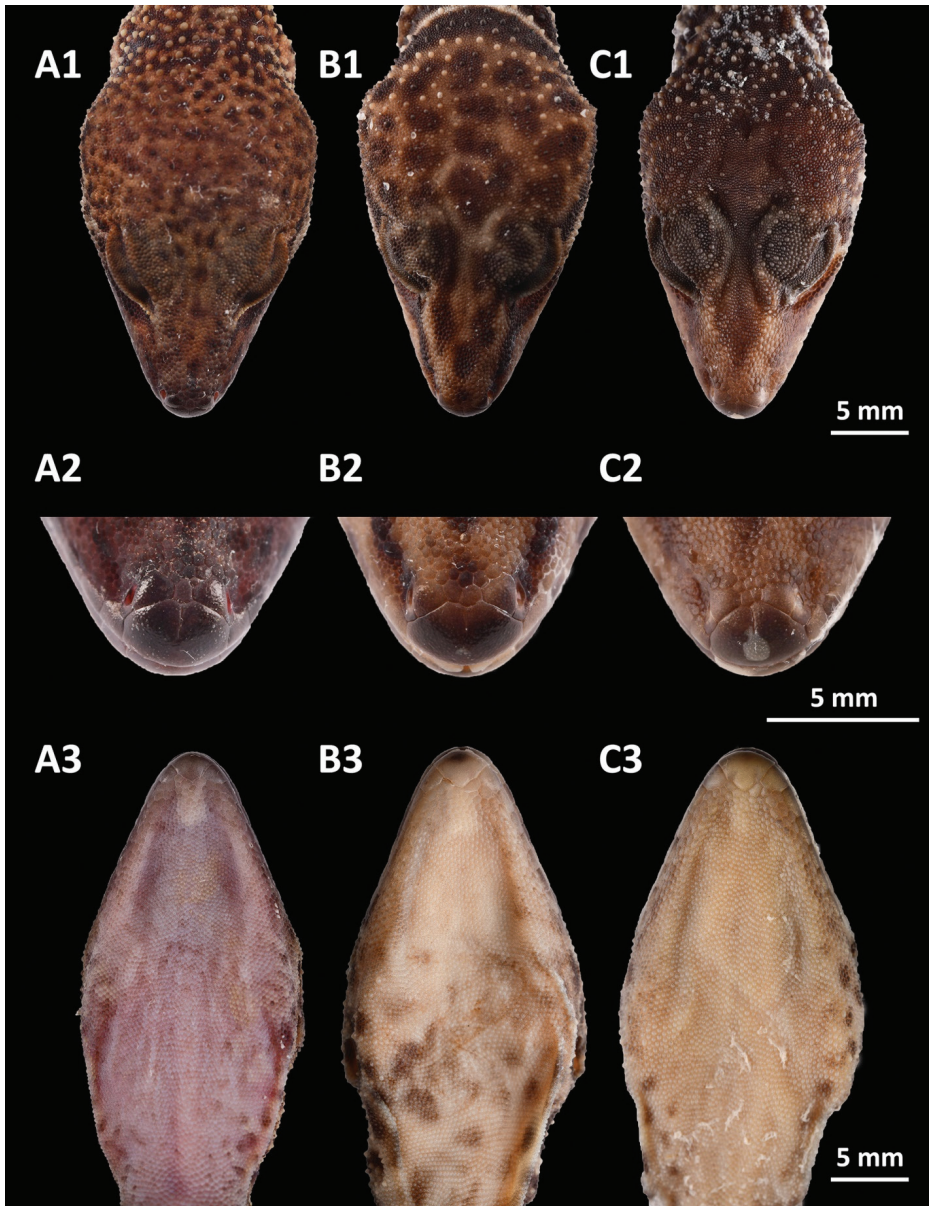


Figure 8. Comparisons of head morphological characteristics with two closely related congeners **A** *Goniurosaurus varius* sp. nov. (holotype, SYS r002333) **B** *Goniurosaurus yingdeensis* (SYS r001943) **C** *Goniurosaurus zbelongi* (holotype, SYS r000770) **1** dorsal view **2** close-up of dorsal snout **3** Ventral view. Photographs by Shuo Qi.

ence of ten precloacal pores in males, and absent in females; (13) dorsal ground color of head, body, and limbs in adults reddish brown, mottled with varied spots and stripes; (14) nuchal loop usually incomplete, if complete, posteriorly rounded; (15) presence



Figure 9. Comparisons of iris color with two closely related congeners **A** *Goniurosaurus varius* sp. nov. (holotype, SYS r002333) **B** *Goniurosaurus yingdeensis* (holotype SYSr000504) **C** *Goniurosaurus zhelongi* (holotype, SYS r000770). Photographs by Shuo Qi and Ying-Yong Wang.

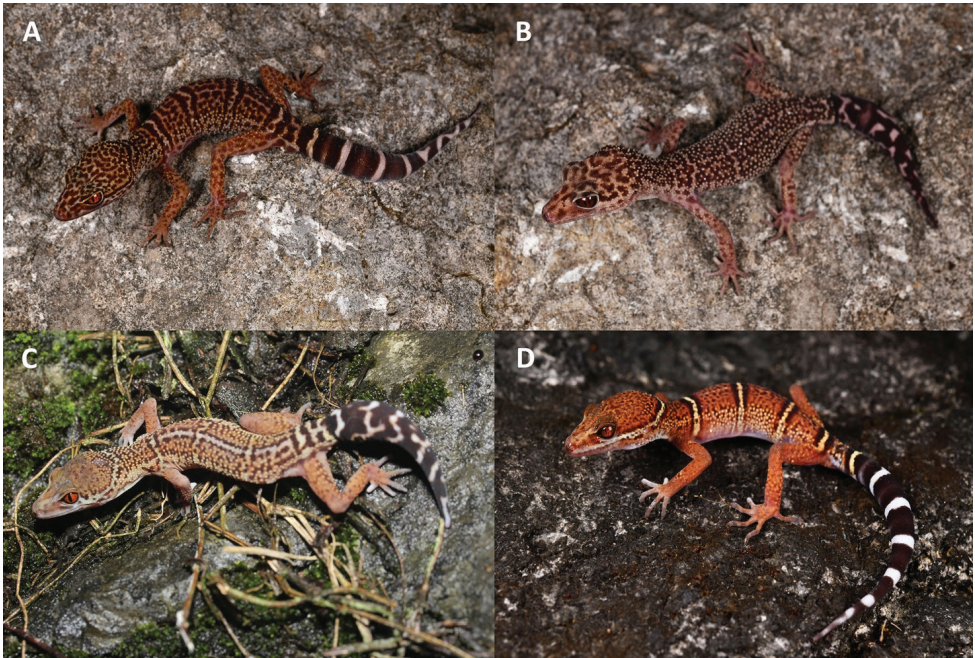


Figure 10. Differently patterned morphs of adult and juvenile coloration in *Goniurosaurus varius* sp. nov. **A** cross-banded morph **B** mottled morph **C** striped morph **D** juvenile coloration. Photographs by Shuo Qi and Peng Cen.

of four thin dorsal body bands with dark spots, bordered with black anteriorly and posteriorly, sometime last two bands indistinct; (16) usually presence of a longitudinal light colored vertebral stripe on the trunk of body; (17) light pink beneath, with dark brown lateral spots; (18) iris orange-red.

Comparisons. *Goniurosaurus varius* sp. nov. is most similar to *G. yingdeensis* and *G. zhelongi*, two closely related species from north Guangdong Province, but it differs

from them by following characters: paravertebral tubercles between limb insertions 27–29 (25–26 in *G. yingdeensis*, 28–33 in *G. zhelongi*); dorsal tubercle rows at midbody 21–24 (vs. 25–27 in *G. yingdeensis*, 23–28 in *G. zhelongi*); trunk of body usually with a longitudinal light colored vertebral stripe (vs. absent in *G. yingdeensis* and *G. zhelongi*); nuchal loop and body bands with black spots (vs. without black spots in *G. yingdeensis* and *G. zhelongi*); iris orange-red (vs. iris gray, becoming orange near pupil in *G. yingdeensis*, iris gray-white, tinged with orange in *G. zhelongi*). Additional comparisons of morphological characteristics with *G. yingdeensis* and *G. zhelongi* are provided in Figures 8, 9.

Description of holotype. An adult male with regenerated tail; SVL 84.7 mm; HL 22.7 mm; HW 16.0 mm; SE 9.1 mm; EE 13.0 mm; SVL:HL 3.7; HL:HW 1.4; SE:EE 0.7. Head triangular, wider than neck, covered with granular scales, densely interspersed with tubercles in the temporal and occipital regions; area between orbits uniformly covered by small granular scales; supraorbital tubercles with almost uniform size; scales of rostrum slightly larger than those in between orbits; rostral convex and hemi-elliptic, 1.3 times as broad as high, middorsal portion of rostral partially sutured dorsomedially, bordered laterally by first supralabial and prenasal, dorsolaterally by supranasal, dorsally by one internasal; external nares oval, surrounded by 7/8 nasals each, anteriorly by prenasal and supranasal, dorsally by supranasal and a granular scale, posteriorly by 5/5 smaller granular scales, and ventrally by the prenasal; prenasal with long recurved ventral portion; supranasals large, separated by one internasals; supralabials rectangular, 8/10; preorbital scales 15/15; eyes relatively large, pupils vertical; eyelid fringe scales 50/52; outer surface of upper eyelid composed of granular scales of about the same size of those on top of head; external auditory meatus circular, tympanum deeply recessed; mental triangular, bordered laterally by first infralabial and posteriorly by three postmentals; infralabials rectangular, 9/9; gular scales juxtaposed uniform granular, abruptly into flat juxtaposed pectoral scales, and grading posteriorly imbricated larger ventral scales. Tongue with a small notch at tip. Crowns of teeth expanded, occlusal margins bearing multiple ridges.

Dorsal surface of neck and body covered with uniform granular scales, interspersed with densely sharply pointed conical tubercles; scales around midbody 105; dorsal tubercle rows at midbody 24; vertebral row of scales indistinct; paravertebral tubercles between limb insertions 27; dorsal body tubercles surrounded by 9–11 granular scales; dorsal scales grading ventrally into larger flattened imbricate ventral scales; ten precloacal pores in a transverse series; postcloacal region greatly swollen, covered with imbricate flattened scales, containing 2/2 postcloacal tubercles laterally at the level of the vent.

Regenerated tail, short, thin at base, gradually thickening posteriorly, and gradually thinning into an obtuse tip; dorsal scales in regenerated portion of tail flattened, subimbricate, arranged in more or less regular transverse rows; subcaudal scales flattened, smooth, subimbricate, slightly larger than dorsal caudal scales.

Limbs relatively long and slender; dorsal surface covered with granular scales, densely interspersed with tubercles; ventral surface covered by flat scales, juxtaposed,

Table 8. Mensural (mm) and meristic diagnostic characters (minimum/maximum) of type series of *Goniurosaurus varius* sp. nov. See Materials and methods for abbreviations. * holotype, # paratype.

Morphological character	SYS r002330 #	SYS r002331 #	SYS r002333 *	SYS r002362 #	SYS r002363 #
Sex	female	male	male	female	female
SVL	86.3	84.9	84.7	81.5	85.7
TaL	Regenerated	Regenerated	Regenerated	Regenerated	Regenerated
HL	22.3	22.9	22.7	21.5	23.5
HW	14.7	15.0	16.0	14.5	15.6
SE	8.7	8.8	9.1	8.7	10.0
EE	12.4	14.3	13.0	12.8	13.2
SPL	8/8	9/9	8/10	9/8	7/7
IFL	8/8	9/9	9/9	9/8	9/9
N	7/7	8/7	7/8	7/8	7/9
IN	1	2	1	2	1
PostlN	4	4	3	4	4
PM	3	3	3	3	4
GP	7	8	8	6	8
CIL	52/54	54/56	51/53	51/50	53/55
PO	11/12	14/15	15/15	14/15	16/16
GST	9–11	9–11	9–11	8–11	9/12
PTL	28	28	27	28	29
DTR	24	24	24	21	24
MB	104	101	105	107	110
PP	Absent	Injured, unable to count	10	Absent	Absent
PAT	2	2	2	2	2

subimbricate or imbricate; dorsal surface of pes and manus covered with granular scales, interspersed with several conical tubercles on top of pes, lacking tubercles on top of manus; hind limbs slightly larger than forelimbs; ventral surfaces of pes and manus covered with large granular scales; axillary pockets deep; subdigital lamellae wide, 7/7 on Finger I, 12/12 on Finger II, 15/16 on Finger III, 17/15 on Finger IV, 13/13 on Finger V, 8/8 on Toe I, 13/ 13 on Toe II, 17 / 17 on Toe III, 21 / 18 on Toe IV, and 18 / 15 on Toe V; fingers laterally compressed, relative finger lengths $I < V < II < III \leq IV$; toes laterally compressed, third toe nearly as long as the fourth toe, relative toe length $I < II < V \leq III < IV$; base of claws sheathed by four scales, two lateral scales of claw short shell-shaped.

Coloration in life. Dorsal ground color of head, neck, body, and limbs reddish brown, mottled with indistinct faint dark brown-colored markings, scattered with densely light yellow tubercles and a few dark brown and reddish brown tubercles; nuchal loop incomplete, just from posterior corner of eyes to the temporal region, dirty yellow; four narrow body bands between the nuchal loop and the caudal constriction, fourth band inserting into the dorsal thigh, bands dirty yellow, with dark spots, edged in dark-brown anteriorly and posteriorly; a longitudinal light colored vertebral stripe between third and fourth bands; supralabials and infralabials grayish brown; pupils vertical and black; iris orange-red; dorsal surface of limbs deep reddish brown with dirty yellow tubercles and indistinct dark spots; chin, throat, thorax, and ventral surfaces of body pink, tinged brownish, with dark-brown lateral spots; ventral surface

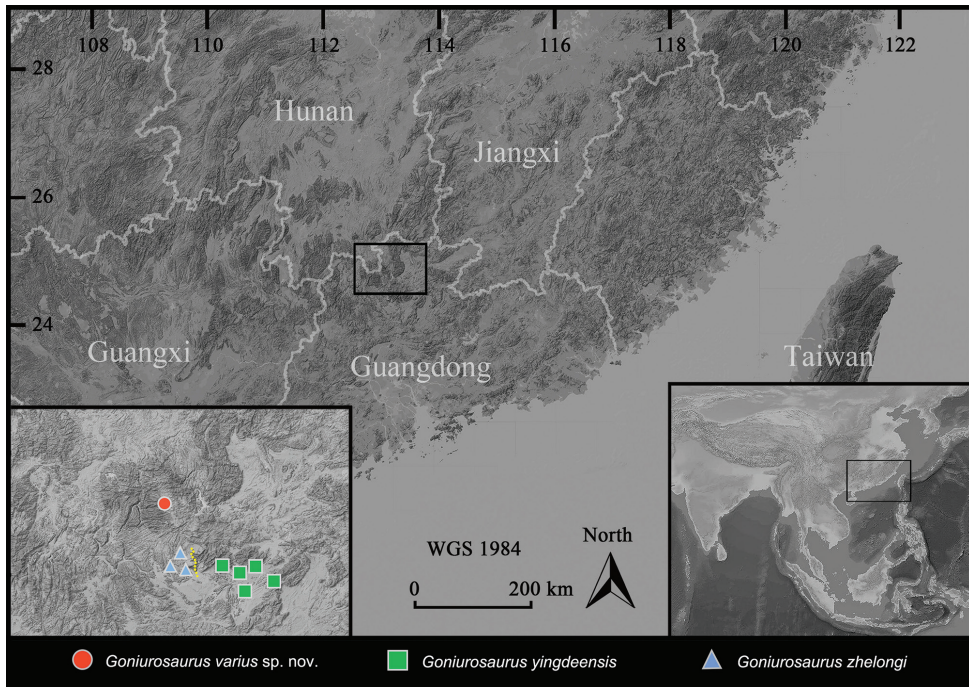


Figure 11. Geographic distribution of three species of *Goniurosaurus yingdeensis* group, the background depicts altitude in the southern China and the provinces of the region (darker shades indicating higher altitudes). The inset on the bottom left shows the detailed distribution, red circle indicates the collecting locality of the *Goniurosaurus varius* sp. nov., green squares and blue triangles indicate that known distributions of *G. yingdeensis* and *G. zhelongi*, respectively. The yellow dotted line indicates the Ruyuan Canyon. The bottom right inset shows the location of the main map in a regional context. Geographical basic map source from Google Maps.

of limbs pink, tinged brownish, with dark-brown spots; digits gray; ground color of the regenerated tail dark-brown, one original white band on the bases of tail, followed by irregularly shaped white markings. The body color becomes darker after capture.

Coloration in preservative. Dorsal ground color of head, body, and limbs black; ventral surface faded to grayish white; all darker spots and bands on the dorsal surface blurred.

Coloration in juvenile. Dorsal ground color of head, neck, body, and limbs dark-orange, mottled with indistinct faint dark-brown-colored markings, scattered with dense light yellow tubercles and a few dark-brown tubercles; nuchal loop from posterior corner of the mouth to the back of head, light yellow; four narrow body bands between the nuchal loop and the caudal constriction, fourth band inserting into the dorsal thigh, band color light yellow with dark spots, edged in dark-brown anteriorly and posteriorly (but not laterally); supralabials and infralabials grayish brown; pupils vertical and black; iris orange-red; dorsal surface of limbs dark orange with orange tubercles and indistinct dark spots; chin, throat, thorax, and ventral surfaces of body pink; ventral surface of limbs pink with dark-brown spots; digits gray; tail black-grey bearing white caudal bands encircling tail.

Variations. Measurements of type series specimens are shown in Table 8. Three paratypes have more complete and distinct nuchal loops than holotype, but SYS r002363 has only half a nuchal loop from the posterior corner of the right eye to the back of head. SYS r002330 has vertebral stripe extending from the posterior edge of the second body band to the anterior edge of third body band. SYS r002331 and SYS r002363 have large dark dorsal blotches on the head and the body band margin are broader than those in the holotype. Also, SYS r002363 has immaculate body bands. An additional female specimen (Fig. 10B) shows a more mottled dorsal pattern than all other types and its bands are mingled with irregular patterns on the body. SYS r002362 (Fig. 10C) has smaller dorsal blotches making it appear almost as if it has a reticulated dorsal pattern and its bands are greatly obscured, it has a distinct white vertebral stripe from the posterior edge of the first body band extending to the anterior edge of the last body band.

Distribution and ecology. *Goniurosaurus varius* sp. nov. is currently known only from the karst environment of the Nanling National Nature Reserve, northern Guangdong Province, China (Figure 11). All individuals were found in crevices of limestone near villages, farmlands, or country lanes at elevations between 180 and 560 m at night after 21:00 hrs.

Discussion

Our continued herpetological surveys coupled with extensive sampling in Guangdong Province, China in the past decades have resulted in discovery of three new species of *Goniurosaurus* from two localities, which all belong to the *G. yingdeensis* group. Topographically, rivers and a canyon form a series of geographic barriers that might lead to the isolations of members of *G. yingdeensis* group. Among them, *G. yingdeensis* is distributed in the lower hill areas on the east side of the Ruyuan Canyon, *G. zhelongi* was found on the west side of canyon. Moreover, microhabitat selection might also play an important role in species differentiation. Nearly all of *G. varius* individuals were found in karst topography but *G. yingdeensis* and *G. zhelongi* were also found in granitic landforms. This suggests they may be saxicolous generalist as opposed to a microhabitat specialist. Future phylogeographic and habitat selection studies are needed to gain a better understanding of their evolutionary history.

As the development of integrated taxonomy, to combine the morphological comparisons and phylogenetic relationships, has become an important and necessary work. In the present study, we propose the morphological definition of the *Goniurosaurus yingdeensis* group, which can be significantly distinguished from all other congeners, consistent with their distinct divergences in phylogeny. Nevertheless, it is worth noting that the species *G. bawanglingensis* and *G. zhoui* can be assigned to the *G. luii* group according to previous morphological diagnoses (Grismer et al. 2002; Zhou et al. 2018), while they were clustered within *G. lichtenfelderi* group in phylogeny based on two mitochondrial and two nuclear genetic segments (Liang et al. 2018). Hence, further comprehensive work with detailed morphological examinations and more genetic data is asked for, to clarify these incongruences or revise the morphological definitions of the *G. luii* group and the *G. lichtenfelderi* group.

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Appendix I

Examined specimens

Goniurosaurus bawanglingensis ($N = 3$): China: Hainan Province: Bawangling National Nature Reserve: SYS r001075, 1670, 2162.

Goniurosaurus hainanensis ($N = 2$): China: Hainan Province: Jianfengling National Forest Park: SYS r000349; Baoting County: SYS r001270.

Goniurosaurus liboensis ($N = 3$): China: Guizhou Province: Libo County: Maolan National Nature Reserve: SYS r000217, 854, 855.

Goniurosaurus luii ($N = 4$): China: Guangxi Zhuang Autonomous Region: Jingxi City: SYS r000255, 256, 859, 860.

Goniurosaurus yingdeensis ($N = 10$): China: Guangdong Province: Yingde City: SYS r000501, 503, 504, 535, 536, 550, 1271, 1272, 1493, 2115.

Goniurosaurus zhelongi ($N = 5$): China: Guangdong Province: Yingde City: SYS r000816, 1491, 1492, 2011, 2108.

Goniurosaurus zhoui ($N = 2$): China: Hainan Province: SYS r002213, 2214.

