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Description of new apterous winter species of *Leuctra* (Plecoptera: Leuctridae) based morphology and DNA barcoding and further records to stonefly fauna of the Caucasus, Georgia

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Abstract

Leuctra adjariae **sp. n**. and *Leuctra georgiae* **sp**. **n.** (Plecoptera: Leuctridae) are described as two new apterous stonefly species from the Meskheti Range (Lesser Caucasus) in southwestern Georgia. Descriptions and illustrations are provided for both sexes and diagnostic characters are discussed. Males and females of the two species are associated by DNA barcodes. Comparisons with corresponding regions of COI between *L. adjariae* **sp. n.** and *L. georgiae* **sp. n**. produced K2P genetic distances of 8.38%, values well associated with interspecific variation. The well-supported monophyly as well as results of an ABGD analysis confirms the validity of both new species. *Capnioneura gouanerae* Vinçon & Sivec, 2011, previously described and known only from Turkey, is reported for the first time for the Caucasus.

Key words: Plecoptera, *Leuctra adjariae* sp. n., *L. georgiae* sp. n, *Capnioneura gouanerae,* apterous, DNA barcoding, Adjaria, Georgia, Caucasus

Introduction

The Caucasian stonefly fauna includes 68 species (Zhiltzova 1981, Cherchesova 2006). The Holarctic genus *Leuctra* Stephens, 1836, is one of the species rich genera of the Caucasian fauna with 18 species (Zhiltzova 1997, Cherchesova 2006). Eight species (*L. dispinata* Balinsky, 1950, *L. dissimilis* Zhiltzova, 1960, *L. meyi* Braasch & Joost, 1981, *L. simplex* Zhiltzova, 1960, *L. svanetica* Zhiltzova, 1960, *L. tarnogradskii* Martynov, 1928, *L. torrenticola* Zhiltzova, 1960, *L. uncinata* Martynov, 1928) are Caucasus endemics (Zhiltzova 2003). Adults of most of *Leuctra* species are active in the spring-summer months, whereas other species emerge in the fall. Recently during a winter field trip to Adjaria, Georgia in vicinity of the town of Kobuleti, where the Kintrishi River drains the Meskheti Range (Western Lesser Caucasus) and into the Black Sea, wingless adults of leuctrids and capniids were found on the snow. The wingless specimens of *Leuctra* represent two new species and the capniid species is a new record for the stonefly fauna of the Caucasus.

DNA barcodes using the mitochondrial DNA (mtDNA) cytochrome c oxidase subunit 1 (COI) gene have been used for identification of stoneflies (Dela Cruz *et al.* 2016, 2018). Also, DNA barcoding has become an effective tool to associate insect stages (Mynott *et al.* 2011) and adults of different sexes (Avelino-Capistrano *et al*. 2016). Therefore, it is important to supplement barcode library of stoneflies in Barcoding of Life Project (Hebert *et al.* 2003) for the molecular identification of these taxa.

Material and methods

Adults were collected with entomological forceps from the snow. All specimens were preserved in 75% ethanol.

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Abdomens of adults were removed and soaked in 10% NaOH overnight and rinsed with distilled water. Specimens were examined with the aid of a compound microscope in transmitted light. Illustrations were produced using digital cameras (Nikon Coolpix 995 and Toup View 3.7) and with the stereomicroscope Olympus SZX16 and digital camera Olympus DP74, and stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software. Photographs were taken in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia (FSC EATB FEB RAS). The holotype and all paratypes of the two new species described herein are deposited in the collection of the FSC EATB FEB RAS. Terminology of species description follows Vinçon & Ravizza (2001).

Total DNA was extracted from five specimens using the Invitrogen PureLink Genomic DNA Mini Kit (Invitrogen Corp, Carlsbad, California, 2007) according with the protocol, and the resultant DNA was eluted in 70 µl. Polymerase chain reaction was performed to amplify the COI barcode fragment using primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), according to Folmer *et al*. (1994). PCR reaction for this fragment was run in total volume of 10 µl with 5 µl Go Taq Green Master Mix (Promega Corp, Madison, Wisconsin, USA), 0.5 µl of each primer (100 ng/µl), 3.5 µl nuclease-free water and 0.5 µl of total DNA. The PCR thermal conditions included an initial denaturation at 95 °C (1 min), followed by 35 cycles at 95 °C (denaturation, 20 s), 50 °C (annealing, 20 s), 72 °C (extension, 60 s), and a final extension step at 72 °C (5 min). All PCR amplifications were verified using electrophoresis on a 1,5% TBE agarose by visualizing on GelDoc XR+ imaging systems (Bio-Rad). PCR products were purified for cycle sequencing using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) by ThermoFisher Scientific. Sequencing reactions had a total volume of 10 µl and included 10 pmol of each primer, PCR product, Nuclease-Free water and reagents of BigDye terminator v3.1 cycle kit. Each sequencing reaction was purified by ethanol precipitation and bidirectional sequenced on an ABI 3130x sequencer (Applied Biosystems). Double stranded sequences were aligned and manually edited in MEGA 7 (Kumar *et al*. 2016). Based on the Kimura-2-Parameter (K2P) model were calculated inter- and intraspecific genetic distances and constructed Maximum likelihood tree (GTR+G) using MEGA7. ABGD analysis (wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html, Puillandre *et al.* 2012) was used for OTU delineation based on DNA barcodes using well proven for stoneflies settings (Morinière *et al.* 2017): relative gap width (X = 1.0), Kimura 2 parameter model and other settings were default. All sequences have been deposited in GenBank (accession numbers MK482355- MK482395) and Barcode of Life Data System (BOLD: PLECO001-19.COI-5P - PLECO005-19.COI-5P).

Results and discussion

We obtained five cytochrome oxidase I barcodes with full length of 658 base pairs (bp), two barcodes for *L. adjariae* **sp. n.** and *L. georgiae* **sp. n.** In total, we have determined 53 synonymous substitutions, most of which were species specific. Among these substitutions were 45 transitions and 8 transversions, and all of them were synonymous, therefore all barcodes had the same protein sequence. Each specimen formed a unique haplotype. The sequences were heavily AT-biased (59.4%), especially in the third position where 74.8 were A or T. The average intraspecific pairwise K2P distance was 0.77% and 0.30% for *L. adjariae* **sp. n.** and *L. georgiae* **sp. n.**, respectively. However, we had a limited data set to accurately calculate this parameter. The interspecies distances (K2P) between two new species were 8.23%. According to Morinière *et al.* (2017) these value is sufficient to maintain the species level for genus *Leuctra*. Obtained barcodes have been compared with other *Leuctra* species. The closest species were *L. geniculata* Stephens, 1836 and *L. prima* Kempny, 1899 from which the two new species differed on average 16.0%.

Species independence was also confirmed by ABGD analysis. We created a dataset of all *Leuctra* sequences from GenBank longer than 595 bp. The ABGD of the entire dataset separated two new species in different groups (OTU) with a prior intraspecific divergence of P=0.0077.

The relationships of *L. adjariae* **sp. n.** and *L. georgiae* **sp. n.** were constructed using maximum likelihood (ML) with *L. geniculata* and *L. prima* as outgroup. The monophyly of the two new species is supported using bootstrap values (Fig. 1).

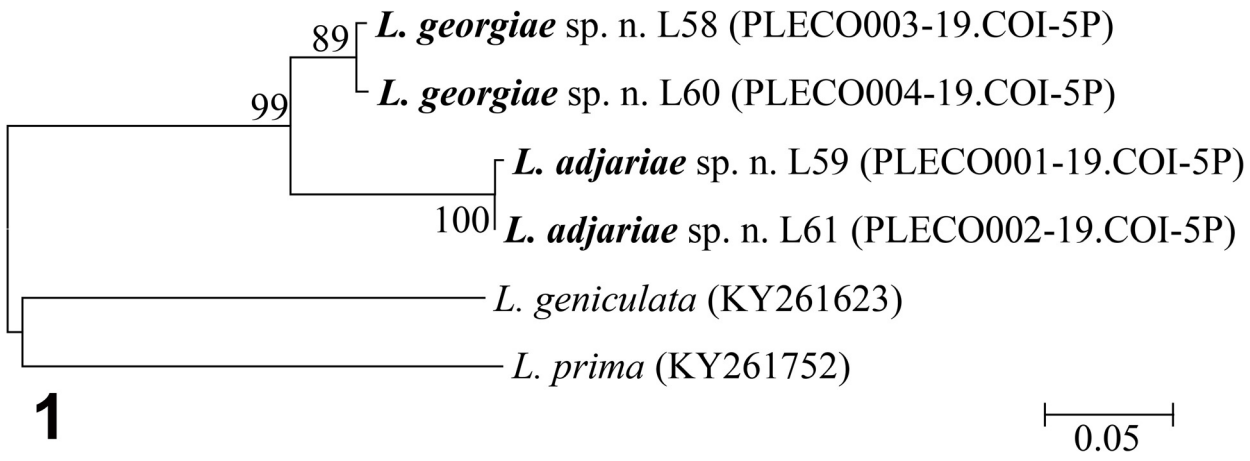


FIGURE 1. Maximum Likelihood (ML) tree (-Ln likelihood = 1785.88) of *Leuctra adjariae* **sp. n.**, *Leuctra georgiae* **sp. n.** and outgroup inferred from the cytochrome c oxidase I (COI) nucleotide sequence data (658 bp). Numbers are bootstrap support of 500 replicates. Specimens obtained in this study are in BOLD.

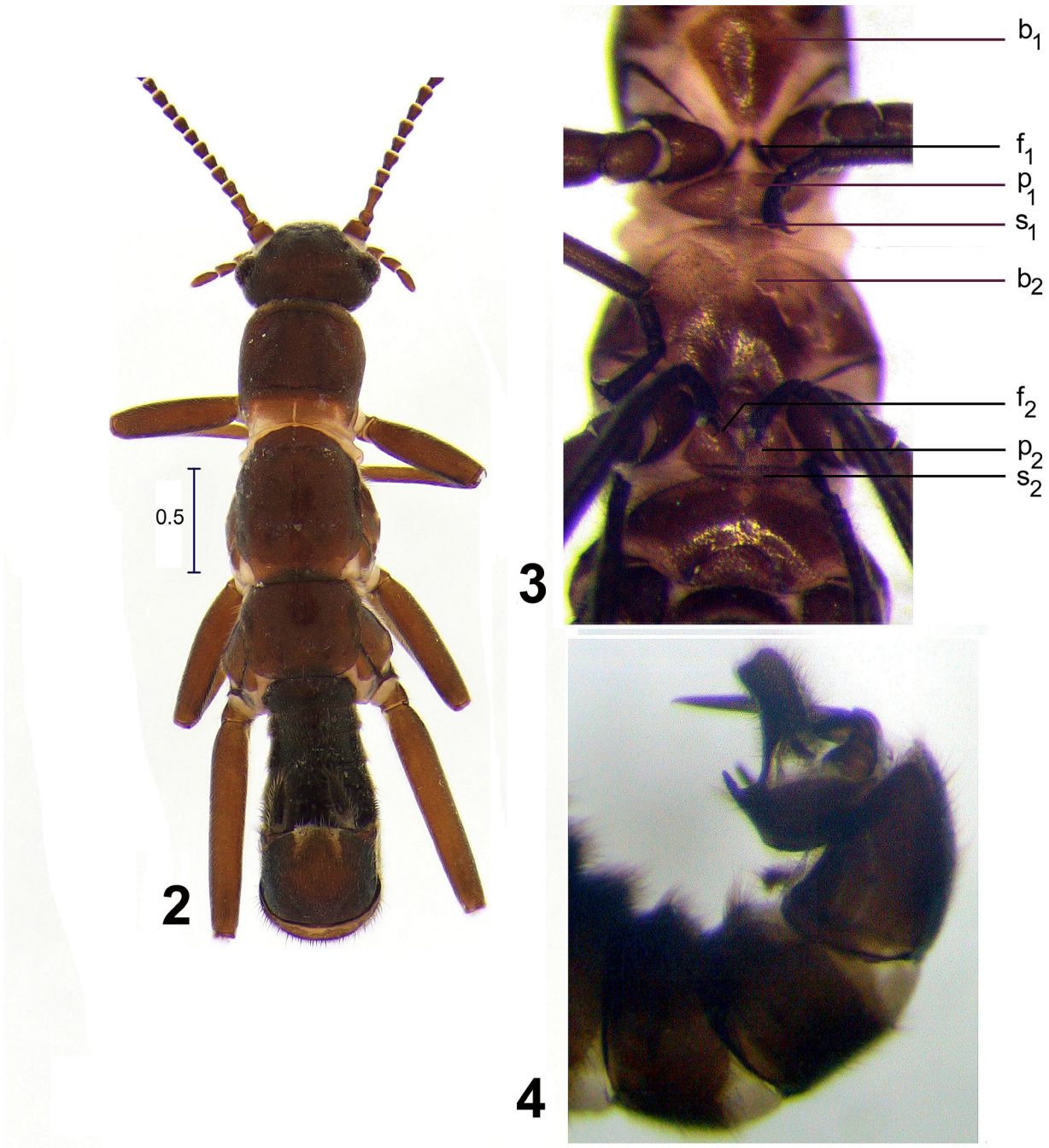
***Lectra adjariae* Teslenko sp. n.**
(Figs 1–13)
urn:lsid:zoobank.org:act:711EAB15-5DB4-4F36-ADFA-F588D1B2B3A8

Material examined. Holotype male. Georgia. Adjaria. Kintrishi River, 16 km upstream from Kobuleti and 4 km upstream from Tchakhati Village, 41°47.192 N 41°57.390 E, 06.02.2017, coll. D. Palatov (FSC EATB FEB RAS). Paratypes: 2 males (one mounted), 3 females (one mounted), same locality and date, coll. D. Palatov (FSC EATB FEB RAS).

Description. Body length of males 3.5–4.0 mm, females 4.0–5.0 mm. Apterous species in both sexes. General color brown, sclerotization heavily, body covered with pilosity, visible mainly in lateral view (Fig. 4). Palpi, head, pronotum, meso-, metanotum and legs uniformly brown, dark brown rugosities on the occiput and pronotum (Fig. 2). Head bears two small, barely visible lateral ocelli, the median ocellus indistinct, difficult to distinguish.

Prothoracic sternum of male with diamond-shaped basisternum (b_1), furcasternum (f_1) is a pair of diverging bars, postfurcasternum (p_1)—a large elliptical and medially subdivided sclerite, spinasternum (s_1) obscure, appears as a small round sclerite surrounded by pleurae, (Fig. 3). Mesothoracic sternum bears basisternum (b_2) much wider than that on the prothoracic sternum, with two triangular anterolateral processes; furcasternum (f_2) separated from the basisternum by distinct arms; postfurkasternum (p_2) as a pair of sclerotized rounded triangle sclerites laterally to furkasternum arms (f_2) not fused to furcasternum; spinasternum (s_2) narrow elongated (Fig. 3).

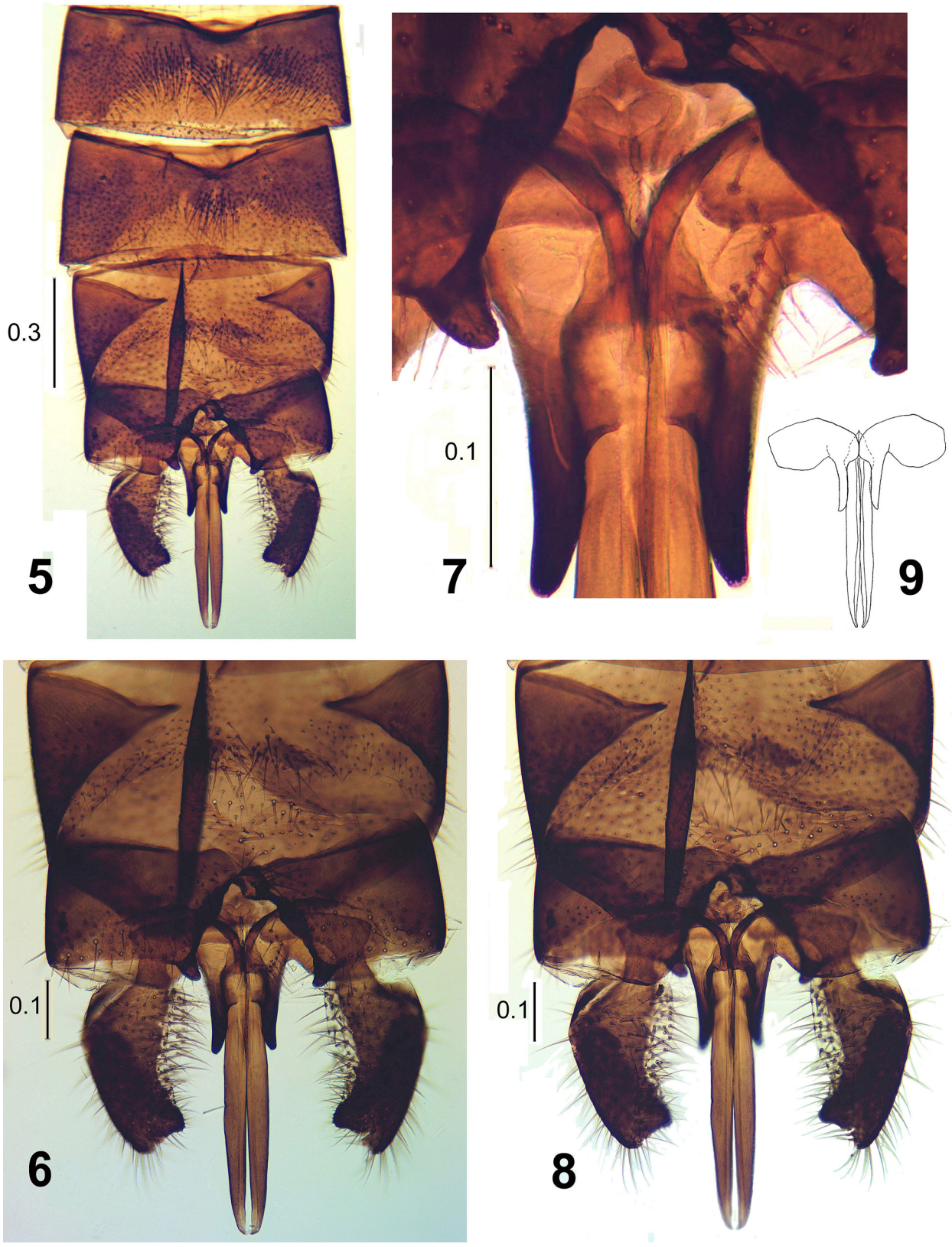
Male. Terga I-VII simple, each tergum with heavily sclerotized anterior margin and semicircular paramedian membranous area, increased in size posteriorly to tergum VIII and covered by dense, long black setae (Fig. 5). Tergum VIII bears indistinct weakly sclerotized anteromedial process looking like a pair of short slightly pigmented fan-shaped spots, closely separated, directed backward and covered by long black setae (Fig. 5). Tergum IX mostly membranous with distinct antecosta, divided medially for more than ⅓ of segment width; posteromedial sclerite consists of paired small prolonged pigmented spots covered with long, black setae (Figs. 5, 6). Posterior margin of tergum X with wide and deep median notch, each lobe bears pointed process at posterior inner margin, directed inward and oblique backward in dorsal view and risen in lateral view (Figs. 4–7). Epiproct rounded, mushroom-shaped, membranous with pigmented stalk (Fig. 7). Cerci are very specific, each cercus large, wide, scoop-shaped, gently bent inward and upward, hardly sclerotized on outer edge; the inner part membranous, covered with long black setae, denser than on the outer edge; apex wide, with slight depression on edge (Figs. 4–6, 8). Paraprocts stout (Figs. 4, 9), styles 2.25X shorter than specilla (Fig. 9). Styles with wide base; narrowed and curved sideward near the apex, and ending in rounded tip (Figs 5–7, 9). Specilla long gently bent inward apically in dorsal view, narrowed to the apex and ending in a sharp point (Figs. 5, 6, 8, 9). In lateral view the apex of specilla pointed and directed upward (but downward as shown in Fig. 4 since the abdomen curved, in mating position). Sternum IX with U-shaped unsclerotized area, no ventral vesicle (Fig. 2).



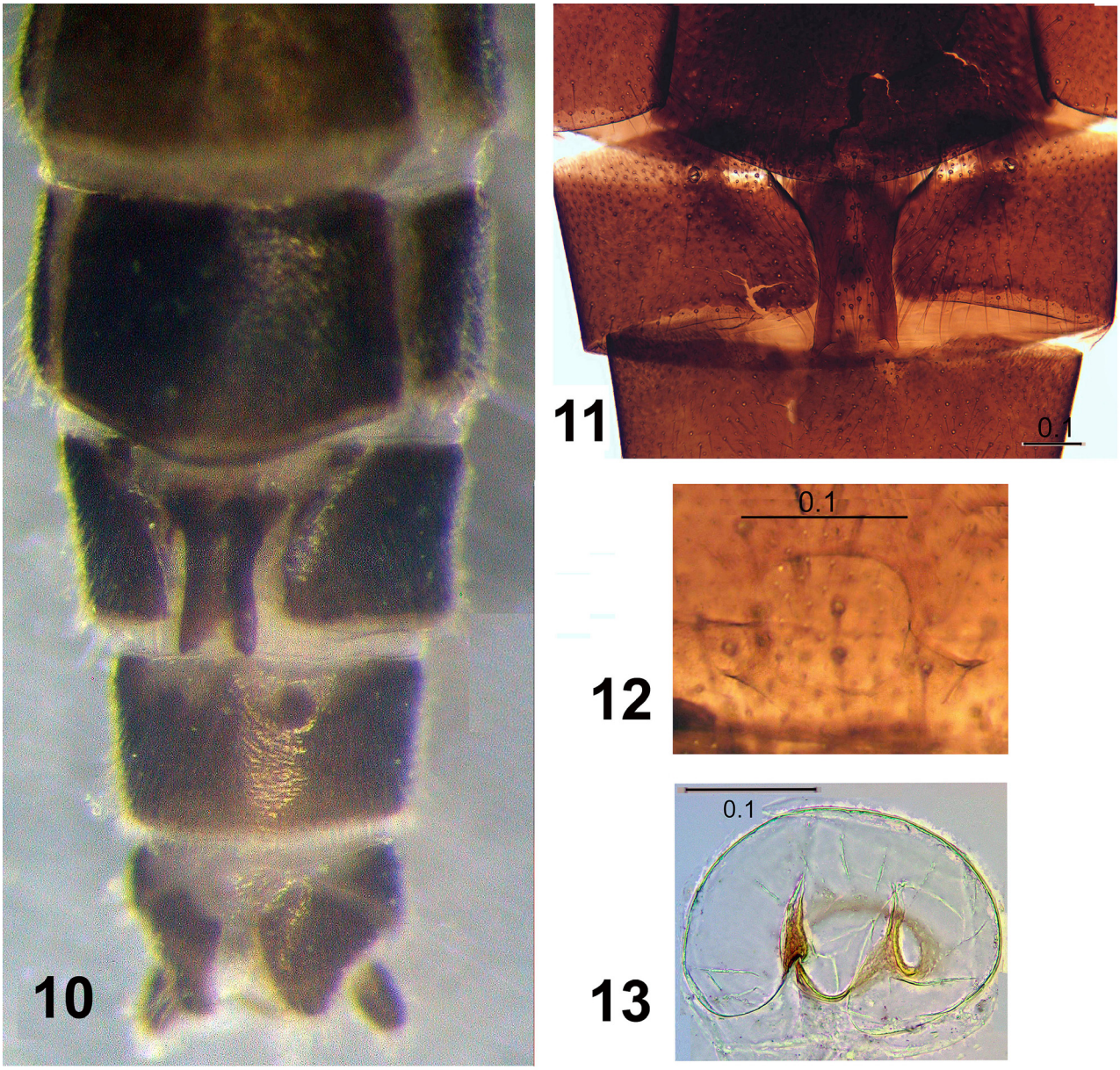
FIGURES 2–4. Male of *Leuctra adjariae* **sp. n.** 2. Habitus, dorsal view. 3. Pro- meso- and methatorax. Prothorax: b_1—basisternum, f_1—furcasternum p_1—postfurcasternum, s_1—spinasternum; b_2, f_2, p_2, s_2—the same on the mesothorax. 4. Abdominal tip, lateral.

Female. Terga II-VI with dorsal sclerite and pair of unsclerotized paramedian bands, the size of dorsal sclerite increasing to tergum VI; terga VII-X completely sclerotized. Sternum VII convex, very large and strongly sclerotized, almost trapezoidal with truncated anterolateral margins, anterior margin slightly concave, posterior margin broadly rounded, overlapping the anterior margin of sternum VIII and partly hiding the base of subgenital plate (Fig. 10). Subgenital plate triangular, prolonged, reaches anterior margin of sternum IX: a wide, triangular, unpigmented base delimited by a pair of small sclerotized sports anterolaterally, the second pair of small pigmented spots supports the plate laterally in the first ⅓ of its length; further to the subgenital plate narrowing, ending in a pair of sclerotized finger-shaped prolonged posterior lobes, shortly bifurcated at the apex and directed slightly sideward; apex with membranous area mesoposteriorly (Fig. 10). Subgenital plate covered with long setae especially in less pigmented part between sclerotized finger-shaped lobes, along inner edges of the lateral sclerites

and on posterior margin (Fig. 11). Sternum IX has two pairs of small triangular, membranous spots and one pair of sclerotized rounded spots between distal tops of each pair of membranous spots, below the subgenital plate (Fig. 10). Seminal receptacle spheroid enclosing spermathecal ring-shaped slightly sclerotized sclerite with pair of lateral pointed arms (Figs. 12, 13).



FIGURES 5–9. Male of *Leuctra adjariae* **sp. n.** 5. Abdominal tip, VII-X terga, dorsal. 6. Abdominal tip, IX-X terga, dorsal. 7. Epiproct, and styles, dorsal. 8. Abdominal tip, IX-X terga, ventral. 9. Styles and specilla, ventral.



FIGURES 10–13. Female of *Leuctra adjariae* **sp. n.** 10. Abdominal tip, subgenital plate, ventral. 11. Subgenital plate, cleared, mounted, ventral. 12. Spermatheca, cleared, mounted ventral. 13. Spermatheca, cleared, mounted, ventrolateral.

Diagnosis. Apterous in both sexes. Terga of male I-VII simple, with heavily sclerotized anterior margin and semicircular paramedian membranous area, increased in size posteriorly to tergum VIII. Tergum VIII bears a pair of anteromedial, short, slightly pigmented fan-shaped spots, covered by long black setae. Tergum IX with distinct antecosta, posteromedial sclerite consists of paired small prolonged pigmented spots covered with long, black setae. Tergum X with wide and deep median notch, each lobe bears very specific pointed process at posterior inner margin. Epiproct large, rounded, mushroom-shaped, membranous with pigmented stalk. Cerci large, wide, scoop-shaped, bent inward and upward, apex wide, with slight depression. Paraprocts strong, styles shorter than specilla, narrowed and curved sideward near the apex. Specilla long, narrowed to the apex, ending in a sharp point. Ventral vesicle is absent. Female subgenital plate also conspicuous: a triangular, prolonged, reaches anterior margin of sternum IX, with a wide, triangular, unpigmented base delimited by two pairs of small sclerotized sports anterolaterally and laterally, with a pair of sclerotized finger-shaped prolonged lateral lobes shortly bifurcated at the apex.

Affinities: According to the shape of processes and pigmented spots on the male abdominal terga, the structure of male epiproct and paraprocts, *L. adjariae* appears to belong to the *hippopus* species group (Ravizza & Vinçon

1998, Ravizza 2002). However, the indistinct, short, fan-shaped processes on tergum VIII, paired pointed process at posterior inner margin of tergum X and other features mentioned in diagnosis, readily distinguish *L. adjariae* males from all congeners. No closely related species are indicated based on external morphology. In the male, a pair of pointed processes of tergum X are unique and they only resemble those of male of Alpine-Carpathian *L. autumnalis* Aubert, 1948. The short styles of the paraprocts of *L. adjariae* are also unusual in the *hippopus* group, that are only shared with *L. tarnogradski* and *L. dispinata,* both species with short styles (Zhiltzova 2003). The triangular, prolonged subgenital plate of female is not similar to other *Leuctra* species and only remotely resembles members of the *brevipennis* sub-group, *hippopus* group, e.g. *L. vinconi aubertorum* Ravizza & Ravizza Dematteis, 1994 or *L. vinconi vinconi* Ravizza & Ravizza Dematteis, 1993, found in the western Alps of Switzerland and Italy (Ravizza, 2002) and another apterous species *L. kumanskii* Braasch & Joost 1977 from the Balkan Mountains, Bulgaria.

Distribution. *Leuctra adjariae* was collected at a single locality along the Kintrishi River near the town of Kobulety in Adjaria, in southwestern Georgia. The Autonomous Republic Adjaria, a historical, geographic and political-administrative region of Georgia, borders Turkey to the south and the eastern edge of the Black Sea (Fig. 25). The Kintrishi River drains the western slopes of Meskheti Range (Lesser Caucasus) into the Black Sea. At the type locality, where the altitude does not exceed 180-190 m a.s.l, the Kintrishi River flows through a canyon, with a width 5-6 m. The water level increased 0.3 m during flooding, but was clear, flowing at 0.3-0.9 m/s (Figs. 27, 28). Adults of *L. adjariae* were walking on the snow (Fig. 26).

Etymology. This species is named after Adjaria, region of Georgia, where it occurs.

***Leuctra georgiae* Teslenko sp. n.**

(Figs. 14–24)

urn:lsid:zoobank.org:act:C6A3B5AB-A3CC-40B0-B8F0-C4ED8C313243

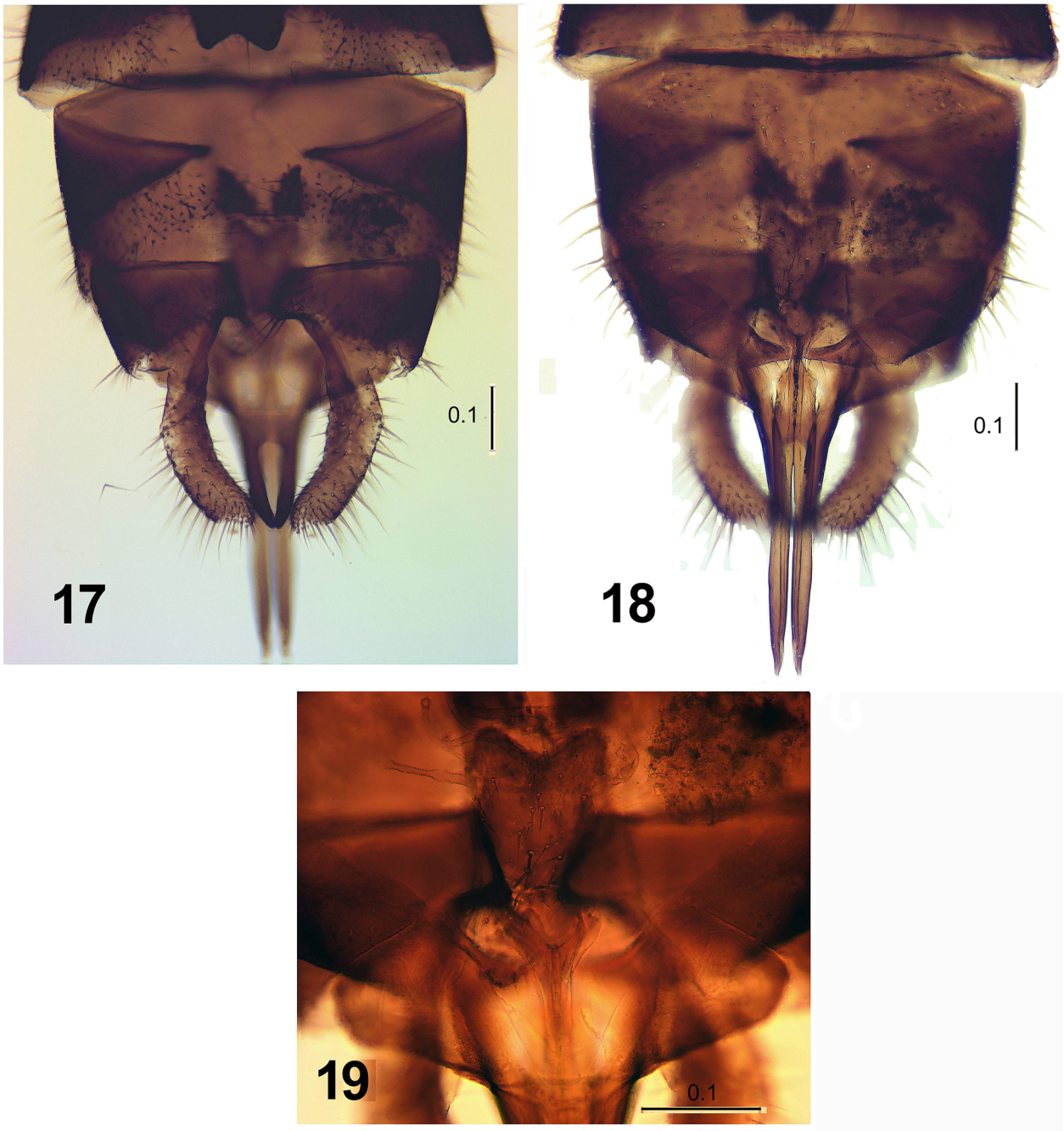
Material examined. Holotype male. Georgia. Adjaria. Kintrishi River, 16 km upstream from Kobuleti and 4 km upstream from Tchakhati Village, 41°47.192 N 41°57.390 E, 06.02.2017, coll. D. Palatov (FSC EATB FEB RAS). Paratypes: 3 males (1 male mounted), 5 females (2 females mounted), same locality and date, coll. D. Palatov (FSC EATB FEB RAS).

Description. Dark brown, apterous species, with tiny rudimentary wings, appearing as dark brown plates on the meso- and mesothorax; body length of males 4.0–4.2 mm, females 5.2−6.2 mm. Setation distinct, sclerotization heavy (Figs. 14, 15). Palpi, head, pronotum, meso- and metanotum, legs uniform black brown without pattern (Fig. 14). Head bears two very small lateral ocelli, poorly visible, the median ocellus indistinct, difficult to distinguish (Fig. 14).

The sclerite configuration on the female prothoracic and mesotoracic sterna of *L. georgiae* is similar to that of *L. adjariae* (Figs. 3, 20), except for prothoracic furcasternum as a pair of diverging bars which is larger and touching basisternum; prothoracic spinasternum of *L. georgiae* is oval, and mesothoracic basisternum having a concave anterior margin (Fig. 20).

Male. Terga I-VII simple, heavily sclerotized, except semicircular paramedian poorly pigmented areas on posterior margins, which are increased in size to tergum VII and covered by long black setae. Tergum VIII bears anteromedial sclerotized process armed with a pair of triangular, heavily sclerotized teeth directed backward and covered by long black setae (Fig. 16). In lateral view teeth raised oblique upward and forward (Fig. 15). Tergum IX mostly unpigmented with distinct antecosta, divided medially on more than one fifth of segment width; posteromedial sclerite consists of pair pigmented triangular spots, disconnected at base (Figs. 16, 17). Tergum X deeply cleft, bilobed, each lobe with oblique mesal edge in dorsal view, bears a small posteromedial process, covered with tuft long black setae (Figs 16, 17). Between the lobes a small rounded membranous epiproct appears; a large conspicuous V-shaped pigmented sclerite is behind the epiproct (Figs. 18, 19). Cerci long, slim (Figs. 16-18), more sclerotized along inner edges than along outer ones, covered with long setae, especially along outer edges; apices round, not pigmented with vestigial terminal segment modified into an ‘eye-spot’ mark. In dorsal view cerci form a circle around styles (Figs. 17, 18). Paraprocts strong, styles more 2.0X shorter than specilla (Figs. 16, 17). Styles base wide with triangular truncate lateral edges (Figs 16, 19), and ending in strong sclerotized tapered to the tips, gently bent inward (Figs 16, 17) processes. Specilla gently tapered to the tip, apices blunt, gently bent outside in dorsal view (Fig. 18). Sternum IX with U-shaped unsclerotized area, ventral vesicle absent, a tiny, indistinct knob on the anteromedial margin weakly visible (Figs. 14, 15).

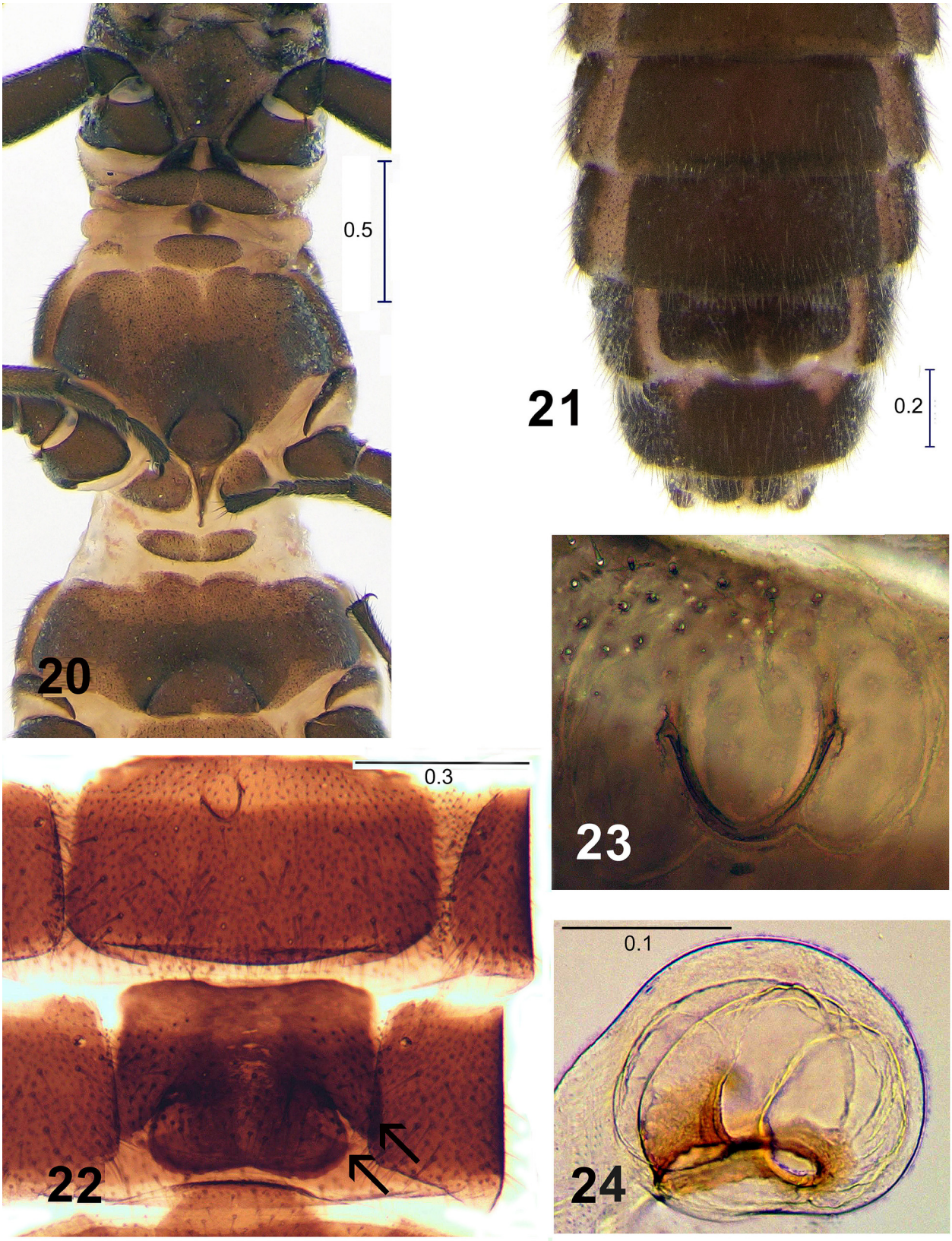
FIGURES 14–16. Male of *Leuctra georgiae* **sp. n.** 14. Habitus, dorsal view. 15. Abdominal tip, lateral. 16. Abdominal tip, dorsal.



FIGURES 17–19. Male of *Leuctra georgiae* **sp. n.** 17. Abdominal tip, IX & X terga, dorsal. 18. Abdominal tip, IX & X terga, ventral. 19. Epiproct, dorsal.

Female. Sterna with wide ventral sclerite and pair lateral membranous bands (Fig. 21). Sternum VIII forms a rectangular subgenital plate with two rounded posteromedial lobes (Figs 21, 22). In ventral view of a cleared specimen (Fig. 22) in ⅔ of the length of the subgenital plate one notices a pair of lateral wing-shaped processes covered with black setae; each process tapered to the tip and directed to outside, makes subgenital plate slightly wider, than at anterior or posterior margin (Fig 22); the last third of subgenital plate bilobed, posteromedian lobes rounded with slightly concaved posteromedian margin (Figs 21, 22). A median less-pigmented area divides the posteriomedian lobes (Fig. 22). Subgenital plate fused with paragenital plate being dark brown that can be seen by transparency (Fig. 22). Paragenital plate with paired fan-shaped processes, directed sideward and slightly overhang from under of posterolateral corners of the subgenital plate (Fig. 22). Sternum IX completely sclerotized, with a produced arch-shaped extension anteromesally and pair of small triangular anretolateral unpigmented spots below subgenital plate (Figs 21, 22). Seminal receptacle spheroid (Figs 23, 24), spermathecal sclerite ring-shaped, with

strongly sclerotized semi-ring which ends in a pair of small teeth; the teeth joined to each other by slightly sclerotized a central arch (Figs 22, 24).



FIGURES 20–24. Female of *Leuctra georgiae* **sp. n.** 20. Pro- meso- and methatorax. 21. Abdominal tip, subgenital plate, ventral. 22. Subgenital plate and spermatheca, cleared, mounted, ventral. Right edge of paragenital plate and right lateral wing-shaped process noted by arrows correspondingly. 23. Spermatheca, cleared, mounted ventral. 24. Spermatheca, cleared, mounted, ventrolateral.

FIGURES 25–28. 25. Collecting place of Plecoptera in Adjaria, Georgia (black dot). Square shows the nearest collecting site of *Capnioneura gouanerae* in Artvin, Turkey. 26. Female *of Leuctra adjariae* sp. n. on the snow. 27 & 28. The Kintrishi River, Meskheti Range, the type locality of *Leuctra adjariae* sp. n. and *Leuctra georgiae* sp. n. Photo by D. Palatov.

Diagnosis. Apterous in both sexes. *Leuctra georgiae* is distinguished from other *Leuctra* species by the pair of short, triangular and heavily sclerotized anteromedial processes on male tergum VIII in combination with pair of pigmented triangular posteromedial spots on tergum IX. The male is also distinguished by tergum X deeply notched with small paired posteromedial processes, covered with long setae; epiproct small, rounded and membranous; styles shorter specilla more than 2X. The female is characterized by a rectangular subgenital plate with two lateral wing-shaped processes, posterior margin of subgenital plate with paired posteromedian rounded lobes.

Affinities. Based on tergal ornamentation of the male, *L. georgiae* possibly belongs to the *prima* species subgroup within the *hippopus* species group according to Ravizza & Vinçon (1998) and Ravizza (2002). Within the *prima* subgroup, the male of *L. ligurica* Aubert, 1962 has similar paired anteromedial sclerotized processes on tergum VIII. In *L. ligurica* they are pointed at the apices, whereas in *L. georgiae*, the apex of each anteromedial process is rectangular. The posteriomedial sclerite on tergum IX is rectangular in *L. ligurica*, the same sclerite of *L. georgiae* appears as a pair of pigmented triangular spots, which are disconnected at the base. The females of both species are only generally similar in rectangular shape of subgenital plates with a bilobed posterior margin. However, the female genitalia differ in detail. The spermatheca of *L. georgiae* has a strongly sclerotized central arch and caudally connected arms, similar to *L. ligurica* and some members of the *prima* sub-group. Both species are apterous and are distributed within a relatively small area in the different mountain systems. *Leuctra ligurica* is known from the western edge of the Ligurian Apennines to the eastern margin of the Ligurian Alps (Ravizza 2002); *L. georgiae* occurs on the western slopes of Meskheti Range (Lesser Caucasus) in Adjaria, Georgia.

Furthermore, the styles of *L. georgiae* resemble remotely the styles of *L. tarnogradski,* which are short. *L.*

georgiae also differs from *L. tarnogradski* by the different shape of the posteromedial sclerite on tergum IX, by the lack of ventral vesicle on sternum IX, by the lack of wings, and by the very different shape of the female subgenital plate (Zhiltzova, 2003).

Distribution. Apparently, larvae of *L. georgiae* occur in the Kintrishi River, near the town of Kobulety located in Adjaria in the southwestern corner of Georgia bordered Turkey to the south and the eastern end of the Black Sea (Fig. 25). The Kintrishi River flows down from western slopes of Meskheti Range (Lesser Caucasus) into the Black Sea. The Kintrishi River at the type locality on altitude 180–190 m a.s.l. flows through a canyon with a width 5–6 m (Figs. 27, 28). Despite the river being at flood stage, the water was clear, flowing at 0.3–0.9 m/s. Adults of *L. georgiae* were collected walking on the snow.

Etymology. This species is named after Georgia, where it occurs.

***Leuctra adjariae* and *L. georgiae* were collected together with other Plecoptera:**

Leuctridae Klapalek, 1905

***Leuctra fusca* (Linnaeus, 1758)**

Material examined. 1♂. Macropterous. Georgia. Adjaria. Kintrishi River, 16 km upstream from Kobuleti town and 4 km upstream from Tchakhati village, 41°47.192 N 41°57.390 E, 06.02.2017, coll. D. Palatov (FSC EATB FEB RAS).

The emerging period of *L. fusca* lasts on the Caucasus from August to December (Zhiltzova 2003). This species was collected in February for the first time. Unfortunately, the single *L. fusca* specimen cannot be assigned to a subspecific identity; the specimen was damaged during slide mounting.

Capniidae Klapalek, 1905

***Capnioneura gouanerae* Vinçon & Sivec, 2011b**

Capnioneura veronicae Vinçon & Sivec, 2011a

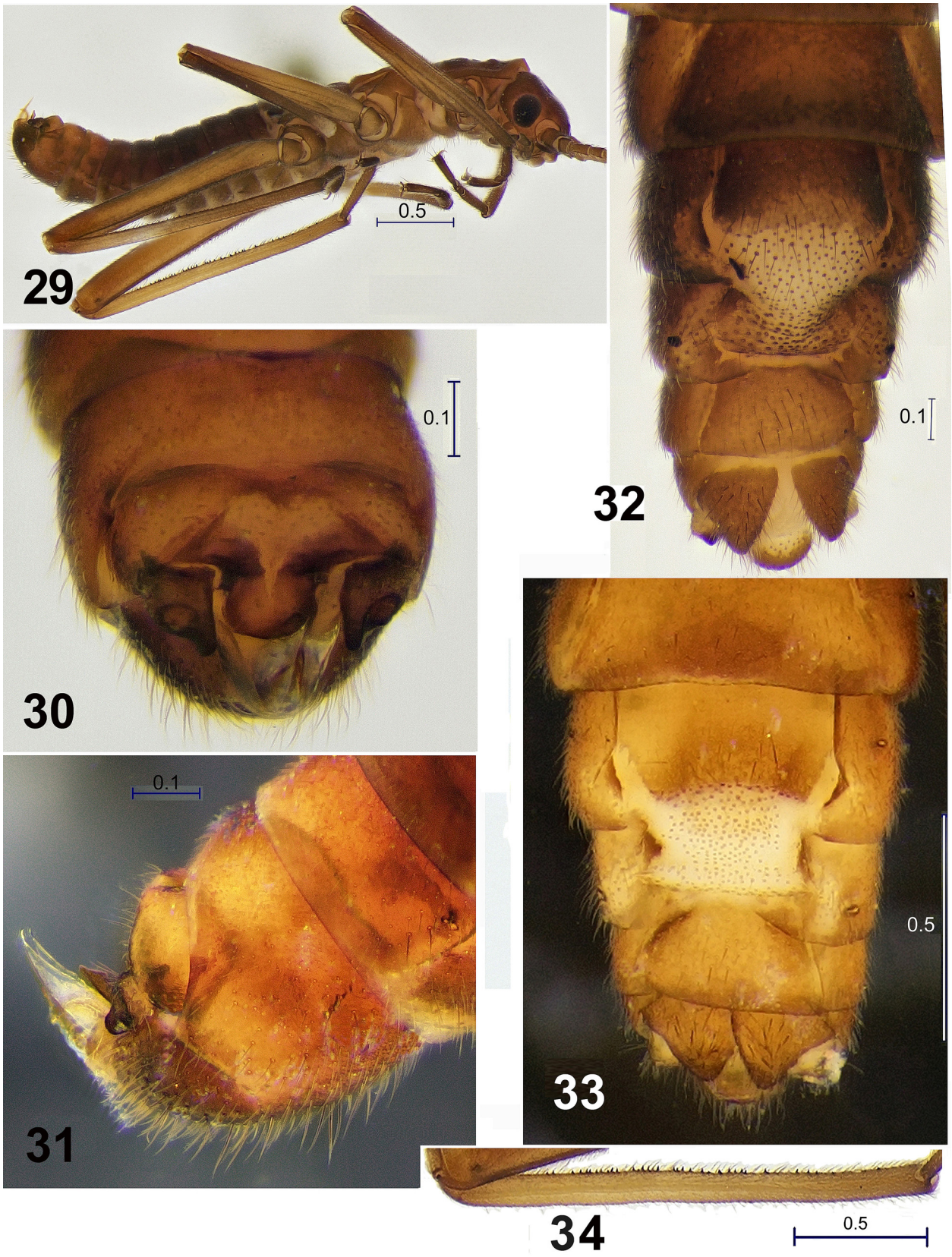
(Figs 29–34)

Material examined. 1♂, 2♀. Georgia. Adjaria. Kintrishi River, 16 km upstream from Kobuleti and 4 km upstream from Tchakhati Village, 41°47.192 N 41°57.390 E, 06.02.2017, coll. D. Palatov (FSC EATB FEB RAS).

Capnioenura gouanerae is reported for the first time for the Caucasian stonefly fauna. Vinçon & Sivec (2011b) did not designate a holotype for *C. veronicae* (Vinçon & Sivec 2011a). Later Vinçon & Sivec (2011b) proposed the replacement name *C. gouanerae*. This species was described from Turkey, Far Eastern Pontic Mountains, Gorgit Yaylası Plateau, District of Borçka, Camili Village, and Province of Artvin. This area is close to the Georgian border (Fig. 25). *Capnioneura gouanerae* is considered as relict or as micro-endemic species (Vinçon & Sivec 2011a, Darilmaz *et al.* 2016) restricted to the Artvin Region (Eastern Pontus). It is a crenophilic and stenothermal coldwater species, occurring in mountain springs and brooklets (1350–1600 m) with emergence in October. From a geographical aspect, the Eastern Pontic Mountains are considered as a western extension of the Lesser Caucasus (Vinçon & Sivec 2001). Therefore, the record of *C. gouanerae* in Georgia bordered to the south by the Turkish Province Artvin, the type locality of *C. gouanerae*, our new record for *C. gouanerae* was predictable. *Capnioneura gouanerae* was collected in Adjaria along the Kintrishi River on the snow in February (Figs. 27, 28). This record suggests an extended period of emergence for this species in autumn and winter.

Structure of genitalia of *C. gouanerae* from Adjaria agrees with description by Vinçon & Sivec (2011a), except for a few details. Specimens from Adjaria are smaller than from Artvin (Fig. 29), sexual dimorphism pronounced, the body length of male 3.6 mm, females 5.2−5.6 mm. Palpi longer, 1.4X than the male body length and 1.2X than the female body length. Legs long and stout, femur and tibia brown, tarsus dark brown (Fig. 29). Anterior margin of male hind tibia covered with conspicuous short and stout black spines and thin brownish setae (Figs. 34). Tergum X of male with a heart-shaped median membraneous field (Fig. 30). Epiproct regularly curved and narrowing towards the tip, the apex is obliquely truncate (Fig. 31). Specillum regularly curved, ending into a pointed tip and hidden between paraprocts (Fig. 31).

The tergum I of the female membranous with an oval dorsal sclerite. Terga II-VIII sclerotized, each tergum with paired paramedian membranous spots, forming V-shaped transversal band narrowed to tergum VIII. Tergum IX-X fully sclerotized. Sternum VI with trapezoid heavily sclerotized ventral sclerite (Fig. 32). Sternum VII



FIGURES 29–34. 31. *Capnioneura gouanerae.* 29. Male, habitus, lateral view. 30. Male abdominal tip, IX-X terga, dorsal. 31. Male abdominal tip, lateral. 32. Female abdominal tip, subgenital plate. 33. Variation of sclotization of female subgenital plate. 34. Hind leg, tibia, lateral.

convex, anteromedian half heavily sclerotized, posteromedian half membranous; a pair of small narrow oblique black spots posterolaterally. Subgenital plate forms by the fusion of the medial membranous part of posterior margin of sternum VII and medial membranous triangular part of anterior margin of sternum VIII. Sternum VIII slightly sclerotized; median area partly crossed by membranous triangular top of subgenital plate; posterior margin bears thin threadlike sclerite medially scarcely visible; a pair of dark belt-shaped spots anterolaterally connected with pair of small round black spots closely to posterolateral margin (Fig. 32). The subgenital plate (Fig. 33) of a second female specimen from the same collecting site was not as heavily sclerotized.

Remarks. The ecological characteristics of the type locality of *L. adjariae, L. georgiae* and *C. gouanerae* is uncommon for other known Palearctic apterous leuctrid and capniid species. The new leuctrids and the capniid were collected in the metharhitral zone of the Kintrishi River, whereas previously known apterous *Leuctra* species are considered mostly crenophilic, occurring in small brooks or brooklets at elevations higher than 800 m a.s.l. The apterous *C. gouanerae* collected at the same site, is considered a "crenophylic, stenothermic, cold water species, inhabiting mountain springs and brooklets (1350–1600 m)" (Vinçon & Sivec 2011). It is not excluded that there is a possibility that our specimens of these species could have emerged not from the Kintrishi River but from inlet brooks or springs nearby.

The Meskhleti Range serves as a barrier to moist air masses from the Black Sea. Most of the precipitation in the form of rain and snow falls in the autumn and winter, with a maximum of snowfall occurring in January–February. The height of the snow cover in the belt of 1000–2500 m a.s.l. can reach 1–3 m. In subtropical conditions, the cyclones are accompanied by extensive thaws, causing melting of snow and resulting flood events. The Kintrishi River flows through a canyon with smaller mountain tributary streams. It is certainly possible that drifting of mature stonefly larvae from other biotopes, especially headwater streams of this drainage may have occurred during snow melt. During adult emergence, larvae usually stay on the surface of the substrate, and are often dispersed by flood waters downstream. Mature larvae of all three species may have been transported from small drainages of peripheral valleys into the river.

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