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To cite this article: A. V. Martynov, D. M. Palatov, J.-L. Gattolliat, J. Bojková & R. J. Godunko (2022) A remarkable finding of *Centroptilum* Eaton, 1869 (Ephemeroptera: Baetidae) in Georgia, Turkey and Iran: one new species evidenced by morphology and DNA, The European Zoological Journal, 89:1, 827-855, DOI: [10.1080/24750263.2022.2090625](https://doi.org/10.1080/24750263.2022.2090625)

To link to this article: <https://doi.org/10.1080/24750263.2022.2090625>



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Published online: 22 Jul 2022.



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# A remarkable finding of *Centroptilum* Eaton, 1869 (Ephemeroptera: Baetidae) in Georgia, Turkey and Iran: one new species evidenced by morphology and DNA

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(Received 27 January 2022; accepted 2 June 2022)

## Abstract

The genus *Centroptilum* Eaton, 1869 is widely distributed in the West Palaearctic region, but poorly diversified as it previously only encompassed two valid species, *C. luteolum* (Müller, 1776) and *C. pirinense* Ikononov, 1962. The discovery of a new congener, *C. volodymyri* Martynov, Godunko and Palatov, sp. nov., is therefore remarkable; the new species is described based on larvae collected in Georgia, Turkey and Iran in 2012–2019. This new species can be distinguished by the structure of the cuticle with flattened, stretched shallow, triangular and semilunar-shaped corrugations; scales and their bases densely scattered on body surface; features of the mouth parts, including the presence of an expanded laterally labrum, in combination with segment III of maxillary palp and superlinguae of hypopharynx rounded apically, without any pointed projections at the tip; numerous small teeth scattered proximally in two rows along inner margin of the pretarsal claw; the shape of denticulation along the posterior margin of the abdominal terga, and the number of prominent posterolateral spines on tergum VII; and the shape of gills II–VII, with a distinctly concave posterior margin. Together with a morphological description, data on the distribution and mitochondrial cytochrome c oxidase subunit I gene (COI) barcode sequences of the species are provided. The species delimitation of *C. volodymyri* Martynov, Godunko and Palatov, sp. nov. and *C. luteolum* is tested using COI barcode sequences. The data obtained show that the new species can be distinctly recognised as a separate lineage. The habitat preferences associated with the different parts of the area of *C. volodymyri* Martynov, Godunko and Palatov, sp. nov. are described and compared. Distribution of *C. volodymyri* Martynov, Godunko and Palatov, sp. nov. confirms the connections between Caucasian and Asian fauna of Ephemeroptera, as already demonstrated in various families and genera. Short notes on the distribution of mayfly species common to the Caucasus, north-western Iran and Central Asia are presented.

<http://www.zoobank.org/urn:lsid:zoobank.org:act:1D2B7557-0DEC-4ACD-8CA3-A6B50FCC4A51>.

**Keywords:** Mayflies, Cloeoninae, Middle East, Central Asia, Palaearctic region, taxonomy, morphology, barcoding, COI

## Introduction

The mayfly fauna of the Caucasus is still largely unexplored, although considerable progress has been achieved recently. Important taxonomic studies, checklists and critical reviews of literature data

were published in the last decade (e.g. Kluge et al. 2013; Godunko et al. 2015; Martynov et al. 2015, 2016; Salur et al. 2016; Martynov & Godunko 2017; Bojková et al. 2018; Gabelashvili et al. 2018; Hrivniak et al. 2018, 2020). The family

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Baetidae Leach, 1815 represents an important component of mayfly diversity; however, it remains incompletely known in the Caucasus and adjacent regions, especially when considering taxa from lentic habitats and low altitudes. They include mainly species belonging to the subfamily Cloeoninae Kazlauskas, 1972. A new species from this group, *Procloeon* (*Pseudocentropilum*) *caspicum* Sroka, 2018, was recently described from Iran based on larvae (Bojková et al. 2018). Other genera of this subfamily, mainly *Cloeon* Leach, 1815 and *Centropilum* Eaton, 1869, remain unexplored or their published records are doubtful. Importantly, the taxonomy of these genera is not fully resolved and a critical review of Cloeoninae is necessary. This study is hence focused on the genus *Centropilum*, the concept of which has greatly evolved since its original description by Eaton (1871).

During more than a century, it conventionally encompassed all species of Cloeoninae with hind wings from all over the world (including Nearctic, Afrotropical, Oriental and Australasian regions). The concept was circumscribed step by step, for instance by excluding all African species (Gillies 1990). Kazlauskas (1972) and Keffermüller and Sowa (1975, 1984) listed the distinguishing characters for the *C. luteolum* species group, including the structure of larval mouthparts and cerci, the shape of the gills and the surface of abdominal terga, and the shape of the hind wing and forceps-base projection in male imago. Jacob (1973, 1991) recognised the polyphyletic origin of the species grouped into *Centropilum* sensu Eaton (1871) and divided them into *C. luteolum* and the *C. stenopteryx* complex. McCafferty and Waltz (1990) discussed diagnostic characters for *Centropilum*, including Nearctic representatives originally described within this genus, *Cloeon* and *Neocloeon* Traver, 1932. *Neocloeon* was considered a junior synonym of *Centropilum*. Kluge and Novikova (1992a, 1992b) summarised previous data and proposed additional diagnostic characters for genera and subgenera within Cloeoninae. Because these authors applied a broader generic concept, several taxa traditionally recognised as genera of Cloeoninae were subordinated as subgenera to *Cloeon*. The presence/absence of hind wings was no longer considered a reliable character to separate genera; *Centropilum* was distinguished from *Procloeon* Bengtsson, 1915 by the absence of spines on the lateral margin of tergites VII to IX and the absence of greatly enlarged spines on the outer margin of cerci, and from *Anafroptilum* Kluge, 2012 by the presence of a patella-tibial suture on fore legs of larvae (Kluge 2012, 2016).

Finally, Jacobus and Wiersema (2014) reinstated the genus *Neocloeon* and transferred seven Nearctic species originally described in *Centropilum* or *Cloeon* to the genus *Anafroptilum*.

Currently, the distribution of *Centropilum* s.s. is limited to the Palearctic region only, encompassing two species, the widely distributed *C. luteolum* (Müller, 1776) and the poorly known *C. pirinense* Ikonomov, 1962. The latter was originally described from the Balkans and only its larval stage is known. Keffermüller and Sowa (1984) considered *C. pirinense* to be closely related to *C. luteolum*. Kluge and Novikova (1992a, 1992b) did not cite this species in the revision of the systematics of the subfamily Cloeoninae. Bauernfeind and Soldán (2012) reviewed the distinguishing characters, distribution and ecology of its larvae. They pointed out its possible synonymy with *C. luteolum* and considered it a *species inquirenda*. Jacobus and Wiersema (2014) considered it a valid Eurasian species in the discussion of species belonging to *Centropilum* s.s. They noted the need for additional studies of *C. pirinense*, and also mentioned a single larva identified as *C. pirinense* by R. D. Waltz, that was partly mounted on the slide and deposited in the collection of the Purdue University Entomological Research Collection (USA). The specimen was labelled as collected in Turkey, without an exact locality – not even an indication of whether it was collected in the European or Asian part of the country.

This study aims to describe a new species of *Centropilum*, *C. volodymyri* Martynov, Godunko and Palatov, sp. nov., based on material collected during field trips to Turkey, Georgia and Iran in 2012–2019; to discuss in detail morphological differences of the new species from *C. luteolum* (Müller, 1776) and *C. pirinense* Ikonomov, 1962; and to briefly discuss affinities of Baetidae faunas of the Caucasus and adjacent regions.

## Material and methods

### *Sampling of material, preservation and morphological study*

The present contribution is based on the larval material collected by A. V. Martynov, D. Palatov and J. Bojková in 2012–2019, in several regions of Georgia, Turkey and Iran. Administrative divisions and geographic coordinates of localities are taken from Google Earth ([http:// earth.google.com](http://earth.google.com)). All specimens were collected by kick sampling in different freshwater habitats and preserved in the field in 96% ethanol.

The holotype (from Georgia) is preserved in ethanol; paratypes from Georgia are preserved in ethanol (one larva), mounted on slides in Canada balsam (three larvae) (see below for the inventory numbers (IN)), preserved on scanning electron microscopy (SEM) stubs for taking SEM photos (one larva), and preserved as the external skeleton only in alcohol (two larvae – used for molecular study); paratypes from Turkey are preserved on a slide (one larva) and in ethanol (48 larvae).

The main part of the type material is housed in several research institutions: the holotype and four paratypes (all from Georgia, three in slides) are deposited in the collection of the National Museum of Natural History, NASU, Kyiv [NMNH NASU]; one paratype from Turkey deposited in the Laboratory of Synecology of the A. N. Severtsov Institute of Ecology and Evolution, RAS, Moscow [IEE]; 44 paratypes from Turkey (one mounted on a slide) are stored in the collection of the Institute of Entomology [IE], BC CAS, České Budějovice; and six paratypes (two from Georgia and four from Turkey) are deposited in the Museum of Zoology Lausanne, Switzerland [MZL].

Also, some non-type material of *Centroptilum volodymyri* Martynov, Godunko and Palatov, sp. nov. from Iran was used in the current study: 19 larvae deposited in IEE; one larva in NMNH NASU; four larvae in MZL; and nine larvae in the Department of Botany and Zoology of Masaryk University, Brno [MU].

The inventory numbers of samples from the type locality (Georgia) deposited in the NMNH NASU are: *Grg24Censp1* and *Grg24Censp2* for vials with larvae in alcohol; and 648, 661 and 662 for larvae mounted on slides.

For morphological analysis using SEM, larval mouthparts, legs and parts of the cuticle, and eggs dissected from a female larva were obtained from *C. volodymyri* sp. nov. (from type locality) and *C. luteolum* (Russian Federation (RF), Moscow Region, Naro-Fominsky District, Turussa River near Kryukovo village, 55.465°N, 36.537°E, 15 August 2017, Palatov D. M. leg.). All parts were subsequently dehydrated through a stepwise immersion in ethanol, dried by critical point drying (Leica EM CPD300), and mounted on SEM stubs. The mounted material of *C. volodymyri* sp. nov. from Georgia was coated with a 5 nm Au/Pd layer (Leica EM ACE200) and subsequently examined and photographed with a Zeiss EVO LS 15 SEM at the State Museum of Natural History Stuttgart, Germany [SMNS]. The material of *C. luteolum* was analysed using Vega3 Tescan in the Paleontological Museum of the Paleontological Institute, Russian Academy of Sciences, Moscow [PIN]. All the photographs were subsequently sharpened and

adjusted for contrast and tonality in Adobe Photoshop™ CS5 and CS6.

Macrophotographs of the larval habitus of *C. volodymyri* sp. nov. (material from Georgia) were taken with a Canon PowerShotA 640 camera and Zeiss Stemi 2000 binocular microscope in the I. I. Schmalhausen Institute of Zoology, NASU, Kyiv. Photo stacks were processed with Helicon Focus Pro 6.4.1 to obtain combined photographs with extended depth of field, and subsequently enhanced with Adobe Photoshop™ CS3. The material was examined using Olympus SZX7 and Leica M205 C stereomicroscopes. Line drawings were made using an Olympus BX41 microscope equipped with a drawing tube.

*Comparative material of Centroptilum luteolum*  
(Müller, 1776)

GEORGIA: 18 larvae, Adjara, territory of Kobulety town, Kintrishi River, 41.804°N, 41.776°E, 4–5 June 2013, Martynov A. V. leg. – IN *Grg6Cenlut*; two larvae, Adjara, territory of Kobulety town, Kintrishi River (near estuary) and Kinkishi River, 41.804°N, 41.782°E, 17 April 2016, Martynov A. V. leg. – IN *Grg23Cenlut*. UKRAINE: five larvae, Donetsk Region, Shahtarsk district, vicinity of Chystiakove (= Torez) town, stream in bayrak, 14 May 2011, Martynov A. V. leg. – IN *Don332*; 25 larvae, Donetsk Region, Bakhmut district, vicinity of Debaltseve town, Bulavina River, 48.315°N, 38.438°E, 2 May 2010–29 April 2011, Martynov A. V. leg. – IN *Don360*, *Don397*, *Don399*, *Don403*, *Don411*, *Don495*, *Don496*, *Don503*; 13 larvae, Luhansk Region, Antratsyt District, vicinity of Khrustal'nyi village, Khryshhtaleva River, 48.182°N, 38.877°E, 30 April 2012, Martynov A. V. leg. – IN *Lug 94*; one larva, Rivne Region, vicinity of Poznan' village, Stvyha River, 51.591°N, 27.478°E, h – 141 m a.s.l., 5 July 2017, Martynov A. V. leg. – IN *Riv4Cenlut*; 16 larvae, Chernivtsi Region, vicinity of Berehomet urban-type settlement, Stebnyk River, 13 May 2008, Martynov V. V. – IN *Chrt25*; four larvae, Chernivtsi Region, vicinity of Berehomet urban-type settlement, slow-flowing reservoir in valley of Stebnyk River, 12 May 2008, Martynov V. V. – IN *Chrt29*.

RUSSIAN FEDERATION: one larva, W Siberia, Yamalo-Nenets Autonomous Okrug, Tyumen Region, Priuralskii District, Chtchouchia River, 67.334°N, 68.432°E, 5 July 2006, Palatov D. M. leg.; 12 larvae, Murmansk Region, Kandalakshkii District, Virma River, 67.169°N, 32.240°E, 24 July 2015, Palatov D. M. leg.; eight larvae, Moscow Region, Stupino City-district, small stream (tributary of Bunchikha River near Pesochnaya village), 54.979°N, 38.204°E, 14 September 2017, Palatov D. M. leg.; one larva,

Krasnodarskii Krai [Province], Tuapsinskii District, Psebe River, 44.287°N, 38.910°E, 29 March 2012, Palatov D. M. leg.

AZERBAIJAN: 19 larvae, Talysh Mts., Lenkaran District, small stream (the tributary of the Vesharyu River near the Dashtatuk village), 38.695°N, 48.757°E, 7 April 2011, Palatov D. M. leg.

TURKEY: 10 larvae, Kastamonu il [Province], Daday District, small stream 1 km southwest of Gekeren Keyu village, 41.450°N, 33.129°E, 15 April 2010, Palatov D. M. leg.

SWITZERLAND: 25 larvae, Vaud, Vallée de Joux, Le Chenit, La Burtignière, Orbe stream, 46.562°N, 6.170°E, 23 August 2001, Wagner A. leg.

### Terminology

The terminology and abbreviations proposed by Godunko et al. (2015) for the subgenus *Rhodobaetis* Jacob, 2003 are used to describe body setation of larvae. Their applicability was confirmed also for the genus *Nigrobaetis* Novikova and Kluge, 1987 (Godunko et al. 2018). The larval characters used to distinguish the the genus *Centroptilum* from its congeners were taken from and adapted based on references cited in the Remarks to Table II.

### DNA extraction, polymerase chain reaction amplification and sequencing, and DNA sequence analysis

Total genomic DNA from XY specimens was extracted using the BioSprint 96 extraction robot (Qiagen Inc., Hilden, Germany), following the supplier's instructions. The non-destructive protocol described in Vuataz et al. (2011), which enables post-extraction morphological study of specimens, was implemented. We then amplified a 658-bp fragment at the 5' end of the mitochondrial cytochrome *c* oxidase subunit I gene (COI), corresponding to the standard animal barcode region, using the HCO2198 and LCO1490 primers (Folmer et al. 1994). Polymerase chain reaction (PCR) was conducted in a volume of 30 µL, consisting of 9 µL of template DNA, 1.5 µL (10 µM) of each primer, 0.24 µL (25 mM) of dNTP solution (Promega), 6 µL of 10X buffer (Promega) containing 7.5 mM of MgCl<sub>2</sub>, 3 µL (25 mM) of MgCl<sub>2</sub>, 1.5 U of Taq polymerase (Promega), and 8.46 µL of sterile ddH<sub>2</sub>O. Optimised PCR conditions included initial denaturation at 95°C for 5 min, 38 cycles of denaturation at 95°C for 40s, annealing at 50°C for 40s, and extension at 72°C for 40s, with final extension at 72°C for 7 min. Purification and automated sequencing were carried out in Microsynth (Balgach, Switzerland).

In order to provide a comprehensive taxon sampling for the species delimitation, we analysed COI sequences from 62 specimens. We included all obtained sequences (three for *C. volodymyri* and three for *C. luteolum*; Table I) and added sequences from related taxa published in the GenBank database (<https://www.ncbi.nlm.nih.gov/>) as well as unpublished sequences from the platform FREDIE (Freshwater Diversity in Europe: <https://www.fredie.eu/>). In total, 43 sequences of *C. luteolum*, one sequence of *C. victoriae* McDunnough, 1938 and the outgroup sequences of *Cloeon dipterum* (Linnaeus, 1761), *Cloeon simile* Eaton, 1870, *Procloeon pennulatum* (Eaton, 1870) and *P. bifidum* (Bengtsson, 1912) were used for the analysis. Sequences were edited and aligned using Geneious Prime v. 2019.0.4 (Biomatters Ltd.). Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018; Stecher et al. 2020; <http://www.megasoftware.net>). We applied the GTR+G + I model of molecular evolution as it produces the lowest Bayesian information criterion (BIC) scores and is therefore considered to describe the substitution pattern the best.

Tree topology was reconstructed with MEGA X using the maximum likelihood method based on the Tamura–Nei model (Tamura & Nei 1993). The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches (bootstrap with 1000 replicates). For grouping sequences, the Automatic Barcode Gap Discovery (ABGD) method was performed on the webserver (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>; 09.09.2021) using as input the multiple sequence alignment obtained below, default values for the prior intraspecific divergences, relative gap width and distance distribution, and a Jukes–Cantor (JC69) distance matrix. The genetic variability between groups of sequences obtained above was calculated using Kimura two-parameter distances (K2P, Kimura 1980), with MEGA X (<http://www.megasoftware.net>).

## Results and discussion

### Taxonomy

#### *Centroptilum volodymyri* Martynov, Godunko and Palatov, sp. nov.

(Tables I–III; Figures 1, 2, 3 (a,c,e), 4 (a–e), 5, 6 (a,c,e), 7 (a,c,e,g), 8, 9 (a,c,e,g), 10, 11 (a,c,e), 12(a,c,e,g))

<http://www.zoobank.org/urn:lsid:zoobank.org:act:1D2B7557-0DEC-4ACD-8CA3-A6B50FCC4A51>

Table I. Codes and origin of new sequences used in molecular study. For each specimen, the specimen catalogue number (GBIF code), the sample information (country, locality, coordinates and date of sampling), the GenBank accession number of the COI sequence, and nomenclature of genetic sequences (GenSeq; follows Chakrabarty et al. 2013) are provided.

Species	Specimen catalogue number	Country	Locality	Latitude	Longitude	Date	GenBank ID	GenSeq nomenclature
<i>Centropitulum volodymyri</i> sp. nov.	GBIFCH00895431	Georgia	Adjara, Kobulety District, vicinity of Chakhati village, valley of the Kintrishi River, small lentic water bodies [Grg24Censp]	41.762222	41.978111	18 April 2016	OL960099	genseq-2 COI
<i>Centropitulum volodymyri</i> sp. nov.	GBIFCH00895432	Iran	Guilan Province, SW from Amlash town, unnamed small brook	37.036944	50.082500	21 May 2016	OL960097	genseq-3 COI
<i>Centropitulum volodymyri</i> sp. nov.	GBIFCH00895437	Iran	Golestan Province, Kalaleh County, vicinity of Zav-e-Bala village, unnamed stream [Iran3 Censp/1]	37.529433	55.791550	23 June 2019	OL960098	genseq-3 COI
<i>Centropitulum luteolum</i>	GBIFCH00895434	Georgia	Adzharia, territory of Kobulety town, Kintrishi River [Grg6Cenlut]	41.803889	41.775833	4 June 2013	OL960101	genseq-4 COI
<i>Centropitulum luteolum</i>	GBIFCH00895435	Ukraine	Luhansk Region, Antratsyt District, vicinity of Khrustal'nyi village, Khryshaleva River [Lug 94]	48.182321	38.877363	30 April 2012	OL960102	genseq-4 COI
<i>Centropitulum luteolum</i>	GBIFCH00895441	Switzerland	Burtigniere, Vallée de Joux	46.561667	6.170000	23 August 2001	OL960100	genseq-4 COI

*Centroptilum* sp.: Martynov et al. 2016: 170 [distribution in Georgia (Kintrishi River valley), with remark on belonging to undescribed new species]

*Centroptilum* sp.: Bojková et al. 2018: 91, 96, tables 1, 2 [distribution in Iran within Zayanderud (based on Mahboobi Soofiani et al. 2012) and Gilan (two localities listed also in present contribution) provinces]

? *Centroptilum* sp.: Mahboobi Soofiani et al. 2012: 137 [Zayandeh-Roud River in the Zayanderud Province; this record should be verified]

Diagnosis [based on larvae]. Larvae of *C. volodymyri* sp. nov. can be distinguished from the two other Western Palearctic representatives of the genus *Centroptilum*, namely *C. luteolum* and *C. pirinense*, by a combination of the following diagnostic characters: (1) the cuticle with flattened, stretched shallow triangular and semilunar-shaped corrugations, and relatively numerous scales and their bases covering the body surface; (2) labrum expanded laterally, with the U-shaped incurvation on the anterior

margin; (3) apically rounded shape of the segment III of maxillary palps; (4) apically rounded shape of superlinguae of hypopharynx without a projection at the tip; (5) pretarsal claw with more than 60 small teeth in basal half of the claw, these teeth arranged into two rows; (6) the posterior margin of terga I–IV(V) with robust equilateral triangle-shaped spines scattered along the segment (equilateral spines sparse on terga (V)VI–X); (7) tergum VII near gill base with 3–4 relatively prominent posterolateral spines and sometimes several small spines; (8) gills II–VII with distinctly concave posterior margin.

Description. Based on specimens from the type locality only.

*Mature larva.* Body length 9.5–10.2 mm (male larvae slightly smaller than female larvae), length of cerci 5.5–5.8 mm (approx. 0.5× body length); length of paracercus 3.0–3.3 mm.

General body colouration intensively brown/dark brown and blackish; base of fore wing pads, lateral and ventral side of meso- and metathorax darker,



Figure 1. Larva of *Centroptilum volodymyri* sp. nov., Georgia, holotype. (a, b), Body in lateral view. Scale bars: 1 mm.



Figure 2. Larva of *Centroptilum volodymyri* sp. nov., Iran. (a) Body in dorsal view; (b) body in lateral view; (c) head and part of thorax in lateral view. Scale bars: 1 mm.

yellow to brownish black and black. Abdominal segments paler than thorax; legs and cerci lightest (Figure 1).

**Head.** Colour yellowish brown to light brown, with markedly darker area between ocelli, diffused light brown maculation on frons and vertex; clypeus and genae slightly darker, brown. Larval compound eyes brown. Antennae unicolor, light brown, distinctly longer than head and thorax. Frontal suture V-shaped. Head cuticle flattened, without pronounced corrugation. Surface of two first antennal segments and frons covered with sparse *B* and *Hr* setae (Figure 3(a,c,e)); clypeus additionally with two groups of long setae grouped on both sides of body axis.

Labrum expanded laterally, approximately 1.8–2.0× broader than long; median notch flattened;

anterior margin concave, with U-shaped median incurvation relatively deep; anterior margin laterally from medial notch relatively symmetrically rounded. Dorsal surface of labrum covered with dense long and short *Hr* not grouped in rows and sparse *B* (Figures 4(a) and 5(b)); ventral surface with row of submarginal short setae (Figure 5(a)).

Superlinguae of hypopharynx rounded apically, without any projections (Figure 5(c)).

Mandible incisors clearly divided into two groups, deeply separated throughout their length. Left mandibular incisor groups terminated by 4 + 2 stout denticles; left prosthema terminated by a group of slender setae (2–3 setae larger than other ones). Right mandibular incisor groups terminated by 3 + 2 stout denticles; right prosthema is stick-like (Figure 4(c,d)).

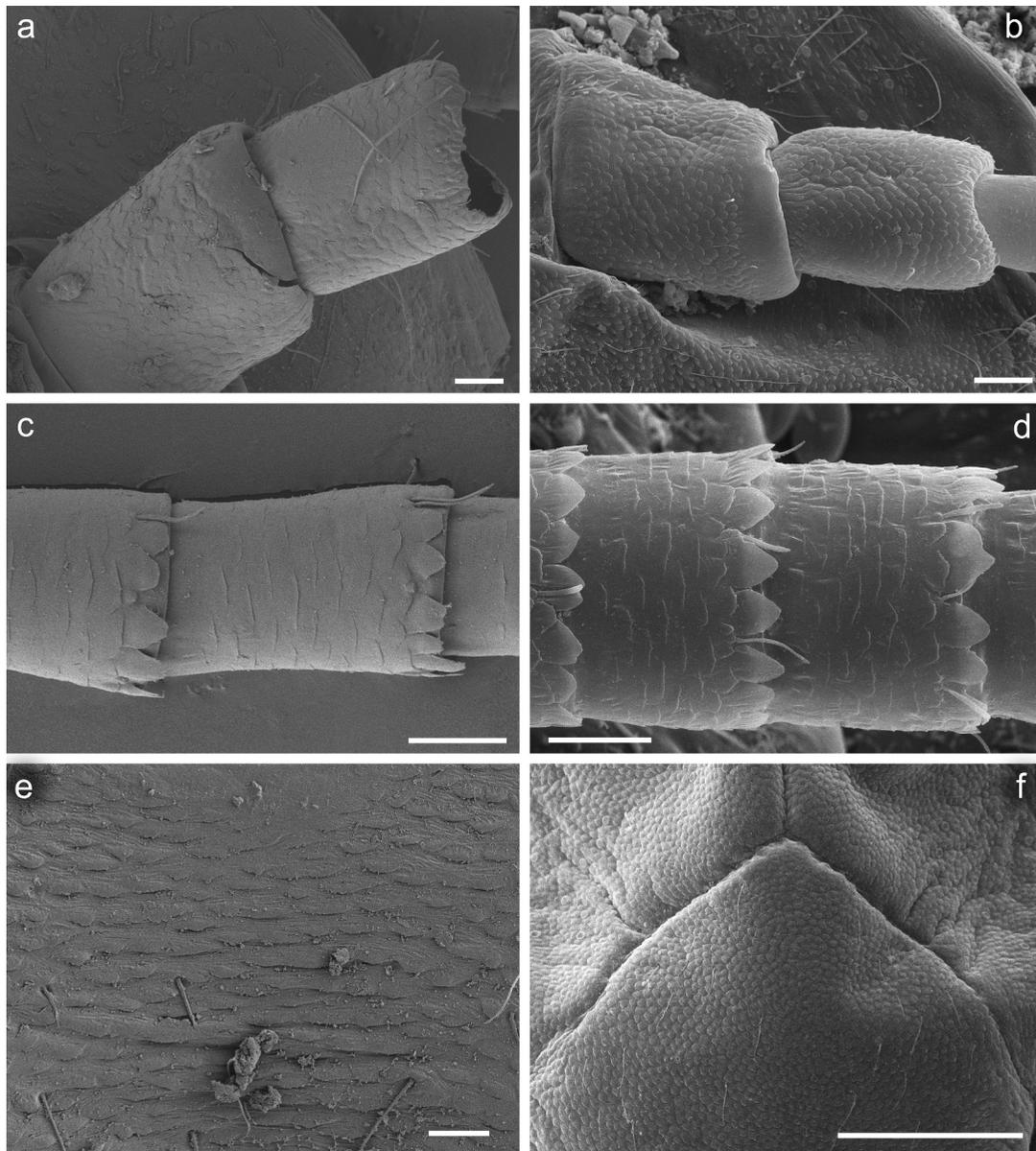


Figure 3. Larvae of *Centropilum volodymyri* sp. nov., paratype (a, c, e) and *C. luteolum* (Müller, 1776) (b, d, f). (a, b) Scapus and pedicellus; (c) flagellum, basal part; (d) flagellum, central part; (e, f) surface of frons. Scale bars: a, c, d = 20  $\mu$ m; b = 30  $\mu$ m; e = 10  $\mu$ m; f = 100  $\mu$ m.

Maxillae with long slender canines and dentisetae; 1st dentisetula is simple, 2nd and 3rd dentisetulae are bifid; dentisetulae not pressed, and well separated from canines. Maxillary palps 3-segmented: segment I distinctly wider than segment II; segment III distinctly longer than segment II, rounded apically, without stout setae apically of surface; surface of maxillary palps covered with sparse *Hr* and *FT* (Figures 4(b,e,f) and 5(f)).

Labial palps 3-segmented. Segment III distinctly expanding apically, nearly trapezoidal with rounded

outer angles; inner margin distinctly concave apically; posterolateral and posteromedian corners of different shape; stout setae along of apical margin and numerous *Hr* scattered on surface. Segment II distinctly wider than base of segment III; ventral side of segments I and II with scattered *Hr*; dorsal side of segment II with 6–10 elongated setae near to apicointernal margin (Figure 5(g,f)).

Glossae nearly as broad as paraglossae (Figure 5(d,e)). Paraglossae slightly longer; dorsal surface with single row of elongated stout setae along inner

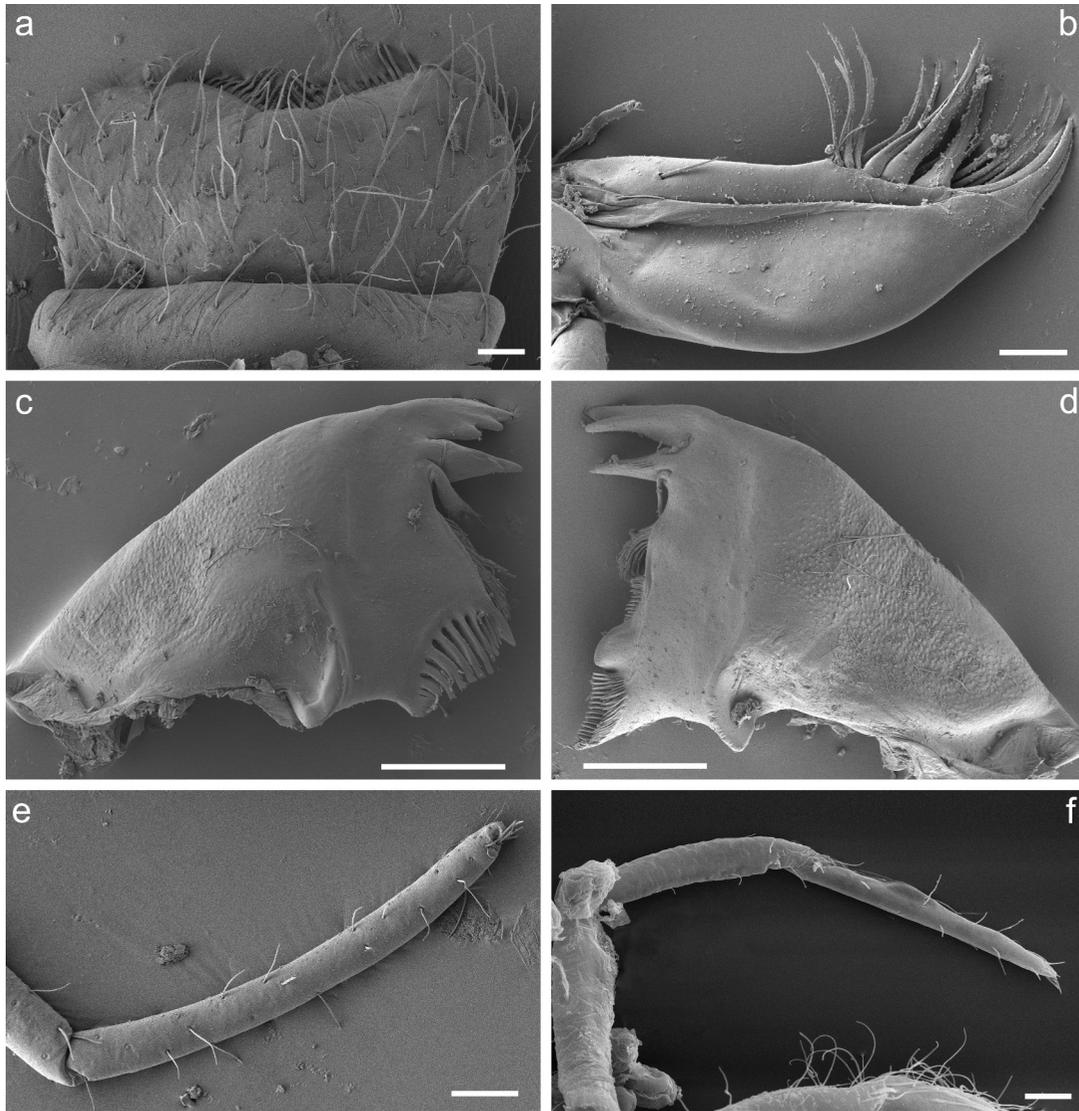


Figure 4. Larvae of *Centroptilum volodymyri* sp. nov., paratype (a–e) and *C. luteolum* (Müller, 1776) (f). (a) Labrum; (b) galea-lacinia; (c, d) mandibles, left (c) and right (d); (e, f) maxillary palp. Scale bars: a, b, e, f = 30  $\mu$ m; c, d = 100  $\mu$ m.

margin; ventral surface with fine long setae scattered over entire surface (apical setae shorter). Glossae with irregular row of submarginal short stout setae along outer and inner margins; denser setation shaped by fine long setae on dorsal side. Basal part of glossae and paraglossae with numerous fine long setae.

**Thorax.** Pronotum narrow, approximately 3.0–3.5 $\times$  longer than broad. Pronotum with two yellowish to light brown maculae centrally; two spreading maculae of same colour on darker background laterally [yellow to yellowish brown, with occasional dirty yellow diffuse spots laterally in specimens from Iran]. Mesonotum with elongated brown strips on light brown background; coxae intensively brown to

black brown [slightly paler in specimens from Iran]. Metanotum with spreading yellowish spot centrally, yellowish brown to dark brown laterally. Thoracic pleura slightly darker than terga (Figure 1(a,b)).

Hind wing pads well developed. Thorax cuticle formed by flattened, stretched shallow triangular and semilunar-shaped corrugations. Surface of pronotum covered with *Hr*, *B*, micropores [*chloride cells*], sparse *SC-et* [6–8  $\mu$ m in length] and *SCS-et* (Figure 6(a)).

Legs relatively pale, whitish yellow to dirty brown [paler in specimens from Iran]; all leg segments slightly darker distally (Figure 1(a,b)). Legs slender; femora parallel-sided; patella-tibial suture developed on all legs (Figure 8(a–c)). Fore leg coxae pale,

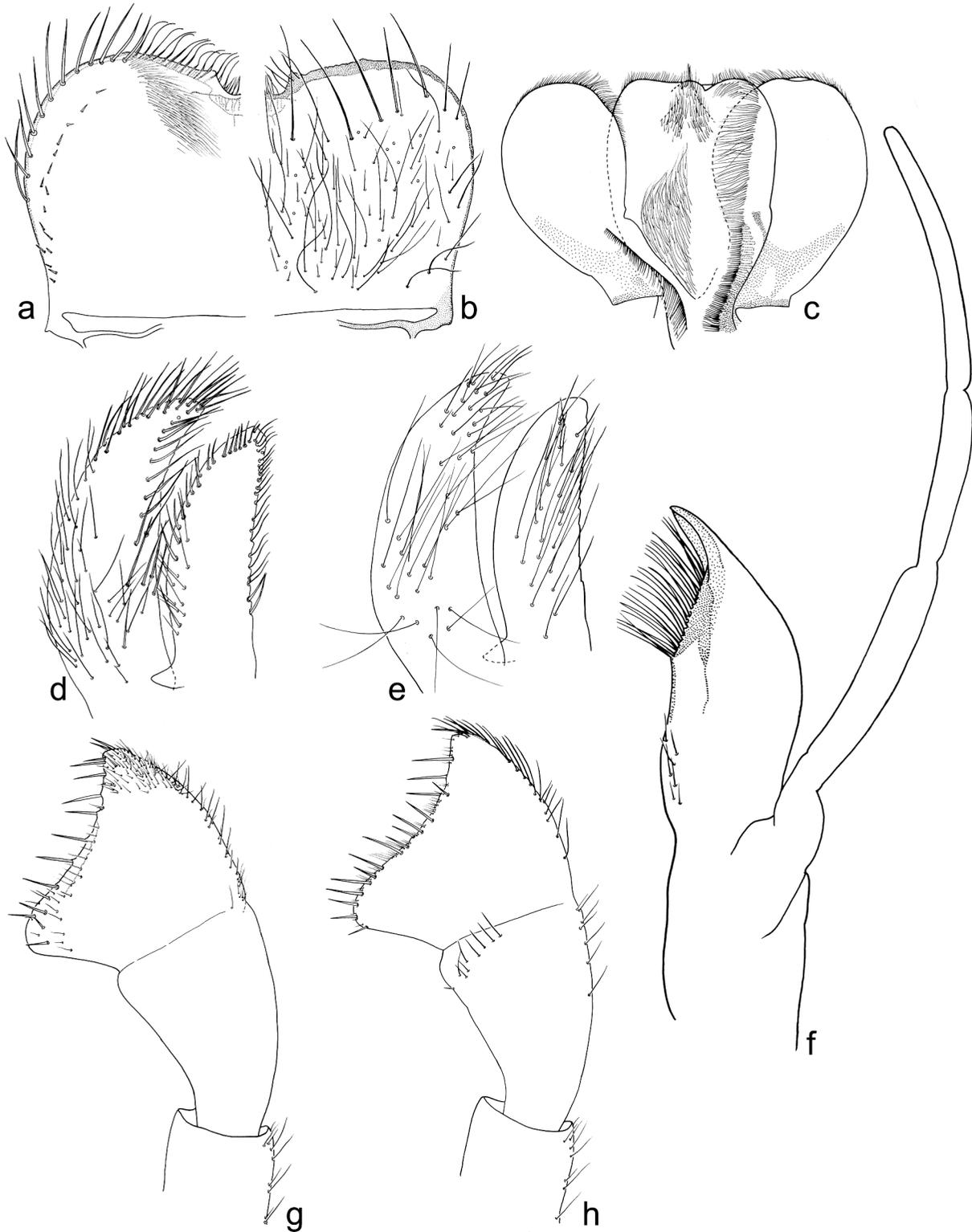


Figure 5. Larvae of *Centropitulum volodymyri* sp. nov., paratypes. (a, b) Labrum, ventral (a) and dorsal (b) view; (c) hypopharynx (left side – dorsal view; right side – ventral view); (d, e) glossa and paraglossa, dorsal (d) and ventral (e) view; (f) maxilla; (g, h) labial palp, ventral (g) and dorsal (h) view.

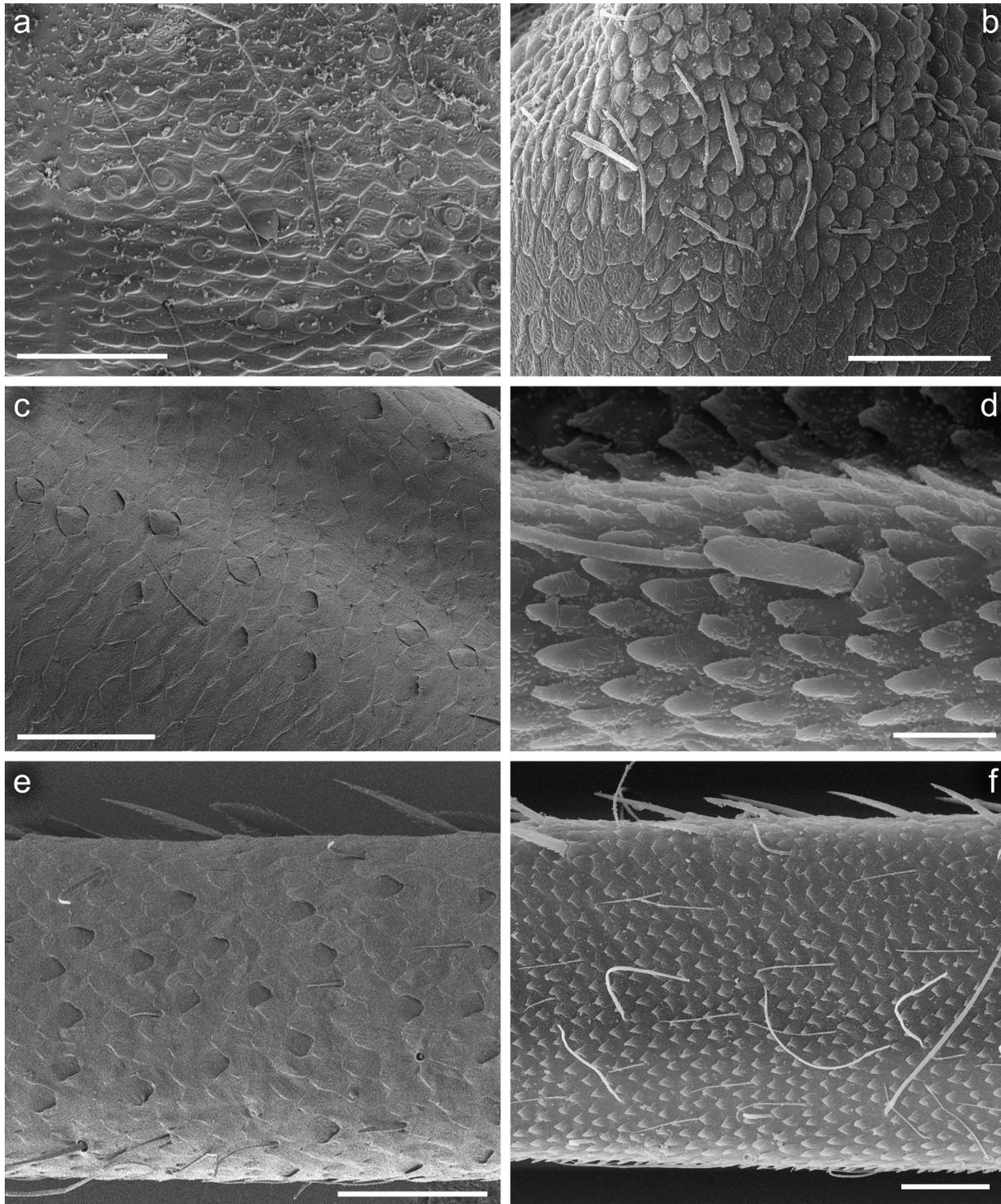


Figure 6. Larvae of *Centroptilum volodymyri* sp. nov., paratype (a, c, e) and *C. luteolum* (Müller, 1776) (b, d, f). (a, b) Pronotum, dorsal surface; (c, d) hind femur, dorsal surface; (e, f) hind tibia, dorsal surface. Scale bars: a–c, e, f = 30 µm; d = 10 µm.

whitish yellow to intensively brown; coxae of middle and hind legs darker, intensively brown to dark brown. Trochanters with diffused brownish spot. Brownish elongated diffused macula along outer

margin of all femora; tibiae yellowish to light brown; tarsi of the same colour as tibiae, occasionally slightly darker distally; claws yellowish brown. Cuticle of the legs formed by flattened equilateral

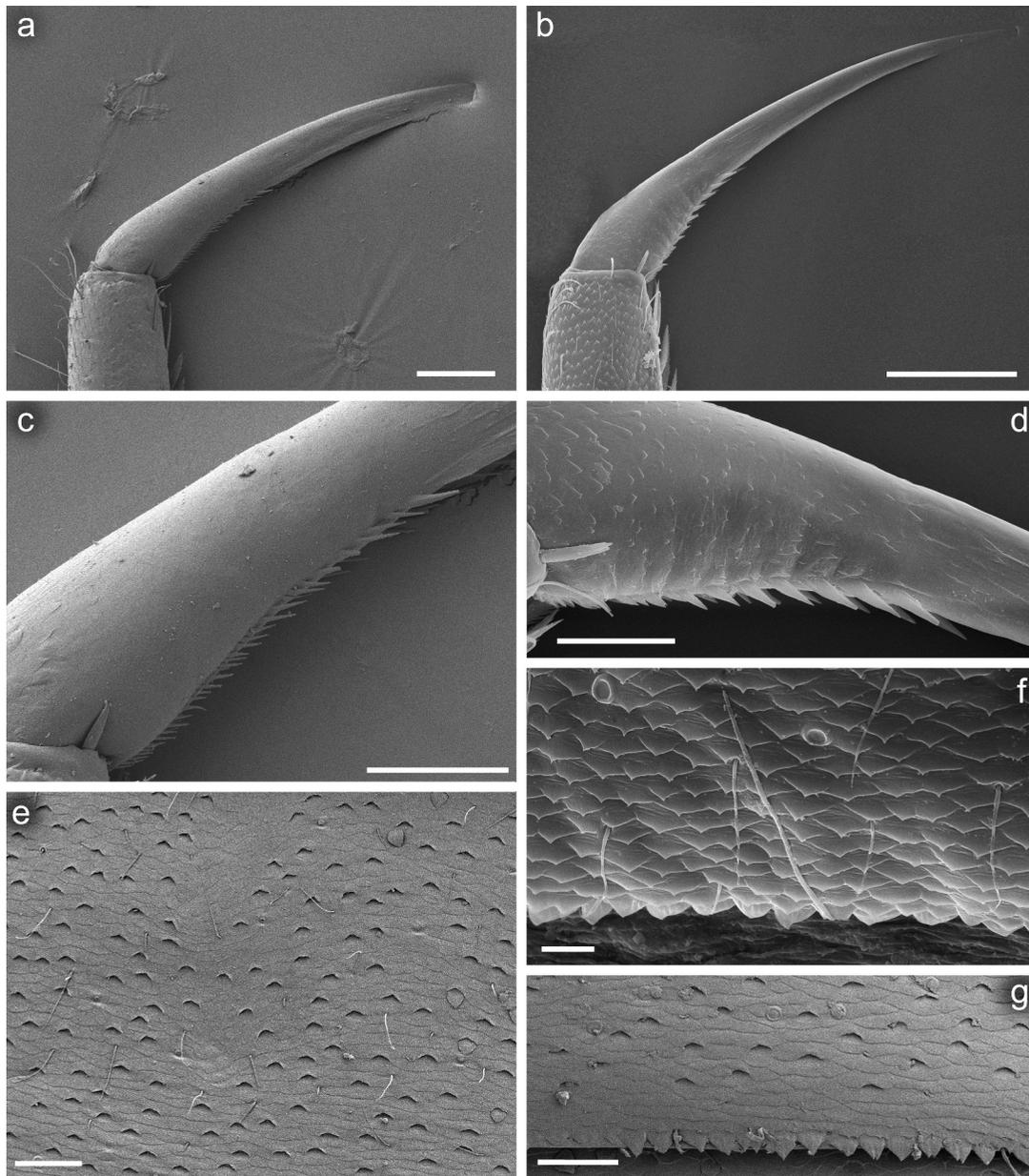


Figure 7. Larvae of *Centropitulum volodymyri* sp. nov., paratype (a, c, e, g) and *C. luteolum* (Müller, 1776) (b, d, f). a, b = hind pretarsal claw (apex of claw not visible in (a)); (c, d) denticles of hind pretarsal claw; (e) surface of sternum IV; (f) surface and posterior margin of sternum VI; (g) surface and posterior margin of sternum VII. Scale bars: a = 50  $\mu\text{m}$ ; b = 100  $\mu\text{m}$ ; c–e, g = 30  $\mu\text{m}$ ; f = 10  $\mu\text{m}$ .

triangle- and semilunar-shaped corrugations. Surface of femora with *SC-et*, *SCS-et*, *Hr*, *B* setae; the same types of scales and setae, with dominance of *SCS-et* distributed of surface of tibiae and tarsi (Figure 6(c,e)). Numerous short, stout, pointed setae along outer margin of femora; numerous longer pointed setae along inner margin of tibiae and tarsi; outer margin of tibiae and tarsi covered with *Hr* and occasional *B* setae. Pretarsal claw length less than or equal to 1/2 of tarsus length (Figure 8(a–c)); more than 60 small teeth arranged

into two rows distributed in basal half to 2/3 of claw length (Figure 7(a,c)).

**Abdomen.** Terga dark, yellowish brown to intensively brown (Figure 1(a,b)); terga I with two broad spots centrally, and two smaller spots anterolaterally. Terga II–V with uniform colour pattern, viz. a small pale spot centrally near anterior margin of segment; large brownish diffused spot centrally, with paler maculae near posterior margin of segment; additionally, yellowish-brown to light brown elongated strips surrounding pale maculae. Tergum VI

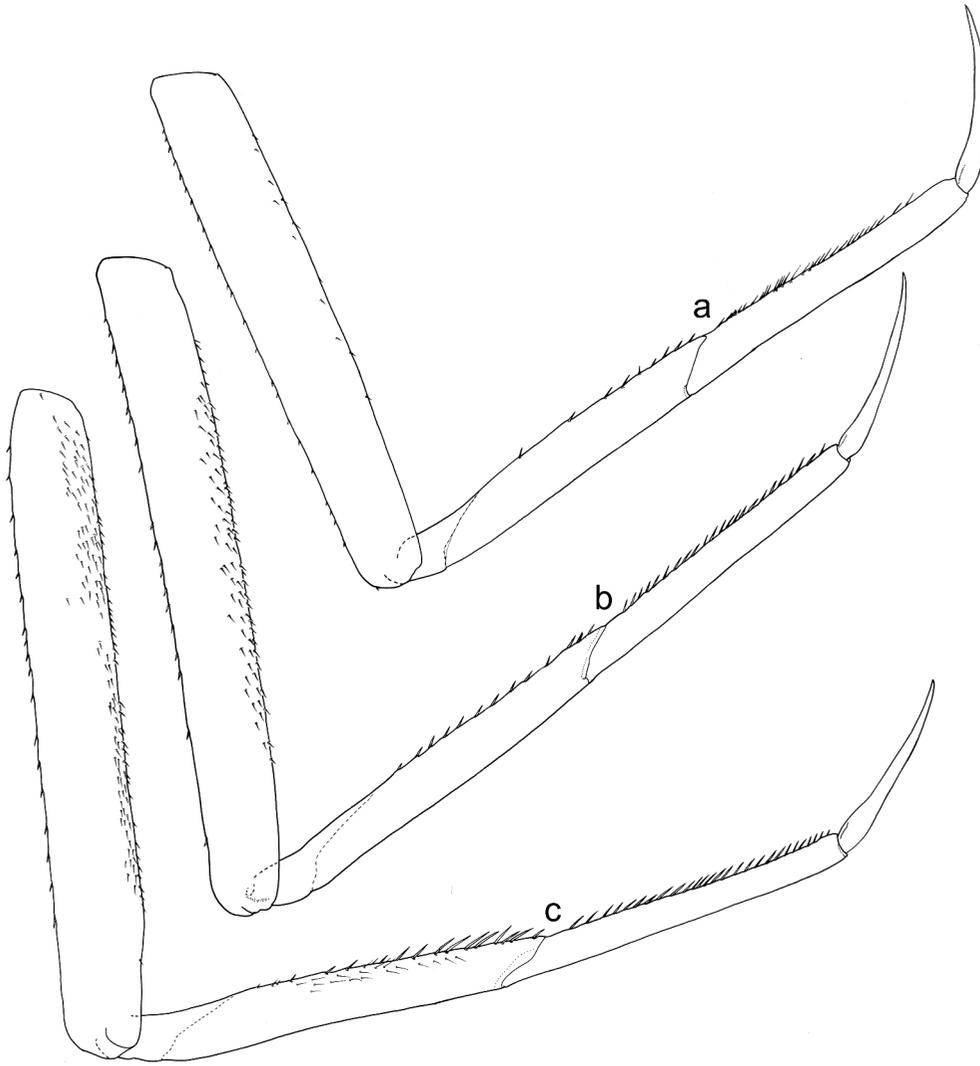


Figure 8. Larvae of *Centroptilum volodymyri* sp. nov., paratypes. (a) Fore leg; (b) middle leg; (c) hind leg.

darkest, with colour pattern similar to those on previous segments, except of more diffused light spot anteriorly, and presence of two pale dots and two oblique strokes centrally. Terga VII–VIII with colour pattern similar to those on terga II–V (occasionally without small pale spot anteriorly), or uniformly light brown. Tergum IX uniformly light brown to brown without conspicuous pattern. Tergum X pale, yellow to light brown, with light transversal macula anteriorly, two unclear maculae centrally near anterior margin of segment and two brown small spots laterally. Anterolateral area of terga I–IX pale, yellowish brown.

Abdominal sterna predominantly uniformly coloured, whitish yellow to light brown, without distinct pattern. Occasionally sterna with contrasting pattern: sternum I whitish yellow, with dark

brown spots anterolaterally; sterna II–V (VI) with diffused triangular brownish spot on yellowish background; sterna VII–VIII darker than previous segments, light brown to brown, with longitudinal pale band and two yellowish spots centrally; sterna VIII–IX darkest, intensively brown, with diffused paler maculation centrally and laterally; sternum X slightly paler than two previous segments, light brown.

Surface of terga with cuticle formed by flattened equilateral triangle- and semilunar-shaped corrugations; terga I–IV covered with micropores, *Hr* and *B*, dominant *SC-et* and more sparse *SCS-et*; terga V–X with the same type of micropores, *Hr* and *B*, sparse *SC-et* and dominant *SCS-et* (Figure 9(a,c,e,g)). Posterior margin of terga I–IV (V) with robust equilateral triangle-shaped

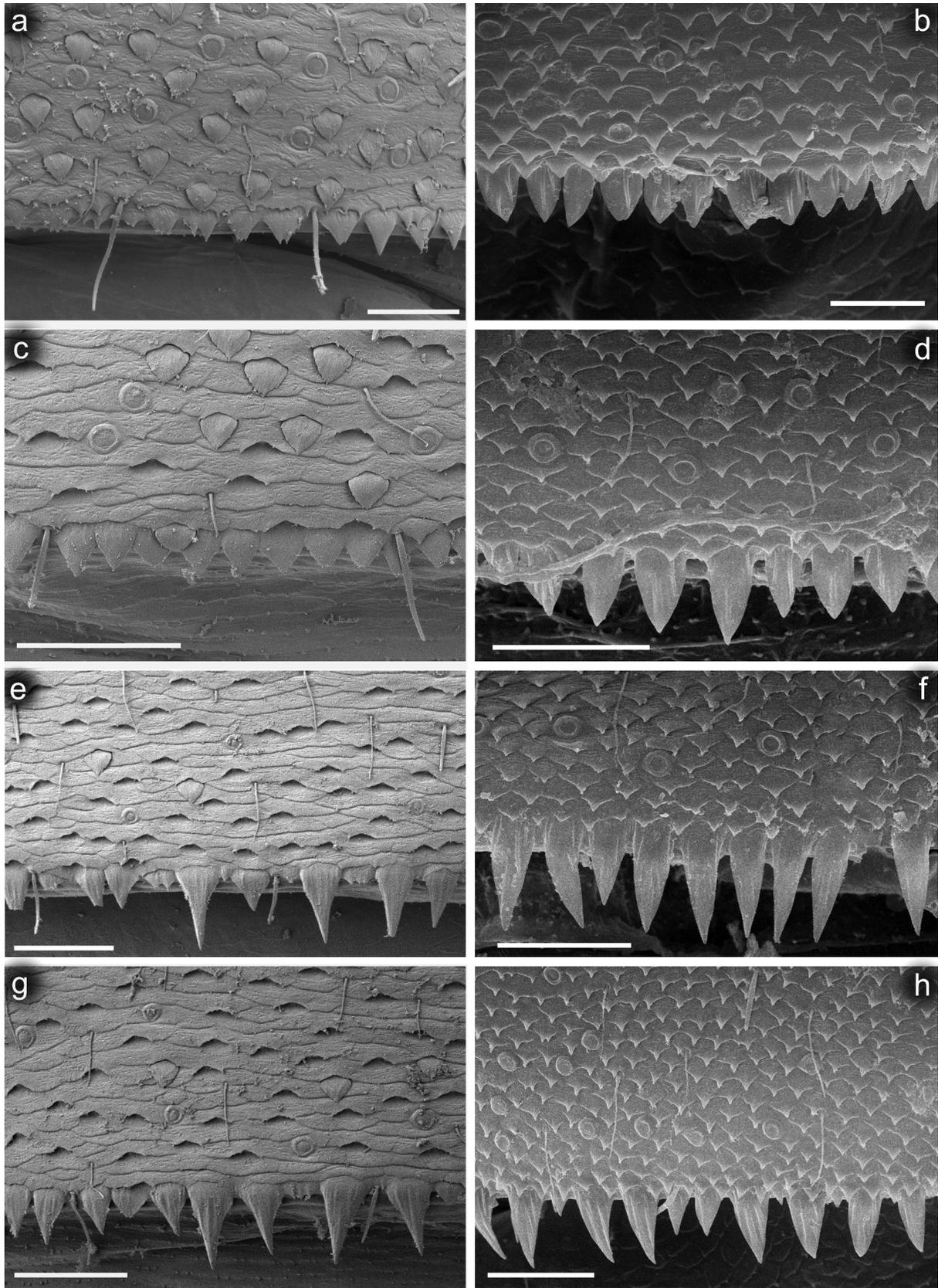


Figure 9. Larvae of *Centroptilum volodymyri* sp. nov., paratype (a, c, e, g) and *C. luteolum* (Müller, 1776) (b, d, f, h). Surfaces and posterior margins of terga: (a, b) tergum II; (c, d) tergum IV; (e, f) tergum VI; (g, h) tergum VIII. Scale bars: a, b = 20  $\mu$ m; c–h = 30  $\mu$ m.

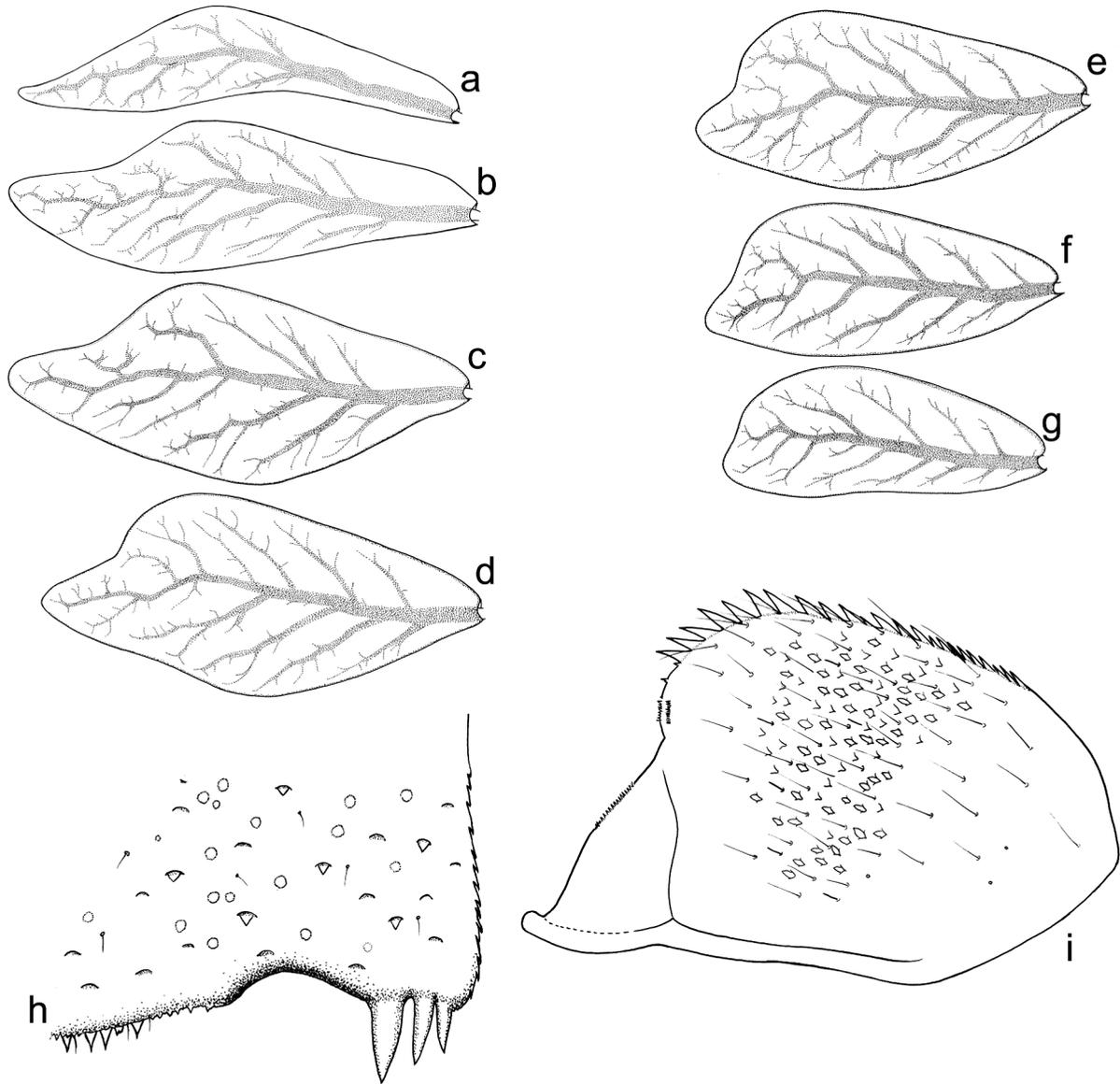


Figure 10. Larvae of *Centropilum volodymyri* sp. nov., paratypes. (a) Gill I; (b) gill II; (c) gill III; (d) gill IV; (e) gill V; (f) gill VI; (g) gill VII; (h) posterolateral part of tergum VII; (i) paraprost plate.

spines, alternating with sparse shorter ones and *B* setae; posterior margin of terga VI–X (occasionally tergum V) with thin and robust isosceles and equilateral triangle-shaped spines, alternating with shorter ones and sparse *B* setae. Tergum VII near gill bases with 3–4 relatively prominent posterolateral spines, and sometimes with several additional small spines; terga VIII–IX with 2–4 relatively prominent posterolateral spines, and sometimes with several additional small spines (Figure 10(h)).

Cuticle of sterna shaped by flattened undulating corrugations covered with micropores, elongated *Hr* and *B* setae, sparse *SC-et* and dominant *SCS-et* (Figure 7(e,g)). Posterior margin of sterna with robust equilateral triangle-shaped spines alternating with occasional short *B* setae.

Gills whitish yellow, with well visible tracheisation, light brown to intensively brown coloured. All gills asymmetrical, rounded apically; gill I distinctly sinuous, narrow, lanceolate; gills II–VII with posterior margin distinctly concave (Figure 10(a–g)).

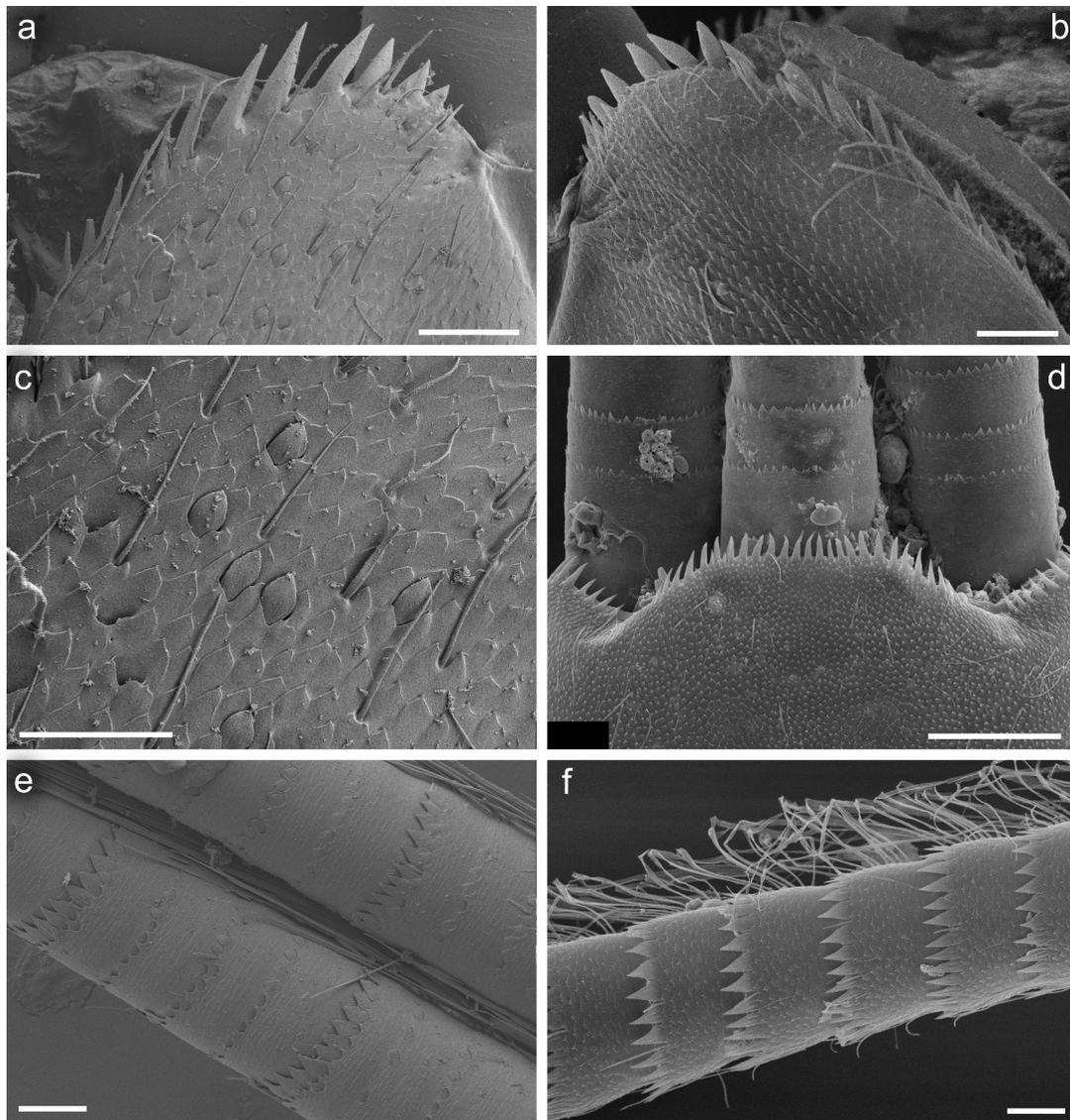


Figure 11. Larvae of *Centroptilum volodymyri* sp. nov., paratype (a, c, e) and *C. luteolum* (Müller, 1776) (b, d, f). (a, b) Apical part of paraproct; (c) surface of paraproct; (d) tergum X and caudal filaments; (e) caudal filaments, basal part; (f) caudal filament, medial part. Scale bars: a, b, e, f = 30  $\mu\text{m}$ ; c = 20  $\mu\text{m}$ ; d = 100  $\mu\text{m}$ .

Paraproct plate with 13–15 pointed strong teeth along inner margin; ventral surface of paraproct plate covered with leaf-shaped SC and their SCS grouped mainly centrally, elongated *Hr* and *B* setae (Figures 10(i) and 11(a,c)).

Cerci and paracercus slightly paler than body, light brown, without coloured rings. Posterior margin of caudal filament segments with combination of robust marginal spines, occasional *Hr* and *B* setae; submarginal area with solitary *SC-et* (Figure 11(e)). Cerci (inner margin) and paracercus (both margins) up to the apex bear well-developed primary swimming

setae, except last one or two segments. Outer margin of cerci with relatively short *Hr* and *B* setae.

*Egg (taken from larva)*. Oval; 130–140  $\mu\text{m}$  long, 65–70  $\mu\text{m}$  wide. Chorionic surface shaped by thin, flat reticulated ridges forming irregular polygonal mesh; cells (2.5–4.2  $\mu\text{m}$  in diameter) of this mesh mostly with rounded margins; no knob-terminated coiled thread inside of chorionic cells (Figure 12(a,c,e,g)). One, occasionally two oval micropyles in equatorial area, 5.5–5.8  $\mu\text{m}$  long and 4.8–5.0  $\mu\text{m}$  wide; no micropylar rim surrounding micropyle.

Imago and subimago. Unknown.

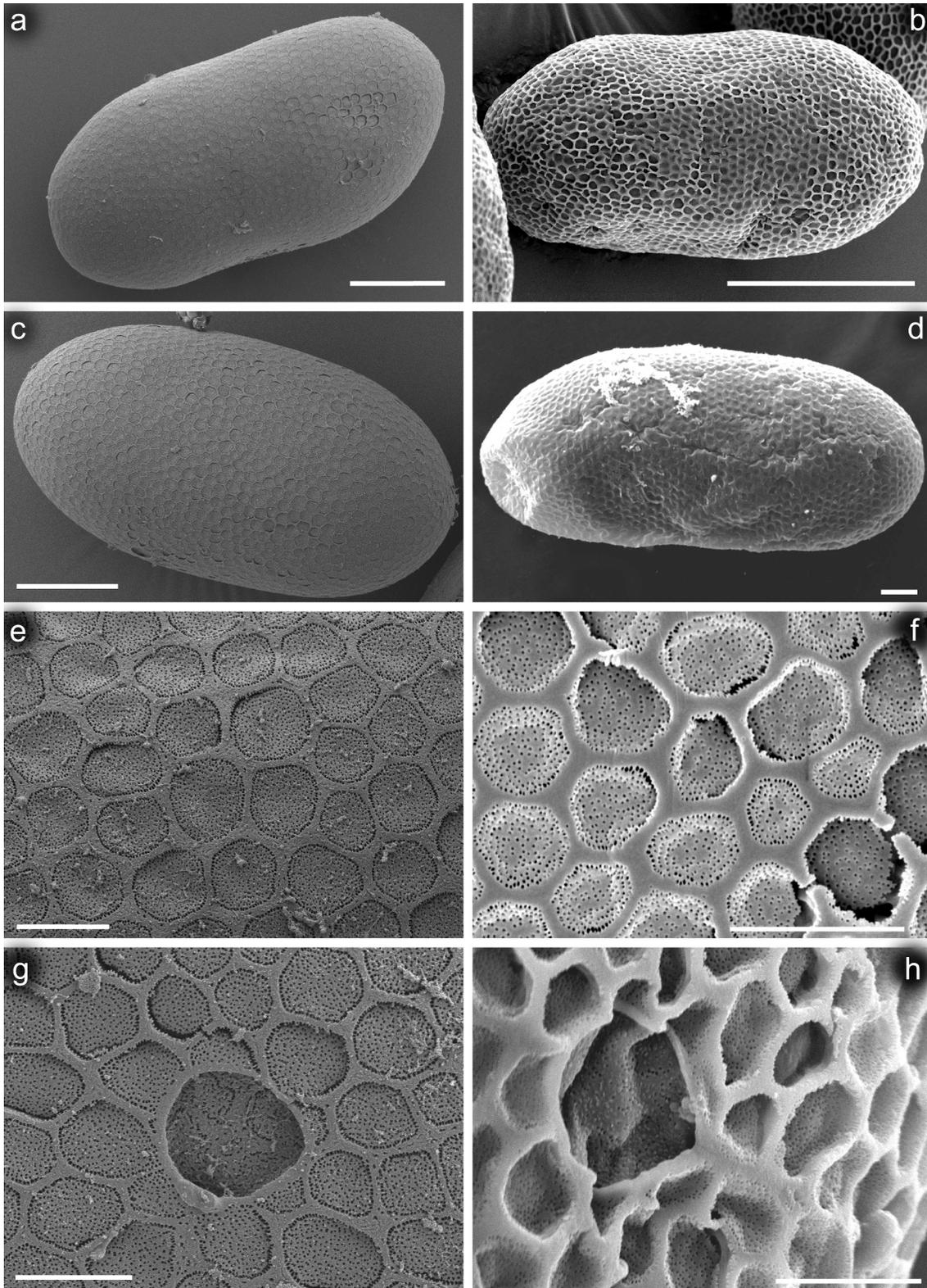


Figure 12. Eggs of *Centropitulum volodymyri* sp. nov., from paratype (a, c, e, g) and *C. luteolum* (Müller, 1776) (b, d, f, h); provided by Nicolás Ubero-Pascal). (a–d) General view of egg; (e, f) chorionic surface; (g, h) micropyle. Scale bars: a, c = 30 µm; b = 80 µm; d = 20 µm; e, g = 5 µm; f = 10 µm; h = 5 µm.

Table II. Morphological characters of larvae that distinguish *Centroptilum volodymyri* sp. nov. from *C. luteolum* (Müller, 1776) and *C. pirinense* Ikonomov, 1962. Distinct differential characters are in bold.

Characters [remarks; No. references; figures]	<i>Centroptilum volodymyri</i> sp. nov. [type material]	<i>Centroptilum luteolum</i> (Müller, 1776) [Europe, Middle Asia, Caucasus]	<i>Centroptilum pirinense</i> (Ikonomov, 1962) <sup>[1]</sup> [Macedonia, Bulgaria]
<b>Larva</b>			
<i>I. Head (colour, shape and setation)</i>			
1 Colour pattern Figures 1(a,b) and 2 (a-c)	Yellowish-brown to light brown; diffused light brown maculation on frons and vertex; clypeus and genae brown	Light brown; clypeus and genae brown	Light brown; diffused pale maculation
2 <b>Antenna length</b> <sup>[2]</sup> Figures 1(a,b) and 2 (a,b)	Distinctly longer than head and thorax	Slightly longer than head and thorax	Reaching approximately the bases of fore wings or longer than head and thorax
3 Surface of scape and pedicel: setation Figure 3(a,b)	Sparse <i>Hr</i> and <i>B</i>	Sparse <i>Hr</i> and <i>B</i>	–
4 <b>Posterior margin of flagellar segment (central part of flagellum): shape</b> Figure 3(c,d)	Sparse row of 10–14 well separated, robust, bluntly pointed or rounded apically spines 1 or grouped by 2 <i>B</i> setae and sparse <i>Hr</i>	Dense row of 16–20 robust, bluntly pointed or rounded apically spines, grouped by 2–3 spines with common basis; 1 or grouped by 2–3 <i>B</i> setae	–
5 Surface of clypeus and frons: setation Figure 3(e,f)	Sparse <i>B</i> and long <i>Hr</i>	Sparse <i>B</i> and long <i>Hr</i>	–
6 Frontal suture: shape <sup>[3]</sup> Figure 3(f)	V-shaped	V-shaped to U-shaped	–
<i>II. Mouthparts</i>			
1 <b>Labrum: width/ length ratio</b> <sup>[4]</sup>	1.8–2.0	1.4–1.6	approx. 2.0
2 Labrum: dorsal surface Figures 4(a) and 5(b)	Dense short and long <i>Hr</i> ; sparse <i>B</i>	Dense short and long <i>Hr</i> ; sparse <i>B</i>	Dense short and long <i>Hr</i> ; sparse <i>B</i>
3 <b>Labrum: anterior margin</b> Figures 4(a) and 5(a, b)	Slightly concave; median incurvation relatively deep, angular, U-shaped	Nearly straight; median incurvation shallow, angular	Nearly straight; small medial semicircular incurvation
4 Ratio of width of maxillary palp segments I/II <sup>[5]</sup> Figures 4(f) and 5(f)	1.3–1.6	1.2–1.8	1.2
5 <b>Segment III of maxillary palp; ratio length segments III/II</b> Figures 4(e,f) and 5(f)	Rounded apically; distinctly longer than segment II – 1.6–1.7	Pointed or bluntly pointed apically; subequal to distinctly longer than segment II – 1.1–1.7	Pointed apically; subequal to segment II – 1.1
6 Right mandible: number of incisors of mandibular incisor groups <sup>[6]</sup> Figure 4(d)	3 + 2	3 + 2	3 + 2
7 <b>Left mandible: number of incisors of mandibular incisor groups</b> <sup>[6]</sup> Figure 4(c)	4 + 2	4 + 2 (occasionally 4 + 3)	4 + 3
8 Mandibles: surface Figure 4(c,d)	Occasionally sparse <i>B</i> and <i>Hr</i>	No setae; occasionally sparse <i>B</i> and long <i>Hr</i> , small prominent isosceles and equilateral triangle-shaped corrugations, and micropores	Occasionally sparse <i>B</i> and <i>Hr</i>

(Continued)

Table II. (Continued).

Characters [remarks; references; figures]	<i>Centroptilum volodymyri</i> sp. nov. [type material]	<i>Centroptilum luteolum</i> (Müller, 1776) [Europe, Middle Asia, Caucasus]	<i>Centroptilum pirinense</i> (Ikononov, 1962) <sup>[1]</sup> [Macedonia, Bulgaria]
9 <b>Hypopharynx: shape of superlinguae</b> <sup>[7]</sup> Figure 5(c)	Rounded; no triangular apicomedial projection	Rounded; small triangular bluntly pointed apicomedial projection	Rounded; no triangular projection
10 <b>Segment II of labial palp [dorsal view]: setation</b> Figure 5(h)	6–10	2–9	6–7
11 <b>Segment II of labial palp: distal end</b> Figures 5(g,h)	Distinctly wider than segment III base	Equal width or slightly wider than segment III base	Equal length or slightly wider than segment III base
12 <b>Segment III of labial palp: shape</b> <sup>[8]</sup> Figures 5(g,h)	Distinctly expanding apically; nearly trapezoidal; inner margin concave; posterolateral and posteromedian corners of different shape	Moderately expanding apically; roughly quadrangular; inner apical margin slightly concave; posterolateral and posteromedian corners of different shape	Slightly expanding apically; roughly quadrangular; inner margin slightly concave; posterolateral and posteromedian corners of very similar shape
<i>III. Thorax and legs (colour, shape and setation)</i>			
1 <b>Colour pattern: pronotum</b> <sup>[9]</sup> Figures 1(a,b) and 2(a,b)	Dark brown to blackish brown maculation centrally; pale diffused spots laterally	Light yellowish brown; brownish maculation centrally and laterally	Light brown; two pairs of large dissolved spots centrally and laterally
2 <b>Surface of pronotum: cuticle structure</b> Figure 6(a,b)	Flattened, stretched shallow triangular and semilunar-shaped corrugations	Prominent rounded and broadly triangular corrugations	–
3 <b>Surface of pronotum: setation</b> Figure 6(a,b)	<i>Hr</i> , <i>B</i> , micropores, sparse <i>SC-et</i> and <i>SCS-et</i>	Sparse <i>Hr</i> and <i>B</i>	–
4 <b>Colour pattern: legs</b> Figures 1(a,b) and 2(b)	Yellowish to dirty brown; all leg segments slightly darker distally	Femora with brown transversal band distally; tibiae darkened proximally	Unicolour light brown
5 <b>Surface of legs: cuticle structure</b> Figure 6(c–f)	Flattened equilateral triangle- and semilunar-shaped corrugations	Prominent isosceles and equilateral triangle-shaped corrugations	–
6 <b>Outer margin of femora: stout setae</b> Figure 8(a–c)	Numerous short, pointed	Occasional short, pointed	Short, pointed setae
7 <b>Surface of femora [dorsal view]: setation</b> Figure 6(c,d)	<i>SC-et</i> , <i>SCS-et</i> , <i>Hr</i> , <i>B</i>	<i>Hr</i> , <i>B</i> , pointed stout setae with feathered margins mainly near inner margin [sparse]	–
8 <b>Surface of tibiae [dorsal view]: setation</b> Figure 6(e,f)	<i>SC-et</i> , <i>SCS-et</i> [dominant], <i>Hr</i> , <i>B</i>	Long <i>Hr</i> , <i>B</i>	–
9 <b>Surface of tarsi [dorsal view]: setation</b>	<i>SC-et</i> , <i>SCS-et</i> [dominant], <i>Hr</i> , <i>B</i>	<i>Hr</i> , <i>B</i> , pointed stout setae with feathered margins mainly near inner margin [sparse]	–
10 Pretarsal claw length relative to tarsus length <sup>[10]</sup> Figures 7(a,b) and 8(a–c)	≤ 1/2	≈ 1/2	1/2–2/3
11 <b>Pretarsal claw: number of small teeth per row</b> Figure 7(a–d)	More than 30 in basal half to 2/3 of claw length	About 18–27 in basal third	About 15–20 in basal half [13–16 in original description]
<i>IV. Abdomen (colour, shape and setation)</i>			

(Continued)

Table II. (Continued).

No.	Characters [remarks; references; figures]	<i>Centropitulum volodymyri</i> sp. nov. [type material]	<i>Centropitulum luteolum</i> (Müller, 1776) [Europe, Middle Asia, Caucasus]	<i>Centropitulum pirinense</i> (Ikononov, 1962) <sup>[1]</sup> [Macedonia, Bulgaria]
1	<b>Colour pattern: terga II–VIII (IX)</b> Figures 1(a,b) and 2(a,b)	Terga dark, yellowish brown to intensively brown; terga II–VIII often with more or less distinct colour pattern consisting of pale spots, maculae and strips centrally, and darker spots laterally	Terga pale, ivory-white, usually with a pair of small, dark brown spots in the middle; tergum VI usually darkest; terga II and VI often with dark spot centrally on paler background	Terga unicoloured, brownish, with indistinct pale diffused area and two unclear spots centrally; clearer colour pattern on terga VII–IX (occasionally VI)
2	<b>Surface of terga: cuticle structure</b> <sup>[11]</sup> Figure 9(a–h)	Flattened equilateral triangle- and semilunar-shaped corrugations	Prominent isosceles and equilateral triangle-shaped corrugations	Prominent isosceles and equilateral triangle-shaped corrugations
3	<b>Surface of terga I–IV: setation</b> <sup>[12]</sup> Figure 9(a–d)	<i>SC-et</i> [dominant], <i>SCS-et</i> [sparse], micropores, <i>Hr</i> and <i>B</i>	Micropores, <i>Hr</i> and <i>B</i>	<i>SC-et</i>
4	<b>Surface of terga V–X: setation</b> <sup>[12]</sup> Figure 9(e–h)	<i>SC-et</i> [sparse], <i>SCS-et</i> [dominant], micropores, <i>Hr</i> and <i>B</i>	Micropores, <i>Hr</i> and <i>B</i>	<i>SC-et</i>
5	<b>Posterior margin of terga I–IV (V)</b> <sup>[12]</sup> Figure 9(a–d)	Robust equilateral triangle-shaped spines, <i>B</i> [occasionally]	Robust isosceles triangle-shaped spines, <i>B</i> [occasionally]	Long and pointed spines alternating with shorter ones
6	<b>Posterior margin of terga (V) VI–X</b> <sup>[12]</sup> Figures 9(e–h) and 11(d)	Thin and robust isosceles and equilateral triangle-shaped spines of different length, <i>B</i> [occasionally]	Slim isosceles triangle-shaped spines of different length, <i>B</i> [occasionally]	Long and pointed spines of different length
7	<b>Posterolateral part of terga VII/VIII–IX</b> <sup>[13]</sup> Figure 10(h)	3–4 relatively prominent spines and several small spines sometimes/2–4 relatively prominent spines and several small spines sometimes	0–6 small spines/3–13 small spines	0/–
8	<b>Surface of sterna: cuticle structure</b> Figure 7(e–g)	Flattened undulating corrugations	Flattened equilateral triangle-shaped corrugations	–
9	<b>Surface of sterna: setation</b> Figure 7(e–g)	<i>SC-et</i> [sparse], <i>SCS-et</i> [dominant], micropores, elongated <i>Hr</i> and <i>B</i>	Micropores, elongated <i>Hr</i> and <i>B</i>	–
10	Posterior margin of sterna: setation Figure 7(f,g)	Robust equilateral triangle-shaped spines, <i>B</i> [sparse]	Robust equilateral triangle-shaped spines, <i>B</i> [sparse]	–
11	<b>Gill I: shape</b> Figure 10(a)	Asymmetrical, distinctly sinuous, narrow, lanceolate	Asymmetrical, distinctly sinuous, narrow, lanceolate	Slightly asymmetrical, nearly straight, narrow, lanceolate
12	<b>Gills II–VI: shape</b> Figure 10(b–f)	Asymmetrical, rounded apically, posterior margin distinctly concave	Nearly symmetrical, leaf-shaped, acutely pointed apically	Nearly symmetrical, leaf-shaped, pointed or bluntly pointed apically
13	<b>Gill VII: shape</b> Figure 10(g)	Asymmetrical, rounded apically, posterior margin distinctly concave	Symmetrical, leaf-shaped, acutely pointed apically	Nearly symmetrical, leaf-shaped, rounded apically
14	<b>Paraproct [inner margin]: stout spines</b> <sup>[14]</sup> Figures 10(i) and 11(a,b)	13–15 pointed strong teeth	More than 17–23 pointed, strong teeth, alternating with smaller ones	Approximately 17 pointed teeth of different lengths
15	<b>Surface of paraproct: setation</b> Figures 10(i) and 11(a–c)	Leaf-shaped <i>SC</i> and their <i>SCS</i> , elongated <i>Hr</i> , <i>B</i>	Elongated <i>Hr</i> , <i>B</i>	–
16	<b>Colour pattern: cerci and paracercus</b> Figures 1(a) and 2(b)	Slightly paler than body, unicoloured light brown; no rings	Slightly paler than body, darker distally; ringed by narrow dark reddish-brown rings	Paler than body, unicoloured brown to dark brown; no rings

(Continued)

Table II. (Continued).

Characters [remarks; No. references; figures]	<i>Centroptilum volodymyri</i> sp. nov. [type material]	<i>Centroptilum luteolum</i> (Müller, 1776) [Europe, Middle Asia, Caucasus]	<i>Centroptilum pirinense</i> (Ikonomov, 1962) <sup>[1]</sup> [Macedonia, Bulgaria]
17 <b>Posterior margin of caudal filament segments: shape</b> Figure 11(e,f)	Robust marginal spines; occasional <i>Hr</i> and <i>B</i> , solitary submarginal <i>SC-et</i>	Elongated marginal spines; occasional <i>Hr</i> and <i>B</i>	Long and pointed spines alternating with shorter ones
18 Cerci length: relative to body length	approx. 0.5	approx. 0.5–0.6	approx. 0.5 [slightly longer than body]
19 <b>Terminal filament length: relative to cerci length</b>	Distinctly shorter	Subequal or slightly shorter	Subequal or slightly shorter
<b>Egg</b>			
1 <b>Length/width [µm]</b> Figure 12(a–d)	130–140/65–70	180–215/120–135	–
2 <b>Chorionic surface: shape of polygonal ridges</b> <sup>[15]</sup> Figure 12(e–h)	Thin and flat	Relatively extended	–
3 Cells of chorionic mesh: diameter [µm] Figure 12(e–h)	2.5–4.2	3.0–6.4	–

## Notes:

<sup>[1]</sup>Based on Ikonomov (1962) and Bauernfeind and Soldán (2012).

<sup>[2]</sup>Character tested by Bauernfeind and Soldán (2012: 180).

<sup>[3]</sup>Character tested by Kluge and Novikova 1992a: 63).

<sup>[4]</sup>For *C. pirinense*, figures by Ikonomov (1962: 115, fig. 25.1), in the original description the shape the of labrum and setation are described as follows: the labrum is almost twice wider than long; lateral side of the labrum with sharp setae that look like a feather; two long sharp setae apically, and 3–4 smaller pointed setae more medially [ventral side]; setae evenly scattered over the entire surface [dorsal side].

<sup>[5]</sup>For *C. luteolum*, figured by Kluge and Novikova 1992: 63, fig. 2.1); for *C. pirinense*, figured by Ikonomov (1962: 117, fig. 27.1).

<sup>[6]</sup>For *C. pirinense*, described and figured in detail by Ikonomov (1962: 114, 115, figs 25.3–7).

<sup>[7]</sup>Apicomedial projection in the middle of anterior margin was considered a generic character of *Centroptilum* by Bauernfeind and Soldán (2012: 178); character also figured for some *Indocloeon* (Kaltenbach and Gattolliat 2017: 52, fig. 4d).

<sup>[8]</sup>For *C. luteolum*, figured by Kluge and Novikova 1992: 63, fig. 2.4); for *C. pirinense*, figured by Ikonomov (1962: 115, figs 25.8, 25.9).

<sup>[9]</sup>For *C. pirinense*, described and figured in detail by Ikonomov (1962: 114, fig. 24).

<sup>[10]</sup>According to Bauernfeind and Soldán (2012: 181), claws as long as 2/3 of tarsus length; Ikonomov (1962: 116, figs 26.9–12) figured the legs with claws as long as about 1/2–2/3 of tarsus length.

<sup>[11]</sup>For *C. pirinense*, the fragmentary cuticle structure figured by Ikonomov (1962: 116, fig. 26.14) and most likely close to that in *C. luteolum*; *C. volodymyri* sp. nov. similar to *Anafroptilum kazlauskasi* (Kluge, 1983) in the presence of transparent scale-shaped bristles on abdominal terga of larvae (Kluge 2012: 373, fig. 43), in contrast to *C. luteolum* which lacks stout setae or scales on surface of terga.

<sup>[12]</sup>For *C. pirinense*, briefly described and fragmentary figured by Ikonomov (1962: 116, 117, fig. 26.14); the structure most likely close to that in *C. luteolum*.

<sup>[13]</sup>Keffermüller and Sowa (1984: 310) reported a lack of posterolateral spines on abdominal terga of *C. luteolum*. McCafferty and Waltz (1990: 784) listed for several Nearctic larvae the presence of posterolateral spines on abdominal terga VIII–IX, as an exception in comparison with all other species of the genus. However, later, Kluge and Novikova 1992: 69, fig. 5.1) clarified this information, and reported small posterolateral spines in *C. luteolum*, typical also for the species of the genus *Cloeon* s.l. According to Bauernfeind and Soldán (2012), posterolateral part of tergum IX in *C. luteolum* occasionally with a group of 3–6 small and rather insignificant spines.

<sup>[14]</sup>For *C. pirinense*, based on fig. 26.13 in Ikonomov (1962).

<sup>[15]</sup>Using scanning microscopy, the chorion surface of the egg was depicted for the first time by Jacob (1991: 284, fig. 9b). Ubero-Pascal and Puig (2007: 332, fig. 4a) and Ubero-Pascal et al. (2005: 266, 268, figs 2b, 4c) depicted a general view of eggs and their chorionic surface.

Distinguishing characters of *C. volodymyri* sp. nov. discussed in the Table II have been analysed based on type material only. Some differences of body colouration between Caucasian and Iranian specimens of *C. volodymyri* sp. nov. are discussed in the text.

*Type material*

*Holotype*. Female mature larva, GEORGIA, Autonomous Republic of Adjara, Kobuleti District, vicinity of Chakhati village, valley of the Kintrishi

River, small lentic water bodies, 41.762°N, 41.978°E, 325 m a.s.l., 18 April 2016, Martynov A. V. leg. (inventory number in NMNH NASU collection: *Grg24Censp1*).

Table III. Genetic distances (COI) between sequenced species of *Centroptilum*, *Cloeon* and *Procloeon* and within them, using Kimura two-parameter.

	<i>C. volodymyri</i>	<i>C. luteolum</i> clade 1	<i>C. luteolum</i> clade 2	<i>C. victoriae</i>	<i>P. bifidum</i>	<i>P. pennulatum</i>	<i>C. dipterum</i>	<i>C. simile</i>
<i>C. volodymyri</i> sp. nov.	0.131							
<i>C. luteolum</i> clade 1	0.254	0.041						
<i>C. luteolum</i> clade 2	0.251	0.206	0.021					
<i>C. victoriae</i>	0.262	0.271	0.257	-				
<i>P. bifidum</i>	0.279	0.271	0.255	0.224	-			
<i>P. pennulatum</i>	0.253	0.263	0.239	0.234	0.181	-		
<i>C. dipterum</i>	0.264	0.282	0.26	0.242	0.184	0.192	-	
<i>C. simile</i>	0.259	0.281	0.262	0.218	0.205	0.188	0.168	-

*Paratypes.* Six larvae, GEORGIA, same locality and date as holotype (three larvae in slides 648, 661, 662; one larva in ethanol deposited in NMNH NASU with inventory number *Grg24Censp/2*); 49 larvae, TURKEY, Rize il [Province], Fındıklı Region, Kaçkar Mountains, Büyük [Çağlayan] stream, 41.236°N, 41.270°E, 337 m a.s.l., 18 August 2012, Palatov D. M. leg.;

*Non-type material.* 10 larvae, IRAN Guilan Province, unnamed small brook (left tributary of Shalmanrud River), SW of Amlash town, 37.046°N, 50.095°E, 185 m a.s.l., 21 May 2016, Bojková J., Soldán T., Imanpour Namin J. leg.;

One larva, IRAN, *ibid.*, SW of Amlash town, 37.037°N, 50.083°E, 287 m a.s.l., 21 May 2016, Bojková J., Soldán T., Imanpour Namin J., leg.;

22 larvae, IRAN, Golestan Province, unnamed small brook (right tributary of Rude-Tengen River), S of Qarangi-ye Jangal town, 37.529°N, 55.792°E, 604 m a.s.l., 23 June 2019, Palatov D. M. leg.

#### Etymology

The species is named in honour of Volodymyr Martynov, senior son of the first author, for his field assistance in mayfly sampling during a series of field trips.

#### Distribution and habitat

In Georgia, the larvae of *C. volodymyri* sp. nov. were collected in small pools, where small brooks flowed in, in the valley of the Kintrishi River at 325 m a.s.l. (Figure 13(a,b)). They were shaded by the ancient colchic forests (chestnut, hornbeam and box trees). Pools were about 0.3 m deep, with bed substrate

composed of silt and detritus, without macrophytes. No other mayflies were found there.

In Iran, the larvae were collected in two small brooks (1–3 m wide) flowing in steep terrain below 300 m a.s.l. on the northern slopes of the Alborz Mts., close to the Caspian Sea coastal area in Guilan Province (Figures 13(c,d) and 14). They were shaded by humid deciduous forest and shallow, with an average depth of 0.05–0.15 m (max. 0.3 m in pools). They were characterised by alternating riffles and pools, where *Centroptilum* larvae were collected. Coarse bed substratum in riffles alternated with finer substrates formed by coarse and fine gravel in pools. Bedrock and boulders were sparsely covered with mosses. Water was relatively cold (16°C) and clear, although one brook was slightly turbid. The mayfly taxocene consisted of the following species: *C. luteolum* (dominant), *Baetis* (*Rhodobaetis*) cf. *gadeai* Thomas, 1999, *Nigrobaetis* (*Alainites*) *muticus* (Linnaeus, 1758) (all Baetidae), *Electrogena pseudaffinis* (Braasch, 1980) (Heptageniidae), and *Caenis macrura* Stephens, 1835 (Caenidae).

In Turkey, larvae were collected in a small stream (1.5–2.5 m width; 0.1–0.2 m depth) flowing in the forest at 337 m a.s.l. (Figure 13(e,f)). Its bed substrate was composed of pebbles, detritus and silted stones and the flow velocity was 0.3–0.6 m.s<sup>-1</sup>. The mayfly taxocene consisted of the following taxa: *Electrogena* sp. (Heptageniidae) (dominant), *Habroleptoides* cf. *confusa* Sartori and Jacob, 1986 (Leptophlebiidae), *B. (R.)* cf. *gadeai*, and *N. (A.) muticus* (Baetidae).

#### Affinities

We attribute *C. volodymyri* sp. nov. to the genus *Centroptilum* based on larval and egg characters depicted by Jacob (1991), Kluge and Novikova (1992a, 1992b), and Bauernfeind and Soldán (2012). The attribution is confirmed by the presence of (1) epicranial suture met at an obtuse angle; (2)



Figure 13. Habitats of *Centroptilum volodymyri* sp. nov. in Georgia, Iran and Turkey. (a, b) Small lentic waterbody, Georgia, Adjara, type locality (April 2016; photos by A. Martynov); (c, d) small brook, left tributary of Shalmanrud River, Guilan Province, Iran (May 2016; photos by J. Bojková); (e, f) small stream, Rize Province, Turkey (August 2012; photos by D. Palatov).

mandibular incisors deeply separated into two groups from their base; (3) segment III of maxillary palp not shortened, longer than segment II; (4) narrow pronotum, approximately  $3.0\text{--}3.5 \times$  longer than its width [4.0 times according to Kluge and Novikova (1992a, 1992b)]; (5) pretarsal claws with two rows of small teeth; (6) simple gills with pinnate

tracheisation [according to Bauernfeind and Soldán (2012), in *Centroptilum* simple gills acutely pointed apically and almost symmetric, except for the very narrow gill I]; (7) abdominal segments almost without strong posterolateral setation, except for tergum VII covered by 1–3 relatively prominent spines [no strong posterolateral setation in *Centroptilum*

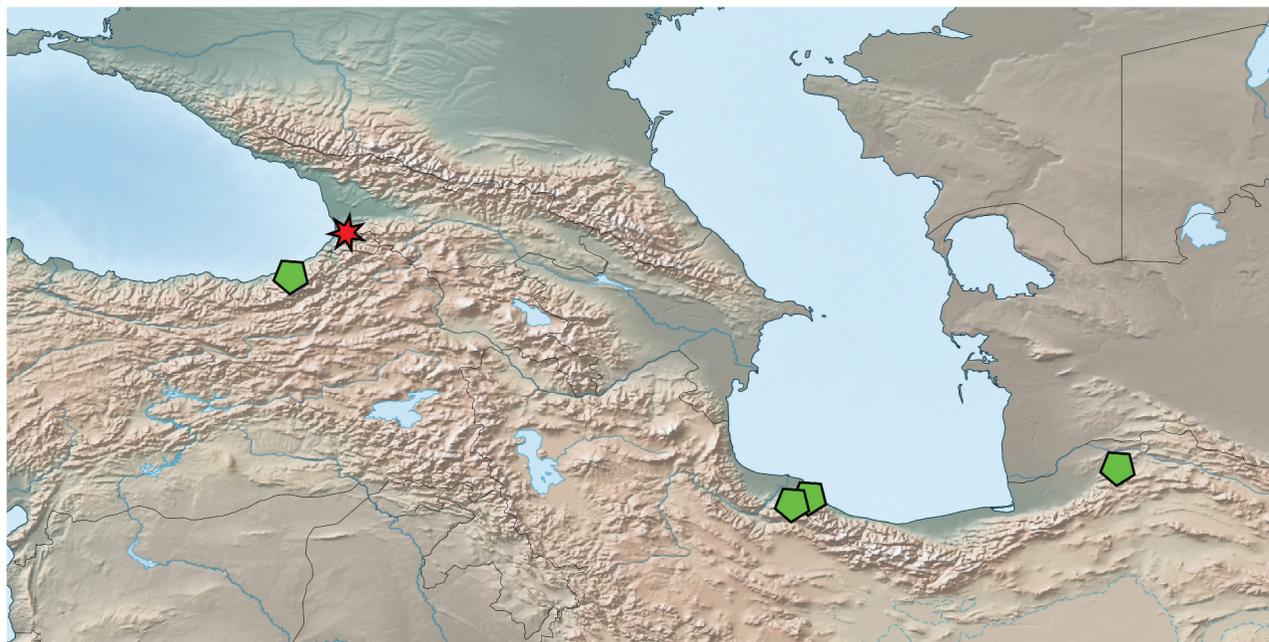


Figure 14. Map of *Centropitulum volodymyri* sp. nov. distribution. Red star – type locality; green pentagams - non-type localities.

according to Bauernfeind and Soldán (2012)]; (8) surface of abdominal terga with flattened, non-pointed corrugation and micropores [pointed cha-grin structure in the form of minute spines, and circular sensillae according to Bauernfeind and Soldán (2012)]; (9) posterior margin of terga with robust spines alternating with shorter ones [the same for some terga of *C. luteolum* (see Figure 9(h)) and *C. pirinense* (according to Ikononov 1962)]; the posterior margin of abdominal terga in *Centropitulum* with spines of subequal length according to Bauernfeind and Soldán (2012)]; (10) no long setation on last two caudal segments [basal and apical 1/5 part of caudal filaments are without setation according to Bauernfeind and Soldán (2012)]; and (11) chorionic surface of eggs with dense uniformly reticulated ridges, forming irregular polygonal cells [“meshes” according to Bauernfeind and Soldán (2012)].

*Centropitulum volodymyri* sp. nov. occupies a relatively independent position within the genus due to characteristics of the cuticle, setation of the posterolateral margin of tergum VII and the gill shape. At the larval stage, it clearly differs from the Western Palearctic representatives *C. luteolum* and *C. pirinense* by numerous scales and scale bases densely scattered on the body surface, some features of the mouthparts (particularly the shape of the labrum and superlinguae of the hypopharynx, and the proportions and setation of maxillary and labial palps; see also Table II), and dense denticulation of claws. Minor differences are found in the size of eggs and

their cells within the chorionic mesh. The structure of the chorionic surface is thus very similar to that of *C. luteolum* (it was not described for *C. pirinense*). A detailed comparative overview of the distinguishing characters for the delimitation of the three species discussed above is given in Table II.

Specimens of *C. volodymyri* sp. nov. collected in the Caucasus and Iran show some variability in colouration and morphology. Caucasian specimens are distinctly darker: their pleural sclerites and particularly anterior part of mesonotum and fore wing pads are markedly brown to dark brown. In contrast, Iranian specimens are distinctly paler, with body whitish yellow to brown. While Caucasian specimens had brown abdominal tergum and sternum of segment IX, Iranian specimens show a whitish-yellow central part of the tergum IX lined by two brownish oblique strips (Figure 2(a)) and small light brown smudges on the sternum IX. Some minor differences between Caucasian and Iranian populations can be recognised in the structure of mouthparts and arrangement of scales and setae along the posterior margin of flagellar segments. Iranian specimens had a more elongated labrum width/length ratio (1.6–1.7, compared to 1.8–2.0 in Caucasian specimens). Some Iranian specimens have fewer setae (fewer than six) on the dorsal side of segment II of the labial palp than the Caucasian specimens (6–8 setae). The setae under the maxillary crown are arranged in a nearly distinct row in specimens from the Caucasus, while they are

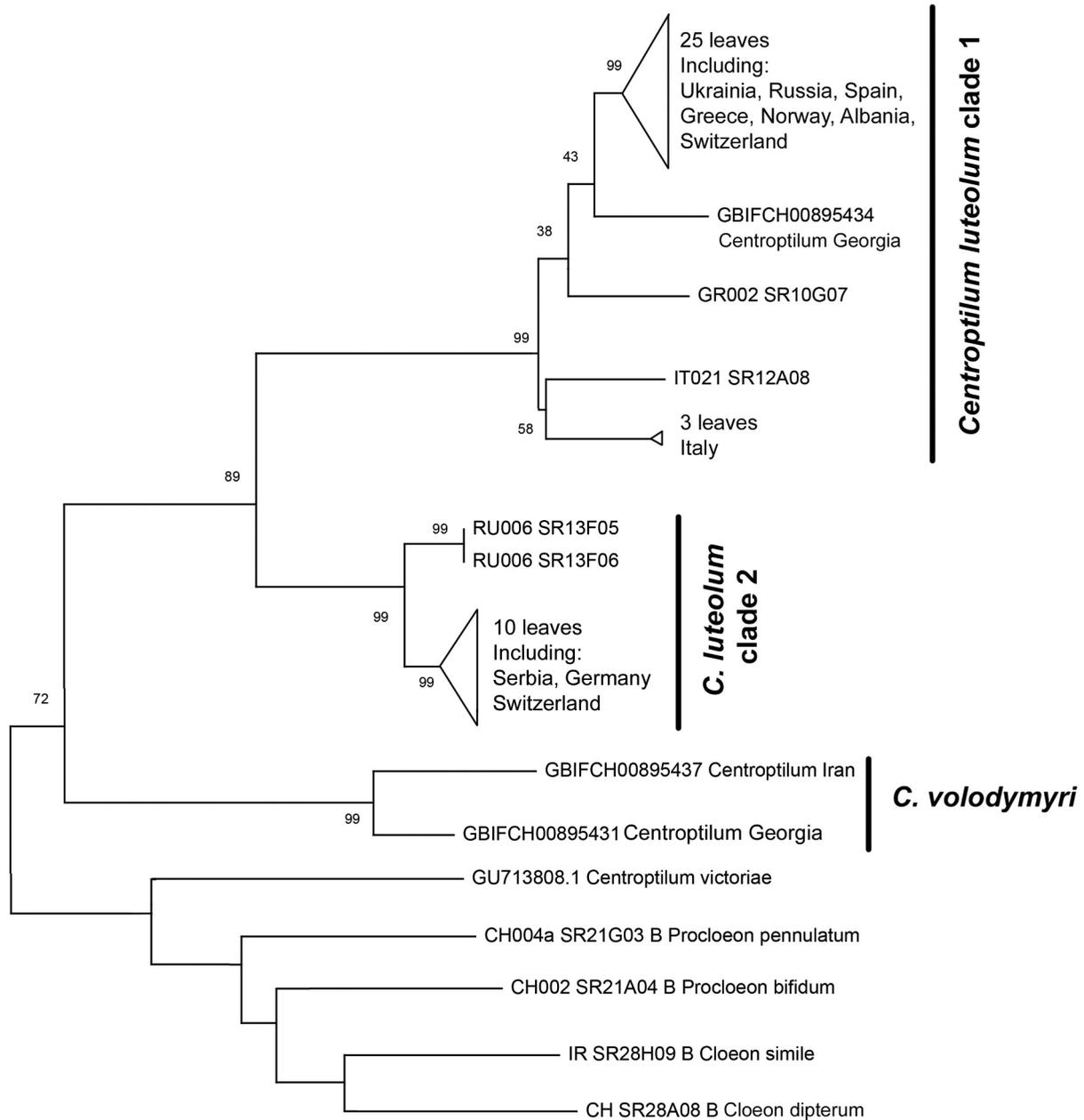


Figure 15. Maximum likelihood tree including several representatives of the genus *Centroptilum*. Bootstrap supports (BS) are indicated on branches for *Centroptilum volodymyri* sp. nov. and *C. luteolum* (Müller, 1776).

mostly scattered in some specimens from Iran. The specimens from Georgia and Turkey have bluntly pointed or apically rounded scales on posterior margins of flagellar segments, while two or three pointed apices are found on each marginal scale in specimens examined from Iran. Nevertheless, these differences are not consistent in all specimens and characters, and we consider the specimens from Caucasus, Turkey and Iran to be conspecific, belonging to *C. volodymyri* sp. nov.

#### Molecular results

The molecular reconstruction highly supported *C. volodymyri* sp. nov. as a monophyletic clade, with a bootstrap support of 99% (Figure 15). Georgian and Iranian sequences are separated by genetic distances (13%, Table III) that are generally considered higher than intraspecific. *Centroptilum volodymyri* sp. nov. is recovered as the sister species of *C. luteolum*; the genetic distances between *C. volodymyri* sp. nov. and the two clades of

*C. luteolum* are higher than 25%. *Centroptilum luteolum* is composed of two monophyletic lineages (clade 1 and clade 2 in Table III and Figure 15); both are highly supported as monophyletic and may represent distinct species (K2P distances higher than 20%).

## Discussion

Integrative approaches clearly confirm the validity of the new species *C. volodymyri* sp. nov. This species is closely related to the type species of *Centroptilum*, *C. luteolum*, both morphologically and genetically. The two species can be morphologically distinguished by the shape and length of the maxillary palp, the length and denticulation of the claws and the shape of the gills. High molecular distances strengthen the validity of the new species. At present, three populations of *C. volodymyri* are known, from Georgia, Turkey and Iran. As these three countries represent the limit of distribution of *C. luteolum*, these two species of *Centroptilum* are partially sympatric. The molecular distances between the Georgian and Iranian population of *C. volodymyri* sp. nov. are rather high and may suggest the presence of two cryptic species. We refrain from considering the two as separate species as no morphological characters support this division. The high molecular distance could also be due the geographical gap between the populations and the absence of gene flow between them. The discovery of additional populations between them might challenge the present interpretation and reduce the average intraspecific distances.

In addition to *C. luteolum* and *C. pirinense* known from the West Palearctic region, Bauernfeind and Soldán (2012) considered three extralimital Palearctic species of *Centroptilum*. Two of them are poorly known species described based on adults, namely *C. chinense* You and Gui, 1995 based on a male imago from China and *C. rotundum* Takahashi, 1929 based on a female imago from Japan. Comparing the species described here with these taxa is impossible because the imago is unknown in *C. volodymyri* sp. nov. The third species, *C. kazlauskasi* Kluge, 1983, distributed in Mongolia, and the Far East and the Republic of Buryatia in the Russian Federation, was designated as the type species of the genus *Anafroptilum* Kluge, 2011. The genus is established for non-African taxa, initially attributed to *Centroptilum* (Kluge 2012). In contrast to *Centroptilum* (incl. *C. volodymyri* sp. nov. as well as the closely related Nearctic genus *Neocloeon*),

the representatives of *Anafroptilum* are characterised by the absence of the patella-tibial suture in larvae and adults of both sexes on the fore legs, and therefore are considered to belong to a completely separate lineage from *Centroptilum*.

There are some other taxa nominally placed within *Centroptilum* and distributed in the Palearctic Region. *Centroptilum hungaricum* Pongrácz, 1913 was described as the female imago from the Trenčianska župa [Trencsén County in the former Kingdom of Hungary; now Western Slovakia] (Pongrácz 1913). *Centroptilum obtusum* Navás, 1915 from Spain is known from the old material only (Navás 1915), but Alba-Tercedor and Peters (1985) reported the absence of the types of this species in the collection of Longinos Navás at the Museo de Zoología del Ayuntamiento (Barcelona, Spain). These two taxa are mentioned as *dubious species* (for additional information and respective references see Jacobus & Wiersema 2014). Additionally, *C. striatum* Bengtsson, 1904 is considered a *nomen nudum* (see Bauernfeind & Soldán 2012) and the generic placement of *C. lituratum* (Pictet, 1843) is considered unclear, because the taxon was described from adult material from Switzerland and the type material is lost (Keffermüller & Sowa 1984). The comparison of the presently discovered species with the taxa referred to above is impossible, again because the imaginal stage of *C. volodymyri* sp. nov. is unknown.

Summarised data on the diversity of the subfamily Cloeoninae of Iran were published by Bojková et al. (2018): five species of this subfamily were reported, belonging to *Cloeon*, *Centroptilum* and *Procloeon*. Regarding biogeographic patterns of Iranian Cloeoninae, the dominant part of the species exhibits a broad Palearctic and Holarctic distribution. This is in clear contrast to the distribution of *C. volodymyri* sp. nov., which is limited to Northern Middle East, Southern Caucasus and Eastern Anatolia. A single species of *Procloeon* (*P. caspicus*) was recorded from Northwest Iran, close to the border with Azerbaijan (Bojková et al. 2018).

The fauna of Caucasian Cloeoninae is more diverse. Altogether, 11 species belonging to three genera were recorded. While *Centroptilum luteolum* is distributed throughout the region (see Cherchesova 2004; Palatov 2013; Martynov et al. 2016; Hrivniak et al. 2018), the taxon listed as *Centroptilum* sp. from the Kintrishi River (AR Adzharia, Georgia) corresponds to the new species described here (see the list of synonymies and Martynov et al. 2016). The species of *Cloeon* are

a typical component of mayfly taxocenes of Caucasian river basins. However, in comparison to the widely distributed *C. dipterum*, three other species were only reported from a restricted number of regions of the Caucasus: *Cloeon simile* Eaton, 1870 listed for Azerbaijan, Georgia (AR Abkhazia) and the Russian part of the Caucasus (Sokolova 1937; Kasymov 1972; Puthz 1978); *Cloeon cognatum* Stephens, 1835 reported by Cherchesova (2004) for the Northern Caucasus within North Ossetia-Alania, Kabardino-Balkaria and Karachay-Cherkessia; and *Cloeon inscriptum* Bengtsson, 1914 known only from a single doubtful record labelled “material from Caucasus”, without additional precision (see Silina 1994). The Caucasian fauna of the genus *Procloeon* (*Procloeon*) Bengtsson, 1915 is represented by *P. bifidum* (Bengtsson, 1912) (the records from Krasnodarskii krai only; see Palatov 2013) and *P. heterophyllum* Kluge and Novikova, 1992a (endemic to the Caucasus, known from the type locality only; see Kluge 1997). There are three species assigned to the subgenus *Pseudocentroptilum* Bogoescu, 1947, within the Caucasus: *P. pulchrum* (Eaton, 1885) and *P. unguiculatum* Tshernova, 1941, occurring in water-flows in Georgia and the Russian Federation (see Palatov 2013; Martynov et al. 2016), and *P. pennulatum* (Eaton, 1870) reported from Armenia and Azerbaijan (Palatov & Sokolova 2016; Hrivniak et al. 2018).

The literature published up to now contains no records of Cloeoninae from the Pontic Mountains within Turkey. Fragmentary information about the distribution of *P. pulchrum* and *C. dipterum* was published by Kazancı (1984) from a neighbouring region in the Ardahan il.

The species composition of Cloeoninae of Turkey, Iran and Central Asia remains only partially established. For now, only *Cloeon dipterum* (Linnaeus, 1761) is known to be common throughout these three territories (Tshernova 1930; Brodsky 1930; Demoulin 1964; Kazancı & Türkmen 2012; Martynov et al. 2016; Hrivniak et al. 2018; Bojková et al. 2018; authors' original data). The species is characterised by wide ecological plasticity. *Cloeon dipterum* inhabits brackish water bodies of deserts and semi-deserts (Hassell et al. 2006; authors' original data). However, it is highly probable that Central Asian limnophilic Cloeoninae, such as *Cloeon tadjikistanicum* Brodsky, 1950, will be found in Iran. *Cloeon tadjikistanicum* was recorded in Pyandzh River, on the border between Tajikistan and Afghanistan. One

more species, *Centroptilum rubidum* Kimmins, 1950, was described from the border of Iran and Afghanistan (Kimmins 1950). Also, most likely *Cloeon karachinensis* Ali, 1970 (described from Western Pakistan) will be recorded within Sistan and Baluchestan Province. *Pseudocentroptilum unguiculatum* (Tshernova, 1941), common within Central Asia and occurring from Eastern Uzbekistan to Mongolia (Kluge & Novikova 1992a, 1992b), also has not yet been recorded from Iran.

Generally, in our opinion, the description of a new valid species of the restricted genus *Centroptilum* s.s., the distribution of which falls within several regions (Southern Caucasus, Eastern Anatolia and Albors Mts), should be of great interest for future investigation of relations and origins of the region's mayfly faunas.

### Acknowledgements

We are grateful to Nicolás Ubero-Pascal (Universidad de Murcia, Spain) for SEM photos of eggs of *C. luteolum* and valuable comments on their structure. We are indebted to Arnold H. Staniczek and Milan Pallmann (SMNS) for macro- and SEM photos of *Centroptilum* species, and provision of Cloeoninae comparative material. We highly appreciate the meaningful insights and reviews made by André Wagner (Switzerland) and Carlo Belfiore (Italy), which significantly improved the manuscript.

### Funding

This research was conducted with institutional support (RVO: 60077344 for RJG) and contains some results of studies supported by a grant of the Czech Science Foundation (Project No. 206/08/1389). Graphic material was prepared during the fellowship stay of RJG in SMNS within the Georg Forster Research Fellowship for Experienced Researchers (Alexander von Humboldt Foundation, Bonn, Germany). Laboratory processing of part of the material was supported by an STSM Grant from COST DNAqua-Net CA 15219 and the CAS Mobility Program [BAS-20] for RJG.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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