

# A new species of *Bungona* in Turkey (Ephemeroptera, Baetidae): an unexpected biogeographic pattern within a pantropical complex of mayflies

Pavel Sroka<sup>1</sup>, Roman J. Godunko<sup>1,2</sup>, Sereina Rutschmann<sup>3,4</sup>, Kamila B. Angeli<sup>5,6</sup>, Frederico F. Salles<sup>7</sup>, Jean-Luc Gattolliat<sup>8,9</sup>

1 Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branišovská 31, 37005 České Budějovice, Czech Republic

2 State Museum of Natural History, National Academy of Sciences of Ukraine, Teatralna 18, 79008 Lviv, Ukraine

3 Department of Biochemistry, Genetics and Immunology, University of Vigo, 36310 Vigo, Spain

4 Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

5 Laboratório de Sistemática e Ecologia de Insetos, Universidade Federal do Espírito Santo, Departamento de Ciências Agrárias e Biológicas, 29933-415, São Mateus, ES, Brazil

6 Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Espírito Santo, 29075-910, Vitória, ES, Brazil

7 Museu de Entomologia, Departamento de Entomologia, Universidade Federal de Viçosa, 36.570-900, Minas Gerais, CEP, Brazil

8 Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland

9 Museum of Zoology, Palais de Rumine, Place de la Riponne 6, 1014 Lausanne, Switzerland

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Corresponding author: Jean-Luc Gattolliat (jean-luc.gattolliat@vd.ch)

## Abstract

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By using an integrative approach, we describe a new species of mayfly, *Bungona* (*Chopralla*) *pontica* sp. n., from Turkey. The discovery of a representative of the tropical mayfly genus *Bungona* in the Middle East is rather unexpected. The new species shows all the main morphological characters of the subgenus *Chopralla*, which has its closest related species occurring in southeastern Asia. Barcoding clearly indicated that the new species represents an independent lineage isolated for a very long time from other members of the complex. The claw is equipped with two rows of three or four flattened denticles. This condition is a unique feature of *Bungona* (*Chopralla*) *pontica* sp. n. among West Palaearctic mayfly species. Within the subgenus *Chopralla*, the species can be identified by the presence of a simple, not bifid right prostheca (also present only in *Bungona* (*Chopralla*) *liebenauae* (Soldán, Braasch & Muu, 1987)), the shape of the labial palp, and the absence of protuberances on pronotum.

## Introduction

The Middle East is regarded as a transitory region containing elements of Western and Eastern Palaearctic faunas (Heller 2007). This explains why also the mayfly fauna of Turkey is very diversified, comprising 157 species reported in the most recent checklist (Salur et al. 2016, with some later comments by Kazancı and Türkmen 2016). Regarding biogeographic patterns of Turkish

mayflies, a large proportion of species exhibits a broad Palaearctic or West Palaearctic distribution. A large part of the fauna is composed of European species with ranges extending into the Middle East (see Bauernfeind and Soldán 2012 for distributional ranges of all European species). Another major group represents strictly Middle East species, including those described and known from only Turkey. Caucasian species also frequently occur, particularly in Eastern Turkey (Kazancı 2009, Türkmen

and Kazancı 2013). However, mayfly taxa usually showing preferences for tropical regions but occur in Turkey outside the tropical realm (and in the Middle East in general) at the same time are extremely rare.

In the present contribution, we describe such a taxon occurring in the Anatolian Peninsula, specifically a new species from the genus *Bungona* Harker, 1957, subgenus *Chopralla* Waltz & McCafferty, 1987. This subgenus currently encompasses seven species and is distributed in Borneo, Vietnam, Hong Kong, and Sri Lanka (Marle et al. 2016). *Bungona* is one of the four genera of the *Cloeodes*-complex (sensu Salles et al. 2016), this complex containing *Cloeodes* Traver, 1938, *Potamocloeon* Gillies, 1990, and *Crassolus* Salles, Gattolliat & Sartori, 2016. This complex is distributed in the Neotropical, Nearctic, Afrotropical, Oriental, and Australasian regions but is most diversified in the Pantropical area. Only a few species are reported from the Nearctic (Waltz and McCafferty 1987) and none from the Palearctic. Consequently, the discovery of this lineage in Asia Minor is extremely surprising, and thus, we discuss the relationships of the new species with other members of the *Cloeodes*-complex together with the evolutionary and biogeographic implications of this discovery. To achieve this goal, we use an integrative approach combining evaluation of morphological characteristics with a molecular analysis of mtDNA sequences. We also provide information on the evolutionary history of the new species and a review of tropical mayfly lineages reported from the Middle East up to now.

## Material and methods

### Taxon sampling

A new species of mayflies *Bungona* (*Chopralla*) *pontica* sp. n. was collected in northern Turkey in 2011 (Fig. 8). Herein, we describe the new taxon including both, morphological and molecular techniques. To analyze the affinities of the new species based on the molecular data, we built a dataset comprising sequences of several mayfly taxa, as summarized in Table 1.

In the selection of taxa, we wanted to include all major clades within the *Cloeodes*-complex, as inferred based on morphological data by Salles et al. (2016). Specifically, the sampling included the genus *Potamocloeon* Gillies, 1990 with the subgenera *Potamocloeon* and *Aquaediva* Salles et al. 2016, which together constitute a monophyletic group sister to all the rest of the *Cloeodes*-complex. We also included the genus *Crassolus* Salles et al. 2016, forming a sister lineage to the clade comprising the genera *Cloeodes* and *Bungona*. These latter two taxa were also included in the analysis, in case of the genus *Bungona*, all three subgenera (*Bungona*, *Centroptella* Braasch & Soldán, 1980 and *Chopralla* Waltz & McCafferty, 1987) were sampled. Therefore, we achieved a comprehensive set of sequences from all major phylogenetic lineages within the *Cloeodes*-complex. We also included some taxa outside of the complex, considered related to the ingroup based on previous morphological investigations (Salles et al. 2016). These encompass the

genera *Crassabwa* Lugo-Ortiz & McCafferty, 1996, *Cheleocloeon* Wuillot & Gillies, 1993, *Dabulamanzia* Lugo-Ortiz & McCafferty, 1996 and *Centroptilum* Eaton, 1869.

A part of the sequences used for the analysis were obtained from the public databases, the rest was newly sequenced within the framework of the present study (see Table 1 for details including sources of data and accession numbers). We also searched in BOLD and GenBank databases for other existing similar sequences. However, no sequences more similar than 84% (and therefore potentially informative for the analysis) were found except those already included in the dataset.

Voucher specimens of the new species, as specified in Results, is deposited in the collection of the Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic (IE CAS: holotype larva, three paratype larvae) and in the Musée cantonal de zoologie, Lausanne, Switzerland (MZL: two paratype larvae).

### Morphological study

Two paratype specimens of *Bungona* (*Chopralla*) *pontica* sp. n. were mounted on slides, using HydroMatrix (MicroTech Lab, Graz, Austria), Canada balsam, or Euparal. Drawings were made using an Olympus SZX7 stereomicroscope and an Olympus BX41 microscope, both equipped with a drawing attachment (camera lucida). Photographs were made using a Leica M205 C stereomicroscope with a Canon EOS 1200D digital camera. All photographs were subsequently enhanced using Adobe Photoshop CS5. For scanning electron microscopy (SEM), the samples in 96% ethanol were transferred in three mixtures of graded series ethanol : acetone (2:1, 1:1 and 1:2) with 15 minutes in each mixture and dried by means of a critical point dryer CPD 2 (Pelco TM). The samples were mounted on an aluminium target by means of carbon tape, coated with gold using a sputter coater Baltec SCD 050. Finally, the samples were examined in a FE-SEM JSM 7401-F scanning electron microscope (JEOL Ltd, Tokyo, Japan) at low accelerating voltage of 4 kV using GB-low mode. The accomplished topographic images provided a resolution of 1.5 nm by an efficient semi-in-lens detector. The morphological terminology follows Salles et al. (2016).

### Molecular study

**DNA extraction, amplification and sequencing.** Genomic DNA was extracted from whole specimens or legs using the NucleoSpin 96 (Macherey-Nagel, Düren, Germany) tissue kit, the BioSprint 96 extraction robot (Qiagen), or the Wizard SV Genomic DNA Purification System Kit (Promega, California, USA), following the manufacturer's protocol. We analyzed the 658 bp fragment of the mitochondrial DNA barcoding gene cytochrome *c* oxidase subunit 1 (*cox1*). Standard polymerase chain reaction (PCR) amplifications were performed with the primer pair: LCO1490 + HCO2198 (Folmer et al. 1994) or C1-J-1718 (Simon et al. 1994) + HCO2198 (for the Neotropical material, see Table 1). The PCR products were pu-

**Table 1.** Taxa used in the molecular dataset.

Species	Location	GBIF or institutional code	GenBank acc. number	Sources
<i>Bungona (Chopralla) pontica</i> sp. n.	Turkey	GBIFCH00272819	MH969424	Present study
<i>Bungona (Chopralla) pontica</i> sp. n.	Turkey	GBIFCH00272820	MH969425	Present study
<i>Bungona (Bungona) narilla</i> Harker, 1957	Australia	See Fig. 9	HM017831 to HM017836	Webb and Suter 2010
<i>Bungona (Bungona) illiesi</i> (Lugo-Ortiz & McCafferty, 1998)	Australia	295442100 295442098	HM017842 HM017842	Webb and Suter 2010
<i>Bungona (Centropsettella) soldani</i> (Müller-Liebenau, 1983)	India	–	LC061855	Selvakumar et al. 2016
<i>Bungona (Centropsettella)</i> sp.	Brunei	GBIFCH00517514 GBIFCH00517515	MH969410 MH969411	Present study
<i>Bungona (Chopralla) ceylonensis</i> (Müller-Liebenau, 1983)	India	–	LC061854	Selvakumar et al. 2016
<i>Bungona (Chopralla)</i> sp.	Sumatra	MG0535 MG0539 MG0544 MG0561 MG0588 MG1083	MH969418 MH969419 MH969420 MH969421 MH969422 MH969423	Present study
<i>Centropsettella elongatum</i> Suter, 1986	Australia	JWA766	HM017830	Webb and Suter 2010
<i>Cheleocloeon soldani</i> Gattolliat & Sartori, 2008	Saudi Arabia	–	HG935111	Salles et al. 2014
<i>Cloeodes aymore</i> Massariol & Salles, 2011	Brazil	Ep2743B	MH969407	Present study
<i>Cloeodes barituensis</i> Nieto & Richard, 2008	Argentina	EP6756B	MH969405	Present study
<i>Cloeodes ioachimi</i> Salles, Massariol & Angeli, 2015	Brazil	Ep6536B	MH969408	Present study
<i>Cloeodes itajara</i> Massariol & Salles, 2011	Brazil	Ep2715C	MH969406	Present study
<i>Cloeodes</i> sp.	Costa Rica	Ep7015A	MH969409	Present study
<i>Crassabwa flava</i> (Crass, 1947)	South Africa	GBIFCH00517544	MH969417	Present study
<i>Crassolus</i> sp.	South Africa	GBIFCH00517535	MH969415	Present study
<i>Dabulamanzia improvida</i> Lugo-Ortiz & McCafferty, 1997	Madagascar	GBIFCH00517533 GBIFCH00517534	MH969413 MH969414	Present study
<i>Potamocloeon (Aquaediva) pseudogladium</i> (Gattolliat, 2001)	Madagascar	GBIFCH00517532	MH969412	Present study
<i>Potamocloeon (Potamocloeon) dentatum</i> (Kimmins, 1956)	Republic of Guinea	GBIFCH00517540	MH969416	Present study

rified using the QIAquick PCR purification kit (Qiagen) or with ExoSAP-IT PCR Product Cleanup (Affymetrix, Ohio, USA), and customary sequenced from both directions by Macrogen (Amsterdam, The Netherlands) or Microsynth (Balgach, Switzerland). Forward and reverse sequences were assembled and edited using Geneious R7 v.7.1.3 (Biomatters Ltd).

**Analyses of molecular data.** To get an overview of the phylogenetic position of the here newly described species and its closely related species, we reconstructed a Bayesian phylogenetic tree. The molecular analysis comprised sequences of 70 specimens, including 21 newly sequenced specimens (Table 1). A multiple sequence alignment was obtained by using MAFFT v.7.050b (L-INS-i algorithm with default settings; Katoh and Standley 2013). The alignment was checked for the occurrence of stop codons and indels using Mesquite v.2.75 (Maddison and Maddison 2011). Identical haplotypes were removed using collapsetypes\_v.4.5.pl (Chesters 2013). An ultrametric gene tree was reconstructed using a coalescent prior sensu Monaghan et al. (2009) and Rutschmann et al. (2014, 2017) in BEAST v.2.4 (Bouckaert et al. 2014). We applied the HKY +  $\Gamma$  model of molecular evolution as this has been shown to fit well *cox1* data sets of mayflies (e.g. Rutschmann et al. 2017, Gattolliat et al. 2018). Moreover, more complex partitioning-schemes resulted in very low effective sample sizes (ESS) for preliminary

tree inferences (see also Gattolliat et al. 2018). Three independent tree inferences each with 50 mio generations were performed in order to check consistency among runs. The convergence of each run and between runs was checked using Tracer v.1.7 (Rambaut et al. 2018), considering effective sample sizes > 200. We summarized the trees using LogCombiner v.2.4 (Bouckaert et al. 2014), discarding 10 % of each tree as burnin and obtained a maximum clade credibility tree using TreeAnnotator v.2.4 (Bouckaert et al. 2014).

## Results

### Taxonomy

#### *Bungona (Chopralla) pontica* Sroka, Godunko & Gattolliat, sp. n.

<http://zoobank.org/78B55194-D8FC-422C-A0C1-5715400FEEAE>

Figures 1–6

**Type material. Holotype.** Male mature larva (IE CAS), TURKEY, Dipsiz Önü Stream, Gemiciler village, 500 m upstream from the village, in forest near Inebolu–Ayancık road, 50 m a.s.l., 41°57.641'N, 33°53.026'E; 06.vii.2011, Sroka & Godunko leg. [locality code: TUR11/52].

**Paratypes.** 2 mature male larvae (IE CAS: 1 larva in EtOH with some body parts mounted on a slide: mouthparts, legs,



**Figure 1.** *Bungona (Chopralla) pontica* sp. n., habitus. **A** Dorsal. **B** Lateral.

gills, tergum X, paraprocts, cerci; 1 larva dried and gilded as a SEM sample), same data as holotype; 1 mature male larva (IE CAS: in EtOH), TURKEY, Ilişi Stream, Inebolu–Ayancık road, Yakaören village, vicinity of Abana town, 50 m a.s.l., 41°56.244'N, 34°13.360'E; 06.vii.2011, Sroka & Godunko leg. [locality code: TUR11/53]; 2 female larvae (MZL: 1 larva in EtOH: GBIFCH00272819 [FREDIE SR24E11] and 1 larva on a slide GBIFCH00272820 [FREDIE SR24E12]), same data as holotype.

**Diagnosis.** The prostheca of right mandible simple (not bifid) with several minute denticles apically; setae on the dorsal margin of the femur reaching 1/4 of the femur width; the surface of pronotum without tubercles. A detailed comparison with related species is presented in the Discussion.

**External morphology of the larva.** Body length approx. 4.5 mm–4.7 mm ( $n = 2$ ). Length of cerci ca 1.5–2.0 mm ( $0.3 \times$  body length), paracercus equal in length to cerci (Fig. 1A).

**Head.** Labrum (Fig. 2A) ca  $1.3 \times$  wider than long, broadly rounded distally, with shallow medial emargination. Dorsal surface of labrum (Fig. 2A, right) with one long seta submedially (sI in Fig. 2A), apicolateral arc of three slightly shorter setae (sII in Fig. 2A); and with few short hair-like setae scattered on surface. Dense row of short branched setae present along anterior margin of labrum, longer setae anteromedially. Ventral surface of labrum (Fig. 2A, left) with group of fine hair-like setae near anterior margin.

Hypopharynx with trilobed lingua apically, slightly longer than superlingua. Distal parts of lingua and superlingua covered with short, hair-like setae.

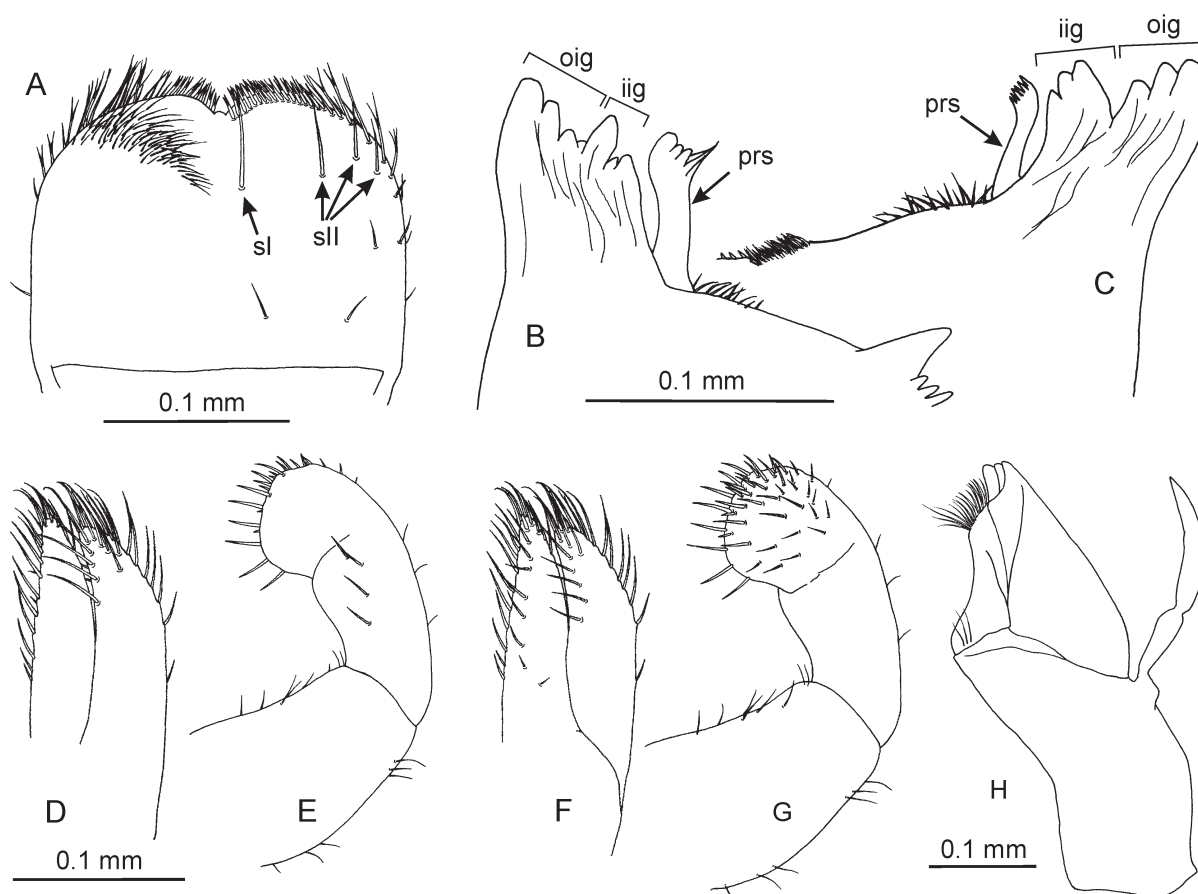
Right mandible (Fig. 2C) with two partially fused incisor groups (outer incisor group (oig) in Fig. 2C, and inner incisor group (iig) in Fig. 2C), each equipped with three denticles. Right prostheca (prs in Fig. 2C) simple, not bifid, with several minute denticles apically. Numerous short setae present between prostheca and mola.

Left mandible (Fig. 2B) with two mostly fused incisor groups, outer incisor group with four denticles (oig) in Fig. 2B, and inner incisor group with three denticles (iig) in Fig. 2B. Left prostheca (prs in Fig. 2B) robust, with about three short rounded denticles and comb-shaped structure apically. Numerous short setae present between prostheca and mola.

Maxilla (Fig. 2H) with two-segmented maxillary palp. Segment II  $1.8 \times$  longer than segment I, narrowing distally, and pointed at apex.

Labium (Figs. 2D–2G) with glossa slightly longer than paraglossa, inner margin of glossa with row of setae increasing in length apically. Second row of shorter setae present submarginally on ventral surface of glossa. Outer margin of glossa mostly without setation except for subapical part.

Paraglossa along outer margin with row of setae, increasing in length apically. Groups of similar setae in subapical region present on both, dorsal and ventral surface. Along inner margin, short rows consisting of ca five setae submarginally also present on both, dorsal and ventral surface.



**Figure 2.** *Bungona (Chopralla) pontica* sp. n., mouthparts. **A** Labrum (right side dorsal, left side ventral). **B** Incisors of left mandible (dorsal, same scale bar for **B** and **C**). **C** Incisors of right mandible (dorsal). **D** Glossa and paraglossa (dorsal, same scale bar for **D**–**G**). **E** Labial palp (dorsal). **F** Glossa and paraglossa (ventral). **G** Labial palp (ventral). **H** Maxilla. Abbreviations: oig—outer incisor group, iig—inner incisor group, prs—prostheca, sl—submedial seta, sII—apicolateral arc of setae.

Labial palp with segment I slightly longer than segments II and III combined. Segment I equipped with sparse short hair-like setae. Segment II with ca four stout setae in central part of dorsal surface, not expanded distoventrally. Segment III quadrangular, slightly distally expanded, with numerous setae on ventral surface, increasing in length and thickness distally.

**Thorax.** Colour whitish with distinct dark brown pattern (Fig. 1A, B). Surface of pronotum with short minute scales and without any protuberance (Fig. 6A).

Legs whitish, tarsi slightly darker (Fig. 1B). Scales abundant on surface of femora, tibiae, and tarsi (Fig. 4A).

Femur in all leg pairs with dorsal and ventral margin subparallel, ca 4 × longer than wide. Dorsal margin with sparse row of 8 or 9 long, apically rounded setae, slightly widened apically (Fig. 3A, C, E). Length of setae ca 0.25 × femur width. Occasional short setae present along anterior margin of femur.

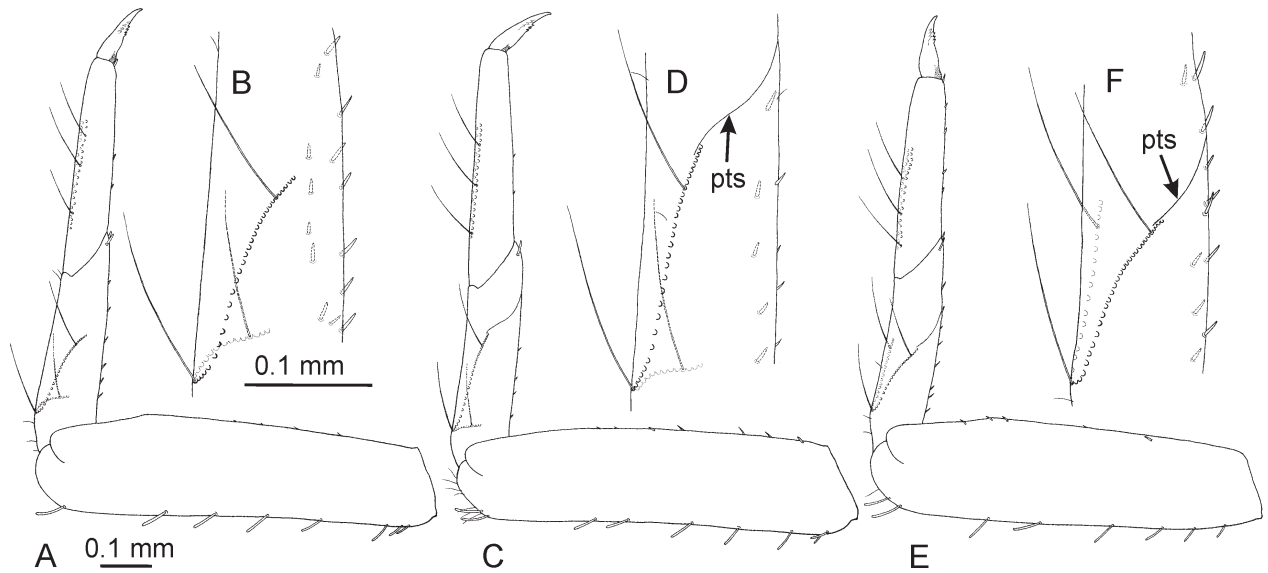
Tibia with patella-tibial suture (middle and hind leg; pts in Figs 3D, F, 4C, D) which is absent on foreleg (Fig. 3B, 4B). Position of patella-tibial suture at middle of tibia length in hind leg, and slightly more distally in middle leg (Fig. 3D, F). Length of row of long setae on

anterior surface of tibia extending for ca 0.5 × length of tibia in all legs. Width of row of long setae on posterior surface of tibia extends ca 0.5 × width of tibia in fore- and middle leg. In hind leg, row of long setae on posterior surface of tibia running parallel to outer margin of tibia, for same distance as row of setae on anterior surface. Angle between rows of setae on anterior and posterior margin of tibia more acute on hind leg compared to fore- and middle leg. (Fig. 3B, D, F). Short, bluntly pointed setae situated along inner margin of tibia.

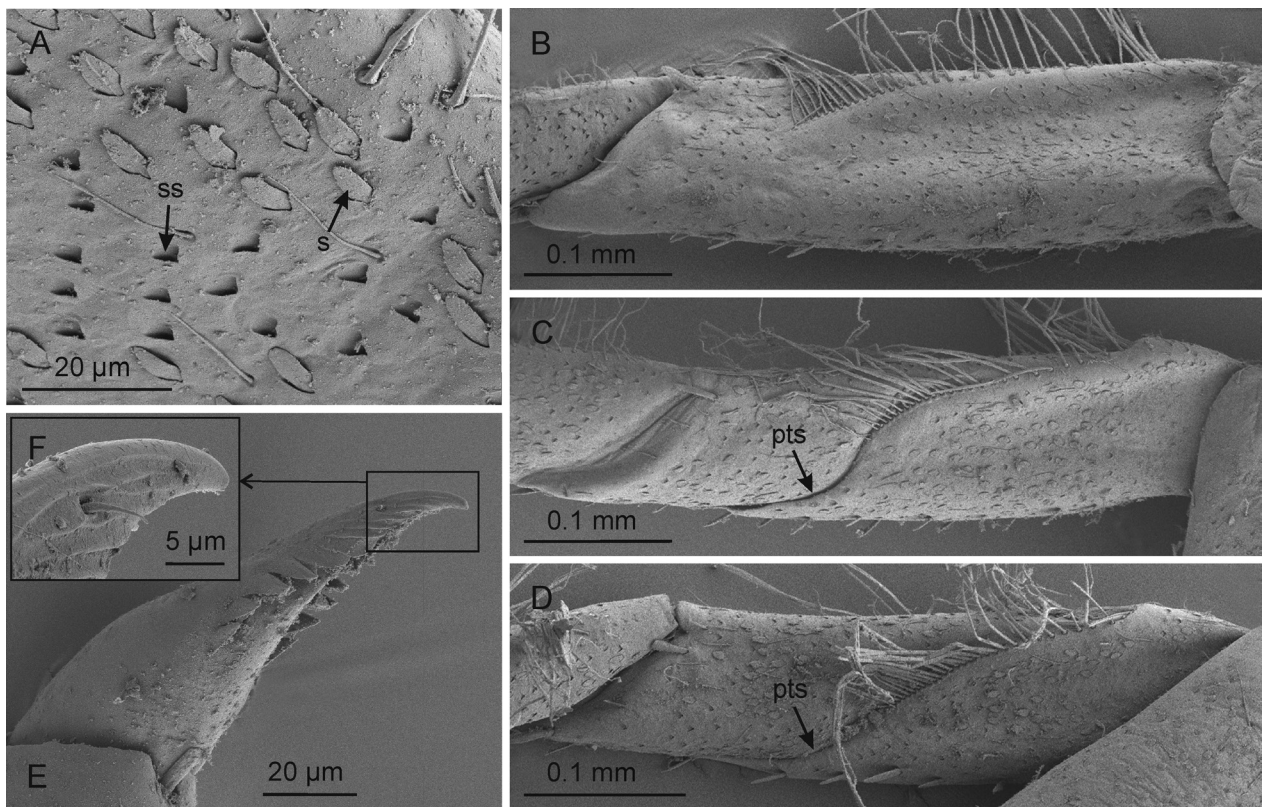
Tarsi equipped with several rows of long hair-like setae along outer margin. Most regular row apparent on anterior surface, and accompanied by more irregular rows posteriorly. Length of rows of setae reaching ca 0.5 × length of tarsus in all legs. Occasional short spine-like setae present along inner margin of tarsus. Claws equipped with two rows of 3 or 4 flattened denticles, subapical striations, and minute subapical setae (Fig. 4E, F).

Hind wing pads vestigial (Fig. 5B).

**Abdomen.** Colour pale whitish with dark brown pattern (Fig. 1A). Tergite I pale, with dark stripe along posterior margin. Tergites II–VI mostly dark, with tiny paired pale dots submedially and several larger pale



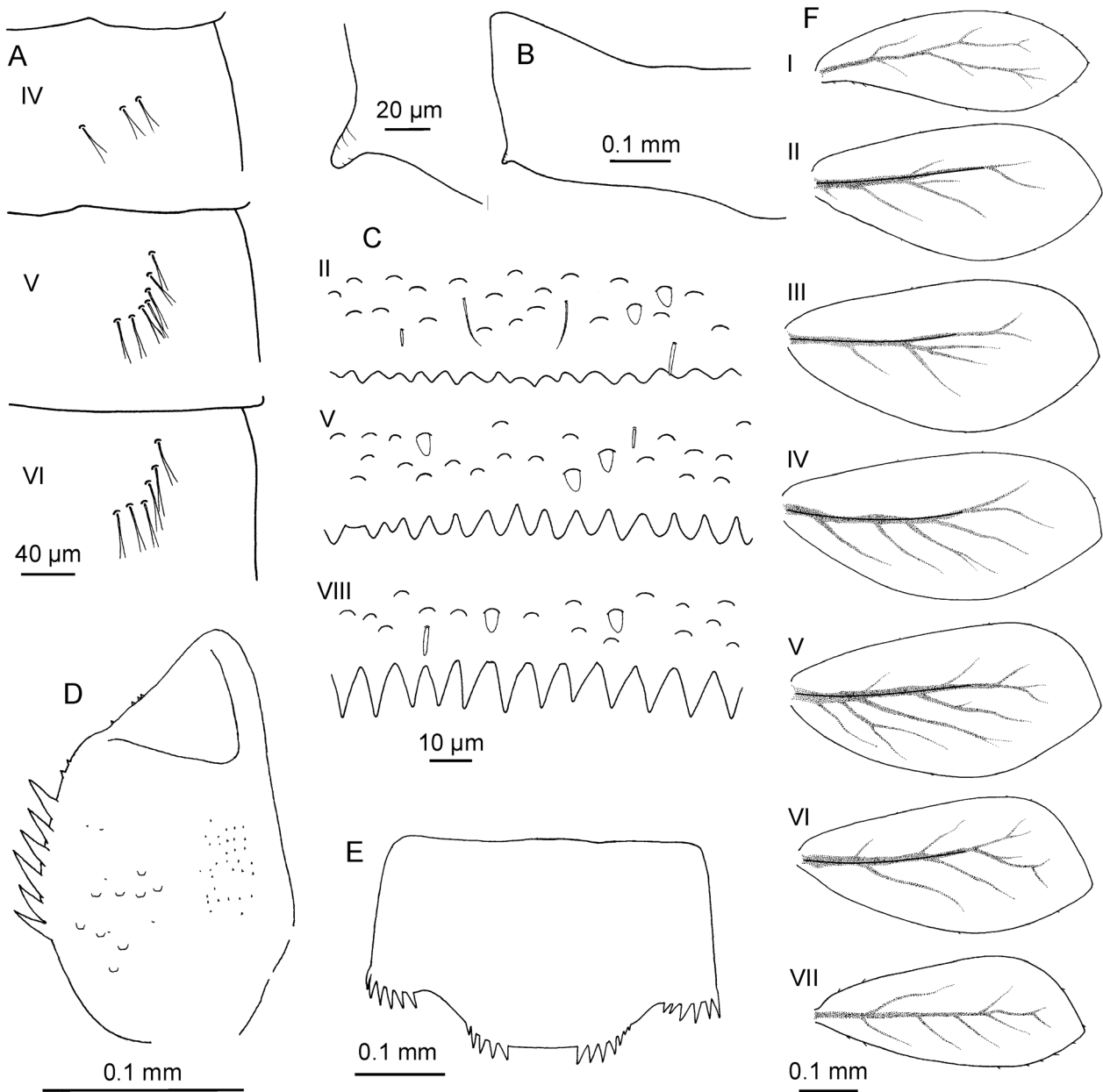
**Figure 3.** *Bungona (Chopralla) pontica* sp. n., legs. **A** Foreleg (dorsal, same scale bar for **A**, **C**, **E**). **B** Basal part of fore tibia (dorsal, same scale bar for **B**, **D**, **F**). **C** Middle leg (dorsal). **D** Basal part of middle tibia (dorsal). **E** Hind leg (dorsal). **F** Basal part of hind tibia (dorsal). Abbreviations: pts—patella-tibial suture.



**Figure 4.** *Bungona (Chopralla) pontica* sp. n., legs. **A** Scales on surface of legs. **B** Foretibia (dorsal). **C** Middle tibia (dorsal). **D** Hind tibia (dorsal). **E** Claw. **F** Detail of claw apex. Abbreviations: s—scale, ss—scale socket, pts—patella-tibial suture.

areas medially, submedially, and laterally. Tergites VII–VIII mostly pale, darker stripes along posterior margins. Tergites IX–X darker, with pale area anteriorly on tergite IX. Sternites pale whitish, with darker longitudinal stripes sublaterally (Fig. 1B).

Tergites equipped with numerous elongate scales, scale bases and short hair-like setae on surface (Fig. 5C). Posterior margin of tergites bear triangular spines (Fig. 5C); limited to lateral side on tergite I, larger and more elongated spines on tergites II–VII generally with median spines



**Figure 5.** *Bungona (Chopralla) pontica* sp. n., thorax and abdomen. **A** Setae on abdominal sternite IV, V and VI. **B** Part of metathorax with vestigial hind wing pad. **C** Surface and posterior margin of abdominal terga II, V and VIII. **D** Paraproct. **E** Abdominal tergum X. **F** Gills.

shorter than lateral, tergites VIII and IX similar to previous ones except central spines more reduced or completely absent. Spines on posterior margin of tergite X in two groups laterally and further two groups submedially (Fig. 5E).

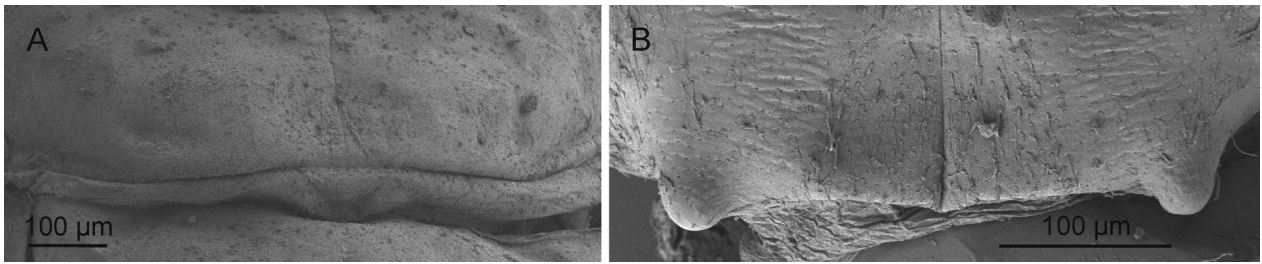
Sternites also equipped with scales and scale bases occasionally scattered over the surface. Posterior margins of sternites IV to IX with triangular spines, very reduced on sternite IV, more distinct on posterior segments; spines absent in segments I–III. Row of conspicuous long setae present on sternites IV–VI (row of shorter setae also present on sternite III; Fig. 5A).

Gills (Fig. 5F) present on segments I–VII, slightly asymmetrical, with indistinct tracheation, apically point-

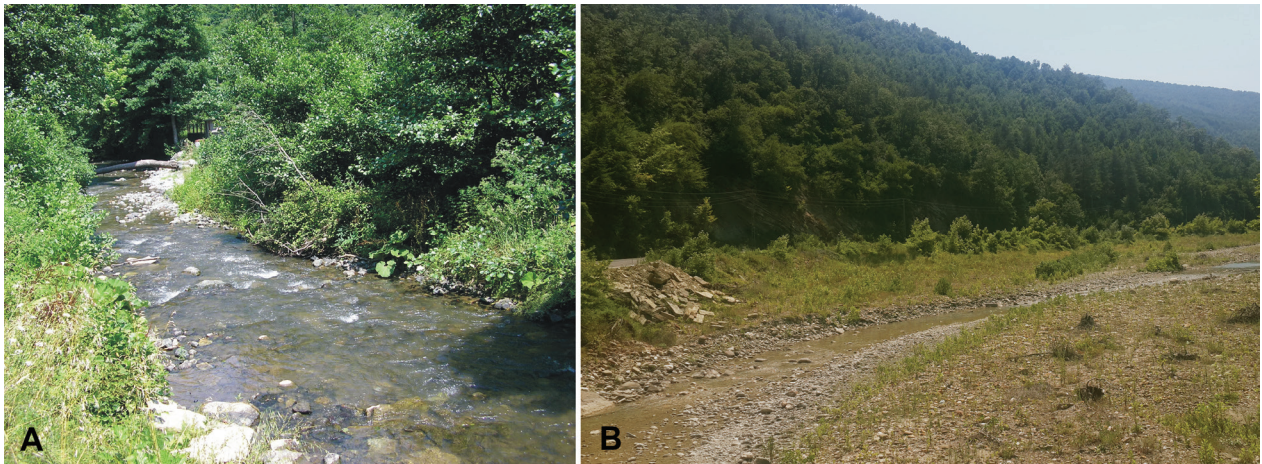
ed, margins occasionally bearing short setae. Brownish line medially on dorsal surface, parallel with medial trachea, not distinguishable on gills I and VII.

Paraprocts (Fig. 5D) with six pronounced marginal spines sometimes accompanied with 1–2 smaller ones. Surface equipped with sparse scales and scale bases. Posterolateral extension with few small marginal spines, absent in some specimens.

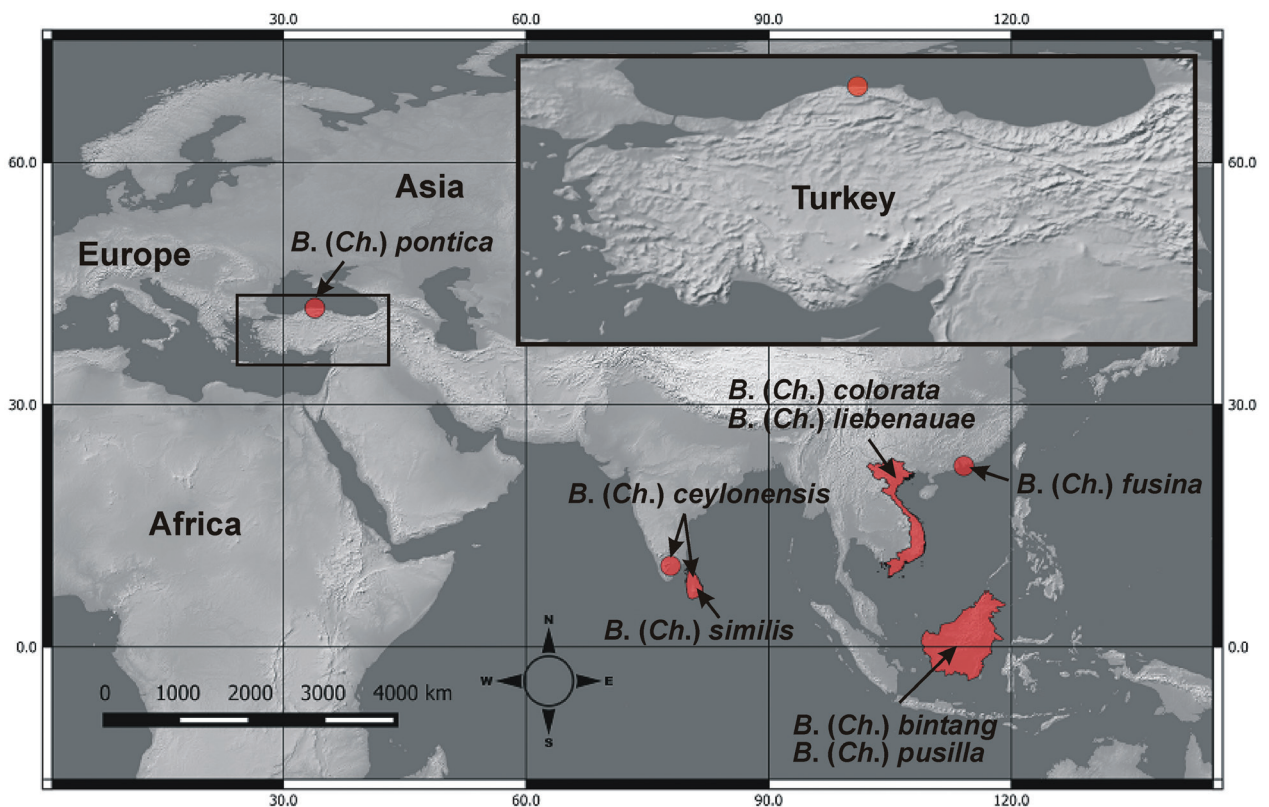
Caudal filaments (Fig. 1A, B) whitish with dark rings on segment margins. Distal margin of each segment equipped with pointed spines and scales. Outer margin of cerci bears enlarged spines on every second segment. Secondary swimming setae present.



**Figure 6.** Difference in the arrangement of the posterior margin of pronotum between *Bungona (Chopralla) pontica* sp. n. (A) and *Bungona (Chopralla) liebenauae* (B).



**Figure 7.** A Type locality of *Bungona (Chopralla) pontica* sp. n. (Dipsiz Önü stream near Gemiciler village). B Valley of Dipsiz Önü stream approximately 400 m downstream from the type locality.



**Figure 8.** Distribution of *Bungona (Chopralla)* spp. Marked occurrence in Turkey encompass the position of both known localities of *Bungona (Chopralla) pontica* sp. n.



**Etymology.** “Pontus” in Latin means “Black Sea” in reference to the geographical region where the type material of the new species was collected.

**Habitat and ecology.** Larvae were found in two slightly eutrophic small streams of different size, the Dipsiz Önü and Ilişi streams. Both small streams flow in northern direction towards the Black Sea within shallow valleys in the westernmost part of the Pontic Mountains (Kuzey Anadolu Dağları). The slopes surrounding both valleys are relatively steep, formed by hills reaching up to 450 m a.s.l. (Fig. 7B) and are densely overgrown by the typical Northern Anatolian conifer and deciduous forests (Euxine-Colchic deciduous forests ecoregion).

The Dipsiz Önü stream at the type locality at 50 m a.s.l. is small, only approximately 0.8–1.5 m wide, and partly shaded by vegetation (Fig. 7A). The bottom consists of relatively coarse stony substratum, partly covered by detritus in the littoral region. The current velocity was approximately 0.5 m/s and the water temperature reached 18 °C (measured ca 5 cm below the water surface).

The Ilişi stream, at the collecting site, was up to 4–4.5 m wide, had a relatively high velocity current (up to 0.7 m/s), well-expressed stream discharge, and a bottom structure consisting of relatively coarse stony substratum with a low concentration of detritus.

We can assume that the new species is probably very rare at the studied localities as well as in all Turkey. During extensive collecting trips in the Sinop Province in 2011 and 2017, only six larvae were found. *Bungona pontica* sp. n. larvae co-occurred with mayfly larvae of *Epeorus* sp., *Electrogena* sp., *Procloeon bifidum* (Bengtsson, 1912), *Baetis fuscatus* (Linnaeus, 1761), *B. (Rhodobaetis) rhodani* (Pictet, 1843), *B. vardarensis* Ikononov, 1962, *Nigrobaetis digitatus* (Bengtsson, 1912), and *Serratella ignita* (Poda, 1761). Additional information on the species composition of the mayfly fauna within the Sinop and Kastamonu provinces was published by Tanatmış (2004), Ertorun and Tanatmış (2004) and further east, in the rivers of the Trabzon Province by Aydınli (2017). The presence of mature larvae at the beginning of July, indicates a flight period of *B. (Ch.) pontica* n. sp. during the first half of the summer.

**Molecular reconstruction.** In total, 61 haplotypes were reconstructed, including 19 previously unknown haplotypes. The new sequences were deposited at GenBank (Acc. nos in Table 1). The *cox1* tree detected *B. (Ch.) pontica* sp. n. as a discrete lineage which is not nested within other *Bungona* (*Chopralla*) species (Fig. 9). Two other *Bungona* (*Bungona*) species, namely *B. (B.) narilla* and *B. (B.) illiesi*, formed a paraphyletic group. The *Cloeodes* representatives from South America (i.e., *C. aymore*, *C. barituensis*, *C. ioachimi*, and *C. itajara*) together with *C. pseudogladus* from Madagascar formed a monophyletic clade. However, as the calculated branch support was very low, the phylogenetic relationships between species/genera remain mostly unsolved.

## Discussion

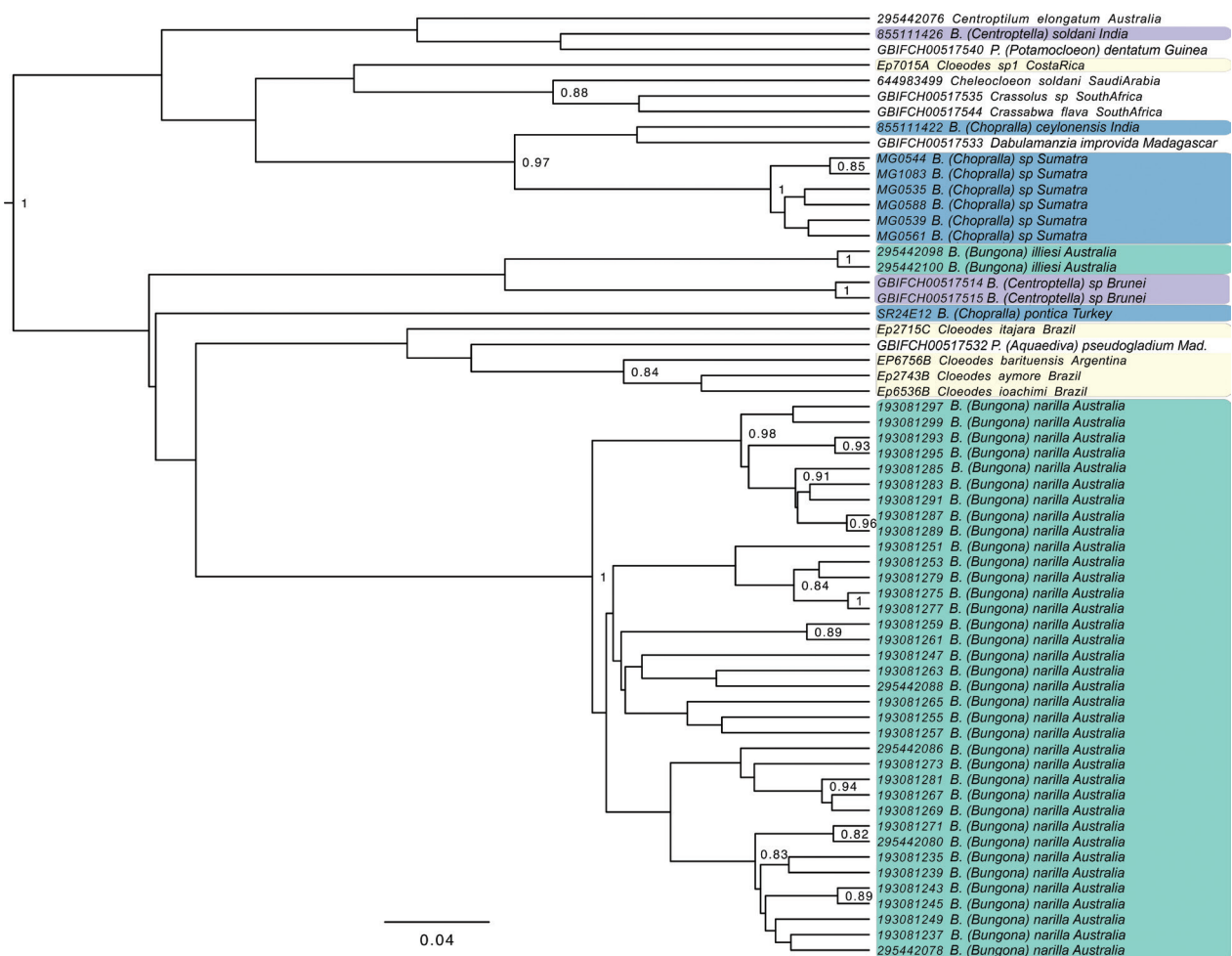
### Phylogenetic affinities

Recently, Salles et al. (2016) performed a comprehensive phylogenetic analysis of *Cloeodes* and related taxa. This reconstruction had considerable impacts on the systematics of the group: *Cloeodes* is restricted to Neotropical and Nearctic regions; all the African species were re-assigned to *Potamocloeon* or *Crassolus*; Oriental species were assigned to one of the three subgenera of *Bungona*. Based on its morphology, the new species is attributable to the *Cloeodes*-complex sensu Salles et al. (2016), sharing all diagnostic characters (apex of maxillary palp slightly narrowing between one-half and apical one-third, presence of a row of long conspicuous setae at the base of tibiae and on tarsi, presence of a row of conspicuous long setae on the abdominal sterna, and apex of gill III or IV pointed).

Within the *Cloeodes*-complex, *B. (Ch.) pontica* sp. n. shows all the features delimiting the genus *Bungona* as defined by Salles et al. (2016): antenna short (Fig. 1B); spine-like setae between prostheca and mola of right mandible present (Fig. 2C); labial palp segment III distally expanded (Fig. 2E, G); setae on the margin of femur long (Fig. 3A, C, E); patella-tibial suture absent on fore tibia and located at middle length of the middle- and hind tibia (Fig. 3B, D, F); a row of conspicuous setae on the dorsal surface of the fore tibia exceeding half of the length of tibia (Fig. 3B); a row of conspicuous long setae present on abdominal sterna IV or V–VI (Fig. 5A); distance between the insertions of conspicuous long setae on the abdominal sterna greater than diameter of insertions (Fig. 5A); and secondary swimming setae on the outer margin of cerci present (Fig. 1A).

*Bungona (Ch.) pontica* also exhibits a characteristic shape of the claw with two rows of flattened denticles (Fig. 4E), a contrasting body colour pattern (Fig. 1A), and lanceolate scales on the surface of the body (Fig. 4A); all these characters are typical for the subgenus *Chopralla* (Salles et al. 2016). In fact, the only synapomorphy of *Chopralla* which is absent in the new species is the bifid shape of the right prostheca (Salles et al. 2016: fig. 4C–E). In *B. (Ch.) pontica* sp. n., as in *B. (Ch.) liebenauae* (Soldán, Braasch & Muu, 1987) from Vietnam, the slender medial process of the right prostheca is absent (Fig. 2C) and might represent a synapomorphy of this possibly closely related species or might be the result of independent reduction in both species. Despite their similarities, *B. (Ch.) pontica* can be separated from *B. (Ch.) liebenauae* by having shorter setae on the dorsal margin of the femur (their length reaching two-thirds of the femur width in *B. (Ch.) liebenauae* and one-quarter of the femur width in *B. (Ch.) pontica*) and by the absence of characteristic tubercles on the pronotum (present in *B. (Ch.) liebenauae*; Fig. 6).

According to the molecular reconstruction, it is clear that *B. (Ch.) pontica* sp. n. represents an isolated lineage, not forming a well-supported clade with any of the taxa



**Figure 9.** Molecular reconstruction including representative set of taxa of the *Cloeodes*-complex (comprising *Bungona (Chopralla) pontica* sp. n.) and additional taxa of other lineages. Bayesian inference was used to reconstruct the tree based on the mitochondrial DNA barcoding gene cytochrome *c* oxidase subunit 1. Bayesian posterior probabilities > 0.8 are indicated. Scale bar represents substitutions per site. Geographic origins of the specimens are indicated. Colours: green = *Bungona (Bungona)*; blue = *Bungona (Chopralla)*; purple = *Bungona (Centroptella)*; yellow = *Cloeodes*; white = other genera.

included in the analysis (Fig. 9). Due to the general lack of support especially in the deeper nodes of the cladogram, the phylogenetic position of *B. (Ch.) pontica* sp. n. within the *Cloeodes*-complex remains unsolved. The presumed position based on the morphological data is near the clade containing *Bungona (Chopralla)* sp. from Sumatra and *B. (Ch.) ceylonensis* from India. These two species formed a well-supported monophyletic group together with *D. improvida* from Madagascar (Fig. 9). This study represents the first analysis of molecular data within the *Cloeodes*-complex. A fine-scaled geographic taxon sampling combined with the use of additional markers will be needed to resolve the phylogenetic position of *B. (C.) pontica* sp. n. and more in general to solve the phylogenetic relationships within the *Cloeodes*-complex.

#### Biogeography: presence of tropical elements in the Middle East mayfly fauna

*Bungona (Chopralla) pontica* sp. n. represents a tropical mayfly lineage in the Asia Minor fauna. Within the *Cloe-*

*odes*-complex, *B. (Ch.) pontica* sp. n. exhibits morphologically high similarity to the Oriental genus *Bungona*, which is distributed in Australia, New Guinea, and southern, eastern and southeastern Asia (Marle et al. 2016, Salles et al. 2016). The closest morphological relative is *B. (Ch.) liebenauae*, described from Vietnam (Soldán et al. 1987). Although not supported by our molecular approach, an Oriental origin of *B. (Ch.) pontica* sp. n. seems most likely, based on morphological data.

The discovery of this new species in Asia Minor is rather unexpected, as other Turkish mayfly taxa have Palaearctic or West Palaearctic distributions (Salur et al. 2016). Only a few taxa with tropical affinities have been reported from Turkey and the Middle East until now; however, most of them should be considered as misidentifications or at least as questionable observations (see below).

The heptageniid *Thalerosphyrus determinatus* (Walker, 1853) was recorded from Turkey based on findings of Demoulin (1965) and Berker (1981), although the genus *Thalerosphyrus* Eaton, 1881 is considered endemic to

the Oriental realm (Webb and McCafferty 2008, Sartori 2014). Demoulin (1965) noted the resemblance of two immature larvae from near Ankara with *T. determinatus* as redescribed by Ulmer (1939) from Southeast Asia. Berker (1981) reported *T. determinatus* from the vicinity of the town of Elazığ but based this on the superficial similarity with Demoulin's (1965) figures. These figures (Demoulin 1965: fig. 4) do not show any of the diagnostic characters of *Thalerosphyrus* as defined in Webb and McCafferty (2008), and the specimens might represent some species of *Electrogena* or *Heptagenia*. *Thalerosphyrus determinatus* was included in recent Turkish checklists (Kazancı 2001, Kazancı and Türkmen 2012, Salur et al. 2016) but were highlighted as doubtfully occurring in Turkey. We conclude the occurrence of *T. determinatus* in Turkey is most probably based on misidentifications.

Another tropical mayfly lineage with a distribution pattern extended into the Middle East is the genus *Clypeocaenis* Soldán, 1978 (Caenidae), with most of its diversity occurring in the Oriental (Vietnam, Borneo, Sri Lanka, and India) and Afrotropical (Burkina Faso and South Africa) realms (Malzacher 2013). *Clypeocaenis bisetosa* Soldán, 1978 was partially collected in the Middle East (part of the type series comes from northern Iran, the other from India). Although the species was not recorded in Turkey, it undoubtedly represents an Oriental tropical element in the Middle East mayfly fauna (Soldán 1978).

In the family Baetidae, the fauna of the Arabian Peninsula shows clear affinities with the Afrotropical fauna. *Cloeon smaeleni* Lestage, 1924, is widely distributed in the Afrotropics; its distribution covers practically all of sub-Saharan Africa, including Madagascar and Reunion Island (Gattolliat and Rabeantoandro 2002), and it was recently reported as invasive in South America (Salles et al. 2014). In the Middle East, it was also reported from Yemen (Gillies 1985) and Saudi Arabia (Salles et al. 2014), but these populations probably represent a separate closely related species (Salles et al. 2014). Recent integrative studies have proven that the Afrotropical *Labiobaetis glaucus* (Agnew, 1961) also occurs along the south-western coast of Saudi Arabia (Gattolliat et al. 2018). However, these Afrotropical lineages reach their northern limit in the southern Arabian Peninsula and appear absent in Turkey and the Levant. *Baetis (Rhodobaetis) pseudogemellus* Soldán, 1977, was described from north-eastern Sudan (Soldán 1977) and was subsequently reported from south-eastern Turkey by Kazancı (2009) based on the observation of three larvae. Nevertheless, the identification of this material as *B. (R.) pseudogemellus* is highly questionable, taking into account that numerous similar species of *Rhodobaetis* occur in Turkey and surrounding areas (Godunko et al. 2015, Yanai et al. 2018).

Components of the Turkish fauna with tropical affinities were already described also in other freshwater invertebrates, including anostracans, caddisflies, sponges (Banarescu 1990, 1992), and most probably also gastropods (Heller 2007). Furthermore, many species of freshwater fish from the Middle East show an Oriental

origin (Heller 2001), as well as several lineages of terrestrial invertebrates (Yom-Tov and Tcherov 1988). The tropical Oriental taxa were supposed to have invaded the Middle East westwards from the Euphrates–Tigris basin (Heller 2007).

*Bungona (Ch.) pontica* n. sp. is the first mayfly species showing this unusual biogeographic pattern. The species is probably very rare in Turkey; our results only include six individuals from two neighboring localities, despite our extensive collecting throughout the country. It was also not found by other Turkish researchers (Salur et al. 2016) and has not been recorded in neighboring Georgia (Gabelashvili et al. 2018), Iran (Bojková et al. 2018), or Syria (Koch 1988). It might represent a relict population left after previous connection to the Oriental region.

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