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A new species of *Limnius* ILLIGER, 1802 from the Balkans, and notes on its sister species *L. colchicus* DELÈVE, 1963 (Coleoptera: Elmidae)

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Abstract

A new species of the genus *Limnius* ILLIGER, 1802 (Coleoptera: Elmidae), *L. balcanicus* JÄCH & BROJER sp.n., is described from Bosnia and Herzegovina, North Macedonia, Greece, and Bulgaria. The new species is sister to the allopatric *L. colchicus* DELÈVE, 1963 from Turkey, Georgia and Armenia. The latter is here recorded from seven Turkish provinces for the first time (Kastamonu, Amasya, Ordu, Rize, Erzurum, Artvin, Kars). The DNA barcode sequence for *L. balcanicus*, photographs of the habitus and the aedeagus, as well as a distribution map of both species are provided. For comparison, a habitus photograph of a syntype of *Limnius rambouseki* (MAŘAN, 1939) [= *L. perrisi* (DUFOUR, 1843)], described from North Macedonia, is also included.

Key words: Coleoptera, Elmidae, *Limnius balcanicus, Limnius colchicus, Limnius rambouseki*, new species, taxonomy, faunistics, new records, distribution, Southeast Europe, Turkey, DNA barcoding.

Introduction

The Natural History Museum Vienna, Austria (NMW) houses by far the largest collection of West Palearctic Elmidae. However, only a small portion of the specimens, especially those from Austria, have been securely identified so far. More than 1,000 specimens of *Limnius* ILLIGER, 1802 from Austria are databased, but more than 2,000 specimens of the genus, namely those from SE Europe, Turkey, Caucasus Region, Russia, Kazakhstan and Iran still remain unidentified or have been only tentatively determined.

Due to the remarkable variability of the elmid species, safe identification based on morphological characters is often difficult or even impossible, as long as the inter- and intrapopulational variability has not been thoroughly studied. To provide a profound morphological characterization for a certain species it is necessary to examine as many populations as possible from its entire distribution range. Furthermore, a comprehensive set of mitochondrial and nuclear DNA sequence data has been found to be indispensable for the species delimitation of certain closely related taxa, and has furthermore led to the unexpected discovery of a considerable number of undescribed species, especially in the taxonomically most intricate genera *Elmis* LATREILLE, 1802 and *Limnius* (see BRUVO MAĐARIĆ et al. 2018).

After decades of extensive collecting in poorly investigated areas and with the help of the growing number of molecular data, we are now able to successfully sort at least part of the rich material stored in the NMW by species and to evaluate the diagnostic characters and the populational and geographical variability and to clarify the distribution of these species. This would not be possible without such comprehensive samples, which are covering the entire distribution area.

Recently, we started to sort out specimens of *Limnius colchicus* DELÈVE, 1963, to transfer them to the main collection and to arrange them geographically from west to east. After its description, this poorly known species had hitherto been treated in only one taxonomic paper (BERTHÉLEMY 1979), and it had so far been recorded from Greece, Bulgaria, northeastern Turkey (type locality), Georgia and Armenia (JÄCH et al. 2016, JÄCH & KODADA 2016).

To our big surprise, we noticed that the European populations differed significantly from the Asian ones in constant external and aedeagal features. Furthermore, the European populations were found to be separated from the Asian ones by a wide distribution gap. After re-examination of the two known syntypes of *L. rambouseki* (MAŘAN, 1939), described from North Macedonia, it became evident that the European specimens of "*L. colchicus*" in fact represent a new species, which is described below.

Material and methods

A total of 95 specimens of Limnius balcanicus and L. colchicus was examined.

Abbreviations:

CPB	Coll. A.A. Prokin, Borok, Russia
NMP	National Museum (Natural History), Praha, Czechia
NMW	Naturhistorisches Museum Wien, Wien, Austria

SMTD Senckenberg Naturhistorische Sammlungen Dresden, Germany

DNA extraction, amplification and sequencing of the standard COI barcoding region (HEBERT et al. 2003) were performed as described in JÄCH et al (2023). Specimens of *Limnius balcanicus* and *L. perrisi* (DUFOUR, 1843) sequenced during the present study are deposited in the NMW; details about the vouchers are listed in the public BOLD dataset DS-NMWLIBA. Newly obtained sequences are deposited in BOLD database (BOLD-IDs: NMWEL085-24, NMWEL086-24, NMWEL087-24 and NMWEL088-24). Uncorrected p-distances were calculated with MEGA 7.0 (KUMAR et al. 2016).

Limnius balcanicus JÄCH & BROJER sp.n.

Limnius colchicus DELÈVE, 1963: BERTHÉLEMY 1979: 44 (partim). – GUÉORGUIEV 1991: 86 (partim). – JÄCH & AVTZIS 1999: 13. – JÄCH et al. 2006: 435 (partim). – JÄCH & KODADA 2016: 595 (partim). – JÄCH et al. 2016: 109 (partim).

TYPE LOCALITY: Trišćanski Potok (stream), 43°43'18.9"N 17°39'57.8"E, 530 m a.s.l., ca. 2 km WSW of Gračac, ca. 10 km NW of Jablanica, Herzegovina-Neretva Canton, Bosnia and Herzegovina.

TYPE MATERIAL: Holotype & (NWM): "BOSNIA & HERZEGOVINA Prozor-Rama, Trišćani Trišćanski Potok 43°43'18.9"N 17°39'57.8"E 530m a.s.l., 20.VII.1994 (BiH3)". Paratypes: BOSNIA and HERZEGOVINA: HER-ZEGOVINA-NERETVA CANTON: 19 exs. (NMP, NMW, SMTD), same data as for holotype; 2 o o (NMW, SMTD): Stream near Ploča, 43°48'6.8"N 17°33'53.2"E, 640 m a.s.l., 20.VII.1994 "(BiH2)". NORTH MACEDONIA: SOUTHEASTERN REGION, GEVGELIJA MUNICIPALITY: 1 & (NMW): Stream between Negorci (60 m a.s.l.) and Sermenin (500 m a.s.l.), exact elevation and coordinates not recorded, 12.VIII.1988, leg. M.A. Jäch "(85)". GREECE: EPIRUS: 1 ex. (NMW): Small mountain stream (tributary of Metsovo Brook), ca. 2 m wide, just below the town of Metsovo, ca. 1000 m a.s.l., 21.IX.1999, leg. M. Hess & U. Heckes "(1)"; 1 & (NMW): Source brook (of Kalaritikos River), ca. 50-80 cm wide, ca. 23 km ESE Ioannina, ca. 15 km SSW Metsovo, Mt. Lakmos, ca. 1430 m a.s.l., 24.IX.1999, leg. M. Hess & U. Heckes "(9)"; 1 ex. (NMW): "3 km NNE Greveniti, ca. 23 km NE Ioannina, 39°49.7'N 21°00.8'E, ca. 950 m a.s.l., 29.IV.2000, leg. A. Komarek, H. Schillhammer & H. Schönmann "(23)"; 3 exs. (NMW): Katara Pass, 1700 m a.s.l., 25.-28.VII.1990, leg. R. Schuh & P. Zabransky; 2 exs. (NMW): Zuzuliotiko, 1116 m a.s.l., 40.15065°N 21.08296°E, 2.VII.2024, leg. Birnstiel, Steffano & Schwingshackl "GR24/10"; EASTERN MACEDONIA AND THRACE: 4 exs. (NMW): Small stream, strongly shaded, ca. 1 m wide, on road from Drama to Livadero, ca. 5 km south of Livadero, ca. 400 m a.s.l., 9.VIII.1988, leg. M.A. Jäch "(76)"; 1 J (NMW): River, fast flowing, ca. 10 m wide, ca. 15 km north of Paranestion, ca. 9 km southwest of Dipotama, ca. 300 m a.s.l., 8.VIII.1988, leg. M.A. Jäch "(73)"; 20 exs. (NMW): Stream in valley east of Mt. Sapka, ca. 600 m a.s.l., 30.V.1989, leg. H. Malicky "(217)". BULGARIA: PLOVDIV PROVINCE: 5 exs. (NMW): Tributary of Belishka River, northwest of Belitsa, 41°49'41.8"N 24°53'25.9"E, 715 m a.s.l., 27.V.2015, leg. I. Ribera.

DESCRIPTION: Due to the similarity of *Limnius balcanicus* with its close relatives, *L. colchicus* and *L. perrisi*, we are not going to provide a full description of the new species. Instead, this description is based on differential diagnoses.

Habitus as in Fig. 1. Measurements (n = 31): length (pronotum + elytra): 2.31-2.78 mm (mean: 2.54 mm). Width: 1.19-1.35 mm (mean: 1.27 mm). Index (length/width): 1.86-2.27 (mean: 2.00).

Limnius balcanicus, *L. colchicus* and *L. perrisi* (Figs. 1–3) are characterized by the compact body form and by the lack of distinct strial punctures in the very narrowly impressed medial elytral striae.

The Bosnian specimens of *L. balcanicus* can be distinguished from Bosnian specimens of *L. perrisi*, which were collected together at the type locality of *L. balcanicus*, by the following characteristics:

- 1) Antennae predominantly yellowish brown, only apically darkened (in *L. perrisi* predominantly dark brown, only the basal segments yellowish brown).
- 2) Frons densely micropunctate, surface entirely dull (in *L. perrisi* moderately strongly punctate, interstices predominantly glabrous).
- 3) Pronotal carinae rather evenly curved (in L. perrisi unevenly, somewhat angulately curved).
- 4) Punctation on elytral intervals very fine or replaced by fine transverse microstriolation (in *L. perrisi* punctation on elytral intervals strong and distinct).
- 5) Fourth elytral interval subbasally not modified and not different from adjacent intervals (in *L. perrisi* more convex and slightly wider than the adjacent intervals.
- 6) Punctation of pronotal disc, metaventrite and abdomen stronger in L. perrisi.

These differences cannot generally be applied to other parts of the distribution area, because of the general variability of *L. perrisi*, almost every population seems to be slightly different.

In any case, the genetic distance in the COI barcoding region between *L. balcanicus* (specimens from the type locality and Bulgaria sequenced) and *L. perrisi* (including sequences from Germany and the type locality of *L. balcanicus*) is about 11%, and there is no doubt that *L. balcanicus* and *L. perrisi* are definitely distinct species.

The new species is certainly more closely related to *L. colchicus* than to *L. perrisi*. Externally, *L. balcanicus* can be quite easily distinguished from *L. colchicus* by the body form being narrower and less strongly convex, and by the elytral intervals being slightly flatter (Figs. 1–2).

Aedeagus (Figs. 4, 6; see also BERTHÉLEMY 1979: figs. 131–132, misident. as *L. colchicus*): Ventral sac in its "typical" form basally with numerous oblique elongate meshes usually turning into transverse or oblique curled folds towards the middle, essentially as figured by BERTHÉLEMY (1979: fig. 131); but these meshes and folds are quite variable and in reality very faint (see Fig. 4); in some specimens they are very hard to spot or obviously missing (e.g. in one teneral specimen from Greece (Epirus): 20 km E of Ioannina, 1,430 m a.s.l., 24.VIII.1999, leg. M. Hess & U. Heckes "9", NMW). The parameres are more slender (less spoon-like) in the apical half than in *L. colchicus*; in dorsal view, the inner margin of the parameres is less strongly curved subbasally (see Figs. 4, 6, and BERTHÉLEMY 1979: fig. 132).

Secondary sexual dimorphism: Males are easily distinguished from females by the presence of a short longitudinal median subapical carina on the metaventrite; in addition, the middle of the metaventrite is flat or slightly concave in females but slightly convex in males.



Fig. 1: Habitus of Limnius balcanicus, paratype, J, Greece, Epirus, Zuzuliotiko, NMW. Photo: M. Brojer.



Fig. 2: Habitus of Limnius colchicus, ç, Turkey, Trabzon Province, NMW. Photo: M. Brojer.



Fig. 3: Syntype of Limnius rambouseki, North Macedonia. Photo: J. Hájek.

VARIABILITY: The pronotal punctation varies from faint to distinctly impressed. The pronotal carinae vary from weakly and evenly curved to strongly unevenly curved, depending on the variable gibbosity of the pronotum. The transverse microstriolation on the elytral intervals is not always distinctly developed.

The width of the aedeagal fibula may vary strongly, even in specimens from the same area.

HABITAT: *Limnius balcanicus* lives in clear mountain streams at elevations from ca. 300–1,430 m a.s.l. At the type locality it was collected together with *L. perrisi*, *L. volckmari* (PANZER, 1793) (Elmidae) and *Hydraena pygmaea* WATERHOUSE, 1833 (Hydraenidae).

DISTRIBUTION: The new species is endemic to the Balkan Peninsula. It is so far known from Bosnia and Herzegovina (Herzegovina-Neretva Canton), North Macedonia (Southeastern Region), Greece (Epirus, Eastern Macedonia and Thrace), and Bulgaria (Plovdiv, Smolyan, and Sofia provinces). Very probably it occurs also in Montenegro, Serbia and Albania.

The first specimens of *L. balcanicus* were recorded by BERTHÉLEMY (1979) based on specimens (identified as *L. colchicus*) from Bulgaria (without detailed locality data). This record was obviously based on material collected from two Bulgarian localities in 1957, 1963 and 1977, which were later published by GUÉORGUIEV (1991): 1) Sofia Province: Vitosha, Boyana River; 2) Smolyan Province: Rhodope Mountains, Chudnite Mostove.



Figs. 4–7: Aedeagi, ventral view (4–5) and dorsal view (6–7): 4, 6) *L. balcanicus* (Greece, Epirus, Zuzuliotiko, NMW), 5, 7) *Limnius colchicus* (Armenia, Geghard, NMW).



Fig. 8: Distribution of *Limnius balcanicus* (red circles) and *L. colchicus* (blue circles) based on confirmed identifications of specimens housed in the NMW (the dot for Abkhasia is based on a photograph of a specimen deposited in CPB); grey circles: specimens recorded from Bulgaria by GUÉORGUIEV (1991).

ETYMOLOGY: The name is a Latin adjective and refers to the Balkan Peninsula, where the species is endemic.

Limnius colchicus DELÈVE, 1963

Limnius colchicus DELÈVE 1963: 7 (orig. descr.). – OLMI 1976a: 249, 1976b: 14. – BERTHÉLEMY & OLMI 1978: 317.
 – BERTHÉLEMY 1967: 258 ff., 1979: 44. – JÄCH 1984a: 35, 1984b: 141. – JÄCH & AVTZIS 1999: 13. – JÄCH et al. 2006: 435. – JÄCH & KODADA 2016: 595. – JÄCH et al. 2016: 109. – TAŞAR 2017: 4. – CHERTOPRUD et al. 2020: 280. – GROSSER et al. 2021: 36.

TYPE LOCALITY: Zigana, Gümüşhane Province, northeastern Turkey.

DIAGNOSIS: Habitus as in Fig. 2. Measurements (n = 27): length (pronotum + elytra): 2.27–2.64 mm (mean: 2.45 mm). Width: 1.22–1.41 mm (mean: 1.32 mm). Index (length/width): 1.77–1.97 (mean: 1.86).

Externally, *Limnius colchicus* can be distinguished from *L. balcanicus* by the (on average) shorter, more globular body form and the slightly convex elytral intervals (Figs. 1-2).

Limnius colchicus varies principally in the same characters as *L. balcanicus*. In addition, the interstices of the punctures on the pronotal disc are often rugoseley micropunctate or microreticulate.

The aedeagus (Figs. 5, 7) differs from that of *L. balcanicus* (Figs. 4, 6) in the parameres, which are distinctly wider and somewhat spoon-like in the apical half; in addition, the mesal margins of the parameres are basally distinctly curved in dorsal view.

It must be noted that BERTHÉLEMY (1979: fig. 131) depicted the aedeagus of a specimen from Bulgaria, which therefore belongs to *L. balcanicus* and not to *L. colchicus*.

HABITAT: *Limnius colchicus* lives in streams and small rivers flowing through forest; at higher elevations (Erzurum and Kars provinces) it is found also in unshaded streams flowing through meadows. This species is known from elevations of up to about 2,000 m a.s.l. (SE slope of Ovitdaği Pass, Erzurum Prov., Turkey, 31.V.1989, leg. S. Schödl "35", NMW; 10 km S of Sarıkamış, Kars Prov., Turkey, 8.VI.1989, leg. M.A. Jäch "70", NMW).

DISTRIBUTION: *Limnius colchicus* is so far known from Turkey (Kastamonu, Amasya, Ordu, Gümüşhane, Trabzon, Rize, Erzurum, Artvin, Kars), Georgia (Abkhazia, Adjara, Imereti, Samegrelo-Zemo Svaneti, Samtskhe-Javakheti), and Armenia (Kotayk, Vayots Dzor).

In the original description, this species was recorded only from the Turkish provinces of Gümüşhane and Trabzon, it is here newly recorded from seven additional Turkish provinces (see above).

CHERTOPRUD et al. (2020) recorded *Limnius colchicus* from three caves in Georgia (Gorakha, Kumistavi, Motena). We were able to examine two specimens (now deposited in the NMW) from the first two caves, which turned out to belong to *L. colchicus* (Gorakha Cave, 1 φ) and *L.* cf. *volckmari* (Kumistavi Cave, 1 σ). Unfortunately, we were not able to see any specimen from Motena Cave (the record of *L. colchicus* from this locality was repeated in GROSSER et al. 2021).

Discussion

Limnius balcanicus and *L. colchicus* are separated from each other by a distribution gap of about 600 km in NW Turkey. Despite of extensive collecting efforts by the staff of the NMW in NW Turkey no specimens of *L. balcanicus* or *L. colchicus* could be found in any of the provinces west of Kastamonu.

Remarkably, in the Yedigöller National Park (Bolu Province, northwestern Anatolia), specimens of *Limnius perrisi* were collected together with short convex specimens (1σ , $2 \varphi \varphi$, NMW) of *L*. cf. *volckmari*, which strongly resemble specimens of *L. balcanicus*, not only in the body form, but also in the narrow, indistinctly punctate elytral striae, but they differ in the aedeagus and the usually more distinctly punctate elytral intervals; specimens of *L. colchicus* or *L. balcanicus* could not be traced in the area, although the forest streams in the Yedigöller National Park appear to be ideal habitats for both species.

These remarkable specimens of *L*. cf. *volckmari* from Bolu (Yedigöller) and eight similar females from Istanbul Province (three localities, NMW) obviously refer to the globular form ("individus globuleux") of *L. volckmari* from Lesbos (Greece) and Istanbul Province ("Bosphore (Sariyer)") briefly discussed and illustrated by BERTHÉLEMY (1979: fig. 123).

Including *L. balcanicus*, there are now 15 species recognized in the genus *Limnius*. They are distributed from the British Isles and Northwest Africa eastwards to the Himalaya. With eight recognized species, Turkey is currently the most speciose country. Molecular data revealed a number of undescribed species (see BRUVO MAĐARIĆ et al. 2018). However, as the present paper has shown, the morphological variability is the main challenge in defining and delimiting undescribed species, especially in the genera *Elmis* and *Limnius*. Without such a large set of samples, we would have not been able to justifiably separate *L. balcanicus* from *L. colchicus* based on morphological characters. Although C. Berthélemy was an excellent taxonomist, he was not able to realize that the specimens of *L. balcanicus* from Bulgaria were different from *L. colchicus*, because there were no other specimens than those from Bulgaria and and the type material from NE Turkey available. Obviously, BERTHÉLEMY (1979) credited the external and aedeagal differences, in absence of sufficient samples, to general variability.

Acknowledgements

We are grateful to E. Birnstiel (Zürich, Switzerland), E. Chertoprud (Moscow, Russia), M. Hess & U. Heckes (Munich, Germany), A. Kasapoğlu (Erzurum, Turkey), H. Malicky (Lunz, Austria), I. Ribera (†), and R. Schuh (Wiener Neustadt, Austria), for the donation of specimens, and providing ecological data. Thanks are due to J. Hájek (NMP) for the photograph of the syntype of *L. rambouseki*, A.A. Prokin for sending a photograph of an Abkhasian specimen of *Limnius colchicus* from his collection, and to T. Spasojevic (NMW) for the interpretation of two Bulgarian localities.

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Zeitschrift/Journal: Koleopterologische Rundschau

Jahr/Year: 2024

Band/Volume: 94_2024

Autor(en)/Author(s): Jäch Manfred A., Brojer Michaela, Bruvo Madaric Branka, Freitag Hendrik

Artikel/Article: <u>A new species of Limnius ILLIGER, 1802 from the Balkans, and</u> notes on its sister species L. colchicus DELÈVE, 1963 (Coleoptera: Elmidae) 151-161