



Contribution to the taxonomy and biology of two Balkan endemic *Isoperla* Banks, 1906 (Plecoptera: Perlodidae) species

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Abstract

We contribute new information on the taxonomy and biology of two recently described Balkan endemic stonefly species of the genus *Isoperla* Banks, 1906 (Plecoptera: Perlodidae). The hitherto unknown larva of *Isoperla pesici* Murányi, 2011 can be distinguished from related species on the basis of more laterally positioned abdominal stripes, and the long hairs on the apical half of the cerci. This species has a wide distribution in the Central Balkans, and we add six new localities, including the first record from Albania. It emerges from late spring to mid-summer, and occurs at low to moderately high altitudes. *Isoperla pesici* seems to be associated with springs, especially karst spring outlets, although it occurs in both small and large springs. The hitherto unknown larva of *Isoperla citrina* Murányi, 2011 is distinguished by its brown coloration with an indistinct pattern, quadrangular lacinia and lack of long hairs on the apical half of the cerci. The female lacks distinctive terminalia or egg characters and its habitus is similar to the male and sufficient to distinguish it from other known Balkan taxa. The male mating call is a sequence of beat-groups with a total duration of 3–6 s (at 22 °C); each group generally containing 3 beats, while the beat repetition pattern within beat groups is rather simple. This species is restricted to the high mountains of the western-Central Balkans, where we record four new localities, including the first record from Macedonia. It emerges from June to October, and is associated with very small creeks, lined with bushes at high elevations. Amongst the accompanying species, eleven are reported for the first time from Albania: (*Brachyptera helenica* Aubert, 1956, *B. graeca* Berthélemy, 1971, *Leuctra bronislawi* Sowa, 1970a, *L. hirsuta* Bogoeu & Tabacaru, 1960, *L. hippopoides* Kaçanski & Zwick, 1970, *L. pseudosignifera* Aubert, 1954, *Protonemura hrabei* Raušer, 1956, *P. nitida* (Pictet, 1836), *Nemurella pictetii* (Klapálek, 1900), *Chloroperla russevi* Braasch, 1969 and *Siphonoperla neglecta* (Rostock, 1881)), while one from Macedonia (*Nemoura caligula* Zwick, 1978) and *Leuctra pseudohippopus* Raušer, 1965 is reported from outside the Moesian ranges for the first time.

Key words: *Isoperla pesici* Murányi, 2011, *I. citrina* Murányi, 2011, Albania, Macedonia (FYROM), Montenegro, larval description, bioacoustics, phenology, new records

Introduction

The genus *Isoperla* Banks, 1906 is the most species-rich genus among the stonefly family Perlodidae, with at least 180 valid species recognized from the Holarctic and Oriental Realms (DeWalt *et al.* 2015). The peak of their West Palaearctic diversity occurs in the Mediterranean, where at least 50 valid species are present, of which 38 taxa are restricted to the area (Graf *et al.* 2009; DeWalt *et al.* 2015). Twenty species are reported from the Balkans, 12 of which are endemic to the peninsula (Murányi 2011).

The early taxonomy of the genus was rather unsure, and West Palaearctic species received more definitive identification only by recognition of the importance of penial armatures (Despax 1936; Illies 1952). Thus, from the 19th and early 20th century we have only uncertain faunistical data on the Balkan *Isoperla*. Most

specimens are lost and some, such as the record of the Pyrenean endemic *I. viridinervis* (Pictet, 1865) from Bulgaria (Klapálek 1913), are obviously incorrect. The first precise reports, and the first *Isoperla* species ever described from the Balkans dates back to the 1950s (*I. graeca* Aubert, 1956 - presently synonymized with *I. tripartita tripartita* Illies, 1954a). During the period 1960–1980, our knowledge of the genus in the Balkans was significantly improved, with a further 12 taxa being described: one from Albania (Aubert 1964), five from Bulgaria (Raušer 1962, 1965; Sowa 1970b), two from Bosnia & Herzegovina (Aubert 1964; Kačanski & Zwick 1970), one from Slovenia (Tabacaru 1971), one from Greece (Zwick 1978) and two from Macedonia (Ikononov 1980). Ten of these are still only known from the Balkans. However, the next step was only taken recently, with the description of a further three species and re-description of seven additional taxa (Murányi 2011).

Similar to other stonefly taxa, the distribution of the Balkan endemic *Isoperla* is limited to four main regions: Western (Dinaric), Eastern (Moesian), Southern (Aegean) and Central Balkan ranges (Murányi 2009, 2011). None of them occur throughout the whole peninsula, nor in any more than one of these areas. Most of the endemic species are restricted to special habitats: e.g., large karst springs (*I. inermis* Kačanski & Zwick, 1970), high-mountain brooks (*I. vevčianensis* Ikononov, 1980) and even warm lowland streams (*I. obliqua* Zwick, 1978).

In this paper we contribute new information on the taxonomy and biology of two recently described Balkan endemics, *Isoperla pesici* Murányi, 2011 and *I. citrina* Murányi, 2011, based on new material collected during the last three years and the examination of old collections.

Material and methods

The specimens were stored in 70% ethanol and deposited in the Collection of Smaller Insect Orders, Department of Zoology, Hungarian Natural History Museum (HNHM), in the Invertebrate Collection of the Mátra Museum of Hungarian Natural History Museum (MM) and in the Wien Natural History Museum (WNHM). Morphological terminology follows Murányi (2011). Other stoneflies collected at each sampling site are enumerated in Table 1.

TABLE 1. Coexisting stoneflies found at the habitats of *Isoperla pesici* Murányi, 2011 and *I. citrina* Murányi, 2011—Localities, *I. pesici*: 1: Montenegro, Vidrovan (type locality); 2: Montenegro, Spuž (paratype locality); 3: Montenegro, Redice (paratype locality); 4: Montenegro, Dobrilovina; 5: Montenegro, Manastir Morača; 6: Montenegro, Daljam; 7: Montenegro, Godinje; 8: Albania, Ploshtan; 9: Albania, Shtyllë Pass; 10: Macedonia, Kočuli; 11: Macedonia, Nikolić; 12: Macedonia, Dedeli; *I. citrina*: 1: Albania, Radomirë (type locality); 2: Albania, Mt. Korab; 3: Macedonia, Bozovce; 4: Macedonia, Ehloec; 5: Macedonia, Nižepole.

coexisting species	Isoperla pesici												I. citrina loc.					
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	
<i>Taeniopteryx auberti</i> Kis & Sowa, 1964					+													
<i>Brachyptera risi</i> (Morton, 1896)																		
<i>B. beali beali</i> (Navás, 1924)																		
<i>B. helenica</i> Aubert, 1956					+													
<i>B. seticornis</i> (Klapálek, 1902)																		
<i>B. graeca</i> Berthélemy, 1971					+													
<i>B. macedonica</i> Ikononov, 1983																		
<i>B. tristis</i> (Klapálek, 1901)																		
<i>Rhabdiopteryx doiranensis</i> Ikononov, 1983																		
<i>Leuctra fusca fusca</i> (Linnaeus, 1758)																		

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TABLE 1. (Continued)

coexisting species	Isoperla pesici loc.												<i>I. citrina</i> loc.				
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5
<i>L. mortoni feheri</i> Murányi, 2007	+				+								+				
<i>L. major</i> Brinck, 1949	+				+												
<i>L. bronislawi</i> Sowa, 1970a													+				
<i>L. hirsuta</i> Bogoescu & Tabacaru, 1960	+								+								
<i>L. hippopus</i> Kempny, 1899					+					+							+
<i>L. hippopoides</i> Kačanski & Zwick, 1970									+								
<i>L. pseudohippopus</i> Raušer, 1965																	+
<i>L. olympia</i> Aubert, 1956										+							
<i>L. pseudosignifera</i> Aubert, 1954													+				
<i>L. dalmoni</i> Vinçon & Murányi, 2007																	+
<i>L. inermis</i> Kempny, 1899													+				
<i>L. quadrimaculata</i> Kis, 1963	+																
<i>L. metsovonica</i> Aubert, 1956																	+
<i>L. cf. metsovonica</i> Aubert, 1956									+								
<i>Capnopsis schilleri balcanica</i> Zwick, 1984													+				
<i>Zwicknia acuta</i> Murányi & Orci, 2014 (in: Murányi et al. 2014a)										+							
<i>Zwicknia</i> sp. n.													+				
<i>Capnioneura balkanica balkanica</i> Baumann & Kačanski, 1975					+												
<i>C. balkanica macedonica</i> Ikononov, 1978													+				
<i>C. valandovi</i> Ikononov, 1978													+				
<i>Amphinemura triangularis</i> (Ris, 1902)	+				+												
<i>A. quadrangularis</i> Zwick, 1978												+	+				
<i>A. sulcicollis</i> (Stephens, 1836)					+												
<i>Protonemura praecox praecox</i> (Morton, 1894)										+							+
<i>P. auberti</i> Illies, 1954b					+												
<i>P. intricata intricata</i> (Ris, 1902)	+	+											+				+
<i>P. rauschi</i> Theischinger, 1975										+						+	
<i>P. nitida</i> (Pictet, 1836)													+				
<i>P. autumnalis</i> Raušer, 1956	+												+				
<i>P. hrabei</i> Raušer, 1956					+												+
<i>P. montana</i> Kimmins, 1941	+																

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TABLE 1. (Continued)

coexisting species	Isoperla pesici loc.										<i>I. citrina</i> loc.						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5
<i>Nemoura cinerea cinerea</i> (Retzius, 1783)	+			+					+				+				+
<i>N. subtilis</i> Klapálek, 1896																+	+
<i>N. caligula</i> Zwick, 1978													+			+	
<i>N. anas</i> Murányi, 2007									+								
<i>N. peristeri</i> Aubert, 1963																	+
<i>N. uncinata</i> Despax, 1934																	+
<i>Nemoura</i> sp. n. (<i>brevipennis</i> group)					+												
<i>Nemoura</i> sp. n. (<i>marginata</i> group)									+								
<i>Nemurella pictetii</i> (Klapálek, 1900)	+		+						+								+
<i>Perla abdominalis</i> Burmeister, 1839										+							
<i>P. cf. pallida</i> Guérin-Méneville, 1838						+				+			+				+
<i>Dinocras megacephala</i> (Klapálek, 1907)	+																
<i>Perlodes intricatus</i> (Pictet, 1841)				+													
<i>Isoperla oxylepis balcanica</i> Raušer, 1962													+				
<i>Isoperla albanica</i> Aubert, 1964													+				
<i>I. cf. tripartita</i> Illies, 1954a													+				+
<i>Isoperla</i> sp. (larva only)					+												
<i>Chloroperla russevi</i> Braasch, 1969									+								
<i>Siphonoperla torrentium torrentium</i> (Pictet, 1841)					+												
<i>S. graeca</i> (Aubert, 1956)				+													
<i>S. neglecta</i> (Rostock, 1881)									+								+

Bioacoustic studies were done on a single male *I. citrina* collected in the Pelister Mts on 2.x.2013. Drumming signals were recorded using a small, 10 cm diameter dynamic speaker (SAL YD78, 8 Ohm) as a vibration transducer. The specimen was placed on its diaphragm, and the voltage fluctuations generated by the vibrational signal of the animal were recorded using a Zoom H4n digital recorder. Recordings were made indoors at an air temperature of 22 °C. The recorded signals were analysed using the software Adobe Audition 1.5 and oscillograms were generated using the R package seewave (Sueur *et al.* 2008).

Results

Isoperla pesici Murányi, 2011

Isoperla pesici Murányi, 2011—Murányi 2011: 33. (original description of male, female and egg); Murányi *et al.* 2014b: 73. (first record from Macedonia).

Isoperla tripartita tripartita Illies, 1954a—Murányi 2011 (*partim*): 24. (misidentification of a specimen from Ploshtan, Albania).

Isoperla graeca Aubert, 1956—Aubert 1964 (*partim*): 295. (misidentification of the above specimen from Ploshtan, Albania).

Type locality: Montenegro, Nikšić municipality, Vidrovan, outlet of the Vukovo Vrelo Spring, 675 m, N 42° 51.47' E 18° 56.59'.

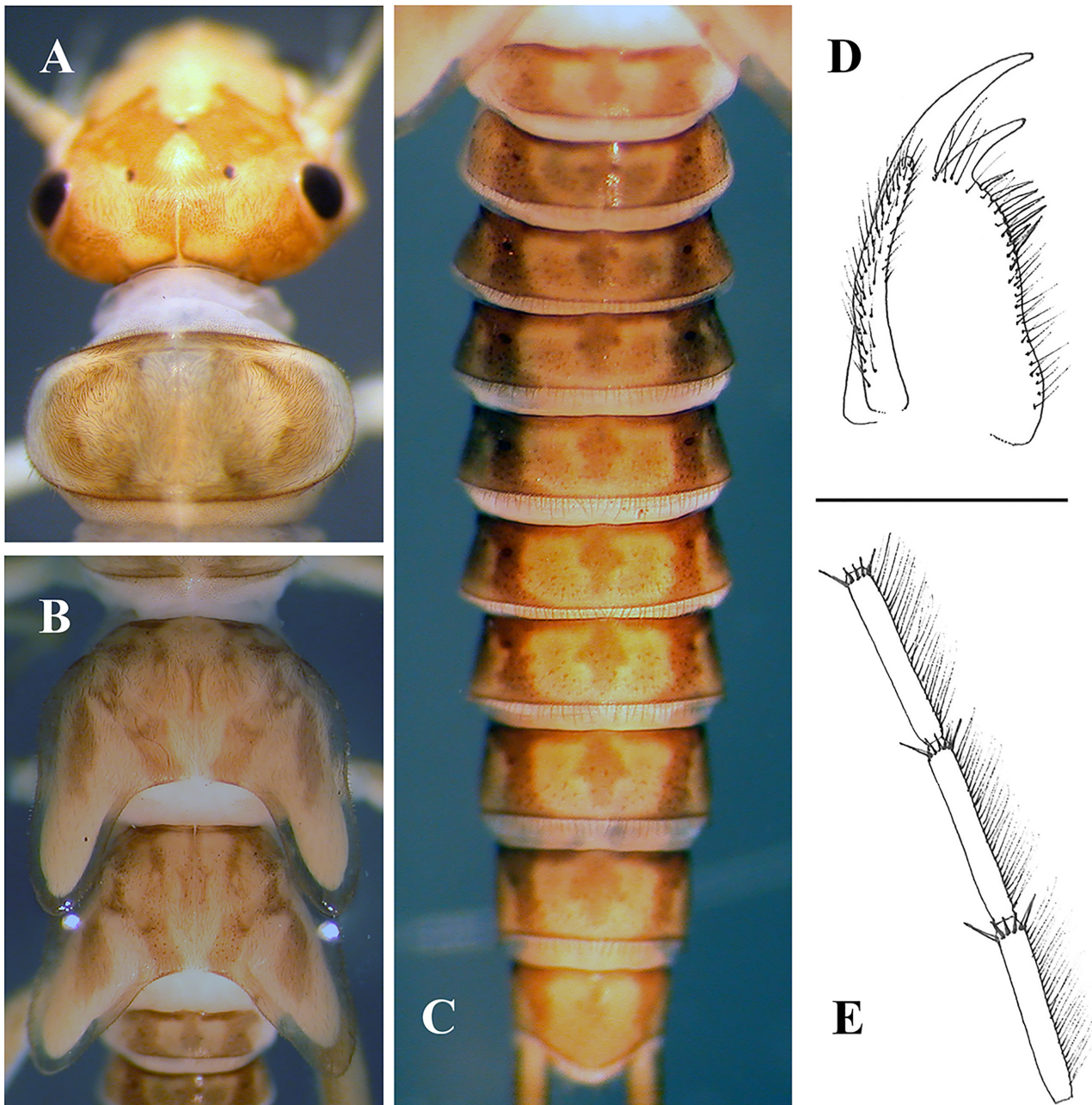


FIGURE 1. Larva of *Isoperla pesici* Murányi, 2011, Montenegro, Rumija Mts, Godinje—A: head and pronotum, dorsal view; B: mesonotum and metanotum, dorsal view; C: abdomen, dorsal view; D: left maxilla, dorsal view; E: distal segments of cerci, lateral view—scale 0.5 mm, Figs. A–C not to scale.

This species can be easily distinguished from other species of the *tripartita* species group *sensu* Murányi (2011) on the basis of reduced upper medial penial armature, unique collar-less eggs and the distinct coloration of the imagoes. It was described from karst spring outlets and thought to be a summer species restricted to Montenegro, until in 2014 we found it in southeast Macedonia in early May (Murányi *et al.* 2014b). The larva, lobes and fine structures of the penis, and the drumming calls hitherto remained unknown. Herein we describe the larva, give complementary description of some male imago structures, and outline its biology and distribution on the basis of new records.

New records: MONTENEGRO: Žabljak municipality, Sinjajevina Mts, Dobrilovina, forest stream at the monastery, 765 m, N 43° 01.652' E 19° 24.086', 14.vi.2012, leg. Zoltán Fehér, Tibor Kovács, Dávid Murányi: 2♀ (HNHM), 3♂, 3♀ (MM); same locality, 25.v.2013, leg. Péter Juhász, Tibor Kovács, Gábor Magos, Gellért Puskás: 5♂, 3♀ (MM); Kolašin municipality, Manastir Morača, karst spring outlet at the monastery, 300 m, N 42° 45.942' E 19° 23.436', 14.vi.2012, leg. Zoltán Fehér, Tibor Kovács, Dávid Murányi: 1♀ (HNHM), 1♀, 3

larvae (MM); Danilovgrad municipality, Daljam, Mareza Spring beneath the village, 30 m, N 42° 28.804' E 19° 10.905', 16.vi.2012, leg. Zoltán Fehér, Tibor Kovács, Dávid Murányi: 1♀ (HNHM); Bar municipality, Rumija Mts, Godinje, macchia brook at the village, 30 m, N 42° 13.245' E 19° 06.705', 16.vi.2012, leg. Zoltán Fehér, Tibor Kovács, Dávid Murányi: 1♂, 1♀, 4 larvae (HNHM), 2♂, 2♀ (MM).



FIGURE 2. Habitats of *Isoperla pesici* Murányi, 2011—A: Montenegro, Dobrilovina, forest stream; B: Montenegro, Daljam, Mareza Spring; C: Montenegro, Rumija Mts, Godinje, macchia brook; D: substrate of the same; E: Macedonia, Nikolić, substrate of a forest brook; F: Albania, Gropë Mts, brook N of Shtyllë Pass; G: substrate of the same.

ALBANIA: Kukës district, Ploshtan, 22.vii.1918, leg. Alban. Exp.: 1♂ (WNHM); Kukës district, Ploshtan, 2.viii.1918, leg. Alban. Exp.: 1♀ (WNHM); Mat district, Gropë Mts, brook along the Klos-Elbasan road, N of Shtyllë Pass, 1505 m, N 41° 22.455' E 20° 05.073', 20.vi.2012, leg. Zoltán Fehér, Tibor Kovács, Dávid Murányi: 3♂, 5♀ (HNHM), 7♂, 1♀ (MM).

Description of the larva: Body length of the mature larva: 13.5–16.0 mm (n=5, further larvae are penultimate). General colour yellow but with dark markings and pale legs (Figs. 1A–C, 7C). Pilosity usual for the genus, pronotal, posterior tergal and cercal fringes relatively long and acute; long hairs present on the femora, tibiae and metatarsi, distal half of the cerci with distinct dorsal hair fringe of erect hairs half as long as their segment, distal whorl of setae scarce and short (Fig. 1E). Head yellow anterior to the M-line, interocellar spot small, closed off posteriorly by pigment, large spot between the posterior ocellus and the compound eye on each side, connected to a pair of spots posterior to the complete occipital row of setae (Fig. 1A). M-line distinct, tentorial callosities barely visible; eyes normal sized. Scape, pedicel and the following two or three antennomeres are light brown, distal part of the antenna yellowish; palpi and mouthparts yellowish. Lacinia bidentate, triangular, with 6–7 strong setae situated on a shallow mound beneath subapical tooth, thin hairs present all along the inner margin to near base; galea setose on the whole outer, but only the apical half of the inner margin (Fig. 1D). Pronotum rounded, brown but with a wide medial yellow stripe along the medial suture, a pair of elongate dark brown areas and light inclusions present, lateral margins pale yellow (Fig. 1A). Mesonotum and metanotum mostly brown but with pale, marbled pattern; wingpads whitish, with an elongated central brown spot (Fig. 1B). Ventral surface of thorax pale, furcasternites and furcal pits slightly darker. Legs pale, dorsal surface of femora and tibiae slightly darker. Abdominal terga yellow with two more or less continuous, dark brown, longitudinal stripes along the abdomen, and medial, rhomboid brown patches on each segments (Fig. 1C). Transverse row of pigmented spots present on all segments. Ventral surface of abdomen pale, the distal segments usually darker. Paraprocts light brown; cerci light brown in the basal segments, distal part yellowish.

The larva of *I. pesici* can be distinguished from related species on the basis of more laterally positioned abdominal stripes, and apical half of the cerci has long hairs.

Complementary description of the male: Paraprocts brown, sharp, thin and recurved in dorsal view (Fig. 101 in Murányi (2011)). In caudal view, its base is wide and bear only small membranous portion; apex bluntly triangular, slightly diverging (Fig. 4B).

Lobes of the extruded penis, and the distribution of non-coloured scales, hairs and sensilla are similar to other members of the species group (Fig. 4A; compare with details depicted for different forms of *I. tripartita tripartita* and *I. autumnalis* Murányi, 2011 in Murányi (2011): Figs. 72–84, 114–120). The only probable specific difference is the wider distribution of small ciliated scales on the medial lobe. The presence of small lateral penial armatures on the Albanian specimens can be regarded as intraspecific variation: these are lacking on the types, and from most of the new specimens.

Distribution and biology: *Isoperla pesici* has a wide distribution in the Central Balkans, herein we report it from Albania for the first time (Fig. 7A). It seems to be associated with springs and headwater streams, but occurs in both small and large springs at different elevations. In the southern ranges of the Dinarids (Montenegrin localities), all of its known habitats are permanent karst springs. These include smaller outlets in moderately high elevation beech forest (Fig. 2A) to low elevation macchia (Fig. 2C–D) and large spring complexes in the foothills (Fig. 108 in Murányi (2011)), but we also found the species in channelized remnants of the outlet of a water-piped large spring on the plain (Fig. 2B). The only known Central Albanian habitat is quite different, being an autumn-dry spring brook that originates in beech forest, then flows through a subalpine meadows at considerably higher elevation (Fig. 2F–G). The southeast Macedonian habitats include both permanent and ephemeral habitats in beech forest, dense secondary forest and open macchia (Fig. E; and Figs. 5B, D in Murányi *et al.* (2014b)).

Isoperla pesici emerges from late spring to mid-summer. All the Montenegrin data are from June and the last days of May. Recent Albanian specimens were also caught in June, while the old specimens were caught late July and early August 1918. The Macedonian imagos were caught considerably earlier, in early May. However, that particular region in southeast Macedonia has already been characterized by the earlier emergence of spring stoneflies (Murányi *et al.* 2014b).

Unfortunately, the habitat of the old Albanian specimens from Ploshtan is unknown. They were overlooked and the male was enumerated as *I. tripartita tripartita* in Murányi (2011), but we confirmed their identity during a subsequent visit to the WNHM.

We found *I. pesici* together with 49 other stonefly species. Eighteen of these are Balkan endemics, the rest have wider distributions; most of them inhabit smaller streams (Graf *et al.* 2009). Types were collected together, or in the same habitat as 12 other species (Murányi 2011). We found a further autumnal species at the type locality in November 2011. In Macedonia, we reported *I. pesici* together with 17 species (Murányi *et al.* 2014b). Amongst the new Montenegrin localities, we found *I. pesici* together with six species at Dobrilovina. When we revisited the site in May 2013, we found a further three species. At Manastir Morača, *I. pesici* was collected together with four species, while during several previous and later visits, we found a further six species. Most of these taxa were already reported from this well-known site (Baumann & Kačanski 1975; Kačanski & Baumann 1981; Murányi 2008). In contrast, at the Mareza Spring we found only *Brachyptera tristis* (Klapálek, 1901) besides *I. pesici*, and it was the only stonefly we found at the macchia brook in the Rumija Mts. In the WNHM collection, *I. pesici* is also the only stonefly specimen bearing the locality label 'Ploshatan'. Finally, at the Central Albanian locality, *I. pesici* was found together with eight other stonefly species, though during October visits in 2002, 2004 and 2012 we found three further species (Murányi 2007). Among the co-occurring species, *Brachyptera helenica*, *B. graeca*, *Leuctra hirsuta*, *L. hippopoides*, *Nemurella pictetii*, *Chloroperla russevi* and *Siphonoperla neglecta* are new for the fauna of Albania. Most of these are common species in the Balkans or at least already reported from neighboring countries. *Brachyptera helenica*, *L. hippopoides* and *Chloroperla russevi* are Balkan endemics.

Isoperla citrina Murányi, 2011

Isoperla citrina Murányi, 2011—Murányi 2011: 12. (original description of male).

Type locality: Albania, Dibër district, Korab Mts, Radomirë, torrent E of the village, 1460 m, N 41° 49.131' E 20° 30.160'.

The male of this species can be easily distinguished from any other European *Isoperla* on the basis of the tricarinated medial penial armature and the pale coloration that is bright yellow in life (Fig. 7D). It was described from a single stream and thought to be a summer species restricted to the Korab Mts, the highest range of the western-Central Balkans. The female, egg, larva and the drumming calls were unknown. These are now described and an outline of its biology and distribution is given on the basis of new records.

New records: ALBANIA: Albania, Dibër district, Korab Mts, 3.5 km SE of Radomirë, spring area of right tributary of Elbini Stream, 1835 m, N 41° 48.180' E 20° 31.456', 11.x.2014, leg. Péter Juhász, Tibor Kovács, Gellért Puskás: 1♂ (MM).

MACEDONIA: Polog region, Šar Planina, Bozovce, open brook W of the village, 1810 m, N 42° 03.031' E 20° 47.059', 24.vi.2014, leg. Péter Juhász, Tibor Kovács, Dávid Murányi: 1♂, 1 larva (HNHM); Southwestern region, Stogovo Mts, Ehloec, beech forest SW of the village, 1190 m, N 41° 27.065' E 20° 44.505', 6.vi.2014, leg. V. Dusanek, R. Kundra, Tamás Németh: 1♂ (HNHM); Pelagonia region, Pelister Mts, Nižepole, bushy brook at the ski station, 1375 m, N 40° 58.787' E 21° 15.218', 2.x.2013, leg. Tibor Kovács, Dávid Murányi: 1♂, 1♀ (HNHM), 4♀, 2 larva (MM).

Description of the larva: Body length of the mature larva: 11.5 mm (n=1, the two larvae from Nižepole are immature). General colour brown, with small and indistinct paler markings (Figs. 3A–C). Pilosity usual for the genus, pronotal, posterior tergal and cercal fringes relatively long and mostly blunt; long hairs present on the femora, tibiae and metatarsi, distal half of the cerci lack long hairs, distal whorl of setae dense but short (Fig. 3E). Head uniformly brown with only four, small yellow spots: one pair lateroapically to posterior ocelli and one pair by the compound eyes; interocellar spot lacking, occipital row of setae scarce (Fig. 3A). M-line indistinct, tentorial callosities barely visible; eyes normal sized. Scape, pedicel and the following two antennomeres are light brown, distal part of the antenna paler; palpi and mouthparts yellowish. Lacinia bidentate, quadrangular, with 5 strong setae situated on a pronounced mound beneath subapical tooth, thin hairs scarce on the inner margin and finishes well before base; galea setose on the whole outer, and most of the inner margin (Fig. 3D). Pronotum wide and short, lateral sides rounded; brown but with yellow margins, rugosities indistinct (Fig. 3A). Mesonotum and metanotum mostly brown but with pale, marbled pattern; wingpads brown, with wide, pale stripes along inner margins (Fig. 3B). Ventral surface of thorax pale, furcasternites and furcal pits slightly darker. Legs brown, outer surface of femora and tibiae slightly darker. Abdominal terga brown with indistinct pattern; two wide, continuous paler longitudinal stripes can be observed, marking a medial dark line of rhomboid patches on each segments (Fig. 3C). Transverse row of pigmented spots present but hardly seen on all segments. Ventral surface of abdomen pale, the distal segments usually darker. Paraprocts brown; cerci light brown.

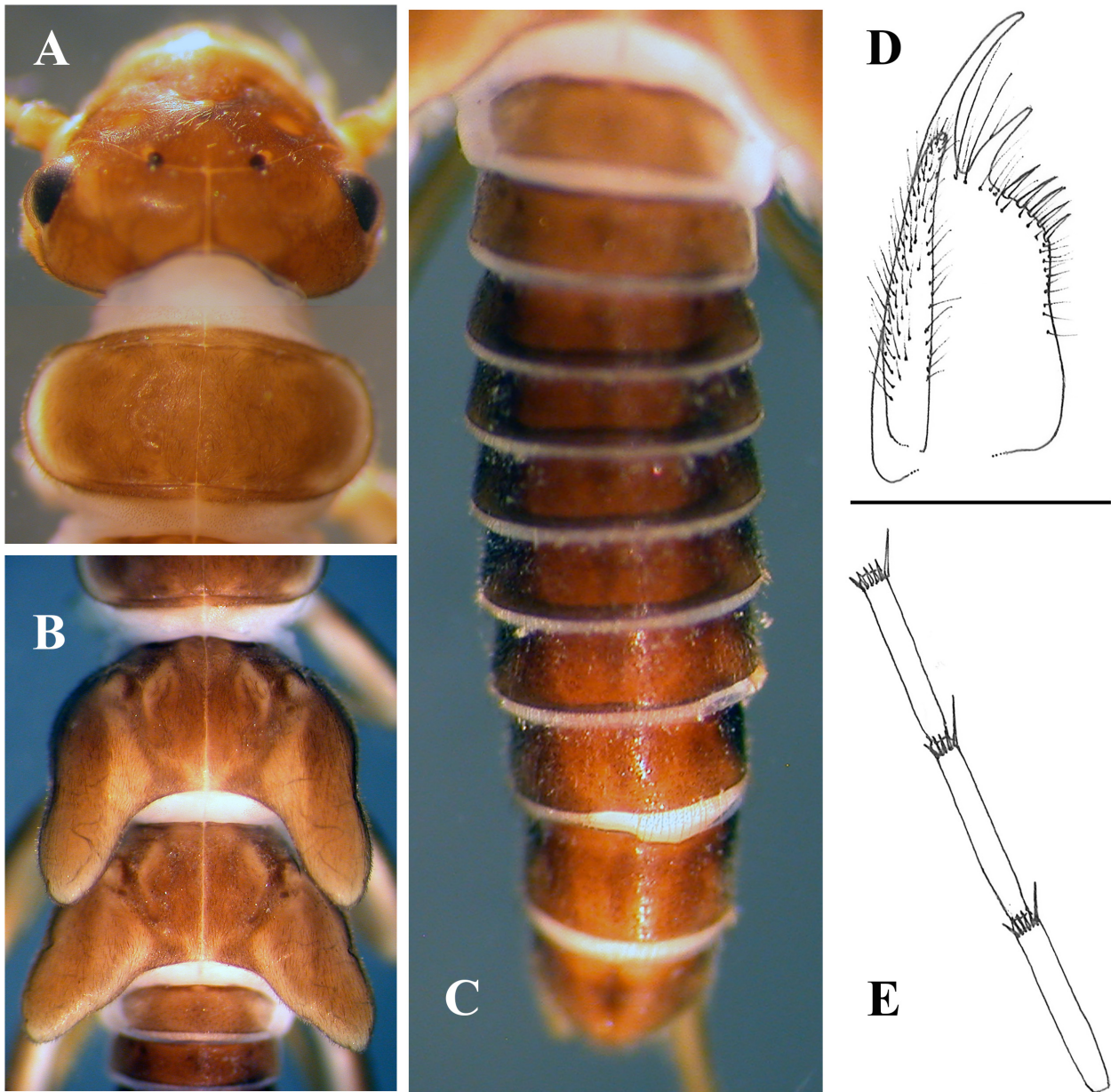


FIGURE 3. Larva of *Isoperla citrina* Murányi, 2011, Macedonia, Šar Planina, Bozovce—A: head and pronotum, dorsal view; B: mesonotum and metanotum, dorsal view; C: abdomen, dorsal view; D: left maxilla, dorsal view; E: distal segments of cerci, lateral view—scale 0.5 mm, Figs. A–C not to scale.

The larva of *I. citrina* can be distinguished from the known Balkan *Isoperla* larvae on the basis of brown coloration with an indistinct pattern, quadrangular lacinia and lack of long hairs on apical half of cercus.

Description of the female and egg: Female: Forewing length: 12.0–12.5 mm (n=5); habitus and coloration is similar to the male. Subgenital plate covers most of sternum VIII and slightly overlaps anterior part of sternum IX, most of the plate yellow, but with small basomedial darker patch; posterior margin rounded with a weak tip (Fig. 4E). Sternum IX yellow, bearing two small, brown lateral patches on the posterior half. Sternum X and the paraprocts yellow; the first two cercal segments yellow but distal parts of the cerci are dark brown.

Egg: The only available female lacked fully mature eggs. The immature eggs already had a distinct collar, and slightly quadrangular cross section. The mature eggs are likely to be similar to the type common amongst European *Isoperla* and probably also having the marked penta- or hexagonal pattern of FCIs on the chorion (this type of eggs are depicted for *I. albanica* Aubert, 1964 and the different forms of *I. tripartita tripartita* in Murányi (2011): Figs. 59–61, 85–90).

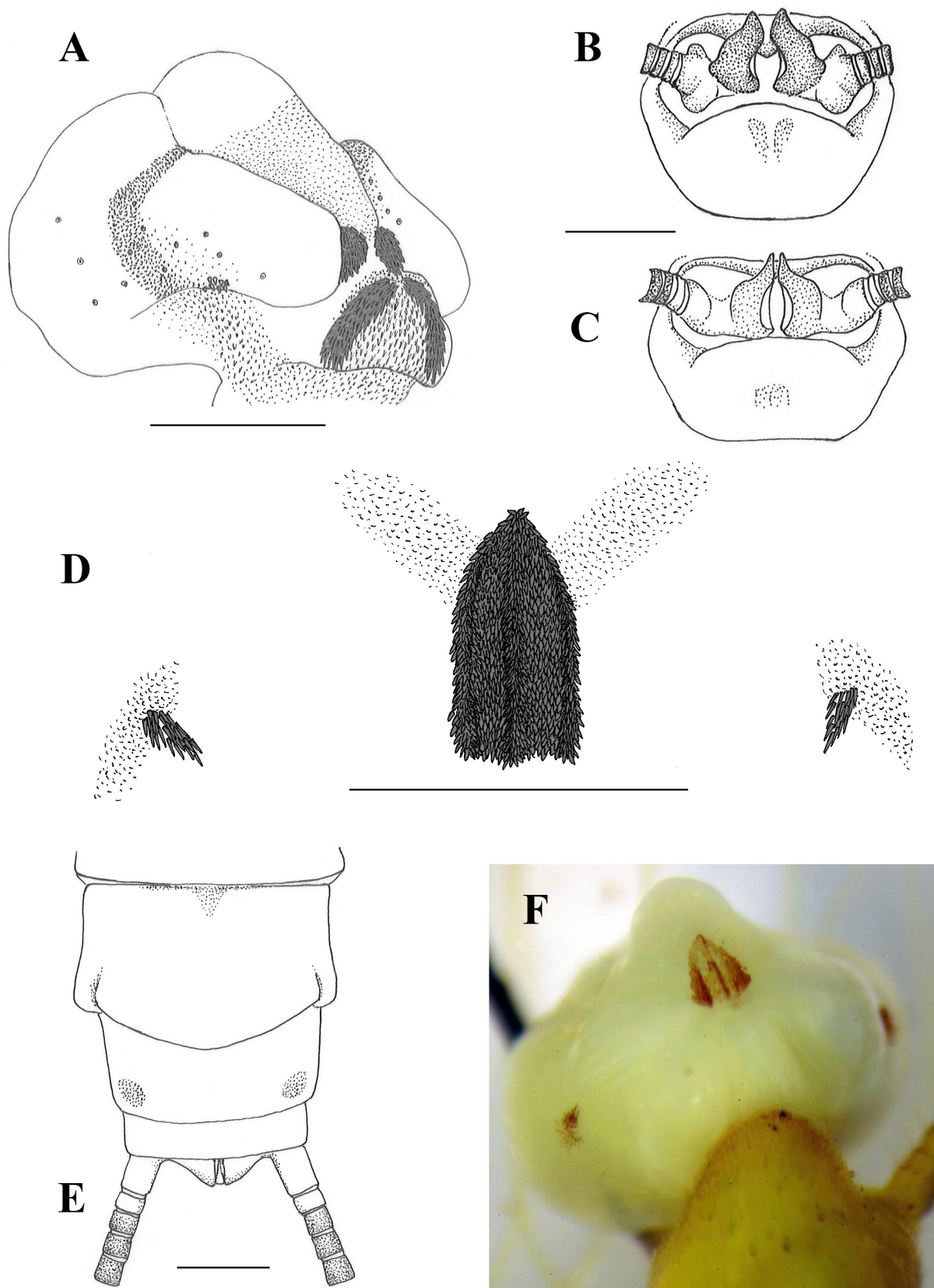


FIGURE 4. Imago of *Isoperla pesici* Murányi, 2011 (A–B: Albania, Gropë Mts, Shtyllë Pass) and *I. citrina* Murányi, 2011 (C–F: Macedonia, Pelister Mts, Nižepole)—A: extruded penis, ventrolateral view; B–C: male terminalia, caudal view; D: penial armatures, slide mounted; E: female terminalia, ventral view; F: extruded penis, ventrolateral view—scales 0.5 mm, Fig. F not to scale.

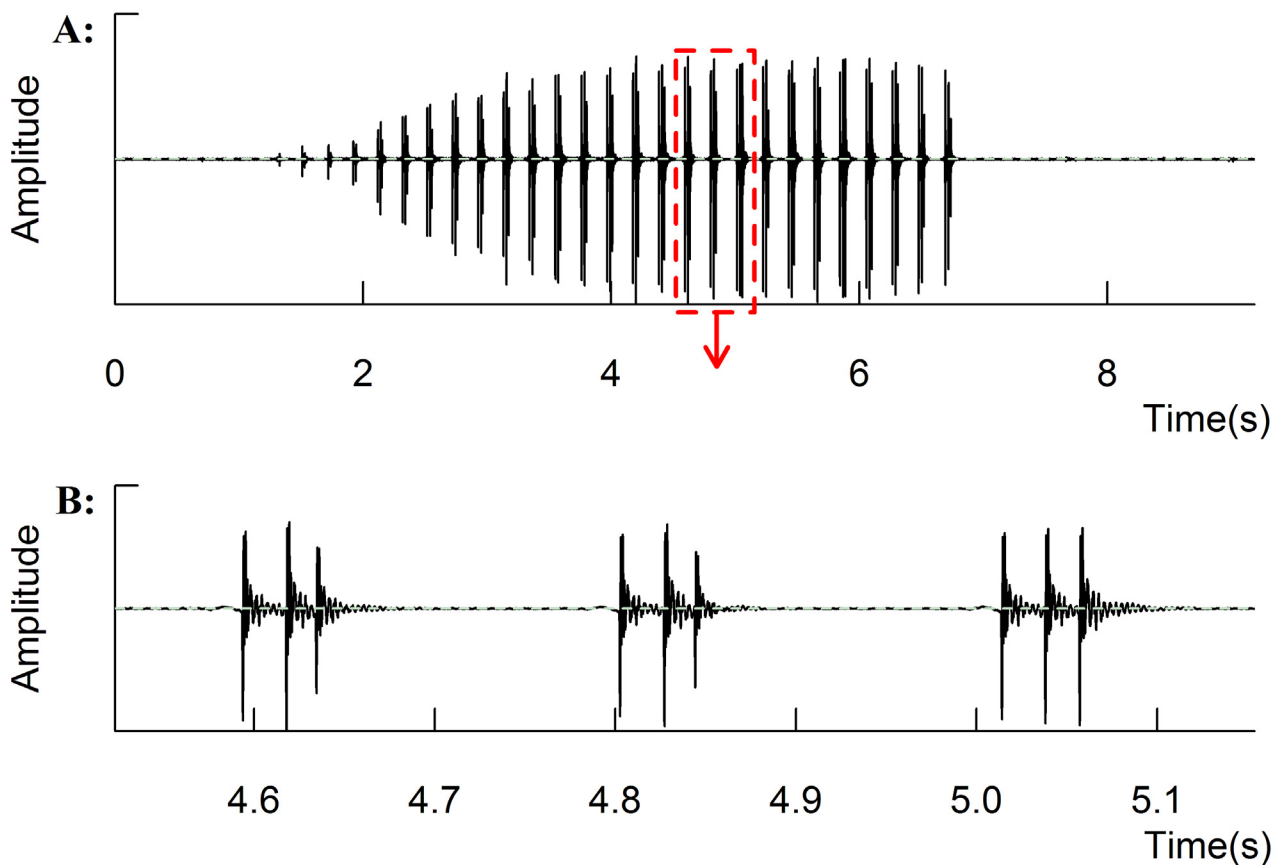


FIGURE 5. Male drumming call of *Isoperla citrina* Murányi, 2011, Macedonia, Pelister Mts, Nižepole—A: an oscillogram showing the whole beat-group series of a call; B: a faster oscillogram presenting the beat repetition pattern of three beat-groups from the middle section of the call—air temperature was 22 °C.

Although the female lacks distinctive terminalia or egg characters, its characteristic habitus and coloration are similar to the male and sufficient for distinction from other known Balkan taxa.

Complementary description of the male: Paraprocts brown, sharp, thin and slightly recurved in dorsal view (Fig. 27 in Murányi (2011)). In caudal view, its base is thin and curved around an inner membranous portion; apex simple, thin, erect and acute (Fig. 4C).

In the original description, penial armatures were depicted by SEM photography and diagrammatic figure on the extruded penis (Figs. 30–40 in Murányi (2011)). For comparative reasons, we figure the stretched armatures as they appear slide mounted (Fig. 4D), and a color photograph of the extruded penis (Fig. 4F).

Description of the male drumming call: We recorded 18 drumming calls of a single male of unknown age. The specimen, however, was apparently in good condition frequently producing calls.

The male produced drumming calls with a duration of 2.2–5.4 seconds (mean=4.35 s) consisting of a series of 11–27 beat-groups (mean=21.7 groups) (Fig. 5A). Within a call, beat-groups are repeated with a rather constant interval (slightly longer beat-group intervals can be observed at the beginning and end of each call), that varied between 159–191–(225) ms in the study specimen (65 inter beat-group interval measurement in 3 calls). Beat-groups contain typically 3 beats following each other with a longer and a shorter inter-beat interval as is observable in Fig. 5B. This signal pattern is a grouped call (Stewart & Sandberg 2006).

Amongst the European *Isoperla* species where grouped calls are reported, the drumming call of *I. citrina* is most similar to the calls of *I. rivulorum* (Pictet, 1841) (Rupprecht 1969; Luzón-Ortega *et al.* 2010) and *I. claudiae* (Graf & Konar 2014 (in: Graf *et al.* 2014)), since all these three species produce a low number of beats/beat-group and use a relatively simple beat repetition pattern within the beat-groups. However, the call pattern of *I. citrina* seems to differ from the calls of those species with regard to inter beat-group interval: 159–191 ms in *I. citrina* vs. 103–163 ms in *I. rivulorum* (Luzón-Ortega *et al.* 2010) and 230–350 in *I. claudiae* (Graf *et al.* 2014). Comparing to *I. claudiae*, it differs also in the number of beats/beat-group (3 in *I.*

citrina, 2 in *I. claudiae*). All these results and comparative notes have to be taken with caution due to the small samples. We have presently no information on the intra-specific variation pattern of the above described signal parameters, which will be necessary to have a better understanding of the taxonomic diagnostic value of these signal characters in the case of *I. citrina* and related species.

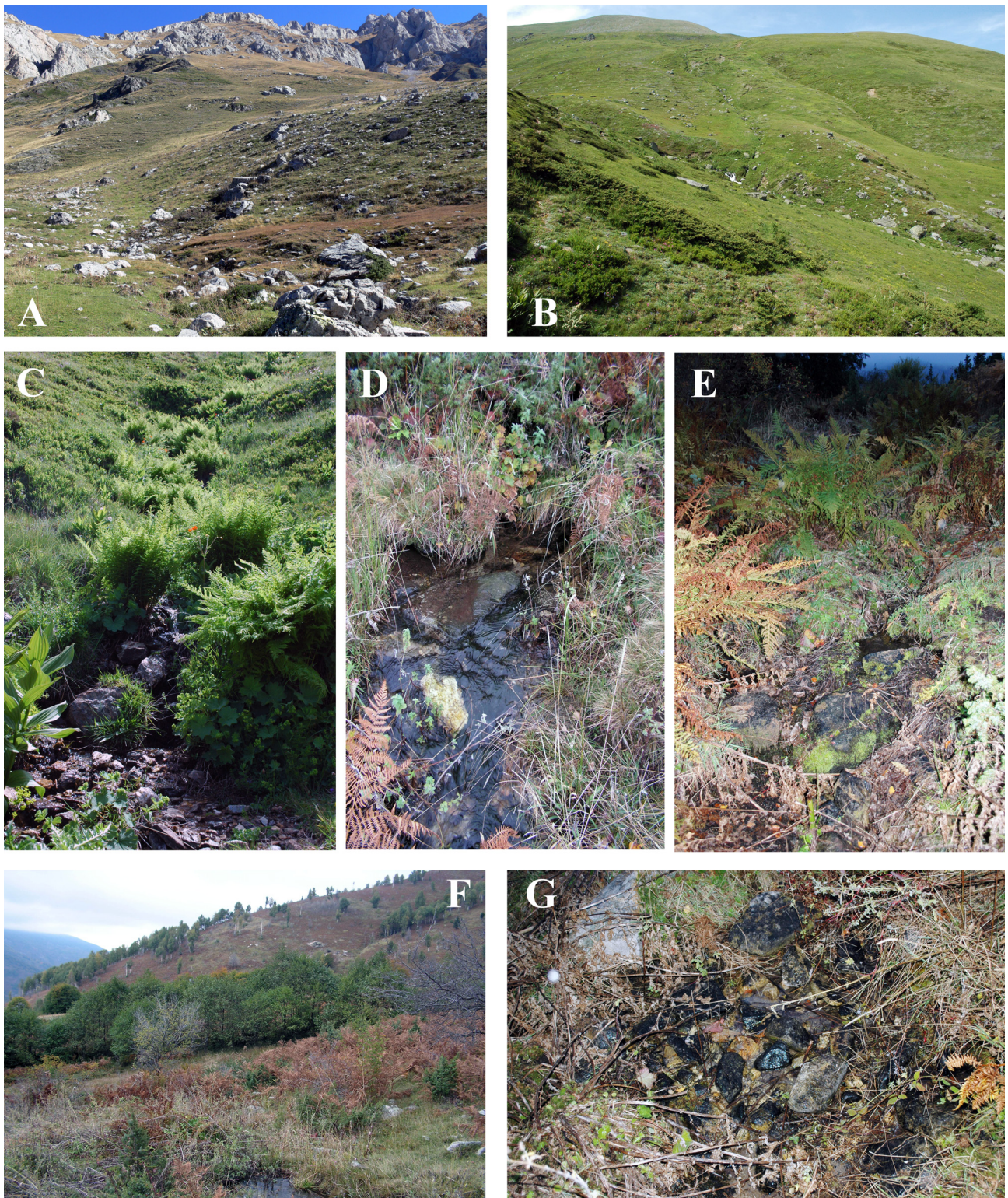


FIGURE 6. Habitats of *Isoperla citrina* Murányi, 2011—A: Albania, Mt. Korab, open brook and boggy source; B: Macedonia, Šar Planina, Bozovce, open brooks; C: close-up of the same; D: close-up and substrate of brook Fig. 6F; E: close-up of brook Fig. 6F; F: Macedonia, Pelister Mts, Nižepole, brook with riparian bush; G: substrate of the same.

Distribution and biology: *Isoperla citrina* is restricted to the high mountains of the western-Central Balkans, and we report it from Macedonia for the first time (Fig. 7B). It seems to be associated with very small creeks, with riparian bushes at high elevations, usually above the timberline. The type locality, a torrent in the Korab Mts is the largest stream where the species has so far been found (Figs. 41–42 in Murányi (2011)). All the subsequent records are from brooks less than 0.5 m wide, and only a few centimeters deep (Fig. 6). These brooks have a stony substrate with dense organic matter, and usually swampy edges overgrown by semiaquatic plants (Fig 6C–E, G). The specimen from the Stogovo Mts was swept from a beech forest, its habitat was probably a nearby forest seep or brook. In the Pelister Mts, we found *I. citrina* at the edge of a secondary timberline (Fig. 6F). In the Šar Planina, its locality is high in the alpine grasslands (Fig. 6B), and the new locality in the Korab is actually one of the highest water sources in the range, beneath the peak of Mt. Korab (Fig. 6A).

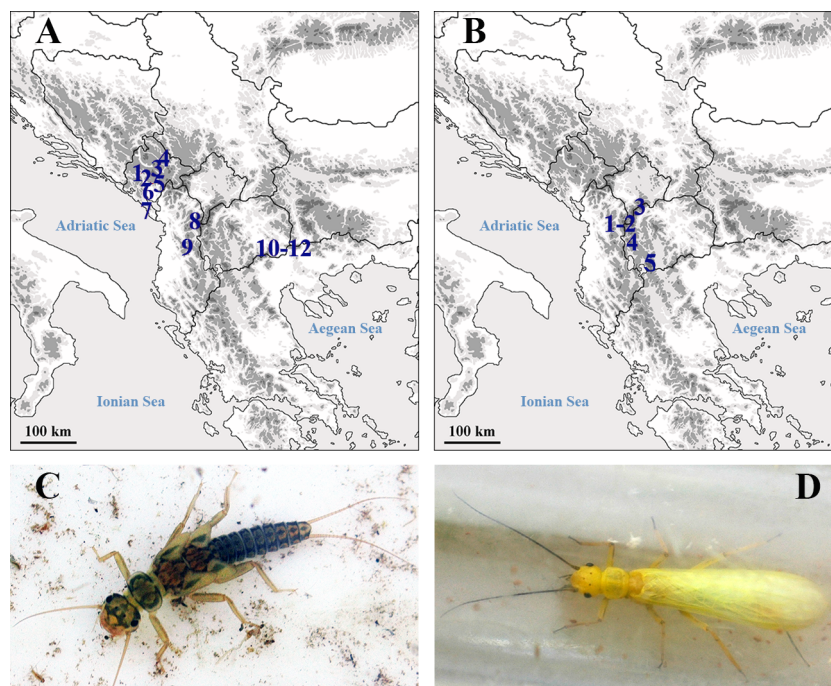


FIGURE 7. Distribution and habitus of *Isoperla pesici* Murányi, 2011 (A, C) and *I. citrina* Murányi, 2011 (B, D)—A–B: known occurrences; C: larva, Montenegro, Rumija Mts, Godinje; D: male imago, Macedonia, Pelister Mts, Nižepole—locality numbers are enumerated in Table 1.

Isoperla citrina emerges from June to October, which covers the whole snow-free period in most of its habitats. At the lowest locality (1190 m) in the Stogovo Mts, it was found in early June and probably adults do not survive there until the autumn. However, at the equally low elevation (1375 m) habitat in the Pelister Mts, we found young males and females in October, though specimens were not found when we visited the site in late June 2014.

We found the species together with 27 other stonefly taxa. Nine of these are Balkan endemics, the rest have wider distribution; nearly all of them inhabit small streams (Graf *et al.* 2009). The types were collected together with five other species (Murányi 2011). When we visited the site in May 2010, October 2012 and June 2013, we found a further ten species. At the high locality on Mt. Korab, the species was the only stonefly collected. We also found no other stonefly at the Šar Planina locality during our limited sampling, but neighboring brooks where we spent more time had a rich fauna, so it is most probably not the only stonefly species in that brook. In the beech forest on Stogovo Mts, it was found together with three species. Finally, in the small brook on the Pelister Mts, the species coexists with a rather rich stonefly fauna, and in October 2013 we found it together with *Protonemura hrabei* Raušer, 1956 and the endemic *Nemoura peristeri* Aubert, 1963. The same two species were found during a previous visit in October 2006, but *I. citrina* was not found on that occasion (Murányi 2007). Upon a return visit in May and June 2014, we found a further thirteen species. Among the co-occurring species, *Leuctra pseudosignifera*, *L. bronislawi*, *Protonemura hrabei* and *P. nitida* are new for Albania, while *Nemoura caligula* is new for the Macedonian fauna. The first four species are

common in the Balkans, but were not yet reported from Albania. *Nemoura caligula* is a Balkan endemic hitherto known only from Greece (Zwick 1978) and Albania (Murányi 2011). In addition, the occurrence of *Leuctra pseudohippopus* in the Pelister Mts constitutes its westernmost locality; this species hitherto was known only from Moesian ranges (Raušer 1965, Ikonov 1986).

Discussion

Despite recent efforts, more than half of the Balkan *Isoperla* must be regarded as poorly known (Murányi 2011). Besides incompleteness in their morphology, not a single molecular sequence has been published, and the present *I. citrina* male mating call is the first bioacoustic data ever reported from the *Isoperla* of the peninsula.

As was suggested in a previous work (Murányi 2011), Balkan populations of *I. tripartita tripartita* do not constitute a variable taxon but a complex of species. Our recent recordings proved that certain populations have rather different male mating calls. Unfortunately, these are not always matched with morphological and ecological differences, and morphological differences apply only to their distinct coloration. The otherwise useful genital characters are useless in this group. At present, these taxa would be diagnosed only as morphologically cryptic species. Drumming signals of the *tripartita* species group members, of which identification is solely on the basis of their genital morphology, all remained unknown: *I. autumnalis*, *I. illyrica* Tabacaru, 1971, *I. obliqua*, *I. pesici* and *I. tripartita recta* Zwick, 1978. Certainly, this work must be complemented with more fieldwork to obtain drumming signals, while molecular studies of these populations are at least underway.

Recent collecting has also shown that *I. russevi* Sowa, 1970b is not a member of the *saccii* species group sensu Consiglio (1967), but forms a species complex with *I. buresi* Raušer, 1962. Bulgarian and Macedonian specimens are morphologically indistinguishable in most cases, and species sometimes present complete transitional clines.

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