A New Species of *Isoperla* (Plecoptera: Perlodidae) from the Southern Carpathians, and Further Contributions to the Fauna of the Tarcu Mts

D. MURÁNYI1*, T. KOVÁCS2 and W. GRAF3

¹Department of Zoology, Institute of Biology, Eszterházy Károly University,
H-3300 Eger, Leányka út 6, Hungary

²Mátra Museum of the Hungarian Natural History Museum,
H-3200 Gyöngyös, Kossuth Lajos utca 40, Hungary

³Institute of Hydrobiology and Aquatic Ecology Management,
University of Natural Resources and Life Sciences,
A-1180 Vienna, Gregor Mendel Strasse 33, Austria

(Received: 30 September 2020; accepted: 18 November 2020)

Isoperla nagyi sp. n. is described on the basis of morphology of male and female adults and eggs. The new species is classified as an isolated species within the West Palaearctic Isoperla. It was found in the lower elevations of the Tarcu Mts that is forming a high range of the westernmost Southern Carpathians. Further contributions are given on the stonefly fauna of the Tarcu Mts, including notes on the Romanian distribution of Brachyptera bulgarica Raušer, and the taxonomy and distribution of Isoperla pusilla (Klapálek).

Keywords: Isoperla nagyi sp. n., Brachyptera bulgarica, Isoperla pusilla, taxonomy.

The genus *Isoperla* Banks is one of the largest group of stoneflies, distributed in the Holarctic and Oriental realms (DeWalt et al., 2020). In the West Palaearctic, the genus consists of closely related species groups, with peak of diversity in the Holomediterranean (Graf et al., 2009; Murányi, 2011). The Carpathians possess a relatively poor set of *Isoperla* species, with occurrence of 13 species confirmed (Kis, 1974; Žiak, 2016). Three of these are Carpathian endemics, all known only from the Southern Carpathians (Kis, 1974).

A decade ago, single male of a peculiar *Isoperla* was found in a lower elevation forest stream of the Tarcu Mts, a high range of the westernmost Southern Carpathians. In 2011, we took a collecting trip to this range, trying to find more specimens of this apparently new species. We found it in low number at the same locality, while collecting in various other localities of different elevations yielded no further specimens. Herein we describe this *Isoperla* as an isolated new species of the genus. In addition, we enumerate the rich stonefly material collected during the short trip to the Tarcu Mts, giving faunistic and taxonomic notes on two rare and poorly known species.

^{*}Corresponding author; e-mail: d.muranyi@gmail.com

Materials and Methods

Specimens were collected by hand, beating sheet or water net and stored in 75% ethanol. All materials were deposited in the Hungarian Natural History Museum, Budapest, Hungary (HNHM), the Mátra Museum of the HNHM, Gyöngyös, Hungary (MM), and the private collection of Wolfram Graf, Vienna, Austria (CWG).

(Fig. 3a-d), (Fig. 4a-f), (Fig. 5a-f) (SEM images) were made using a Hitachi S-2600N scanning electron microscope. Penis for SEM study was critical point dried and sputter coated with gold-palladium. (Fig. 1a-f), (Fig. 2a) were made by a drawing tube mounted on a Nikon SMZ1500 microscope. To avoid destruction of the paratype male, penial armature was not slide mounted and (Fig. 2b) was made on the basis of SEMs, counting how the structure may seen when flattened.

Morphological terminology follows our previous papers (Murányi, 2011; Murányi et al., 2016; Murányi et al., 2021).

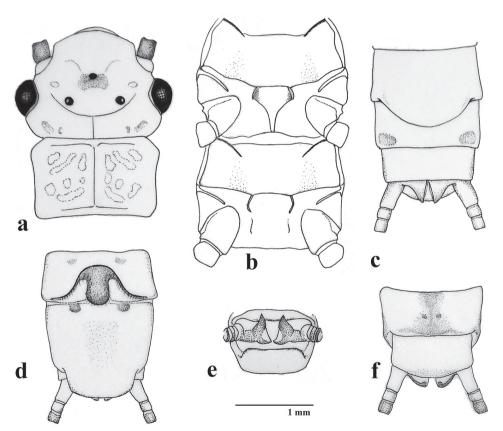


Fig. 1. Morphology of *Isoperla nagyi* sp. n. — a: head and pronotum, dorsal view; b: meso-and metathorax, ventral view; c: female terminalia, ventral view; d: male terminalia, ventral view; e: same, caudal view; f: same, dorsal view

Results

Isoperla nagyi sp. n. (Figs 1–7)

Type material. Holotype male: ROMANIA, Caraş-Severin county, Țarcu Mts, Poiana Mărului, forest stream NW of the village, 655 m a.s.l., N 45°25.031' E 22°29.167', leg. T. Kovács, D. Murányi, G. Puskás, 8.vi.2011 (HNHM: PLP3715). Paratypes: same locality and date: 1 female (HNHM: PLP3716); same locality and collectors, 10.vi.2011: 1 female (MM: PLETYP-27); same locality, leg. M. Bálint, P. Neu, 16.vi.2008: 1 male (CWG).

Diagnosis. Medium sized, pale species with yellow head and pronotum. Male medial penial armature stripe-like consisting of spike-like scales, lateral penial armatures lacking, inner portion of lateral penial lobes with dense uncoloured scales, ventral penial lobe with uncoloured patch of strong scales. Female subgenital plate widely rounded, egg oval, with marked ornamentation of FCIs, collar with double row but rim not flanged, micropyles not raised.

Description. Medium sized species, macropterous in both sexes (Fig. 6a). Body length: holotype male 11.0 mm, paratype male 10.5 mm, paratype females 12.5–13.0 mm; forewing length: holotype male 12.0 mm, paratype male 12.0 mm, paratype females 13.0–14.0 mm. General colour bright yellow in life, whitish in ethanol; brown pattern occur on dorsum of meso- and metanotum, abdomen and legs. Pilosity of the body and legs short and dense. Head yellow with a single dark spot around anterior ocellus; tentorial callosities and M-line barely visible, occiput with two indistinct rugosities (Fig. 1a). Eyes smaller than the area delimited by the three distinct ocelli. Scape dark brown, pedicel and the following three or four antennomeres brown but the remainder dark brown; palpi dark brown. Pronotum rectangular with rounded corners, narrower than head and entirely

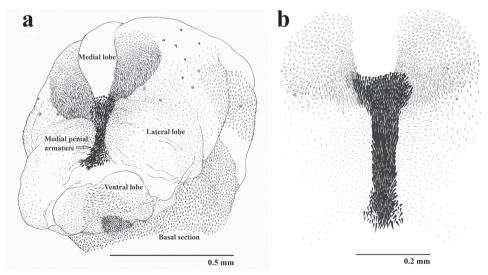


Fig. 2. Penial structure of *Isoperla nagyi* sp. n. — a: extruded penis, dorsolateral view; b: medial penial armature, mounted slide view

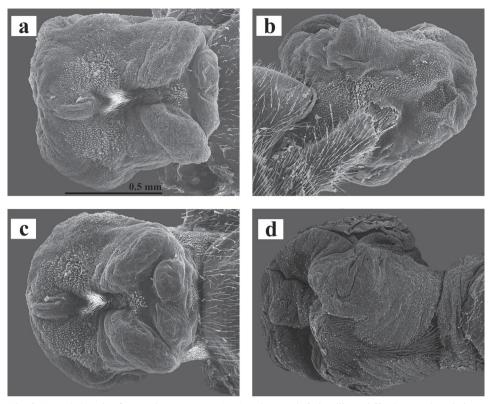


Fig. 3. Extruded penis of *Isoperla nagyi* sp. n. (a–c) and *Isoperla belai* Illies, 1963 (d) — a: dorsal view; b: lateral view; c: basal view; d: dorsolateral view

yellow; rugosities are large and few, inconspicuous (Fig. 1a). Mesonotum mostly yellow with brown scutellum, metanotum has larger brown pattern, distributed also on the medial portion of scutum. Wings yellowish, venation yellow in the anterior half, gradually turning light brown then brown towards the apex. Ventral surface of thorax yellow, meso- and metabasisternum with indistinct brownish hint in the line with the base of coxae; furcasterna light brown, furcal pits brown (Fig. 1b). Femora and tibiae yellow with longitudinal dark brown stripe on the dorsal surface; tarsi brown.

Male abdomen: First six terga mostly brown, the next three gradually turn lighter, with dark pattern limited to medial and posteromedial portion; tergum X is entirely yellow, with brownish hints on the posterior edge (Fig. 1f). Transverse row of four pigmented spots on terga II–VIII, tergum IX has only two spots (the medial pair), tergum X lack spots. Ventral surface of abdomen yellow, sterna II–VII have a brown transverse anterior line interrupted in the middle; sterna II–VIII with a medial transverse row of two spots, lateral ones not recognizable. Vesicle of sternum VIII large and brown, longer than wide, its posterior margin is rounded; longer than half of the segment's length (Fig. 1d). Sternum IX yellow with a pair of brown patch on the anterior edge, and brownish hint in the medial portion; apex subdivided from the anterior two thirds of the plate, posterior edge widely rounded. Paraproct brown, acute, relatively thin and recurved in dorsal view (Fig. 1f); in caudal view, its slightly widened base bear only small membranous portion,

apex triangular and slightly diverging (Fig. 1e). The first two cercal segments yellow but further cercomeres gradually turn to light brown then brown.

Penis: Divided into four lobes and a basal section in extruded position (Fig. 2a), (Fig. 3a-c). The medial penial armature located between the lateral lobes, on the basal section of medial lobe adjacent to ventral lobe; lateral penial armatures lacking. The medial penial armature elongated, stripe-like, upper portion hardly delimited from colourless scales of the lateral lobes; length 420-440 µm, width 120-140 µm (Fig. 2b). The scales are spike-like, but those on the lower part are generally shorter and wider; width 5-8 μm, length 25-50 μm (Fig. 4a-b). The ventral lobe is hemispherical, most of the surface covered with branched hair-like scales, gradually turn into simple hair-like and ciliated scales laterally; lateral area left bald; ventromedial section bears a central patch of strong triangular scales that may refer to a lower portion of medial penial armature but are uncoloured (Fig. 4d). The medial lobe is small and elongated, bald besides the medial penial armature and the diverse scales that spread on the lateral edges from the lateral lobes. The lateral lobes are strong and elongated, bent above basal section; portion above ventral lobe is nearly bald, bearing only scattered ciliated and simple hair-like setae, while those gradually turn into branched hair-like setae towards the inner lateral portion meeting the medial penial armature; inner portion above the medial penial armature is densely covered with variable scales, junction to the armature consist of triangular scales that gradually turn into elongated, then short hydra-like scales (Fig. 4c); further inner portion by the medial lobe has a wrinkled bald surface with a few sensillae (Fig. 4e); the outer lateral portion is armed with a transverse stripe of dense hydra-like scales mixed with sensillae, spreading to the edge of basal portion (Fig. 4f). The basal section is covered all with triangular scales.

Female abdomen: Terga I–VIII brown, terga VIII–IX light brown, tergum X yellowish. Terga II–IX with a medial transverse row of two spots, lateral ones not recognizable. Sterna entirely yellow, transverse line and row of spots not recognizable, but sternum IX bear light brown to brown posterolateral patches. Subgenital plate covers most of sternum VIII and the anterior half of sternum IX; the plate is yellow, posterior margin evenly rounded (Fig. 1c). Paraproct light brown. The first two cercal segments yellow but further cercomeres gradually turn into light brown then brown.

Egg: Chorion brown to dark brown, 0.32–0.38 mm long and 0.28–0.32 mm wide (n = 32). Shape oval, lateral sides sometimes depressed, hatching line inconspicuous (Fig. 5a-b). Micropyles placed in a transverse row on the opercular third, not raised, each located at the meeting of carinae between follicular cell impressions (FCIs) (Fig. 5e-f). Chorion with marked ornamentation of hexa- and pentagonal FCIs with fine inner punctuation, depth and width of their rim depends on egg condition (compare Fig. 5a,c,e with Fig. 5b,d,f). Collar round, bearing two distinct rows of FCIs, rim not flanged but the ridge between the two FCIs rows is rather strong (Fig. 5c-d). Anchor flat, anchor surface structure in development stage 1 (unmodified), according to Isobe (1997), globular bodies distributed in the whole area.

Larva: unknown.

Affinities. The new species cannot be assigned to any of the West Palaearctic species groups as defined by Consiglio (1967) and Murányi (2011), and should be treated as an isolated species. The position and scales of the medial penial armature may suggest affinity towards the *silesica* group, but the recently described fine structures of the penis of *Isoperla vevcianensis* Ikonomov differs greatly from those of the new species (Murányi et

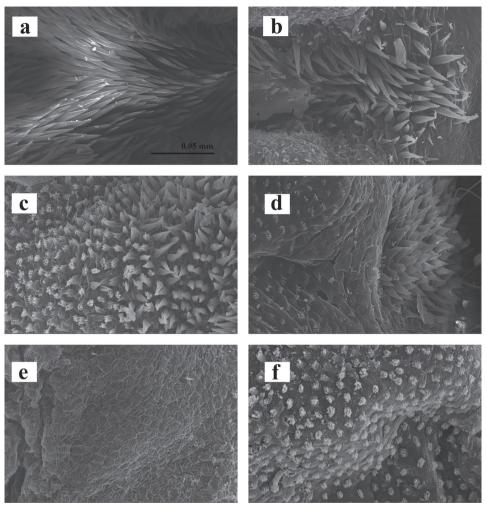


Fig. 4. Fine penial structures of *Isoperla nagyi* sp. n. — a: upper portion of the medial penial armature; b: lower portion of the medial penial armature; c: triangular and hydra-like scales of the lateral lobe at the junction to the medial penial armature; d: triangular scale patch and branched hair-like scales on the ventral lobe; e: bald wrinkled surface and sensillae on the apical portion of lateral lobe; f: hydra-like scales on the medial portion of lateral lobe

al., 2021). The medial penial armature shares some superficial resemblance with *I. belai* Illies, however, that species belongs to the *albanica* group since the medial penial armature is located on the ventral lobe of the penis (Fig. 3d). In addition, *I. belai* possesses lateral penial armatures, and has a very different colour pattern. Regarding outer morphology, bright yellow habitus of the new species resembles to the South Carpathian endemic *I. flava* Kis and the western-Central Balkan endemic *I. citrina* Murányi. Both of these species belong to the *rivulorum* group, having very different penial structures and armatures than those of the new species. The egg of *I. nagyi* has the usual structure of European *Isoperla*, hence identification of the female must rely on colour pattern.

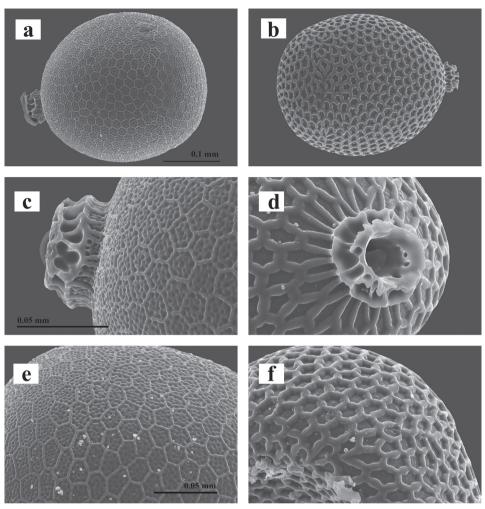


Fig. 5. Eggs of *Isoperla nagyi* sp. n. in different condition— a–b: whole egg; c: collar, lateral view; d: same, apical view; e–f: chorion with micropyles

Ecology and distribution. The species is known only from the type locality, where it was collected in June of 2008 and 2011. The habitat is a submontane stream that flows in beech forest, and crossing a dirt road at the collecting site where dense riparian vegetation consists mostly of *Petasites hybridus* (Fig. 6b). The stream is located on the lower elevations of the Tarcu Mts that is forming a high range of the westernmost Southern Carpathians, connected to the Retezat and Godeanu Mts (Fig. 7). The mostly crystalline Tarcu Mts is separated from the southeast located Godeanu Mts by valleys of the streams Râul Rece and Şes, while from the east located Retezat Mts by the valley of River Râul Mare. The new species is apparently rare, we did not found it at other localities during our collecting in the Tarcu Mts, and the number of adults of two other congeners, *I. buresi* Raušer and *I. pusilla* (Klapálek) far exceeded *I. nagyi* at the type locality. The stream owns a rich stonefly fauna, besides the three *Isoperla*, we collected *Brachyptera*

bulgarica Raušer, B. seticornis (Klapálek), Leuctra hippopus Kempny, L. nigra (Olivier), L. quadrimaculata Kis, Protonemura illiesi Kis, P. intricata (Ris), Nemoura cinerea (Retzius), Nemurella pictetii (Klapálek), Perla pallida Guérin-Méneville and Siphonoperla neglecta (Rostock).



Fig. 6. Habitus and habitat of $Isoperla\ nagyi$ sp. n. — a: holotype male; b: type locality

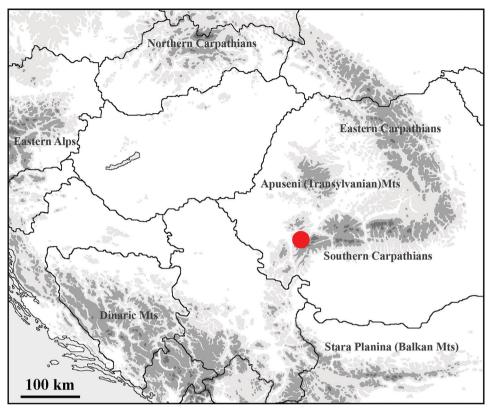


Fig. 7. Situation of the type locality (red dot) of *Isoperla nagyi* sp. n. in the Carpathian-Pannonian Region

Etymology. The species is dedicated to our recently departed colleague, Dr. Barnabás Nagy (1922–2020), renowned orthopterologist and plant protection expert. He showed us an example of active work even in his 90s. Used as the genitive of a noun of male gender.

Plecoptera specimens collected in the Țarcu Mts by T. Kovács, D. Murányi and G. Puskás

Brachyptera bulgarica Raušer, 1962:

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 1Q (MM); Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 1Q (HNHM), 1Q (MM).

Remarks: This species is known from Bulgaria, North Macedonia and Romania (Kis, 1972b; Ikonomov, 1986; Tyufekchieva et al., 2019). The Romanian occurrence was overlooked in most catalogues (e.g. DeWalt et al., 2020), since it was only briefly reported in a least known faunistic paper but was not included neither in the review of the Romanian occurrence.

nian *Brachyptera* species, nor in the widely used Romanian Plecoptera monograph (Kis, 1972a; Kis, 1974). The previous Romanian records are all from the Banat region (southern Apuseni (Transylvanian) Mts range: Zarand Mts; and western Southern Carpathians range: Poiana Ruscă Mts, Valea Mara) (Kis, 1972b). Our recent collecting confirms its presence in the lower and medium elevations of the Banat.

Brachyptera seticornis (Klapálek, 1902):

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 1Q dead specimen (MM); Open stream on the N slope of Mt. Țarcu, 1500 m, N 45°17.678' E 22°31.742', 9.vi.2011: 10° 1Q (MM).

Leuctra hippopus Kempny, 1899:

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011:4Q (HNHM).

Leuctra inermis Kempny, 1899:

Open stream on the N slope of Mt. Țarcu, 1500 m, N 45°17.678' E 22°31.742', 9.vi.2011: 1Q (HNHM).

Leuctra nigra (Olivier, 1811):

Poiana Mărului, forest stream NW of the village, 655 m, N $45^{\circ}25.031$ 'E $22^{\circ}29.167$ ', 8.vi.2011: $8^{\circ}7^{\circ}$ (HNHM), $5^{\circ}5^{\circ}$ (MM); Poiana Mărului, forest brook SW of the village, 1215 m, N $45^{\circ}20.329$ ' E $22^{\circ}29.144$ ', 9.vi.2011: $14^{\circ}7$ (HNHM), $6^{\circ}7$ 40 (MM); Spring and its outlet at Cuntu Meteorological Station, 1465 m, N $45^{\circ}18.008$ ' E $22^{\circ}30.059$ ', 9.vi.2011: $9^{\circ}7$ $16^{\circ}7$ (HNHM), $10^{\circ}7$ $19^{\circ}7$ (MM).

Leuctra quadrimaculata Kis, 1963:

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 8° 5° (HNHM), 3° 5° (MM); Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 1° 1° (HNHM).

Leuctra rauscheri Aubert, 1957:

Open stream on the N slope of Mt. Tarcu, 1500 m, N 45°17.678' E 22°31.742', 9.vi.2011: 3σ 9Q, 6 larvae (HNHM), 4σ 7Q (MM).

Leuctra rosinae Kempny, 1900:

Open stream on the N slope of Mt. Țarcu, 1500 m, N 45°17.678' E 22°31.742', 9.vi.2011: 1Q (HNHM), 1Q (MM).

Protonemura auberti Illies, 1954:

Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 10' (MM); Spring and its outlet at Cuntu Meteorological Station, 1465 m, N 45°18.008' E 22°30.059', 9.vi.2011: 10' (MM).

Protonemura illiesi Kis, 1963:

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 1Q (HNHM); Spring and its outlet at Cuntu Meteorological Station, 1465 m, N 45°18.008' E 22°30.059', 9.vi.2011: 1O 1Q, 2 larvae (HNHM).

Protonemura intricata intricata (Ris, 1902):

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 1°C (HNHM), 1°C 2°C (MM).

Protonemura pseudonimborum Kis, 1965:

Open stream on the N slope of Mt. Tarcu, 1500 m, N 45°17.678' E 22°31.742', 9.vi.2011: 5Q (HNHM), 1° 6Q (MM).

Nemoura cinerea cinerea (Retzius, 1783):

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 2Q (HNHM), 3Q (MM); Spring and its outlet at Cuntu Meteorological Station, 1465 m, N 45°18.008' E 22°30.059', 9.vi.2011: 1O (HNHM).

Nemoura longicauda Kis, 1964:

Spring and its outlet at Cuntu Meteorological Station, 1465 m, N 45°18.008' E 22°30.059', 9.vi.2011: 15' 49 (HNHM), 15' 109 (MM).

Nemoura marginata Pictet, 1836:

Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 10' (MM).

Nemoura transsylvanica Kis, 1963:

Spring and its outlet at Cuntu Meteorological Station, 1465 m, N 45°18.008' E 22°30.059', 9.vi.2011: 2Q (HNHM), 2O 7Q (MM); Open brook on the W slope of Mt. Tarcu, 1770 m, N 45°17.329' E 22°31.081', 9.vi.2011: 1O 1Q, 5 larvae (HNHM), 4O 1Q (MM).

Nemurella pictetii (Klapálek, 1900):

Poiana Mărului, forest stream NW of the village, 655 m, N $45^{\circ}25.031$ ' E $22^{\circ}29.167$ ', 8.vi.2011: 1Q (HNHM); Open stream on the N slope of Mt. Tarcu, 1500 m, N $45^{\circ}17.678$ ' E $22^{\circ}31.742$ ', 9.vi.2011: 1Q (HNHM), 1Q (MM); Spring and its outlet at Cuntu Meteorological Station, 1465 m, N $45^{\circ}18.008$ ' E $22^{\circ}30.059$ ', 9.vi.2011: 1Q (MM).

Perla pallida Guérin-Méneville, 1843:

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 2Q larva (HNHM); Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 10 larva (HNHM).

Arcynopteryx dichroa (McLachlan, 1872):

Spring and its outlet at Cuntu Meteorological Station, 1465 m, N 45°18.008' E 22°30.059', 9.vi.2011: 10' larva (HNHM), 20' 10, 10' larva, 20' exuviae (MM); Open stream on the N slope of Mt. Tarcu, 1500 m, N 45°17.678' E 22°31.742', 9.vi.2011: 10' 10' larva (HNHM), 10' larva (MM).

Isoperla buresi Raušer, 1962:

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 5°C 1°Q (HNHM), 1°C 1°Q (MM).

Isoperla pusilla (Klapálek, 1923):

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 3° 15°Q (HNHM), 2° 3°Q (MM); Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 2°Q (HNHM).

Remarks: Generic and specific identity, as well the type locality of the inadequately known *Chloroperla pusilla* Klapálek was clarified in the late eighties by Zwick (1989). On the basis of syntypes, it proved to be conspecific with the Southern Carpathian species *Isoperla minima* Illies, described from the same massif (Iezer Mts) of the eastern Southern Carpathians (Illies, 1963; Kis, 1974; Zwick, 1989). This species is probably endemic to the Southern Carpathians, though was reported also from Slovenia (Sivec, 1979). Since it is not known from the relatively well explored northern Balkans, this rather isolated occurrence should be confirmed. The species is rather uniform and morphologically distinct in the eastern Southern Carpathians, and typical specimens occur in the western Southern Carpathians as well. However, in the Țarcu and Semenic Mts we found much bigger and differently coloured specimens, sometimes co-occurring with, or applying different habitat than the typical specimens. SEM examination of their penises showed no distinct difference between the two forms. Bioacustic and molecular studies will be needed to reveal if the different forms are having taxonomic value.

Siphonoperla neglecta (Rostock, 1881):

Poiana Mărului, forest stream NW of the village, 655 m, N 45°25.031' E 22°29.167', 8.vi.2011: 2° 3°Q (HNHM) 5°Q (MM); Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 7°C 2°Q (HNHM), 3°C 3°Q (MM).

Siphonoperla transsylvanica (Kis, 1963):

Poiana Mărului, forest brook SW of the village, 1215 m, N 45°20.329' E 22°29.144', 9.vi.2011: 10' (HNHM), 10' (MM).

Discussion

The herein described *Isoperla nagyi* sp. n. explain morphological affinities towards the *silesica* group, and more superficially to the *albanica* and *rivulorum* groups of the genus *Isoperla*. However, it cannot be placed in any of these groups and must be treated as an isolated species within the West Palaearctic *Isoperla*. It is apparently rare, and probably endemic to the Southern Carpathians, presently known only from a single low elevation forest stream of the Tarcu Mts.

Besides the new species, the Țarcu Mts owns a rather rich stonefly fauna. Hitherto there are 38 species reported, among them 8 that are firstly reported in the present paper (Brachyptera bulgarica, B. seticornis, Leuctra hippopus, L. quadrimaculata, L. rauscheri, L. rosinae, Isoperla nagyi, Siphonoperla transsylvanica). We did not find 14 species that were previously reported from the range (Kis, 1974): Leuctra digitata Kempny, L. fusca (Linnaeus), L. major Brinck, Amphinemura triangularis (Ris), Nemoura cambrica Stephens, N. carpathica Illies, N. fusca Kis, Protonemura autumnalis Raušer, P. hrabei Raušer, P. montana Kimmins, P. nitida (Stephens), Perla marginata (Panzer), Perlodes microcephalus (Pictet) and Chloroperla kisi Zwick. About half of the 38 species are typical montane species, with the most orophilic high montane species Leuctra rosinae, Nemoura transsylvanica, Protonemura pseudonimborum, P. montana and Arcynopteryx dichroa. Seven of the known species are Carpathian endemics (Protonemura pseudonimborum, Nemoura carpathica, N. fusca, N. transsylvanica, Isoperla nagyi, I. pusilla, Chloroperla kisi, Siphonoperla transsylvanica).

Acknowledgements

We are indebted to Miklós Bálint (Senckenberg Institute, Frankfurt am Main, Germany), Peter Neu (Kasel, Germany) and Gellért Puskás (HNHM, Budapest, Hungary) for taking part in the collecting, and to Krisztina Buczkó (HNHM, Budapest, Hungary) for help in using the Hitachi S-2600N scanning electron microscope of the HNHM.

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