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ISOPERLA PROKOPOVI, A NEW PRESUMEDLY PARTHENOGENETIC STONEFLY FROM CRIMEA (PLECOPTERA, PERLODIDAE)

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ABSTRACT

Isoperla prokopovi sp. n. from Crimea is described from females, larvae, and eggs. Males or male larvae were not found, despite concerted efforts. The species is therefore believed to be parthenogenetic. The life cycle appears to be univoltine, possibly with a long egg diapause. The thick egg chorion differs from other Perloidea in that it disintegrates in KOH. *Isoperla prokopovi* is an isolated species without apparent close relatives among the West Palaearctic *Isoperla* spp.

Keywords: Taxonomy, Ukraine, Crimea, new species, parthenogenesis, life cycle

INTRODUCTION

For many years, unusually large *Isoperla* sp. larvae were collected in various streams on Crimea, mainly in the Great Canyon of Crimea, near the junction of the rivers Almachuk and Auzun-Uzen'. In 2009 G. Prokopov succeeded to rear adults, which were all females. Numerous attempts by G. Prokopov to collect or rear males or to detect male larvae failed. We therefore assume that the new species lacks males completely and is parthenogenetic.

Isoperla prokopovi Zhiltzova et Zwick, sp. n.

(Figs. 1-10)

Material examined. All specimens are from Ukraine, Autonomous Republic of the Crimea.

Holotype ♀, 15.06.2004 Riv. Small Salgir (Malyi Salgir). Adult reared in cage. G. Prokopov, adult with exuviae and eggs on microscope slide [in Zoological Institute, Russian Academy of Sciences, St. Petersburg). *Paratypes*: 1♀, 7.06.89, Crimea, Riv. Small Salgir (Malyi Salgir). Sample II. L. Zhiltzova. 2♀, 9 last instar L, Crimea, end of May 2008, brook Almachuk (basin of river Bel'bek) G. Prokopov, plus 1 exuviae without type status. 5 last instar L, 29.05.89. Crimea. Spring Hasta-Bash. L. Zhiltzova [in Zoological Institute, Russian Academy of Sciences, St. Petersburg, except 1♀, 4 last instar larvae in coll. Zwick].

Additional material. 13 young L, River Guva (Derekoika) upper part (near Yalta), 14.10.2011 [G. Prokopov, coll. Zwick]. 16 young L, River Auzun-Uzen' (Grand Canyon of Crimea), 18.11.2011 [G. Prokopov, coll. Zwick].

Additional material communicated by G. Prokopov, collectors' names in square brackets. Spring Hasta-Bash (under Ai-Petry mountain): 3L, 29.05.1984 [G. Kiseleva]; 6L, 19.04.1988 [L. Zhiltzova]; 11L,

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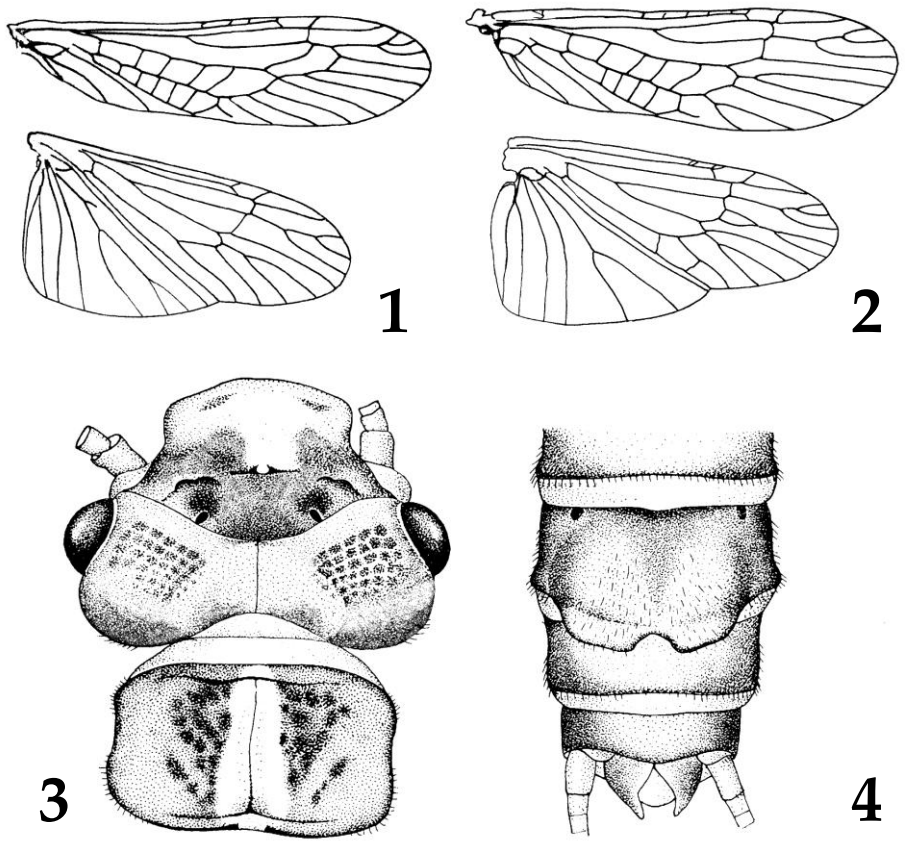
29.04.1988 [L. Zhiltzova]; 5L, 29.05.1989 [L. Zhiltzova]; 2L, 31.05.1999 [G. Prokopov]; 2L, 5.06.2010 [G. Prokopov]. - River Guva (Derekoika) upper part (near Yalta): 20 juv. L, 19-21.09.1999 [G. Prokopov]; 6L, 23-25.05.2000 [G. Prokopov]. - River Ulu-Uzen' Alushtinskyi basin (near Alushta): Riv. Uzen'-Bash, 1L, 7.05.2000 [G. Prokopov]; Riv. Sofu-Uzen', 2 juv. L, 31.10.1999 [G. Prokopov]. - River Eastern Ulu-Uzen' (near village General'skoe): 1L, 11.08.1964 [R. Kazlauskas]; 4 juv. L, 31.10.1999 [G. Prokopov]; 1 juv. L, 28.07.2000 [G. Prokopov]; 7 juv. L, 15.10.2000 [G. Prokopov]; 1L, 1.05.2001 [G. Prokopov]. - River Belbek basin (upper part), Riv. Sary-Uzen': 2L, 22.04.1988 [L. Zhiltzova]; 2L, 1.05.2002 [G. Prokopov]. - Riv. Auzun-Uzen' (Rozovaja) (Grand Canyon of Crimea): 4L, 11.08.1964 [R. Kazlauskas]; 3L, 27.04.1988 [L. Zhiltzova]. Spring Almachuk: 9L, 22.04.1988 [L. Zhiltzova]; 2L, 5.05.2003 [G. Prokopov]; 3♀, 17L, 5.05.2003 [G. Prokopov]; 25L, 10♀, 30.05.2009 [G. Prokopov]. Riv. Kokkozka, 2L, 22.08.2000 [G. Prokopov]. - River Alma basin (upper part), spring near Koz'ma and Damian monastery, 2L, 26.06.2001 [G. Prokopov]. River Salgir basin: Riv. Angara (upper part of Salgir river): 1L, 6.06.1984 [G. Kiseleva]; 1L, 25.05.2002 [G. Prokopov]; Riv. Kizil-Koba (upper part of Salgir river): 2L, 14.04.88 [L. Zhiltzova]; 1L, 4.05.1988 [L. Zhiltzova]; 1L, 17.02.2001 [G. Prokopov]; 2L, 25.05.2006 [G. Prokopov]; 2L, 25.05.2007 [G. Prokopov]; Riv. Small Salgir (tributary of Salgir river) near vill. Drujnoie: 2L, 20.03.2003 [G. Prokopov]; 1♀, 5L, 20.06.2004 [G. Prokopov]; 6L, 28.05.2006 [G. Prokopov]; 6L, 01.06.2007 [G. Prokopov]; Riv. Burulcha (tributary of Salgir river) near village Mejgor'e, 4L, 26.04.1988 [L. Zhiltzova]; Riv. Suat (tributary of Burulcha river) upper part: 2 juv. L, 3.09.1999 [G. Prokopov]; 4L, 1.05.2001 [G. Prokopov].



Figs. 1-4. *Isoperla prokopovi* sp. n., adult female. 1-2. Examples of the variable wing venation. 3. Head and thorax. 4. Abdominal tip, ventral view. Not to scale.

The material is in the respective collectors' collections, except specimens taken by G. Kisela which are kept in the collection of the Zoological Institute of the Russian Academy of Sciences.

DESCRIPTION

Adult female. (Figs. 1-4). Body length 13.5 – 16.0 mm, length of forewing 12 – 15 mm. External morphology typical of the genus.

Wings clear with thick brown veins in forewing, paler and finer in hindwing. Forewing with 8 – 10 branches entering the wing margin between R and Cu, details variable (Fig. 1) between specimens as well as between the two wings of a given specimen. For example, a large extra cell may be formed by a crossvein between Rs and M which is only occasionally present (Fig. 1, forewing). Also, branches of Rs and M may fuse for a short distance and then separate again (Fig. 2, hindwing). In the hindwing, A2 and A5 branched.

Head very large, area behind occipital suture unusually large, appearing inflated. Genae in dorsal view about as long as eyes, convex, protruding. Antennae and mouthparts lack structural particularities.

Ground colour of body yellowish, with brown marks, appendages light brown. Head in front of the M-Line yellow, behind it brown to the occipital suture, with small paler areas laterally from the paired ocelli and the tentorial calluses. Occiput light except darker muscle attachment scars. Small paramedian and large caudolateral areas at the posterior margin of head brown.

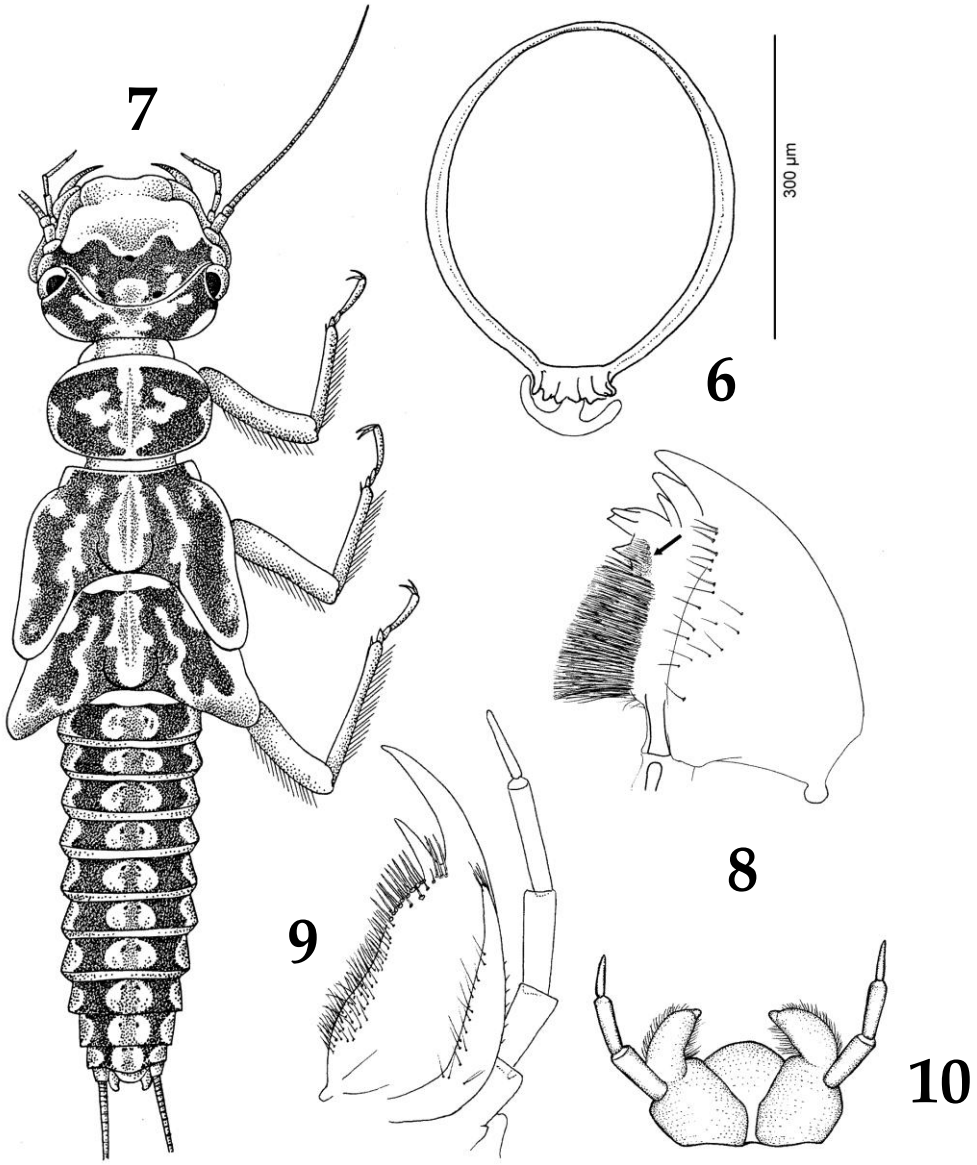
Pronotum light brown with wide pale median band and dark brown muscle attachment scars. Mesonotum with light prescutum and a light brown median area in anterior part of scutum, remainder dark brown. The abdominal integument seems to be light brown but appears dark brown because of egg masses visible through the integument. Abdominal sterna mainly pale yellow, except brown basi- and furcasterna and brown hind areas of posterior abdominal segments.

Sternite 8 with short subgenital plate occupying the entire width of segment, with U-shaped medial notch. Sternite 9 brown, 10 pale. Paraprocts wide with concave medial edge producing a narrow apex. Ventrally sclerotized, dorsally unsclerotized. Cerci with ca. 15-17 segments, segment length changing from half as long as wide at base to about 4 times longer than wide at apex. Setal ring on each segment with one or a few very long ventral setae.

Fig. 5. *Isoperla prokopovi* n. sp., last instar larva (photography by G. Prokopov).

Egg. (Fig. 6). Abdomen of last instar larvae and adult females packed with fully formed eggs. Egg subspherical, cross section circular, ca. 365 µm long, longer than wide largely because of the projecting collar. The egg contents is ca. 0.014 mm³. Collar with irregular longitudinal crests, recurved edge irregular. Anchor large, mushroom-shaped. Micropyles not found. Chorionic surface irregularly and minutely finely punctate. Operculum with slightly larger punctations in the usual hexagonal arrangement left by follicular cells.

In optical cross section and in egg fragments the egg shell consists of an inner, apparently structureless layer where the diameter abruptly decreases at the level where the eclosion line would occur in other *Isoperla* species. Under the operculum and above the anchor attachment inside the collar the inner layer is very thin. The outer layer is densely and finely radially striated and appears spongy. The chemical nature of egg shell is unknown (see discussion).



Figs. 6-10. *Isoperla prokopovi* n. sp., egg and last instar larva. 6. Egg. 7. Habitus. 8. Left mandible, ventral view; an arrow points at the dense patch of small spicules. 9. Left maxilla, ventral view. 10. Labium, ventral view (courtesy G. Prokopov). Only Figure 6 to scale.

Last instar larva (Figs 5, 7-10). Body length 15-19 mm, head capsule width ranging from 3.0-3.8 mm (Fig. 14). External morphology typical of genus *Isoperla* (Zwick 2004).

Dorsum yellow with dark brown marks. Anterior portion of head yellow. Median round pale mark posterior to transverse dark band behind the M-line and anterior to the occipital suture, smaller light areas lateral to ocelli. Oblique dark band extending from behind the eyes and posterior to occipital suture extending to posterior margin of head meeting it paramedially. Anteromedially the band has several extensions into the pale central area behind the occipital suture. Caudolateral corners of head pale.

Pronotum dark with narrow pale edge. A wide median line widens and forms a pale central area resembling a clover leaf. Anterior part of meso- and metanotum with pale median strip flanked by paramedian dark bands connected to oblique bands over the wing pads. All bands with lobes and excisions, shape complex (Figs. 5, 7).

Abdomen banded. Pale sides and a series of round maculae along midline separated by two brown paramedian bands. Borders of the dark areas on each segment medially and laterally concave. The large pale circular areas in the centre are divided by a greyish median band. Tergite 10 essentially pale.

Ventrum largely pale. Mouthparts, antennae, legs and cerci yellowish to light brown.

Mouthparts generally similar to congeners. Left mandible dorsally, behind the cusps and in front of the setal band, with a dense patch of spicules arising from cuticle with polygonal pattern. The right mandible has the polygonally structured area but no spicules. Lacinia with two large cusps basally from which begins a row of about 10-12 large spines. The row is locally bilinear and blends basally with an elongate densely setose area. Because of transitions in shape and size, the number of lacinial spines cannot be precisely determined. Dorsally between the two cusps stand 3-4 very slender, hyaline sensory setae. Galea dorsally with several thin slender setae, a few smaller hairs along ventral edge.

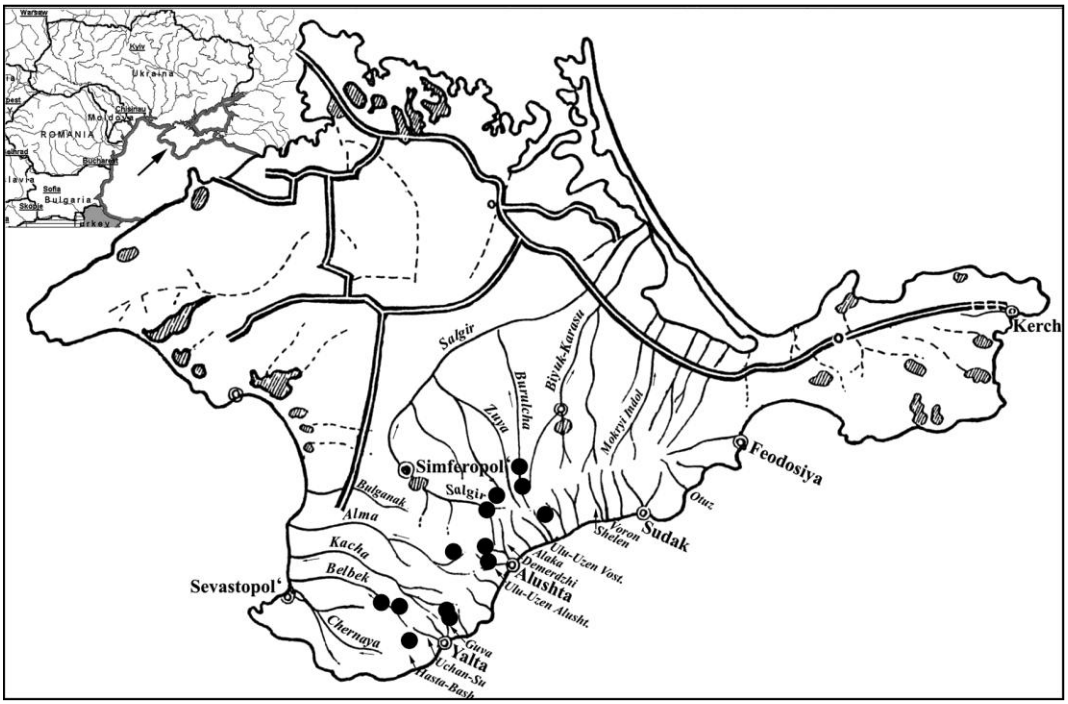


Fig. 11. *Isoperla prokopovi* n. sp., distribution map (courtesy of G. Prokopov).

Setation. A curved plurilinear band of short spines in the brown occipital area is medially widely interrupted. Femora and tibiae with, antennae and cerci without long silky hair fringes. The slender pointed marginal setae on sides of pronotum, along lateral edges of wingpads and along rear margins of abdominal tergites are unusually short, shorter than the intersegmental areas covered with chloride cells. Intercalary setae numerous but smaller. The exceedingly fine procumbent hairs typical of *Isoperla* larvae are dark on the fore body but pale on the abdomen, same as the other setae.

Etymology. It is our pleasure to name this remarkable new species in the honour of Grigoriy Prokopov, Simferopol, in recognition of his valuable contributions to our study.

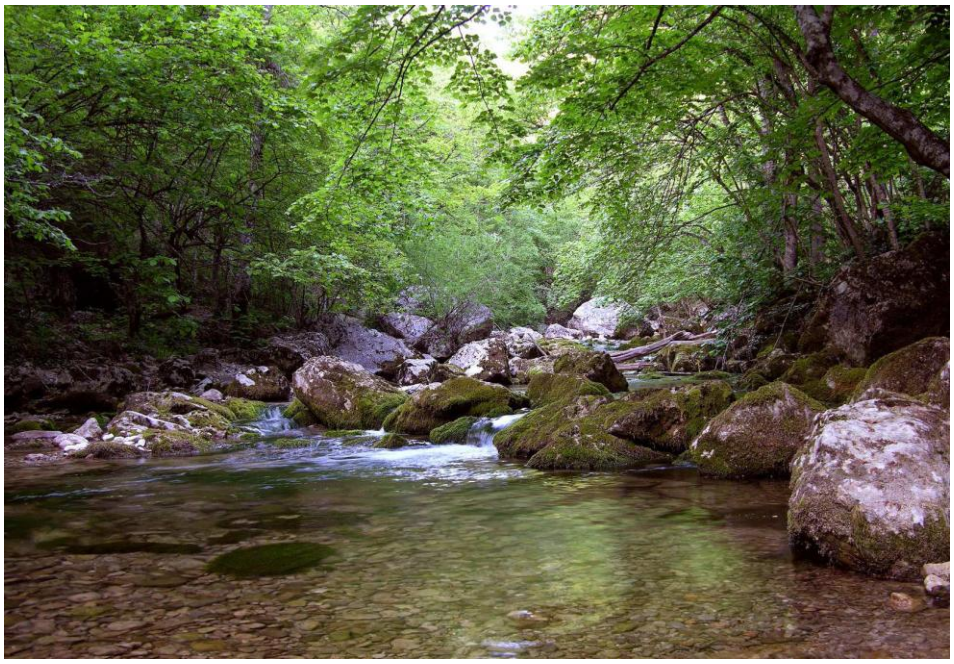


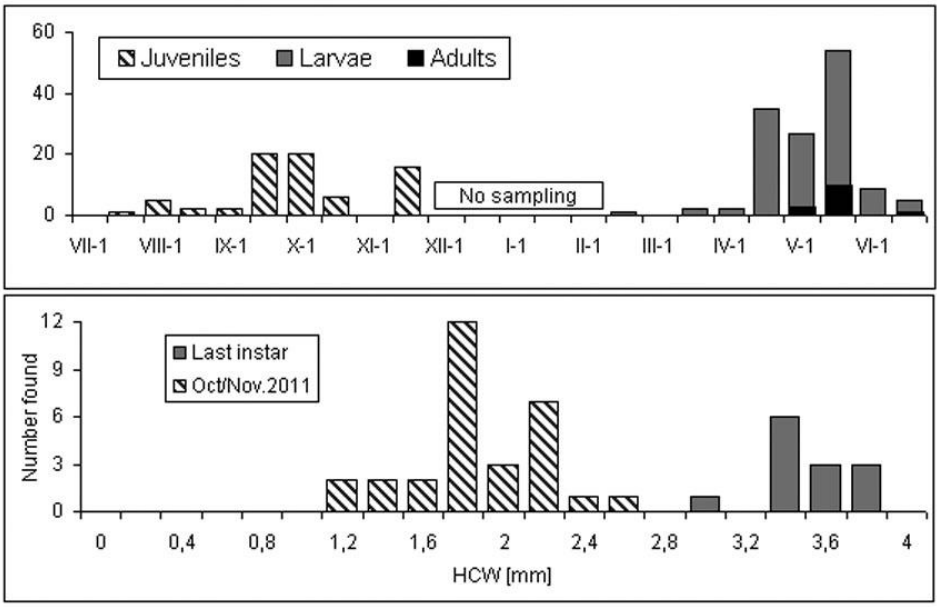
Fig. 12. *Isoperla prokopovi* n. sp., typical habitat at the confluence of rivers Auzun-Uzen' and Almachuk, Grand Canyon of Crimea (photography by G. Prokopov).

Distribution and biology.

Isoperla prokopovi sp. n. inhabits the upper sections of rivers on the northern and southern main slopes of the Crimean Mountains between 400 and 900 m a.s.l. (Fig. 11). The species occurs where the stream bed is rocky and the current fast, at water depths of 0.2-0.5 m (Fig. 12). The mineralization of water fluctuates between 340 and 700 mg/l. The dominant ions are HCO_3^- (177-300 mg/l) and Ca^{2+} (44-110 mg/l). Water temperature at these localities fluctuates from 6°C to 20°C. The inhabited sites may have no surface flow for two to three months in summer, beginning in July. However, the subsurface flow continues.

Isoperla prokopovi is part of the epi- and metarhithral community of the Crimean rivers. It coexists with, for example, *Siphonoperla taurica* (Pictet) (Plecoptera), *Baetis milani* Godunko, Prokopov & Soldán, *Electrogena braaschi* (Sowa) (Ephemeroptera), *Agapetus ajpetriensis* Martynov, *Plectrocnemia intermedia* Martynov, *Silo alupkensis* Martynov (Trichoptera), the blackflies *Nevermannia brevidens* (Rubtsov), *N. chodakovi* (Panchenko), *N. karajimae* (Panchenko), *N. tauricum* (Rubtsov), the beetle *Hydraena chersonesica* Jäch, Diaz & Przewozny, and *Dugesia gonocephala taurocaucasica* Porfirieva (Tricladida) (Prokopov 2004).

Life cycle. (Figs. 13, 14). The list of material distinguished juvenile larvae, larvae without specification, and adults. The collection data were classified as falling into the 1st or 2nd half of the



Figs. 13, 14. *Isoperla prokopovi* n. sp. 13 (top). Seasonal occurrence of numbers of juvenile larvae, larvae, and adults as evidenced by the lists of material studied. 14 (bottom). Frequency of size classes (HCW, head capsule width) among larvae collected in October / November 2011, and among the available last instar larvae.

respective month. Numbers collected per half month were pooled, irrespective of year or site of collection. The seasonal distribution of the different life stages suggests the life cycle is univoltine. The size spectrum of larvae collected in October-November 2011 agrees perfectly with this. Larvae appear to hatch from eggs in the middle of year and grow to last instar and adulthood by May-June the next year.

It is unknown whether the hatchlings were from freshly laid eggs or from eggs that had spent much time in diapause. Direct fast development is improbable in view of a general positive relationship between egg volume and length of development time. The eggs of *I. prokopovi* are larger than those of other Perloidea which require more time to develop (Gillooly & Dodson 2000) than remains between presence of adults and appearance of the new generation of *I. prokopovi*. A long egg diapause appears probable since it is not unusual among Perlodidae, also in European *Isoperla* spp. (Schwarz 1970, Lillehammer 1987).

Notes and discussion. *Isoperla prokopovi* seems to be parthenogenetic. All available large larvae and adults were females. Twenty-nine young larvae (head capsule width 1.0-2.4 mm, body length 4.2-10.3 mm) taken in October and November 2011 all exhibited secondary female characters.

We are therefore convinced that males have not been overlooked, as might happen if the species were strongly sexually dimorphic. *Isoperla difformis* (Klapálek) offers an example. Its macropterous females are readily found in littoral vegetation but the much smaller micropterous males are fast runners dwelling mainly on the ground. Male larvae of *I. difformis* (and other Perlodidae that lack secondary male sexual characters) resemble juvenile specimens, except in the absence of secondary female characters. Females can be recognized long before they attain half final body size, several moults before the last instar (Zwick & Zwick 2010). The setal fringe on sternite 8 is medially interrupted, and, in slightly larger individuals, a minute scar of the prospective genital opening appears in the anterior third of sternite 8. This scar moves stepwise back at moults until it reaches the caudal segment edge (Zwick & Zwick 2010).

We know of no other Perlodidae emerging with fully developed eggs. We found no micropyles in the eggs. If they indeed lacked them our assumption of parthenogenesis would be strongly supported. In eggs of other Perloidea, the micropyles are easily seen on the clean chitinous chorion that remains after the egg content was removed in cold KOH. However, the eggs of *I. prokopovi* behaved in a unique way. The shell was hardly more resistant than the egg content to treatment in cold KOH. Within an hour it turned into a shape- and colourless mass of approximately hexagonal small fields, each surrounded by some gelatinous material. Later the egg fell completely apart. It seems to differ structurally and/or chemically from known eggs of other Perloidea (for example, Rosciszewska 1991).

There is no confirmed case of natural parthenogenesis in stoneflies but species of which many females but no or very few males are known (for example, *Baikaloperla* spp.; Zhiltzova 2003) are probably at least facultatively parthenogenetic. Parthenogenetic development of eggs during laboratory incubation is known to occur (Degrange 1958, Harper 1973).

Affinities. *Isoperla prokopovi* is an isolated species without close relatives in the West Palaearctic fauna. The venation of its front wing is richer than in other European *Isoperla* spp. which have only 2 branches of Rs, except *I. difformis* which may have three (Illies 1955). None of the other species has a subgenital plate as wide as the segment, and none has a large U-shaped notch in it. At first glance, the larva reminds one of genus *Bulgaroperla* certainly also because of its large size.The shortness of larval pilosity is unique among *Isoperla*, and so are shape and chorion structure of the egg.

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REFERENCES

Degrange, C. 1958. Premiers cas de parthénogenèse chez les Plécoptères. Comptes Rendus Hébdomadaires des Séances de l'Académie des Sciences, 246:1100-1102.

Gillooly, J.F. & S.I. Dodson. 2000. The relationship of egg size and incubation temperature on embryonic development time in univoltine and multivoltine aquatic insects. Freshwater Biology, 44:595-604.

Harper, P.P. 1973. Emergence, reproduction and growth of setipalpian Plecoptera in Southern Ontario. Oikos (Copenhagen), 24 (1):94-107.

Illies, J. 1955. Steinfliegen oder Plecoptera. *In:* Dahl, F. (ed.), Die Tierwelt Deutschlands und der angrenzenden Meeresteile, 43:I-IV; 1-150.

Lillehammer, A. 1987. Diapause and quiescence in eggs of Systellognatha stonefly species (Plecoptera) occurring in alpine areas of Norway. Annales de Limnologie, 23:79-184.

Prokopov, G.A. 2004. Trophic relations in communities of macrozoobenthos fauna in epirhithral of the rivers of Crimea. Uchenye zapiski TNU. Series: Geography. Vol. 17 (56). No 3:91-98.

Rosciszewska, E. 1991. Ultrastructural and histochemical studies of the egg capsules of *Perla marginata* (Panzer, 1799) and *Dinocras cephalotes* (Curtis, 1827) (Plecoptera: Perlidae). International Journal of Insect Morphology and Embryology, 20:189-204.

Schwarz, P. 1970. Autökologische Untersuchungen zum Lebenszyklus von Setipalpia-Arten (Plecoptera) Teil II und Schluß. Archiv für Hydrobiologie, 67 (2):141-172.

Zhiltzova, L.A. 2003. Plecoptera Gruppe Euholognatha. Russian Academy of Sciences, Zoological Institute, St. Petersburg, 537 plus 7 unnumbered pages.

Zwick, P. 2004. Key to the West Palaearctic genera of stoneflies (Plecoptera) in the larval stage. Limnologica, 34:315-348.

Zwick, P. & H. Zwick. 2010. Life history and development of *Dictyogenus fontium* (Plecoptera: Perlodidae) in two thermally contrasting streams at Lunz am See, Lower Austria. – pp.:459-475 *in* Denisia 29, Festschrift zum 75. Geburtstag von Hans Malicky, 478 pp.

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