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Glossiphonia balcanica n. sp. and *Dina prokletijaca* n. sp. (Hirudinida: Glossiphoniidae, Erpobdellidae) - two new leeches from Montenegro and Kosovo

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Abstract
Two new leech species (Hirudinida) representing the families Glossiphoniidae (*Glossiphonia balcanica* n. sp.) and Erpobdellidae (*Dina prokletijaca* n. sp.) are described. The populations from Montenegro assigned by Utevsky *et al*. (2013) and Grosser *et al*. (2015) to *Glossiphonia nebulosa* Kalbe, 1964 represent an new species, here described as *G. balcanica* n. sp. The new species can be easily be separated from *Glossiphonia nebulosa* by the reduction of the papillae. *Dina prokletijaca* n. sp. closely resembles *D. dinarica* Sket, 1968 and *D. montana* Sket, 1968 from which can be distinguished in the combination of the small and stocky body, dorsal surface with two wide and dark paramedian longitudinal stripes and ovisacs reaching the fourth somite after the female genital pore, and curled in their entire course. A key to the species of *Glossiphonia* Johnson, 1816 and *Dina* R. Blanchard, 1892 from the Western Balkans is presented.

Key words: leeches, taxonomy, new species, Kosovo, Montenegro.

Introduction

In last decade a number of data were published on leeches from the Western Balkans (i.e. Montenegro, Bosnia, Serbia, Kosovo, Albania and Mecedonia) including some species described as new for science (Grosser *et al*. 2007, 2014). Recently all published and new data on the leeches from Montenegro and Serbia (including Kosovo) were summarized, respectively in two checklists published by Grosser *et al*. (2015a, b). However, the hirudinean fauna of the Western Balkans still remains relatively unknown taxonomically and biogeographically. The leech fauna of Montenegro includes 29 species and subspecies (Grosser *et al*. 2015a). On other hand the leech fauna of Kosovo is poorly documented and includes only 11 species (Sket 1968, Šapkarev 1975, Grosser *et al*. 2015b), so there is a good reason to assume that more species are expected to be found in the future.

In this paper, two new species of the families Glossiphoniidae (*Glossiphonia balcanica* n. sp.) and Erpobdellidae (*Dina prokletijaca* n. sp.) are described.

Material and Methods

Leeches in this study were collected by Surber net (material from Kosovo), or by hand or with pincers from the underside of roots and stones in water, as well on the banks. The external morphology (number and position of eyes, annulation, colouration, papillation and the position of genital pores) was examined on several specimens. The characters of sexual organs of *Dina prokletijaca* n. sp. (location, shape and extension of the genital atrium with the cornua, shape of the ovarian sacks and vasa deferentia), were checked on the three adult specimens, which show well developed sexual organs with visible oocytes inside the ovisacs. The digestive system of *Glossiphonia balcanica* n. sp. was examined on several particularly transparent specimens.

Measurements were taken with a ruler (in the authors opinion the precision of such measurement is sufficient, because they anyway largely depend on the body contraction). Material was examined using a stereomicroscope (Novex); photographs of the internal anatomy, mouth and annulation were taken with a microscope camera (Euromex, VC 3031C), and the photographs of the habitus and colour were taken with the camera Canon EOS 400D.

The holotype and paratypes are deposited in the Senckenberg Museum Frankfurt (SMF); the rest of the material is kept in the collection of the first author.

Taxonomical part

Family Glossiphoniidae Vaillant, 1890

Subfamily Glossiphoniinae Autrum, 1939

Genus *Glossiphonia* Johnson, 1816

Glossiphonia balcanica Grosser & Pešić n. sp.

(Figure 1A-E)

Synonymy. *Glossiphonia nebulosa* Utevsky *et al*. 2013 nec Kalbe, 1964: p. 123, fig. 2.

Glossiphonia cf. *nebulosa* Grosser *et al*. 2015a nec Kalbe, 1964 partim: p. 21, fig. 1B.

Type material. Holotype (SMF 19961), KOSOVO, KS40 Dečani/Decan, Lebush, spring Toplla, 42°34'19"N, 20°17'26"E, 10.5.2014, leg. Berlajolli, body length 10 mm, width 8 mm. Paratypes (SMF 19962): eight specimens (body length/width: 8/7.5, 8/7, 8/6, 9/8, 8/7, 7/5.5, 7/7[breeding], 5/4.5[breeding] mm), same locality and data as holotype, 21.12.2014, leg. Berlajolli, 2 specimens; 24.1.2015, leg. Berlajolli, 6 specimens; 27.2.2015, leg. Berlajolli, 2 specimens; 30.4.2015 leg. Berlajolli, 4 specimens; 26.9.2015, leg. Berlajolli, one specimen. Five paratypes are deposited in the SMF, other paratypes in the first author's collection

Further records (published in Grosser *et al*. 2015a as „*Glossiphonia nebulosa*"). MONTENEGRO: Podgorica, Mareza spring, 42°28'47.92"N, 19°10'56.09"E, 08.iv.2005 Pešić & Grosser, 9 specimens; ibid., 08.10.2005 Pešić, 2 specimens; Podgorica, Mareza canal, 42°28'26.86"N, 19°10'47.37"E, 04.2.2006 Pešić, one specimen; ibid., 08.4.2005 Pešić & Grosser, 8 specimens; Skadar Lake, sublacustrine spring Karuč, 42°21'29.80"N, 19°6'20.84"E, 09.8.2006 Pešić & Grosser, 6 specimens; Cetinje, River Crnojevića, stillwater channel near River Crnojevića, 42°21'17.78"N, 19°1'11.14"E, 09.8.2006 Pešić & Grosser, 16 specimens; little stream and pool near Crno Jezero (Black Lake), 43°9'6.87"N, 19°6'6.89"E, 11.8.2006 Pešić & Grosser, 5 specimens. Danilovgrad, River Zeta near village Martinići, 42°32'10.59"N, 19°9'38.66"E, 13.8.2015 Pešić, one specimen.

Comparative material examined. *Glossiphonia nebulosa* Kalbe, 1964: KOSOVO, KS40 Dečani/Decan, Lebush, spring Toplla, 42°34'19"N, 20°17'26"E, 10.5.2014, leg. Berlajolli, 25 specimens (length/width: 11/6, 10/5, 9.5/7, 9.5/7, 9/6.5, 9/6, 9/6, 9/6, 9/6, 8/7, 8/6, 8/6, 8/6, 8/6, 8/6, 8/5, 8/5, 8/5, 8/5, 7.5/6, 7.5/5, 7.5/5,

7/6, 7/5, 7/4.5 mm); ibid., 16.11.2014, 5 specimens; ibid., 21.12.2014, one specimen; ibid., 24.01.2015, 8 specimens; ibid., 27.02.2015, 7 specimens; ibid., 30.04.2015, one specimen; ibid., 29.08.2015, 2 specimens; ibid., 26.09.2015, 3 specimens.

Diagnosis. Dorsal surface amber-coloured; prominent paramedian papillae on annulus a2, further small and inconspicuous papillae irregularly arranged on dorsal surface; three pairs of distinct and well visible eyes in two parallel rows; the cranial sucker without median fold.

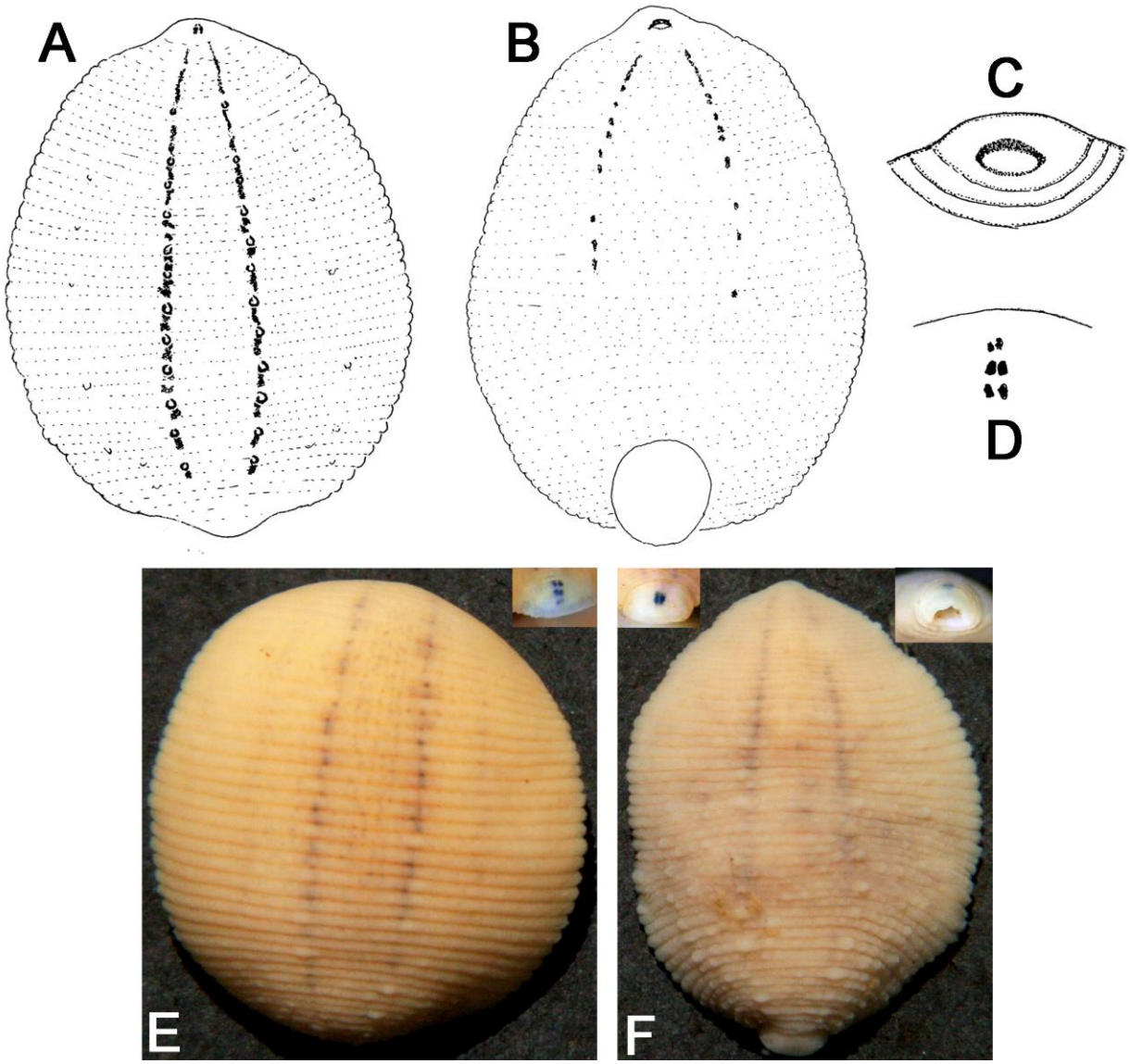


Figure 1. *Glossiphonia balcanica* n. sp., paratype A = dorsal view; B = ventral view; C = cranial sucker; D = eyes. Photographs: E = *G. balcanica* n. sp., holotype, dorsal view (inset: eyes). F = *G. nebulosa*, spring Toplla, Kosovo, dorsal view (insets: left – eyes, right - cranial sucker).

Description

Habitus: Small leeches, preserved specimens up to 10 mm in lenght and 8 mm in width, only slightly longer than width (Fig. 1A). The body dorsoventrally flattened, oval in shape and with a slightly bulbous head. Dorsally there are prominent paramedian papillae on annulus a2, further small and inconspicuous papillae irregularly arranged on dorsal surface. The ventral surface without papillae. The cranial sucker small, transverse oval, without median fold (Fig. 1C).

Annulation: The midbody somites typically triannulate; the annuli equally wide. Gonopores separated by two annuli.

Colour: The colour of the dorsal surface in living specimens bright brownish (amber-coloured). Dorsally there is one pair of continuous dark paramedian longitudinal stripes; sometimes the paramedian stripes are interrupted by papillae on annulus a2. Dark or yellow spots absent, a yellow spotted sideband absent. The ventral surface one-coloured and brighter than the dorsal surface.

Eyes: Three pairs of distinct and well visible eyes in two parallel rows, the frontal pair of eyes smaller than the following two pairs, the middle pair usually largest (Fig. 1D); reductions or fusions of eyes not observed.

Digestive system: The crop has six pairs of caeca.

Etymology. The species is named after its presence in the Western Balkan.

Differential diagnosis. *Glossiphonia balcanica* n. sp. resembles to *G. concolor* (Apathy, 1888) and *G. nebulosa* Kalbe, 1964. With the later species, *G. balcanica* n. sp. share similar colour and presence of two narrow, uninterrupted dark paramedian lines on one-coloured dorsal surface. However, in *G. nebulosa* the stripes are sometimes absent (see: Nesemann and Neubert 1999). The both species can be separated by the colour which is bright brownish (amber-coloured) in *G. balcanica* n. sp. but more greyish in the specimens of *G. nebulosa* (compare Fig. 1E and - F). Due to the colour of body *Glossiphonia balcanica* n. sp. is similar to *G. concolor*, but the latter species can be distinguished by the presence of three pairs of dark stripes on dorsal surface with the inner paramedian one very prominent and interrupted irregularly on annulus a2 (see: Nesemann and Neubert 1999). Moreover the new species resembles *G. concolor* in the position of eyes. On the other hand in *G. nebulosa* the frontal pair of eyes is very small (sometimes reduced) while the middle and posterior pairs are often more or less fused (Fig. 1F inset). A further distinctive difference between the compared species refers to the arrangement and shape of dorsal papillae. Dorsal surface of *G. balcanica* n. sp. is covered by a few small irregularly arranged papillae and the prominent paramedian papillae located only on annulus a2. *G. nebulosa* has three pairs of prominent papillae situated on two annuli (a2 and a3) while *G. concolor* has a smooth body surface without prominent papillae (Nesemann and Neubert 1999). A further difference between *G. balcanica* n. sp. and *G. nebulosa* regard the median fold on the cranial sucker. This median fold, lacking in *G. balcanica* n. sp., is prominent in the population of *G. nebulosa* from Kosovo (Fig. 1F inset) but only slightly developed in the population of the later species from its *locus typicus* in Germany.

Remarks. Variability was found in body dimensions (5-10 mm in length, 4.5-8 mm in width); some breeding specimens with almost half of maximum size were found.

Discussion. Utevsky *et al*. (2013) reported *Glossiphonia nebulosa* from Skadar Lake and mentioned that the dorsal surface of examined specimens bears small papillae. Later on Grosser *et al*. (2015a) stated that the specimens from Skadar Lake clearly differ from the typical *nebulosa* specimens by the reduced papillae. The records of Utevsky *et al*. (2013) and Grosser *et al*. (2015a) should be ascertained to species described here as *G. balcanica* n. sp.

The finding of a new species living sympatrically with *G. nebulosa* in the *locus typicus* clearly demonstrated that we are dealing with two separate species. The occurrence of two or more *Glossiphonia* species, living sympatrically in the same water bodies is not unusual. Nesemann (1990) demonstrated that association of three *Glossiphonia* species (*G. verrucata, G. complanata, G. paludosa* or *G. concolor*) is frequent in the Danubian regions of Austria and Hungary.

Habitat: *Glossiphonia balcanica* n. sp. occurs in small to medium-sized fast running waters, but also in large standing waters (Skadar Lake - Utevsky *et al*., 2013). At the *locus typicus* (spring Toplla, Kosovo) leeches were collected from March to December, with peak in winter and spring.

Distribution: Montenegro and Kosovo, widespread and common in the Mediterranean part of Montenegro.

Key to the *Glossiphonia*-species from the Western Balkans.

1 Dorsal surface smooth, without visible papillae; dark paramedian stripes interrupted iregularly .....*Glossiphonia concolor* (Apathy, 1888)
- Dorsal surface with well visible papillae; dark paramedian stripes various…..... 2
2 Dorsal surface rough, with numerous very small, not prominent papillae; dark paramedian stripes absent; usually four fused eyes .... *Glossiphonia paludosa* (Carena, 1824)
- Prominent papillae at least in two paramedian rows on annulus a2; two dark paramedian stripes present; eyes various 3
3 Prominent papillae only in two paramedian rows; dark paramedian stripes continious or interrupted by papillae on annulus a2; six distinct eyes .....*Glossiphonia balcanica* n. sp
- Prominent papillae in more rows; dark paramedian stripes and eyes various 4
4 Prominent papillae only on annulus a2 of midbody somites; a yellow spotted sideband present; dark paramedian stripes always interrupted on annulus a2; six distinct eyes .... *Glossiphonia complanata* (Linnaeus, 1758)
- Prominent papillae on two annuli (a2, a3) of midbody somites; a yellow spotted sideband absent: dark paramedian stripes usually not interrupted; usually four fused eyes ..... *Glossiphonia nebulosa* Kalbe, 1964

Family Erpobdellidae R. Blanchard, 1894

Subfamily Trochetinae Perrier, 1897

Genus *Dina* R. Blanchard, 1892

***Dina prokletijaca* Grosser & Pešić n. sp.**
(Figures 2A-D, 3A-C)

Type material. Holotype (SMF 19963), KOSOVO KS16, Istok town, Zatra, spring Nenqershi, 42°47'20.7"N, 20°28'54.4"E, 23.3.2014, leg. Berlajolli, body length 23 mm, width 4,5 mm. Paratypes (SMF 19964): 18 specimens, ten of them are adults with a well visible clitellum (length/width: 23/4, 27/5, 24/5, 26/5, 23/5, 20/5, 20/4, 22/5, 21/4, 20/3.5 mm); eight paratypes without a visible clitellum (length/width: 22/4, 21/4.5, 18/4, 15/3, 15/3, 13/3, 15/3.5, 16/3 mm); 8 specimens, same locality as holotype, 29.3.2015, leg. Berlajolli; 5 specimens, same locality as holotype, 23.8.2015, leg. Berlajolli; 4 specimens. 08.11.2015, leg. Berlajolli. Ten paratypes are deposited in the SMF, other paratypes in the first author's collection.

Further records: KOSOVO: Peje/Peć, Zatra, spring Kroni i Kadrushit, 42°38'52"N, 20°16'50" E , alt. 659 m asl, 05.3.2014 Berlajolli, 2 specimens; Peje/Peć, Zatra, spring Kroni i Bajram Brales, 42°38'36" N, 20°16'39" E alt. 781 m asl, 7.4.2014 Berlajolli, 14 specimens; Peje/Peć, Kuqishte, spring Gurra e Hakes, 42°41'00.8"N, 20°04'57.4"E, alt. 1441 m asl, 30.4.2014 Berlajolli, 70 specimens; Peje/Peć, Rugovo, Boge, spring Gurra e Dreshajve, 42°45'41.2"N, 20°03' 24.0"E, alt. 1599 m asl, 11.5.2014 Berlajolli 33 specimens.

Comparative material examined. *Dina montana* Sket, 1968: MONTENEGRO, Štavna, leg. et det. P. Trontelj, 02.07.2007, 4 specimens (length/width: 40/4.5, 30/4, 25/3, 24/3 mm).

Diagnosis. Leeches with a typical *Dina*-annulation (quinqueannulate, b1, b2, a2, b5 < b6 (c11, c12); small and stocky leeches; the dorsal surface bright greyish with two wide and dark paramedian longitudinal stripes; ovisacs reaching the fourth somite after the female genital pore, and curled in their entire course.

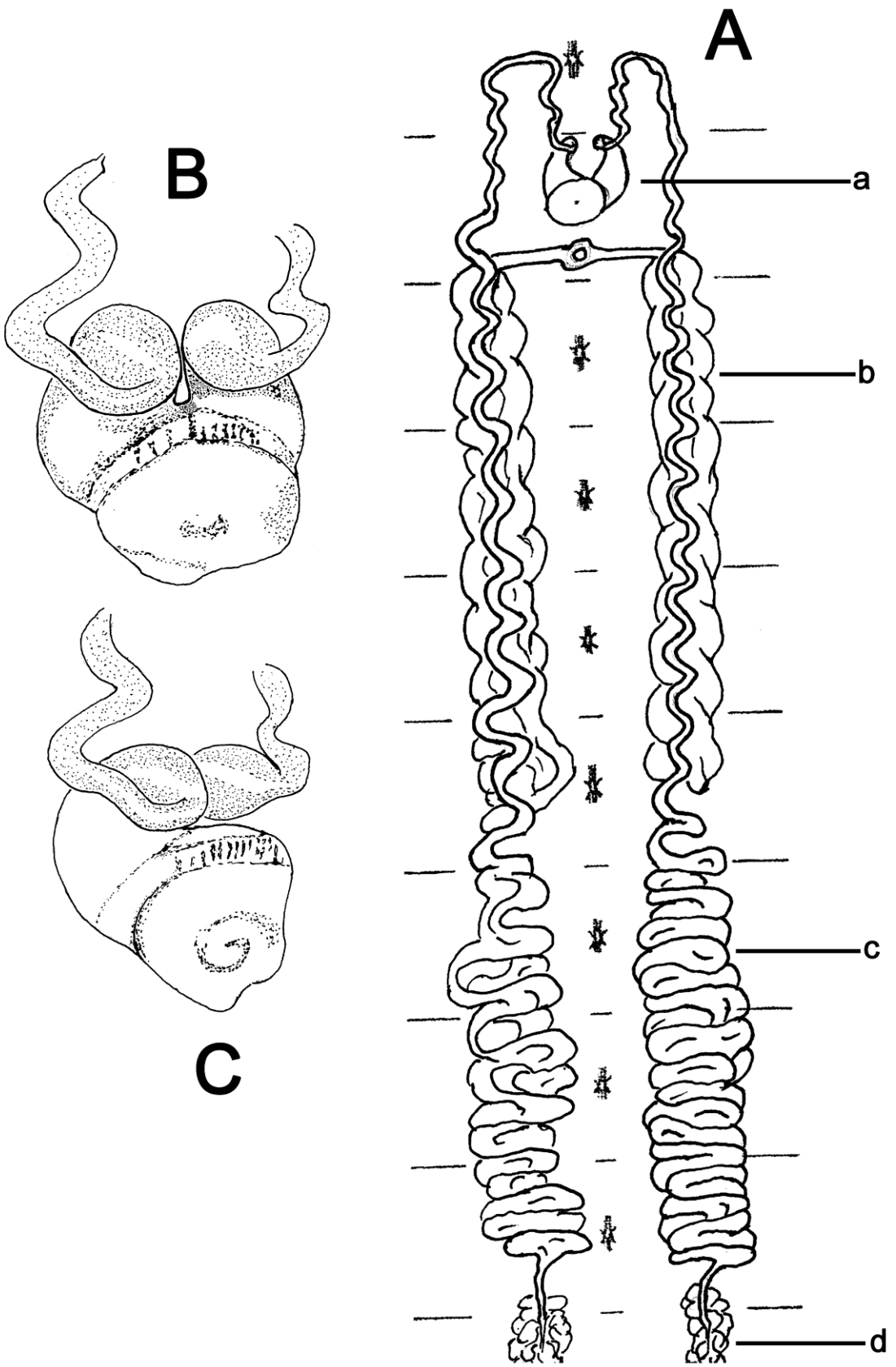
Description
Habitus: Small and stocky leeches. The body is cylindrical along the entire length, with small but not raised lateral keels in the last third (Fig. 2B). The cranial sucker large with a wide mouth opening; the upper lip not

Figures 2. *Dina prokletijaca* n. sp., holotype: A = dorsal view; B = lateral view; C = Habitus: left – *D. prokletijica* n. sp., holotype; right – *D. montana*, Štavna, Montenegro.

elongated. The caudal sucker of medium size, clearly narrower than the width of the body. Conspicuous papillae are absent; numerous very small inconspicuous papillae are dorsally and ventrally visible on annulus a2.

Size: Small leeches. Preserved adult specimens up to 27 mm in the length and 5 mm in width.

Annulation: Leeches with a typical *Dina*-like annulation. The midbody somites quinqueannulate with annulus b6 only slightly broadened, more clearly broadened in the last half of the body. Annulus b6 not or only very slightly subdivided into annuli c11 and c12, other annuli not subdivided. The male genital pore situated in the furrow b2/a2, the female in the furrow b5/b6. The genital pores separated by two annuli.



Figures 3. *Dina prokletijaca* n. sp., paratype: A = internal anatomy (Abbreviations: a, atrium; b, ovarian sacks; c, vas deferens; d, testisacs). B-C = atrium: B = ventral view, C = ventrolateral view.

Colour: The dorsal surface of preserved specimens is bright greyish with two wide dark paramedian longitudinal stripes, along the entire length. The paramedian stripes forms the boundary of a bright median stripe (Fig. 2A). The ventral surface plain and brighter than the dorsal surface.

Eyes: The eyes in studied preserved specimens strongly reduced or not visible.

Sexual organs: The male sexual organs are characterized by a small atrial body with short and thick cornua. The cornua sharply bending to the ventral side, in proximal elongated to the border of b1/b6 or on b6 of the previous somite, the apical part not coiled (Fig. 3B-C).

The paired vasa deferentia extending to the seventh ganglion after the female genital pore, after the fourth ganglion the vasa deferentia thickened and strongly curled.

The female sexual organs are characterized by the ovarian sacks (paired structure which are lying dorsally to the vas deferens) reaching the fourth somite after the female genital pore. The ovarian sacks are strongly curled in their entire course (Fig. 3A).

Etymology: The species is named after the mountain (Prokletije – a mountain range on the western Balkan peninsula, extending from northern Albania to Kosovo and eastern Montenegro) from which the type specimen was collected.

Differential diagnosis: The new species is closely related to *Dina montana* Sket, 1968 and *D. dinarica* Sket, 1968. The both species live in the same area as the new species (see: Grosser *et al*. 2015a, b). From the latter species *Dina prokletijaca* n. sp. differs in the size, reaching approximately the half of size of *D. dinarica*. The latter species has more pronounced annulation than the new species with all annuli apart from b6 may be subdivided. Further difference includes the extension of the ovarian sacks which is one somite shorter in *D. dinarica*. Due to the shape and extension of the sexual organs *Dina prokletijaca* n. sp. resembles *D. montana* Sket, 1968, a species originally described from the Prokletije Mountain (Sket 1968). The latter species differs from *Dina prokletijaca* n. sp. in: 1) slender and dorsoventrally more flattened body, dorsal surface smooth without papillae, 2) cranial sucker elongated with a small mouth opening (Fig. 3C right), 3) the dorsal colour of freshly preserved specimens of *D. montana* is very dark greyish to brownish, therefore the dark paramedian stripes are not clearly visible, and 4) eight eyes visible, occasionally only a single eye absent.

Dina lineata (O. F. Müller, 1774) a medium sized to large leeches can be easily distinguished from *D. prokletijaca* n. sp., by the slender body with a small mouth opening. The small sized *Dina lineata lacustris* Sket, 1968 differs in the female genital pore shifted on b6 (in the middle or at the end of the annulus), never in the furrow b5/b6 (Sket 1968).

The reduction of visible eyes make the new species similar to *Dina minuoculata* Grosser, Moritz & Pešić, 2007, a medium to large yellow spotted leech known from the mountain area of Montenegro (Grosser *et al*. 2007). The latter species is characterized by the conspicuous papillae and less curled ovarian sacks. Moreover, the reduction of pigmentation of eyes is much further progressed in *D. prokletijaca* n. sp.

Variability. A strong variability was observed in eyes and extension of ovarian sacks. In the holotype eyes are not visible; in some young specimens a small single eye can be present. The extension of ovarian sacks also vary; in one specimen the both ovarian sacks reaches the ganglion of the fourth somite, in another specimen the ovarian sacks extends to the furrow b6/a1 between the third and fourth somite after the female genital pore. However, intermediate position was also found and in one specimen, the right ovarian sack reaches to the ganglion of the fourth somite but the left one reaches to the furrow b5/b6 of the third somite after the female genital pore.

Habitat: *Dina prokletijaca* n. sp. was collected in rheocrene springs in deciduous forests dominated by the common beech (*Fagus sylvatica* L.). The leeches were collected from March to December, with peak in spring.

Distribution: Kosovo (Prokletije Mts.).

Key to the *Dina*-species from the Western Balkan (excluded the species of the so-called *Dina ohridana*-complex which contains species endemic to lake Ohrid, characterized by genital pores separated by three annuli)

1 Living specimens white, without any dark or yellow spots or stripes; eyes absent; caudal sucker large, reaching the maximum body diameter; genital pores separated by 3 annuli; troglobiont. ..... *Dina absoloni* Johansson, 1913
- Living specimens coloured with dark paramedian stripes; caudal sucker, eyes and separation of genital pores various; not typical troglobiont. *2*
2 *Trocheta*-like annulation, somite with 8 to 9 annuli, 2 or 3 broadened annuli following 5 or 7 narrow annuli; cranial sucker elongated with a rostelloid upper lip; large leeches, > 100 mm in length..... *Dina krasensis* (Sket, 1968)
- *Dina*-like annulation, somites quinqueannulate with b6 broadened..... 3
3 Dorsal surface with yellow or whitish spots..... 4
- Dorsal surface without yellow or whitish spots..... 5
4 Male genital pore in furrow b1/b2, the female in furrow b5/b6, genitale pores separated by three annuli; dorsal surface with numerous inconspicuous papillae; ovisacs curled in posterior parts ..... *Dina latestriata* Neubert & Nesemann, 1995
- Male genital pore situated in furrow b2/a2, the female in furrow b5/b6, genitale pores separated by 2 annuli; dorsal surface with numerous well visible yellowish papillae; ovisacs not curled .....*Dina minuoculata* Grosser, Moritz & Pešić, 2007
5 Male genital pore in furrow b2/a2, the female usually on b6; ovarian sacks elongated 2 somites after the female genital pore.....*Dina lineata* (O. F. Müller, 1774) 6
- Male genital pore in furrow b2/a2, the female in furrow b5/b6, the genitale pores separated by two annuli; ovarian sacks various..... 7
6 Female genital pore is usually situated on b6, in furrow c11/c12, the genital pores separated by 2.5 annuli; medium to large sized leeches up to 55 mm in length ....*Dina lineata lineata* (O. F. Müller, 1774)
- Female genital pore always situated on b6, but the position in the annulus is variable; small sized reaching a maximum 30 mm in length .....*Dina lineata lacustris* Sket, 1968
7 Mouth opening small, upper lip of the oral sucker elongated .... *Dina montana* Sket, 1968
- Mouth opening wide, upper lip of the oral sucker not elongated 8
8 Caudal sucker slightly narrower than the maximum body width; dorsal surface in anterior part of body medially with one bright stripe, bordered by two wide and dark greyish areas, in posterior part of body with one dark median and two dark paramedian longitudinal stripes; ovisacs curled in posterior parts..... ..... *Dina sketi* Grosser & Pešić, 2014
- Caudal cucker narrower than the maximum body width; longitudinal paramedian stripes on the dorsal surface well visible along the entire length, the mid-dorsal stripe with a brighter and darker part lacking; ovisacs curled in their entire course..... 9
9 Annulus b6 not or only very slightly subdivided, other annuli not subdivided; eyes reduced, only single eyes could be visible; small leech up to 27 mm in length (preserved); ovisacs reach to the fourth somite after the female genital pore ....*Dina prokletijaca* n. sp.
- All annuli apart from b6 may be subdivided; eight eyes in pairs visible (a single eye could be reduced); medium to large up to 50 mm or more in length; ovisacs reach to the third somite after the female genital pore .....*Dina dinarica* Sket, 1968

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