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Mideopsis milankovici sp. nov. a new water mite from Montenegro based on morphological and molecular data (Acariformes, Hydrachnidia, Mideopsidae)

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Original research

ABSTRACT

Water mite species of the genus *Mideopsis* Neuman, 1880 are common in running and standing waters in the Palaearctic. In the present study we used an integrative taxonomic approach by applying partial COI sequences (DNA-barcodes) and morphological characteristics to describe a new species, *Mideopsis milankovici* sp. nov. from the Mediterranean region of Montenegro. A high genetic distance (18.8-26% K2P) from three other known European congeners, *M. crassipes* Soar, 1904, *M. orbicularis* (Müller, 1776), and *M. roztoczensis* Biesiadka & Kowalik, 1979, support *M. milankovici* sp. nov. as a distinct species. From *M. persicus* Pešić & Saboori, 2015, a species known from South Iran, which resembles the new species in the shape of the ejaculatory complex, *M. milankovici* sp. nov. differs by the morphology of dorsal shield.

Keywords Acari; DNA-barcoding; new species; running waters; Montenegro

Zoobank <http://zoobank.org/1627D1BA-DBD7-4FFE-820A-AB390A3B5FFE>

Introduction

Water mites of the genus *Mideopsis* Neuman, 1880 are known from the Holarctic and Neotropical regions (Cook 1974; Pešić *et al.* 2013). Recently, the genus was revised by Pešić *et al.* (2013). The distribution is disjunct, with one group of species with a Holarctic distribution extending with a few species into the Oriental region, and a few species extending into Costa Rica in the New World, and another group limited to South America. According to Pešić *et al.* (2013) it is likely that the South American taxa which are characterized by extensive setal patches in the male genital field area could represent a distinct genus.

So far, only five species of the genus *Mideopsis*, i.e., *M. crassipes* Soar, 1904, *M. orbicularis* (Müller, 1776), *M. roztoczensis* Biesiadka & Kowalik, 1979, *M. rossicus* Tuzovskij, 2002 and *M. persicus* Pešić & Saboori, 2015 have been reported from the Western Palearctic (Tuzovskij 2002; Pešić and Saboori 2015; Gerecke *et al.* 2016).

The present study, from a methodological point of view, is based on techniques established in the past decade (e.g., Fisher 2015, Pešić *et al.* 2019b and bibliography cited therein). Thus, with the exception of *Xystonotus willmanni* (K. Viets, 1920) (see Blattner *et al.* 2019) so far molecular methods have not been used in studies of Palaearctic mideopsid mites.

In this paper, *Mideopsis milankovici* sp. nov. is described from Montenegro. In order to gain insight into the position of the new species in relation to other *Mideopsis* species from the Palaearctic region, we combined morphological and molecular analyses to infer molecular similarities among the studied species.

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Materials and methods

Sampling

Water mites were collected by hand netting, sorted live in the field, and immediately preserved in 96% ethanol. Specimens for molecular analysis were examined without dissecting under a compound microscope in ethanol, using a cavity well slide with a central depression. After DNA extraction, some specimens were dissected and slide mounted in Hoyer's medium. DNA sequences prepared in the course of this study were deposited in Bold with voucher codes and accession numbers indicated in Table 1. The holotype and paratypes of the new species will be deposited in Naturalis Biodiversity Center in Leiden (RMNH).

All measurements are given in μm . The following abbreviations are used: Ac-I = first acetabulum; Cx-I = first coxae; COI = cytochrome *c* oxidase subunit I; dL = dorsal length; H = height; I-L-4-6 = fourth-sixth segments of first leg; L = length; mL = medial length; P-1-P-5 = palp segment 1-5; RMNH = Naturalis Biodiversity Center, Leiden; W = width.

Table 1 List of sequenced specimens used in this study.

Locality (country, name)	Lat/Long	Voucher code	BOLD Acc. nos.
<i>Mideopsis crassipes</i> Soar, 1904			
The Netherlands, Middelsgraaf at crossing with Doorderweg	51°5.190 N, 5°51.468 E	RMNH.ACA.812	NLACA125-15
The Netherlands, Middelsgraaf at crossing with Doorderweg	51°5.190 N, 5°51.468 E	RMNH.ACA.356	NLACA126-15
The Netherlands, Middelsgraaf at crossing with Doorderweg	51°5.190 N, 5°51.468 E	RMNH.ACA.357	NLACA127-15
The Netherlands, stream Verloren Beek	52°19.845 N, 5°59.396 E	RMNH.ACA.812	NLACA396-15
The Netherlands, stream Verloren Beek	52°19.845 N, 5°59.396 E	RMNH.ACA.813	NLACA397-15
The Netherlands, stream Verloren Beek	52°19.845 N, 5°59.396 E	RMNH.ACA.814	NLACA398-15
The Netherlands, stream Verloren Beek S van Epe	52°20.484 N, 5°59.250 E	RMNH.ACA.517	NLACA249-15
<i>Mideopsis milankovici</i> sp. nov.			
Montenegro, Međurječka river	42°01.353 N, 19°13.198 E	22. M19_24_2_E12	DNAEC059-20
<i>Mideopsis roztoczensis</i> Biesiadka & Kowalik, 1979			
The Netherlands, stream Ruenbergerbeek E of Overdinkel	52°14.657 N, 7°2.287 E	RMNH.ACA.1102	NLACA073-15
The Netherlands, stream Ruenbergerbeek E of Overdinkel	52°14.657 N, 7°2.287 E	RMNH.ACA.1103	NLACA074-15
The Netherlands, Lake Maarseveen	52°8.643 N, 5°5.084 E	RMNH.ACA.374	NLACA139-15
The Netherlands, stream Roggelsche Beek	51°15.224 N, 5°56.213 E	RMNH.ACA.876	NLACA450-15
The Netherlands, stream Roggelsche Beek	51°15.224 N, 5°56.213 E	RMNH.ACA.877	NLACA451-15
<i>Mideopsis orbicularis</i> (Müller, 1776)			
The Netherlands, Lake Maarseveen	52°8.643 N, 5°5.084 E	RMNH.ACA.1248	NLACA116-15
The Netherlands, Ditch along E-side Hoge Weg	52°48.450 N, 5°57.569 E	RMNH.ACA.373	NLACA138-15
The Netherlands, Lake Maarseveen	52°8.643 N, 5°5.084 E	RMNH.ACA.537	NLACA262-15
The Netherlands, Dompekolck, Kamperveen	52°30.367 N, 5°55.828 E	RMNH.ACA.546	NLACA269-15
<i>Xystonotus willmanni</i> (K. Viets, 1920)			
The Netherlands, Spring Mosbeek Maatmansweg	52°26.754 N, 6°51.854 E	RMNH.ACA.889	NLACA457-15
The Netherlands, Spring Mosbeek Maatmansweg	52°26.754 N, 6°51.854 E	RMNH.ACA.892	NLACA459-15

Molecular analysis

For the methods used for COI gene amplification and sequencing see Pešić *et al.* (2017, 2019a). For this study, DNA was extracted from 16 specimens of the genus *Mideopsis* from Montenegro and The Netherlands (Table 1). *Xystonotus willmanni* (K. Viets, 1920) from The Netherlands was used as an outgroup.

Phylogenetic analysis

Sequences were aligned by MUSCLE 3.8.425 algorithm as implemented in Geneious Prime 2020.1.1 (Biomatters Ltd.). The neighbor-joining (NJ) and maximum-likelihood (ML) were constructed using the MEGA X software (Kumar *et al.* 2018). Pairwise distance calculations between nucleotide sequences were computed using Kimura's 2-parameter (K2P) distance model (Kimura 1980) for all codon positions and transition/transversion ratio was calculated using MEGA X (Kumar *et al.* 2018). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). We used ML analyses with 500 bootstrap replicates using GTR+I as the most appropriate model of sequence evolution for our data set based on the Bayesian (*BIC*) and corrected Akaike Information Criterion (*AICc*) in the ML model selection feature of MEGA X. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Results and discussion

COI sequences analysis

The analysis involved 18 nucleotide sequences. The nucleotide sequences could be translated into amino acid sequences without any stop codons. The final alignment for the species delimitation using COI sequence data comprised 668 nucleotide positions (nps) for five species including *Xystonotus willmanni* to root the tree. In the analysis of our COI data, the neighbor-joining (NJ) and maximum-likelihood (ML) result trees gave similar results. Other than minor differences in bootstrap support values, no notable differences were found. The ML result tree is presented in Figure 1.

The COI sequence found in the *Mideopsis* specimen collected in Montenegro was recovered as a sister clade of the clade formed by *Mideopsis orbicularis* and *M. roztozensis* (Figure 1). The genetic distance between the COI sequence of the specimen from Montenegro here assigned to *M. milankovici* **sp. nov.** and *M. orbicularis* was 18.8%. Only one specimen of the new species could be acquired for use in molecular analysis, so intraspecific differences in COI sequence could not be investigated.

The mean genetic distance between congeneric COI sequence groups recovered in the molecular analysis ranged from 18% between *M. orbicularis* and *M. roztozensis*, to 29% between *M. orbicularis* and *M. crassipes* (Table 2). The intraspecific distance of *M. orbicularis* was 1% whereas the other two species, i.e., *M. crassipes* and *M. roztozensis* showed no intraspecific variation.

Systematics

Family Mideopsidae Koenike, 1910

Genus *Mideopsis* Neuman, 1880

Mideopsis milankovici **sp. nov.**

Zoobank: [B619AD87-81F8-4156-B86E-3360053AF3F8](https://zoobank.org/B619AD87-81F8-4156-B86E-3360053AF3F8)

Figures 2–3, 4a–b, e–g

Material examined — Holotype ♂ (RMNH), sequenced [22. M19_24_2_E12], dissected and slide mounted, Montenegro, Bar, Međurječka rijeka stream, downstream, between villages

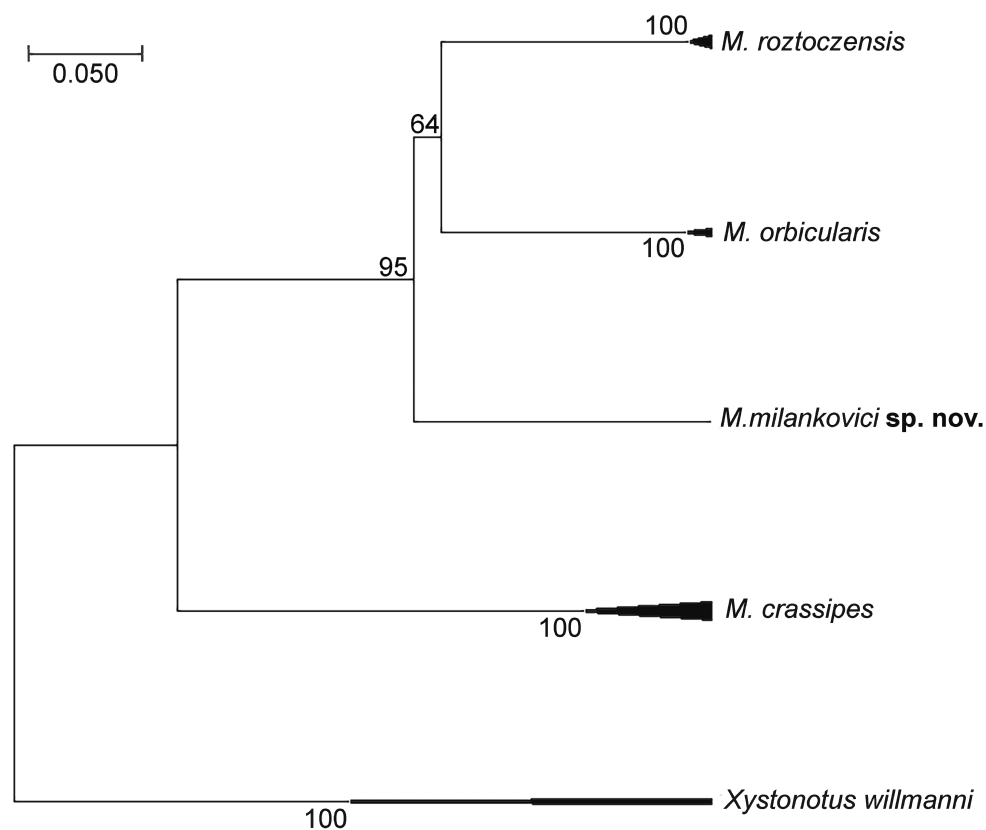


Figure 1 Maximum Likelihood tree based on the barcode region of the COI marker. The numbers near the branches represent the bootstrap probabilities.

of Pečurice and Krute, 42°01'21.21"N, 19°13'11.92"E, 26.vi.2019, leg. Pešić. Paratype: 5♂♂, 17♀♀, Bar, Međurječka rijeka stream, upstream, 42°2'10.80"N, 19°13'4.30"E, 16.vi.2020, leg. Pešić, 1♂, 1♀ dissected and slide mounted (RMNH).

Other material — Montenegro, Bar, Stari Bar old town, Rikavac steam, 42°6'1.72"N, 19°8'33.03"E, 3.vi.2021, leg. Pešić 1♀.

Diagnosis — Dorsal shield flattened, in the centre slightly elevated, V-shaped area formed by anteriorly diverging lines of fine porosity little evident. Ejaculatory complex with well sclerotized anterior keel, anterior ramus wedge-shaped. Postgenital area short (males 116–125 μm, 18% dorsal shield L; females 85 μm, 12% dorsal shield L), excretory pore closer to posterior idiosoma margin (distance 25–35 μm).

Description — Idiosoma rounded; colour dark yellowish to brown. Dorsal shield in the

Table 2 Estimates of genetic distance (K2P) of the mtCOI gene fragment between (lower matrix) and within (diagonal) studied mideopsid species.

	<i>M. roztoczensis</i>	<i>M. milankovici</i>	<i>M. crassipes</i>	<i>M. orbicularis</i>	<i>X. willmanni</i>
<i>M. roztoczensis</i>	0				
<i>M. milankovici</i>	0.208	n/c			
<i>M. crassipes</i>	0.267	0.26	0		
<i>M. orbicularis</i>	0.18	0.188	0.29	0.01	
<i>X. willmanni</i>	0.261	0.283	0.272	0.263	0.00

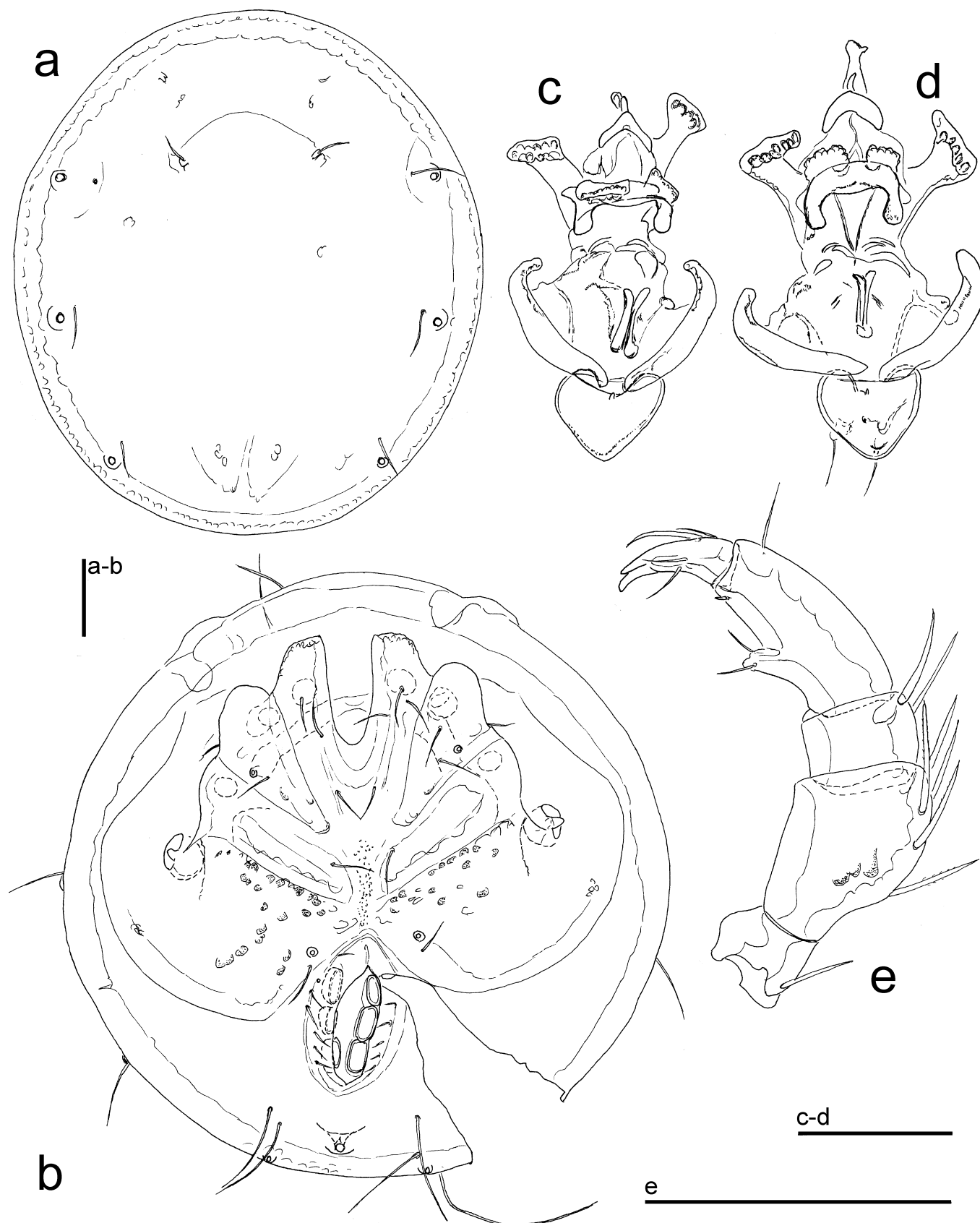


Figure 2 *Mideopsis milankovici* sp. nov., ♂ (a-c, e, holotype; d, paratype), Međurječka rijeka stream, Montenegro. a – dorsal shield; b – ventral shield; c-d, ejaculatory complex; e – palp. Scale bars = 100 μm.

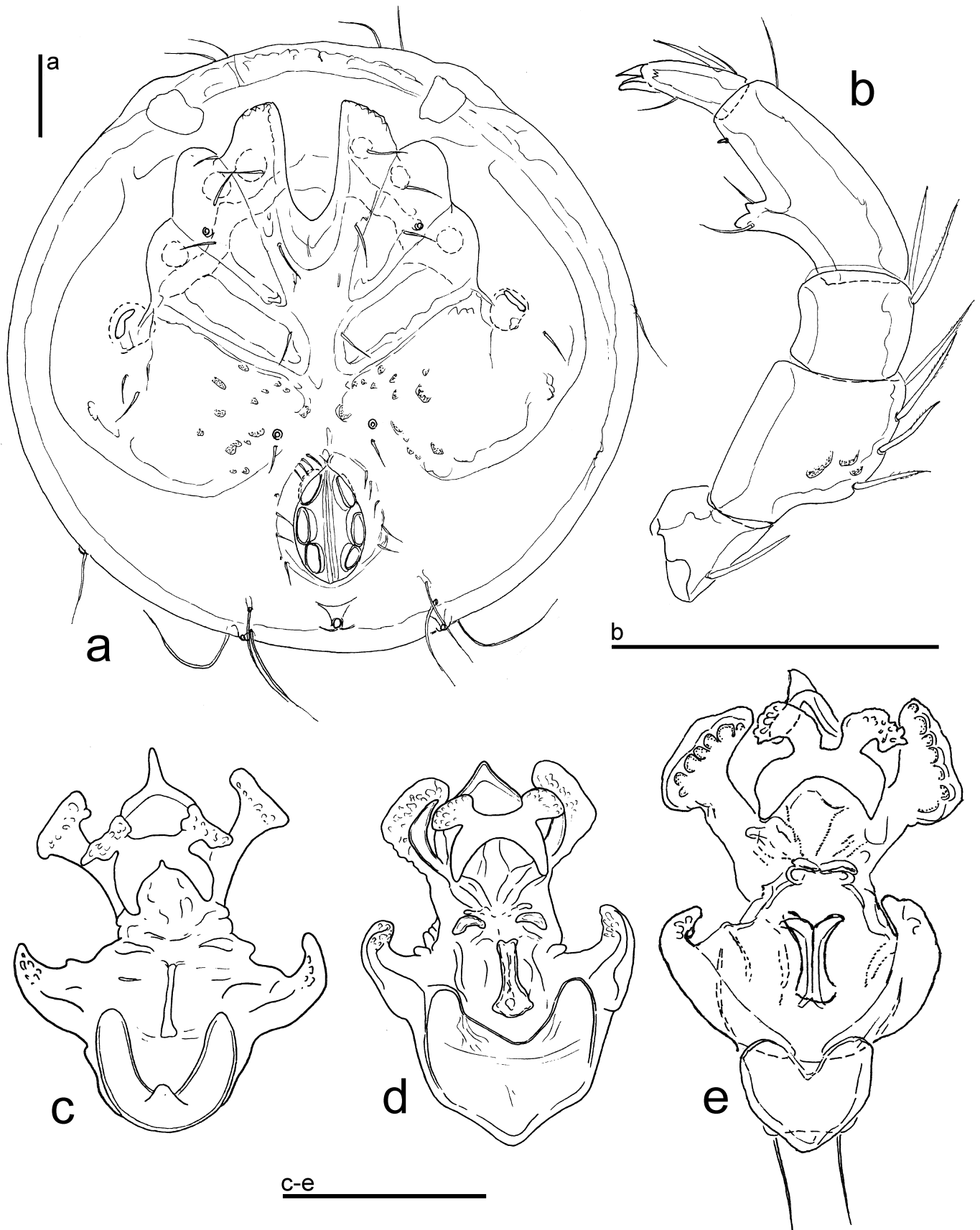


Figure 3 a–b *Mideopsis milankovici* sp. nov., ♀ paratype, Međurječka rijeka stream, Montenegro. a – ventral shield; b – palp; c–e, ejaculatory complex (c–d, from Biesiadka and Kowalik 1979; e – from Pešić and Saboori 2015): c – *M. orbicularis* (Müller, 1776); d – *M. roztoczensis* Biesiadka & Kowalik, 1979; e – *M. persicus* Pešić & Saboori, 2015. Scale bar = 100 µm.

centre slightly elevated, with anteriorly diverging lines of particularly fine porosity forming a V-shaped area slightly evident (Figures 4a–c). Postgenital area short (about 12–18% dorsal shield L), excretory pore close to posterior idiosoma margin (distance 25–35 μm). Ejaculatory complex with a strongly sclerotized anterior keel, anterior ramus wedge-shaped (Figures 2c–d). Palp: P-1 with a seta in its proximal part; P-2 ventral margin almost straight; P-3 ventral margin concave, distal margin convex; P-4 ventral projection directed ventrally, with two tips, both flanked by a fine subterminal seta; distal part of P-4 slightly narrower than basal part, with 3 subapical setae, one ventrally, slightly thicker and stiff, and two fine, located dorsally and laterally (Figures 2e and 3b). *Male* – P-2 L/H ratio 2.5. *Female* – P-2 more slender than in male, L/H ratio 2.7.

Measurements — *Male* (Holotype; in parentheses paratype, n = 1) – Dorsal shield L 684 (641), W 606 (575), L/W ratio 1.13 (1.12). Ventral shield L 800 (772), W 800 (747); gnathosomal bay L 149 (141), Cx-III W 416 (378), distance between IV-L insertions 466 (419). Genital field: gonopore L/W 153/50 (156/50), ratio 3.1 (3.1), L Ac-1-3: 44 (41), 44 (45), 44 (41). Distance genital field-excretory pore 77 (75), excretory pore-caudal idiosoma margin 34 (30). Ejaculatory complex L 244 (275). Capitulum vL 130; chelicera: total L (163), claw L (92). Palp: total L 240 (227), dL/H, dL/H ratio: P-1, 28/31, 0.92 (30/30, 1.0); P-2, 67/45, 1.48 (59/42, 1.41); P-3, 31/37, 0.84 (31/34, 0.93); P-4, 73/29, 2.5 (69/28, 2.46); P-5, 41/16, 2.65 (38/16, 2.4); L ratio P-2/P-4 0.93 (0.86). dL of I-L-1-6: 61 (61), 63 (68), 65 (62), 77 (67), 103 (98), 127 (128); I-L-6 H 36 (36); dL/H I-L-6 ratio 3.5 (3.6). dL of IV-L-1-6: 84 (77), 106 (91), 84 (78), 113 (116), 138 (132), 131 (134).

Female (paratype, n = 1) – Dorsal shield L 708, W 625, L/W ratio 1.13. Ventral shield L 750, W 775; gnathosomal bay L 153, Cx-III W 391, distance between IV-L insertions 447. Genital field: gonopore L/W 141/86, ratio 1.6; L Ac-1-3: 47, 47, 38. Distance genital field-excretory pore 44, excretory pore-caudal idiosoma margin 25. Chelicera total L 164, claw L 84. Palp: total L 242, dL/H, dL/H ratio: P-1, 34/30, 1.16; P-2, 59/44, 1.36; P-3, 31/36, 0.88; P-4, 77/28, 2.73; P-5, 41/15, 2.65; L ratio P-2/P-4 0.78. dL of I-L-1-6: 52, 61, 63, 70, 97, 122; dL of IV-L-1-6: 77, 106, 82, 125, 141, 138.

Etymology — Named after Prof Milutin Milanković (1879–1958), the eminent Serbian astrophysicist best known for developing one of the most significant theories relating to earth movements and long-term climate change.

Discussion — The phylogenetic analysis based on COI data reveals that *M. milankovici* **sp. nov.** is most similar to *M. orbicularis*. The high distance between these two species (18.8% K2P) suggests a long independent history of these two species. The relatively high K2P distance seems to be typical for water mites (Blattner *et al.* 2019) and the obtained data of our study are comparable with the genetic distance between cryptic species of other water mite clades (see Ståhlstedt *et al.* 2013, Martin *et al.* 2010, Pešić *et al.* 2017, 2019a). *Mideopsis orbicularis* is widely distributed in the Palaearctic, inhabiting various types of standing waters such as lakes and canals, occasionally also lowland streams (Gerecke *et al.* 2016), but never recorded from sites with a seasonal flow. From a morphological point of view, *M. orbicularis* can be separated from the new species only in the male sex, based on the shape of the ejaculatory complex (anterior ramus regularly rounded, anterior keel narrow and weakly sclerotized – see Figure 3c). *Mideopsis roztoczensis*, a species widely distributed in running waters in Europe, is characterized by a more elevated dorsal shield, a larger postgenital area (140–190 μm), the excretory pore more distanced from the posterior margin of the idiosoma (distance in general 40–90 μm), and the anterior ramus of the male ejaculatory complex (wedge-shaped as in *M. milankovici* **sp. nov.**) being wider, with a characteristic arrow-shaped delimited area – see Figure 3d).

Due to similarity in shape of ejaculatory complexes (see Figure 3e), *M. milankovici* **sp. nov.** resembles *M. persicus* Pešić & Saboori, 2015. The latter species is known from a single male collected in a stream in Fars Province of South Iran (Pešić and Saboori 2015). *Mideopsis persicus* can be separated by the shape of the dorsal shield with distinct, anteriorly diverging lines of fine porosity forming a well visible V-shaped area. In the new species from

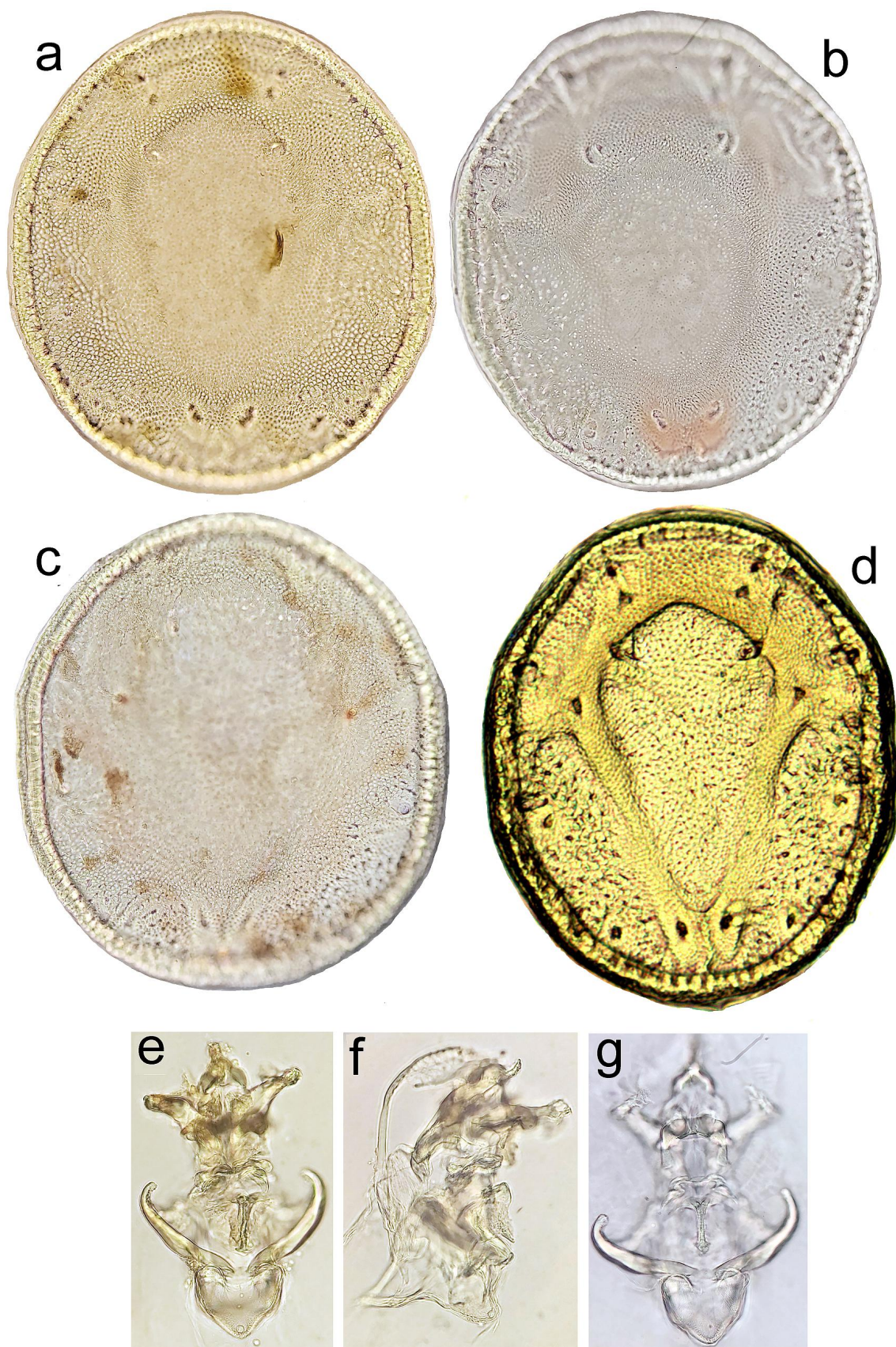


Figure 4 Photographs of dorsal shield (a–d; photographed immediately after dissection) and ejaculatory complex (e–g). a–c, e–g, *Mideopsis milankovici* sp. nov., Međurječka Rijeka stream, Montenegro: a, e, f – holotype ♂; b, g – paratype ♂; c – paratype ♀. d – *M. persicus* Pešić & Saboori, 2015, holotype ♂, Firooz Abad, Iran. Scale bar = 100 µm.

Montenegro this V-shaped area is almost indistinguishable (compare Figures 4a–c with Figure 4d).

Mideopsis crassipes, a species widely distributed in the Holarctic (Gerecke *et al.* 2016) and *M. rossicus*, a species known from Russia (Tuzovskij 2002), can be separated from all above-mentioned species including *M. milankovici* **sp. nov.** in having an egg-shaped idiosoma and a ventral extension of P-4 strongly curved with anteriorly directed tips.

Habitat — Characteristics of sampling sites indicate a preference for intermittent habitats. Both streams in which *M. milankovici* **sp. nov.** was collected are located in the narrow coastal region of Montenegro, their middle and lower courses regularly dry up in summer (for an overview of the species and communities that inhabit intermittent rivers in the southern part of Montenegro see Pešić *et al.* 2020). The upper part of the Međurječka rijeka stream is perennial (Figure 5) but runs dry in its lower reach.

Distribution — Montenegro.

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Figure 5 Međurječka Rijeka stream, the *locus typicus* of *Mideopsis milankovici* **sp. nov.** (inset). Photo by V. Pešić.

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