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Article

Molecular diversity and species delimitation in the genus *Mideopsis* Neuman, 1880 in Europe (Acari, Hydrachnidia, Mideopsidae)

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Abstract
Water mites of the genus *Mideopsis* are often a very abundant member of running and standing water communities whose taxonomy has been based almost exclusively on traditional morphological studies. In this study, we present a species-level phylogeny of the genus in Europe using 71 cytochrome *c* oxidase subunit I (COI) barcodes available in the BOLD database. In addition to morphological identification, we applied two different molecular taxon delimitation approaches (Assemble Species by Automatic Partitioning - ASAP and multi-rate Poisson tree processes). The ASAP molecular delimitation revealed the presence of five molecular operational taxonomic units (MOTU), i.e., *M. crassipes* Soar, 1904*, M. milankovici* Pešić & Smit, 2020*, M. roztoczensis* Biesiadka & Kowalik, 1979 and two distinct *M. orbicularis* MOTUs, both widely distributed and sympatric in Central and Northern Europe. Morphological analysis revealed that specimens of Clade 1 match the description given by Biesiadka & Kowalik (1979) for *Mideopsis orbicularis*. We highlight the need for a thorough revision of the genus *Mideopsis* in Europe by integrating morphological and molecular data.

Key words: Acari, DNA barcoding, taxonomy, running waters, standing waters.

Introduction

Water mites of the genus *Mideopsis* Neuman, 1880 are known from the Holarctic and Neotropical regions (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.

In the Palaearctic, mideopsid mites are common both in running and standing waters. Currently, six species of the genus *Mideopsis*, i.e., *M. crassipes* Soar, 1904, *M. orbicularis* (Müller, 1776), *M. roztoczensis* Biesiadka & Kowalik, 1979, *M. rossicus* Tuzovskij, 2002, *M. persicus* Pešić & Saboori, 2015 and *M. milankovici* Pešić & Smit, 2020) have been reported from the Western Palaearctic (Tuzovskij 2002; Pešić & Saboori 2015; Gerecke *et al*. 2016; Pešić & Smit 2020).

Mideopsis orbicularis (Müller, 1776), the type species of the genus was described from Denmark under the name of *Hydrachna orbicularis*. Since then, *Mideopsis orbicularis* has often been reported from the Palaearctic, and in the bibliography of the 20th century, it is considered easily distinguishable. However, as demonstrated for some other widely distributed water mite species (see Pešić *et al*. 2017, 2019, 2022b for examples), the reliance on a single morphological character, has led to overlooking the diversity by misunderstanding species complexes as one single species. Biesiadka and Kowalik (1979) were the first to question the status of „*orbicularis*“ populations by studying populations from the Roztoce river and its tributaries in Poland. They erected a new species, i.e., *Mideopsis roztoczensis* Biesiadka & Kowalik, 1979, differing from the sympatric and often syntopic *M. orbicularis* by the characteristics of the dorsal and ventral shield and the shape of the ejaculatory complex.

The large number of standardized cytochrome c oxidase subunit I (COI) sequences that, as a result of current DNA barcoding initiatives, have become available in public databases such as Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) provide an opportunity to check the concordance between morphological and molecular identification and elucidate the geographical distribution of species of the genus *Mideopsis* in Europe, which is the aim of this study.

Material and Methods

Water mites were collected by hand netting, sorted live in the field, and immediately preserved in 96% ethanol for further molecular analyses (see below). After DNA extraction, some specimens were dissected and slide-mounted in Faure's medium, whereas the rest were transferred to Koenike-fluid.

The photographs of selected structures were made using a camera on a Samsung Galaxy smartphone. The voucher material will be deposited in the Naturalis Biodiversity Center in Leiden (RMNH).

Molecular and DNA barcode analyses

Molecular analyses were conducted at the Canadian Centre for DNA Barcoding (CCDB) in Guelph, Ontario, Canada (http://ccdb.ca/). DNA extraction, amplification, and sequencing of the standard barcode region of COI were performed according to established protocols (Ivanova *et al*. 2007, Ivanova & Grainger 2007a, b). Sequences were retrieved and assembled according to their internal pipeline and uploaded to BOLD database. DNA was extracted from 26 specimens of the genus *Mideopsis* listed in Table 1. For all other species (Arabi *et al*. 2012; Macher *et al*. 2020, Pešić *et al*. 2021a, b, 2022a; Pešić & Smit 2022; Klimov *et al*. 2022), 45 COI additional sequences were retrieved from the respective sequence data archives. Relevant voucher information, photos, and newly generated DNA barcodes are publicly accessible through the dataset DS-MIDHYD in BOLD. The DNA extracts were archived in −80 °C freezers at the Centre for Biodiversity Genomics (CBG; biodiversitygenomics.net).

The final *Mideopsis* dataset contained 72 sequences, including *Mundamella germanica* (DNAEC041-20) from Montenegro, which was used as an outgroup. COI barcode sequences were aligned using MUSCLE alignment (Edgar 2004). Intra- and interspecific genetic distances were calculated in MEGA X (Kumar *et al*. 2018) employing the Kimura-2-Parameter (K2P) (Kimura 1980). The latter software was used to produce the Maximum Likelihood (ML) tree (models selected by the BIC (Bayesian Information Criterion) implemented in MEGA X: GTR + I with an initial Neighbour-Joining (NJ) tree and using the Subtree- Pruning-Regrafting - Extensive heuristic search (SPR level 5). The support for tree branches was calculated using the nonparametric bootstrap method (Felsenstein 1985) with 1000 replicates and shown next to the branches. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair.

Table 1. Details on specimens used in this study, including data and coordinates of sampling sites, and the barcode index number.

Locality	**Coordinates**	**Voucher Code**	**BOLD/GenBank Acc Nos**	**BIN BOLD**	Reference
***Mideopsis crassipes* Soar, 1904**					
Netherlands, Gelderland, Epe	52.33° N, 5.989° E	RMNH.ACA.355	NLACA125-15	BOLD:ACR9760	Pešić & Smit 2020
		RMNH.ACA.356	NLACA126-15		
		RMNH.ACA.357	NLACA127-15		
		RMNH.ACA.517	NLACA249-15		
Netherlands, Limburg, Echt	51.086° N, 5.857° E	RMNH.ACA.812	NLACA396-15		
		RMNH.ACA.813	NLACA397-15		
		RMNH.ACA.814	NLACA398-15		
Norway, Trondelag, Orkdal, Orklavassdraget, Sagbekken, st. 1	63.2461° N, 9.67573° E	HYDCA197	MMHYD063-19/ OR918849		Public
Norway, Vestland, Osteroy, Saetretjorna, peatbog	60.574° N, 5.56° E	MARB_UIB_614	MARBN1101-23		This study
		MARB_UIB_615	MARBN1102-23		
Russia, Sverdlovsk Region, Visimsky Nature Reserve, Sulyom River	57.43° N 59.74° E	VZ19077	MW369222.1		Klimov *et al*. 2022
Poland, River Bukowa in Szewce	50.5905° N, 22.509° E	201021-011_I07_1F41SAA001_E4.ab1_1379Mideopsis_crassipes.	VPHYD005-23/ OR918845		This study
		201021-011_G07_1F41SAA001_D4.ab1_1309Mideopsis_crassipes.	VPHYD006-23/ OR918841		
		201021-011_C07_1F41SAA001_B4.ab1_1320Mideopsis_crassipes.	VPHYD007-23/ OR918827		
		201021-011_E07_1F41SAA001_C4.ab1_1384Mideopsis_crassipes.	VPHYD008-23/ OR918837		
		201021-011_K07_1F41SAA001_F4.ab1_1353Mideopsis_crassipes.	VPHYD009-23/ OR918835		

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TABLE 1

***Mideopsis milankovici* Pešić & Smit, 2020**					
Montenegro, Bar, Medjurjecka rijeka stream	42.0226° N, 19.22° E	22. M19_24_2_E12	DNAEC059-20/ OL870166	BOLD:AED2191	Pešić & Smit 2020
***Mideopsis orbicularis* Clade** 1					
Netherlands, Overijssel, Overdinkel: Ruenbergerbeek	52.244° N, 7.038° E	RMNH.ACA.1102 RMNH.ACA.1103	NLACA073-15 NLACA074-15	BOLD:ACS0476	Pešić & Smit 2020
Netherlands, Overijssel, Kamperveen: Dompekolk	52.506° N, 5.93° E	RMNH.ACA.1248	NLACA116-15		
Netherlands, Utrecht: Maarseveense Plas	52.144° N, 5.084° E	RMNH.ACA.374	NLACA139-15		
Netherlands, Limburg, Heythuizen: Roggelssche Beek	51.253° N, 5.936° E	RMNH.ACA.876 RMNH.ACA.877	NLACA450-15 NLACA451-15		
Germany, Baden-Württemberg, Lustnau, Ammer near Aeule Bridge	48.5268° N, 9.0794° E	CCDB 38392 E01 CCDB 41824 H03	DCBDJ049-21/ OR918834 HYDBH087-22/ OR918830		This study
Norway, Viken, Nittedal, Nitelva, river	59.988° N, 10.979° E	MARB_UIB_823 MARB_UIB_824 MARB_UIB_825	MARBN1215-23 MARBN1216-23 MARBN1217-23		
Poland, River Bukowa in Szewce	50.5905° N, 22.509° E	201021-011_A07_1F41SAA001_A4.ab1_1348Mideopsis_roztoczensis. 201021-011_M05_1F41SAA001_G3.ab1_1432Mideopsis_roztoczensis. 201021-011_I05_1F41SAA001_E3.ab1_1525Mideopsis_roztoczensis. 201021-011_G05_1F41SAA001_D3.ab1_1459Mideopsis_roztoczensis.	VPHYD001-23/ OR918832 VPHYD002-23/ OR918848 VPHYD003-23/ OR918833 VPHYD004-23/ OR918828		
Russia, Sverdlovsk, Pripyshminskiye Bory National Park, Urginsky pond	56.97° N, 63.68° E	SV20230	OL343047.1		Klimov *et al.* 2022

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TABLE 1

***Mideopsis orbicularis* Clade 2**					
Netherlands, Utrecht: Maarseveense Plas	52.144° N, 5.084° E	RMNH.ACA.373 RMNH.ACA.546	NLACA138-15 NLACA269-15	BOLD:ACR9763	Pešić & Smit 2020
Netherlands, Overijssel, Weerribben	52.807° N, 5.959° E	RMNH.ACA.537	NLACA262-15		
Norway, Vest-Agder, Vennesla, Drivenesvannet, lake, at outlet	58.2926° N, 7.92888° E	HYDCA267 HYDCA268	MMHYD133-19/ OR918846 MMHYD134-19/ OR918829		Public
Norway, Vest-Agder, Kristiansand, Nedre Jegersbergvann, upper part at Malaisefelle, lake	58.169° N, 8.0° E	HYDCA594	MMHYD460-21/ OR918838		
Norway, Vestland, Osteroy, Saetretjorna, river	60.574° N, 5.56° E	MARB_UIB_1050	MARBN777-23		This study
***Mideopsis roztoczensis* Biesiadka & Kowalik, 1979**					
Norway, Vest-Agder, Songdalen, Stavbekken, ved Dynamitten, creek	58.1585° N, 7.81138° E	HYDCA290 HYDCA291	MMHYD156-19/ OR918847 MMHYD157-19/ OR918836	BOLD:ACI1492	Public
Montenegro, Cetinje, Poseljanski stream at Poseljane	42.3095° N, 19.0518° E	CCDB-38679-A02	DNCBD002-20/ OL870113		Pešić *et al.* 2021a
Montenegro, Tuzi, Cijevna river near Dinosa	42.4057° N, 19.3569° E	CCDB-3867-G05	DNCBD077-20/ OL870298		
Montenegro, Danilovgrad, River Zeta near Spuz	42.5112° N, 19.1991° E	CCDB38233 C11 CCDB38233 C12	DCCDB035-21/ OL870122 DCCDB036-21/ OL870179		
Türkiye, Bingol Province, Cevrimpinar stream	38.9092° N, 40.4744° E	CCDB 38361 D10	DCDDJ046-21/ OM321051		Pešić *et al.* 2022a
Türkiye, Bingol Province, Capakcur stream	38.8936° N, 40.4772° E	CCDB 38361 E02	DCDDJ050-21/ OM321084		
Montenegro, Podgorica, River Zeta at Vranjske Njive	42.4683° N, 19.2579° E	CCDB 38363 D07	SEPTA043-21/ OL870110		Pešić *et al.* 2021a

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TABLE 1

Serbia, Zlatibor, Katušnica river below the Gostilje waterfall	43.6576° N, 19.8392° E	CCDB 38363 E08	SEPTA056-21/ OL874908	BOLD:ACI1492	Pešić *et al.* 2021b
Türkiye, Burdur Province, Kizilli village, stream	37.3425° N, 30.9256° E	CCDB 38363 F05 CCDB 38363 F06	SEPTA065-21/ OM321088 SEPTA066-21/ OM321048		Pešić *et al.* 2022a
Bosnia and Herzegovina, Trebinje, Jazina stream	42.7031° N, 18.5059° E	CCDB 41824 B01 CCDB 41824 B02 CCDB 41824 B03	HYDBH013-22/ OR918831 HYDBH014-22/ OR918839 HYDBH015-22/ OR918840		This study
Norway, Vestland, Osteroy, Saetretjorna, peatbog	60.575° N, 5.561° E	MARB_UIB_740 MARB_UIB_741	MARBN657-23 MARBN658-23		
Norway, Rogaland, Sandnes, Kvitlana, river	58.922° N, 5.883° E	MARB_UIB_758	MARBN1150-23		
Germany	-	MNHN-JAC 76	JN018102.1		Arabi *et al.* 2012
Cyprus	34.769801° N, 32.911568° E	-	MT671492.1		Macher *et al.* 2020
Norway, Arendal, bekk i oest mellom oevre og Midtre Sagvann, bekk	58.46° N, 8.695° E	HYDCA389	MMHYD253-20/ OR918843	BOLD:AEN6785	Public
Türkiye, Bingol Province, Capakcur stream	38.8936° N, 40.4772° E	CCDB 38361 E01	DCDDJ049-21/ OM321061		Pešić *et al.* 2022a
Serbia, Zlatibor, Katušnica river below the Gostilje waterfall	43.6576° N, 19.8392° E	CCDB 38363 E07	SEPTA055-21/ OL874890		Pešić *et al.* 2021b
Norway, Vestland, Bergen, stream near Asane	60.487° N, 5.327° E	MARB_UIB_1070	MARBN797-23		This study
Serbia, Stara Planina Mt., Crnovrška reka stream	43.4045° N, 22.5131° E	44. SR_CRNOVRSKA_D5	DNAEC038-20/ OL874867	BOLD:AEA2936	Pešić *et al.* 2021b
Belgium, Holzwarche E of Hunningen, stream	50.4056° N, 6.33775° E	CCDB-44301-F09 CCDB-44301-F10	HYDAL069-23/ OR918842 HYDAL070-23/ OR918844		This study

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TABLE 1

France, Corsica, Ruisseau de Sattu	41.6907° N, 9.1525° E	CCDB 38559 B10	NOVMB022-21/ ON002586	BOLD:AEO2944	Pešić & Smit 2022
		CCDB 38559 B12	NOVMB024-21/ ON002583		
France, Corsica, Ruisseau Tavulella	42.255° N, 8.76658° E	CCDB 38559 D07	NOVMB043-21/ ON002585		

We displayed the haplotype relationship within the species through a Minimum Spanning Network (MSN) using PopART software (Leigh & Bryant 2015). For the MSN visualization, sequences were trimmed to 474 bp, and the five shortest sequences were removed from the analysis (DCDDJ049-21, MMHYD253-20, SEPTA065-21, SEPTA066-21, DCBDJ049-21) to avoid further shortening of the alignment.

We employed three distinct species delimitation methods from two different approaches. Initially, we utilized two distance-based strategies: a) The ASAP procedure, a hierarchical clustering algorithm based on pairwise distance distribution designed for species partitioning (Puillandre *et al*. 2021). We employed the online ASAP version (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) with default settings and the K2P distance model; b) Barcode Index Number (BIN) method (Ratnasingham & Hebert 2013), integrated within BOLD, where COI DNA sequences, both newly submitted and existing, are indexed and clustered into distinct BINs based on their genetic distances. Subsequently, we applied a tree-based approach, mPTP (https://mcmc-mptp.h-its.org/mcmc/) (Kapli *et al.* 2017). This method delimits species by identifying the transition from between-species to within-species processes, accounting for varying levels of intraspecific genetic diversity due to differences in evolutionary history or species sampling (Reid & Carstens 2012). We used Maximum Likelihood (ML) trees calculated with IQTREE (W-IQTREE webserver - Trifinopoulos *et al*. 2016) as input for analysis.

Results

We present 71 DNA barcode sequences of the water mites of the genus *Mideopsis* from different parts of Europe (see Table 1 for details) available in BOLD database. Both the distance-based ASAP analysis and the tree-based method (mPTP) revealed similar groupings of molecular operational taxonomic units (MOTUs), with the ASAP method being the most conservative, grouping *M. roztoczensis* sequences together, while (mPTP) grouped these sequences into four MOTUs. Both methods confirmed the presence of the following MOTUs within our *Mideopsis* dataset, i.e. *M. crassipes, M. milankovici, M. roztoczensis,* and two different *M. orbicularis* MOTUs tentatively named here as *M. orbicularis* Clade 1 and *M. orbicularis* Clade 2. (Fig. 1).

The intraspecific K2P distance varied from < 1.0 (0.58±0.13% and 0.58±0.16% in *M. crassipes* and *M. orbicularis* Clade 2, respectively) to a maximum of 5.56±0.59%, for *M. roztoczensis* for which four BINs were detected. Interspecific distances varied from 16.3±1.8% (between *M. orbicularis* Clade 1 and *M. orbicularis* Clade 2) to 24.5±2.5% K2P between *M. roztoczensis* and *M. milankovici* (Table 2). The number of BINs per species ranged from one (*M. crassipes, M. milankovici, M. orbicularis* Clade 1, *M. orbicularis* Clade 2) to four BINS for *M. roztoczensis*. Minimum Spanning Networks shown (Fig. 2) for the species with multiple BINs (*M. roztoczensis*) revealed the presence of geographical patterns, which will be analyzed below together with morphological and molecular features of the identified *M. orbicularis* clades.

Mideopsis roztoczensis **Biesiadka & Kowalik, 1979**

Remarks — *Mideopsis roztoczensis* was originally described by Biesiadka & Kowalik (1979) from Szum River, Biała Łada River, Wieprz River and their tributaries in Roztocze area, east-central Poland. After that, it has been reported from different parts of Europe (Gerecke *et al*. 2016) and Türkiye (Pešić *et al*. 2022a). According to the original description by Biesiadka & Kowalik (1979), *Mideopsis roztoczensis* in the first line is characterized by a more elevated dorsal shield with distinctly visible anteriorly diverging lines of particularly faint fine porosity (Fig. 3A), and the shape of the male ejaculatory complex with the wedge-shaped anterior ramus being wider, with a characteristic arrow-shaped delimited area - see Figure 3K).

In the analysed material, *M. roztoczensis* was represented by 21 barcoded individuals assigned to four BINs (Fig. 2). BIN ACI1492, which is the most widespread in Europe, contained most of the sequences from Montenegro (n = 5), Norway (n = 5), Russia (n =1), Bosnia and Herzegovina (n = 3), Türkiye (n = 4), Serbia (n = 1), and Cyprus (n = 1). BIN AEN6785 consists of sequences from Türkiye, Serbia, and Northern Europe (Norway). The BIN AEA2936 includes sequences from Serbia and two from Belgium, while BIN AEO2944 contains sequences from Corsica deposited in BOLD. Some BINs, were found together at the same locality (e.g., BIN ACI1492 and BIN AEN6785 in Katušnica River in Serbia).

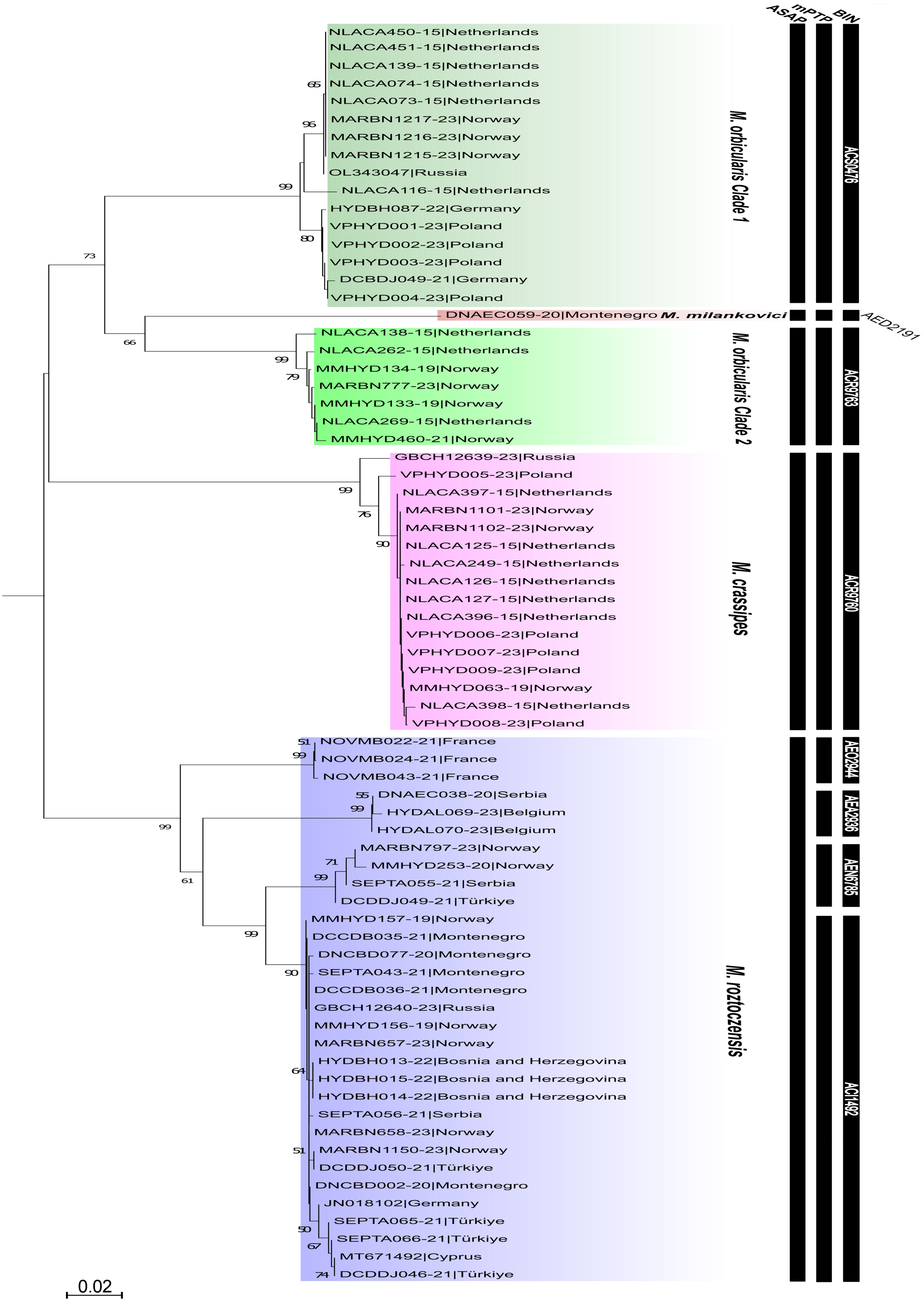

Figure 1. Maximum Likelihood tree (GTR+I model) of the *Mideopsis* spp. obtained from 71 nucleotide COI sequences. The results of the three species delimitation methods are indicated by vertical bars (BIN number given). The outgroup was removed from the figure. Only a bootstrap that supported more than 50% is reported.

Mideopsis orbicularis Clade 1

Material examined — Poland, River Bukowa near Szewce, 50.5905° N, 22.509° E, leg. Stryjecki, 3♂, two of them dissected and slide mounted (201021-011_M05_1F41SAA001G3.ab1, 201021-011_M05_1F41SAA001A4.ab1). Netherlands: Maarseveense Plas, Prov. Utrecht, 13.vi.2011 52.14403° N, 5.08472° E, leg. Smit, 1♂ dissected and slide mounted (RMNH.ACA.374); Dompekolk, Kamperveen, Overijssel, 52.50611° N, 5.93046° E, 17.viii.2012, leg Smit 1♀, dissected and slide mounted (RMNH.ACA.1248); Ruenbergerbeek E of Overdinkel, Prov. Overijseel, 52.24427° N, 7.03805° E, 18.v.2012 leg. Smit 1♂, 1♀ dissected and slide mounted (RMNH.ACA.1102,-1103); Roggelsche Beek, Prov. Limburg, 51.25371° N, 5.93691° E, 30.iv.2012 leg. Smit, 2♀ dissected and slide mounted (RMNH.ACA.876,-877)

Remarks — In this study, we barcoded three males from the Bukowa River in east-central Poland, and they perfectly match the description given by Biesiadka & Kowalik (1979) for the populations of *Mideopsis orbicularis* from neighbouring Roztocze region. According to the description of Biesiadka & Kowalik (1979) from the sympatric and often syntopic *M. roztoczensis*, *M. orbicularis* can be separated by a less elevated, nearly flattened dorsal shield with evident but less pronounced anteriorly diverging lines of particularly faint fine porosity (Fig. 3B), the comparatively slender and less sclerotized genital acetabula (Figs. 3F-G) and the excretory pore more distant from the posterior margin of the idiosoma (comparatively twice further than in *M. roztoczensis*).

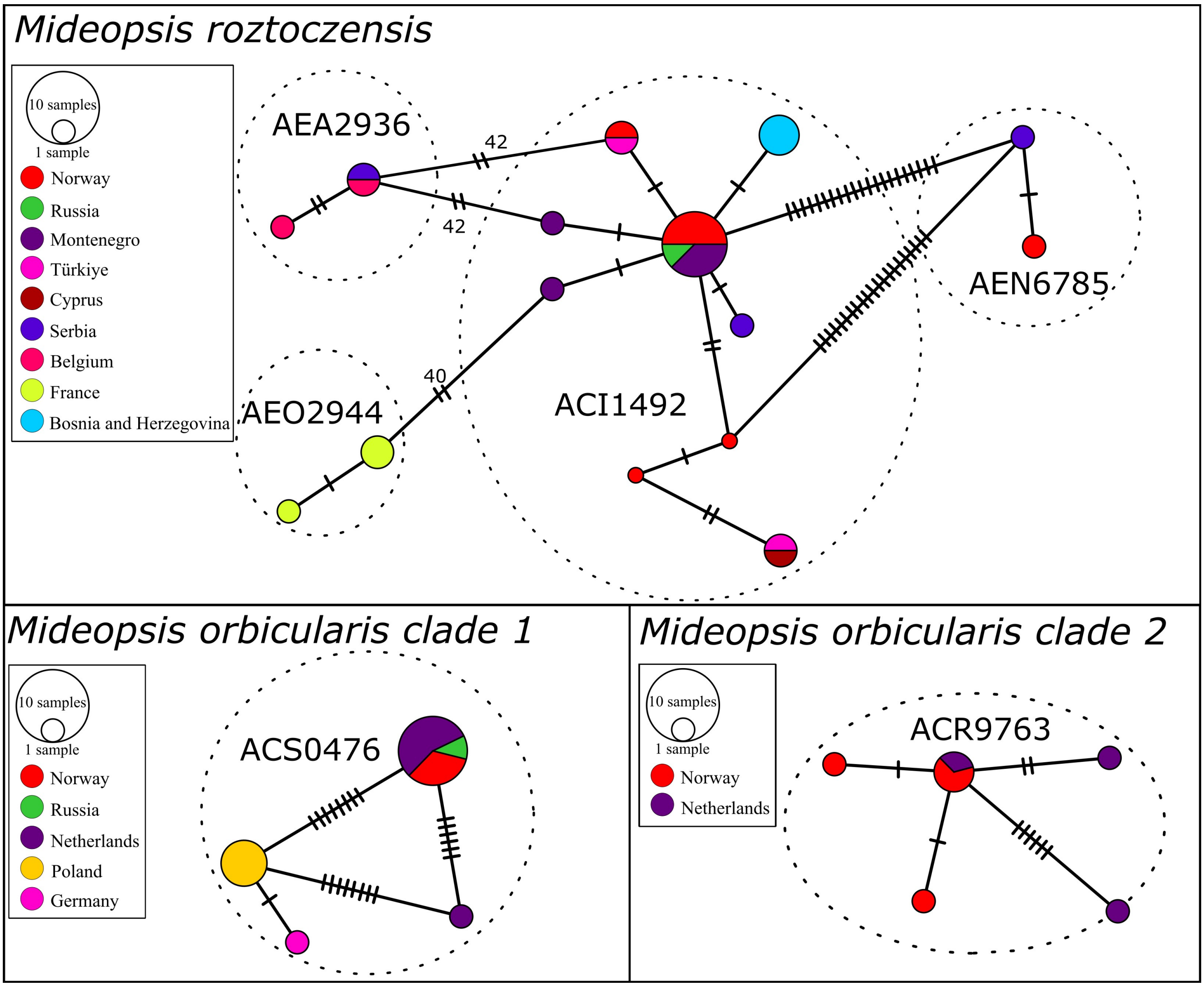

Figure 2. Minimum Spanning Network showing phylogenetic relationships within three *Mideopsis* clades: *M. roztoczensis*, *M. orbicularis* Clade 1, and *M. orbicularis* Clade 2. Each bar represents a single mutational change, and a dashed line encircled by separate BINs. The diameter of the circles is proportional to the number of individuals in each haplotype sampled (see open circles with numbers).

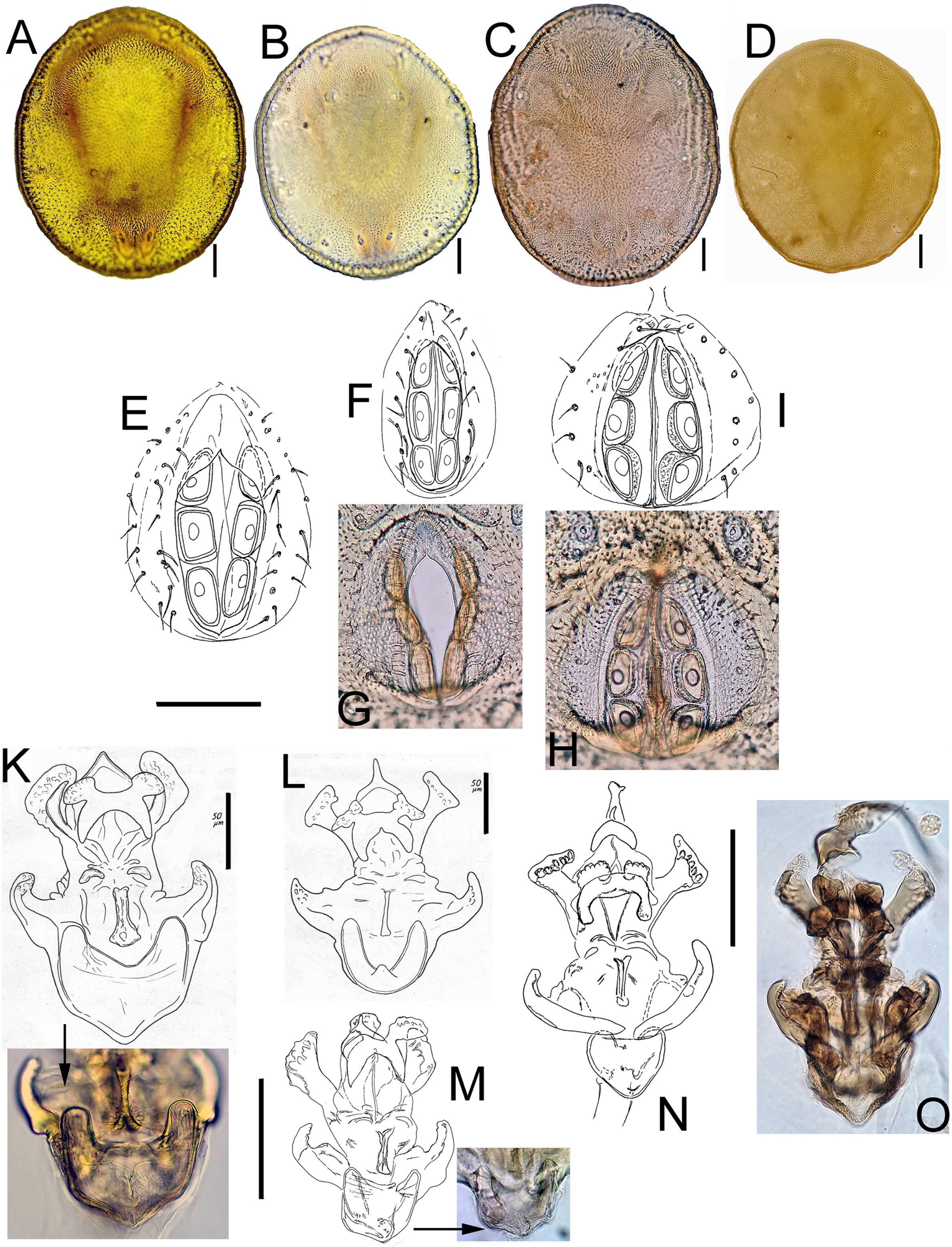

Figure 3. **A-D** Photographs of dorsal shield: **A** – *M. roztoczensis,* ♂, CCDB38233 C11, River Zeta, Montenegro. **B** – *M. orbicularis* Clade 1, ♂, River Bukowa, Poland. **C** – *M. orbicularis* Clade 2, ♀, RMNH.ACA.373, Netherlands. **D** – *M. orbicularis*, ♂, Tjeukemeer, Netherlands. **E-H** Genital field: **E** – *M. roztoczensis,* ♂, CCDB38233 C11, Montenegro. **F-G** – *M. orbicularis* Clade 1, ♂: **F** – River Bukowa, Poland; **G** – RMNH.ACA.374, Netherlands. **H-I** – *M. orbicularis* Clade 2, ♀, RMNH.ACA.373, Netherlands. **K-O** Ejaculatory complex. **K** – *M. roztoczensis,* ♂, Roztocze, Poland (from Biesiadka & Kowalik 1979: fig. 10), inset: photograph of anterior ramus, CCDB38233 C11, Montenegro). **L** – *M. orbicularis* Clade 1, Roztocze, Poland (from Biesiadka & Kowalik 1979: fig. 12). **M** – *M. orbicularis* Clade 1, ♂, River Bukowa, Poland (inset: photograph of anterior ramus). **N** – *M. milankovici*, paratype ♂, Montenegro (from Pešić & Smit 2020). **O** – *M. orbicularis*, ♂, Tjeukemeer, Netherlands. Scale bars = 100 µm (for K-L = 50 µm).

Further differences, according to Biesiadka & Kowalik (1979), are found in the shape of an ejaculatory complex with the anterior keel narrow and weakly sclerotized and a rounded anterior ramus in *M. orbicularis* (see fig. 3L, taken from Biesiadka & Kowalik 1979). Re-examination of males from our Polish material, which belongs morphologically to *M. orbicularis* sensu Biesiadka & Kowalik morphotype, reveals that the shape of the anterior ramus depends on the angle of observation and that it is more or less a wedge-shaped (see Fig. 3M), although not as clearly accentuated as in *M. roztoczensis* (see Fig. 3K).

Molecular data revealed that examined specimens of *M. orbicularis sensu* Biesiadka & Kowalik 1979 from Poland belong to the same BIN ACS0476. The latter BIN also includes sequences from Netherlands (n = 6), Russia (n = 1), Norway (n = 3) and Germany (n =2).

Mideopsis orbicularis Clade 2

Material examined — Netherlands: Ditch along E-side of Hoge Weg, Weerribben Prov. Overijssel, 52.80748° N 5.95947° E, 28.viii.2011 leg. Smit 1♂, dissected and slide mounted (RMNH.ACA.537); Maarseveense Plas, Prov. Utrecht, 52.14403° N, 5.08472° E, 13.vi.2011, leg. Smit, 1♀, dissected and slide mounted (RMNH.ACA.373).

Compared material — Netherlands: Prov. Friesland, Tjeukemeer, E-side, 52.88561° N, 5.84033° E, 6.vi.1992 leg. Smit 2♂, dissected and slide mounted.

Remarks — Molecular data reveals that some of the specimens morphologically assigned to *M. orbicularis* (three from the Netherlands) and/or *M. roztoczensis* (two from Norway) form a unique BIN ACR9763. In this study, we examine two slides of *Mideopsis* specimens from the Netherlands belonging to the BIN ACR9763 which were tentatively morphologically attributed to *M. orbicularis* by the junior author (H.S). The examined female specimen (RMNH.ACA.373) from the Netherlands differs from *M. orbicularis* Clade 1, in anteriorly diverging lines of particularly faint fine porosity not evident on the dorsal shield (Fig. 3C) and comparatively wider and more sclerotized acetabula (Figs. 3H-I).

In the phylogenetic tree based on the COI dataset, the latter clade was placed as a sister clade to a clade containing specimens of *M. milankovici* Pešić & Smit, 2020 from Montenegro (see Fig. 1). The level of COI differentiation between these two species was estimated to be 16.7±2.0% K2P divergence indicating their long genetic isolation.

Unfortunately, the slide of a barcoded male (RMNH.ACA.537) from the Netherlands did not allow for an examination of the morphology of the ejaculatory complex. We examined two males from Lake Tjeukemeer from the Netherlands. The ejaculatory complex of the examined males from Tjeukemeer is characterized by a comparatively much smaller, wedge-shaped, anterior ramus (see Fig. 3O), resembling that of *M. milankovici* (see Fig. 3N), a species recently described from the Mediterranean part of Montenegro (Pešić & Smit, 2020). Nevertheless, to clarify the taxonomic status of specimens from Lake Tjeukemeer and the status of the above-described *M. orbicularis* Clade 2, more specimens, including a male, need to be molecularly and morphologically analysed.

Discussion

In the present study, we showed that the diversity of the genus *Mideopsis* in Europe is underestimated, with more species present than previously recognized. The phylogenetic analysis and the applied delimitation methods revealed at least five distinct lineages of the genus *Mideopsis* in Europe.

For species delimitation, we used two approaches based on distance (ASAP) and coalescence (mPTP), and we found that MOTUs estimated by these methods were not congruent. *Mideopsis roztoczensis* was recognized as a single species by the ASAP method, indicating that the latter approach, which, as numerous studies have shown (e.g. Guo & Kong 2022; Yin *et al*. 2023) is more conservative and is probably the best species-delimitation model for water mites. This is consistent with the previous studies on water mites in which the delimitation approach was used (e.g., Klimov *et al*. 2022; Tyukosova *et al*. 2022; Pešić *et al*. 2023a, b; Pešić & Smit 2023; Pešić & Goldschmidt 2023) which indicates that the use of methods such as ASAP in combination with morphological data can be considered a successful tool for species delimitation in water mites.

Table 2. Estimates of average genetic distance (K2P) (given as distance ± standard error) within (intragroup) and between clades (intergroup) of examined species of *Mideopsis* sequence pairs. Standard error estimates (for intergroup) are shown above the diagonal.

Species	Intragroup	Intergroup				
		(1)	(2)	(3)	(4)	(5)
(1) *M. orbicularis* Clade 1	0.01±0.0026		0.018	0.020	0.022	0.024
(2) *M. orbicularis* Clade 2	0.0059±0.0016	0.163		0.020	0.020	0.025
(3) *M. roztoczensis*	0.0456±0.0049	0.202	0.198		0.025	0.022
(4) *M. milankovici*	n/c	0.191	0.167	0.245		0.024
(5) *M. crassipes*	0.0058±0.0013	0.234	0.252	0.230	0.228	

Our molecular analysis reveals that specimens from Central and Northern Europe, which were often attributed to widely distributed *M. orbicularis*, morphologically, due to a flattened dorsal shield, belong to two well-defined clades with a high COI divergence. The type material of *M. orbicularis* no longer exists, and the original description of Müller (1776) is too scanty to allow any interpretation of the taxonomic characters used in the separation of the two abovementioned genetic and morphological lineages.

Another *Mideopsis* species known from Northern Europe is *M. depressa* Neuman, 1880, a species originally described from Sweden (Neuman 1880) and later synonymized with *M. orbicularis*. In the original description of *M. depressa,* Neuman (1880) did not provide any information on possible diagnostic features that enabled the unequivocal separation of the latter species from *M. orbicularis*. He stated that the species is common in cold lakes (i.e., Mälaren, Vencrn, Asunden, and lakes in Valle county in Vestergötland, Bohuslän and Orust by Hulta). In this study, the molecularly assigned specimens to *M. orbicularis* Clade 2 were collected in standing water such as lakes and ditches. At the same time, *M. orbicularis* Clade 1 specimens were also collected in higher-order lowland streams (Biesadka & Kowalik 1979), suggesting that *M. orbicularis* Clade 2 could possibly be attributed to *M. depressa*.

As mentioned in the introduction, Biesiadka and Kowalik (1979) were the first to question the status of "*Mideopsis orbicularis*" populations in Europe. They provided a set of diagnostic morphological characters that enabled the identification of *M. orbicularis* Clade 1 and *M. roztoczensis* morphotypes. To clarify the taxonomic status within the *M. orbicularis* complex, additional material is needed for *M. orbicularis* Clades 1 and -2 specimens, including barcoded males of the latter clade.

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