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Article

New records of *Torrenticola cf. meridionalis* from Babia Góra Mountain (Poland)

Magdalena Szenejko^{1*} , Andrzej Zawal^{1, 2} , Ewelina Zawal³ , Alireza Saboori² , Zuzanna Krzynówek⁴ , Maja Krakowiak⁴ , Tapas Chatterjee⁵  and Vladimir Pešić⁶ 

1. Institute of Marine and Environmental Sciences, Center of Molecular Biology and Biotechnology, University of Szczecin, Wąska 13, 71–415 Szczecin, Poland; E-mails: Magdalena.szenejko@usz.edu.pl, andrzej.zawal@usz.edu.pl
2. Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran; E-mail: saboori@ut.ac.ir
3. Secondary school No. II in Szczecin, IB School No. 3885, Szczecin, Poland; E-mail: ewcia.zawal55@gmail.com
4. Institute of Biology, University of Szczecin, Wąska 13, 71–415 Szczecin, Poland; E-mails: 224954@stud.usz.edu.pl, 224965@stud.usz.edu.pl
5. Near Hari Mandir Road, Hirapur, Dhanbad 826001, Jharkhand, India; E-mail: drtchatterjee@gmail.com
6. Department of Biology, University of Montenegro, Cetinjski put b.b., 81000 Podgorica, Montenegro; E-mail: vladopesic@gmail.com

* Corresponding author

ABSTRACT

Torrenticola meridionalis Di Sabatino & Cicolani, 1990 was originally described from Italy and later has been noted in different parts of the Balkans. Next researches, based on molecular data, have shown that it is a species-complex. This work provides new data on *Torrenticola cf. meridionalis* based on DNA barcodes of specimens from Poland and confirms previous assumptions about the *Torrenticola meridionalis*-complex.

KEYWORDS: Barcoding, Hydrachnidia, taxonomy, Torrenticolidae, water mites.

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INTRODUCTION

Water mite genus *Torrenticola* Piersig, 1896 has a wide geographical distribution (Di Sabatino *et al.* 2008). Most species inhabiting the Palaearctic are rhitrobionts (Di Sabatino *et al.* 2010). Up to now, with the exception of Pešić *et al.* (2012, 2020c, 2021a) and Pešić and Smit (2022) molecular methods using DNA barcodes have not often been used in studies of Palaearctic torrenticolids. In recent years, molecular approaches to taxonomy have proved to be very helpful in taxonomic decisions in many genera of water mites, and a number of cryptic or overlooked lineages have been described within species previously considered to have a wide distribution (e.g., species of the genus *Hygrobatas*, see Pešić *et al.* 2019a, b, 2020a, b, 2022).

Torrenticola meridionalis Di Sabatino & Cicolani, 1990 was originally described from Italy (Di Sabatino and Cicolani 1990) and later was collected in different parts of the Balkans (Pešić *et al.* 2010, 2018, 2021). This paper is aimed to provide new records of *T. meridionalis* based on DNA

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barcodes of specimens collected in Poland, and to check its genetic identity using COI data from available DNA barcode reference libraries.

MATERIAL AND METHODS

During our 2020 research in Babia Góra Mountain (Poland) four specimens of *Torrenticola* cf. *meridionalis* were collected and three of them were successfully barcoded: two from a stream, and one from a spring (small limnocrone on the meadow) (Table 1). Water mites were collected by hand netting, sorted live in the field, and immediately preserved in 96% ethanol for the purpose of molecular analyses. After DNA extraction, the specimen vouchers were stored in 96% EtOH and morphologically examined. The vouchers were dissected and slide mounted in Faure's medium for morphological identification.

Molecular methods and DNA barcode analyses

DNA was extracted from individual specimens using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany). Qualitative and quantitative analyses of the DNA were performed by separation on 1.0% agarose gels and spectrophotometric measurements using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

The cytochrome oxidase subunit I (COI) gene fragment was PCR-amplified with the universal primers LCO1490-JJ and HCO2198-JJ (Folmer *et al.* 1994). The PCR amplification was carried with 2X Phire Hot Start II PCR Master Mix (Thermo Fisher Scientific, USA). The PCR reactions were performed in a T100™ Thermal Cycler (Bio-Rad, California, USA) according to the thermal profile attached to 2X Phire Hot Start II PCR Master Mix. The PCR products were separated on a 2.0% agarose gel with SimplySafe™ (Eurx, Gdańsk, Poland) added in TAE buffer. To visualize and document the obtained results, Gel Doc™ XR+ system was used (Bio-Rad, California, USA). DNA sequencing of all obtained PCR products was performed by MacroGen Europe (Amsterdam, Netherlands).

DNA sequence assembly, alignment and similarity analyses were completed using BLAST (Altschul *et al.* 1990), T-Coffee (Notredame *et al.* 2000), CLUSTAL W (Thompson *et al.* 1994) and MEGA X (Kumar *et al.* 2018). The DNA barcodes prepared during this study were deposited in the Barcode of Life Data Systems (BOLD Systems) (Ratnasingham and Hebert 2007).

The estimates of sequence COI divergence were conducted in MEGA X software and BOLD database (<https://boldsystems.org>). The genetic distances were calculated based on the K2P model (Kimura 1980). MEGA X was used to calculate Neighbour-Joining (NJ) tree based on K2P distances (standard for barcoding studies) and pairwise deletion of missing data. The support for tree branches was calculated by the nonparametric bootstrap method (Felsenstein 1985) with 1000 replicates and shown next to the branches. Codon positions included were 1st+2nd+3rd+Noncoding. NJ tree was based on the Polish sequences COI (n = 3) identified in this paper and sequences of other *T. meridionalis* (n = 14) retrieved from the GenBank and BOLD database (Pešić *et al.* 2021a). *Torrenticola similis* (GenBank acc. no. OL870123.1, BOLD acc. no. DCDDJ021-21) COI sequence was used as the outgroup (Pešić *et al.* 2021b). To investigate how DNA barcodes might define species boundaries, two different approaches were used: Automatic Barcode Gap Discovery (ABGD) and Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al.* 2012, 2021). We used the online versions of ABGD (<https://bioinfo.mnhn.fr/abi/public/abgd/>) and ASAP (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) with default settings and the K2P distance model.

RESULTS AND DISCUSSION

Torrenticola meridionalis is a rhithrobiontic, eurythermic species, occupying mainly lowland rivers (Di Sabatino and Cicolani 1990), but it was collected also in Prespa Lake (Pešić *et al.* 2021a), a large

lake shared by North Macedonia, Albania and Greece, a habitat that is unusual for torrenticolid mites. During our 2020 research in Babia Góra Mountain (Poland) we collected four specimens of *Torrenticola* cf. *meridionalis* and successfully barcoded three specimens: two from a stream (BGR1 B24a, BGR1 B24b), and one from a spring (BGS1 B14a) (small limnocrone on the meadow) (Table 1). The collection of the species is interesting because there are first records from Poland, very far from the edge of its range, and because limnocrone is an unusual habitat for torrenticolid mites.

Table 1. List of newly sequenced specimens of *Torrenticola* cf. *meridionalis* and the other COI records of *Torrenticola* used in this study.

Locality (country, name)	Lat/Long	ID sample	BOLD code	BIN	Source
<i>Torrenticola</i> cf. <i>meridionalis</i>					
Poland, Babia Góra, spring	49.5701 N 19.5988 E	BGS1_B14a	TMBG001-23	BOLD:AEI3402	Present study
Poland, Babia Góra, stream	49.5699 N 19.5988 E	BGR1_B24a	TMBG002-23	BOLD:AED7519	
Poland, Babia Góra, stream	49.5699 N 19.5988 E	BGR1_B24b	TMBG003-23	BOLD:AED7519	
<i>Torrenticola meridionalis</i>					
N Macedonia, Perspa Lake at Oteševo	40.979 N 20.917 E	15. CG2019_7 B12	DNAEC022-20	BOLD:AED7519	Pešić <i>et al.</i> 2021a
N Macedonia, Perspa Lake at Oteševo	40.979 N 20.917 E	18. CG2019_7 C1	DNAEC023-20	BOLD:AED7519	
Montenegro, Drcka stream at Mateševo	42.7619 N 19.5549 E	CCDB-3867-B09	DNCBD021-20	BOLD:AED7519	
Montenegro, Rikavac stream	42.1001 N 19.1432 E	CCDB-3867-E01	DNCBD049-20	BOLD:AED7519	
Montenegro, Rikavac stream	42.1001 N 19.1432 E	CCDB-3867-E03	DNCBD051-20	BOLD:AED7519	
Montenegro, Orahovštica river	42.2476 N 19.0798 E	CCDB-3867-G01	DNCBD073-20	BOLD:AED7519	
Montenegro, Orahovštica river	42.2476 N 19.0798 E	CCDB-3867-G02	DNCBD074-20	BOLD:AED7519	
Montenegro, Bistrica stream at Crkvine	42.8054 N 19.4456 E	CCDB 38361 D02	DCDDJ038-21	BOLD:AED7519	
Montenegro, Kolašinska rijeka	42.8391 N 19.5749 E	CCDB38233 B10	DCCDB022-21	BOLD:AEI3402	
Montenegro, Bistrica stream at Crkvine	42.9871 N 19.4338 E	CCDB38233 D12	DCCDB048-21	BOLD:AEI3402	
Italy, Sila, Fossiat, stream	39.4011 N 16.5884 E	CCDB 38392 D05	DCBDJ041-21	BOLD:AEK9662	
Italy, Sila, Fossiat, stream	39.4011 N 16.5884 E	CCDB 38392 F07	DCBDJ067-21	BOLD:AEK9662	
Montenegro, Bistrica stream at Crkvine	42.8054 N 19.4456 E	CCDB 38361 B08	DCDDJ020-21	BOLD:AEI3402	
Montenegro, Bistrica stream at Crkvine	42.8054 N 19.4456 E	CCDB 38361 D01	DCDDJ037-21	BOLD:AEI3402	
<i>Torrenticola similis</i> OL870123.1, DCDDJ021-21/outgroup					

The final data set in this study comprised 669 nucleotide positions for 17 specimens of *T. meridionalis* group and one specimen of *T. similis* as outgroup. Phylogenetic analyses based on COI data recovered three haploclades: first one grouped specimens from North Macedonia (Prespa Lake) and Montenegro (TorrM-I), second one grouped specimens from Italy (TorrM-II) and third one which includes specimens from Montenegro (TorrM-III) (Pešić *et al.* 2021a).

In our study we obtained three haplotypes, each 622 bp long, or specimens of *Torrenticola* cf. *meridionalis* from Poland (Table 2, Fig. 1). Interestingly, the phylogenetic analysis showed that specimens (BGR24a,b) from the Babia Góra stream were grouped in the same clade (TorrM-I) with water mites from North Macedonia (Prespa lake) and Montenegro, while the individual (BGS1 B14a) from the limnocrenic spring fell into Clade TorrM-III with specimens from Montenegro. The observed genetic distance between BGS1 B14a and specimens of the third haplogroup (TorrM-III) was only 1.4%. Low genetic divergences were also obtained for two Polish specimens from stream and the first haplogroup of *T. meridionalis* (TorrM-I) (Table 2, Fig. 1).

Table 2. Estimates of average genetic distance (K2P) between identified haplogroups of *T. meridionalis* and COI sequences analyzed in this study.

Haplogroups/ Specimens	BGS1 B14a	BGR1 B24a	BGR1 B24b	TorrM I	TorrM II	TorrM III	<i>T. similis</i> outgroup
BGS1 B14a	0.000						
BGR1 B24a	0.063	0.000					
BGR1 B24b	0.063	0.007	0.000				
TorrM-I	0.065	0.013	0.011	0.000			
TorrM-II	0.066	0.080	0.084	0.082	0.000		
TorrM-III	0.014	0.072	0.068	0.071	0.071	0.000	
<i>T. similis</i> outgroup	0.145	0.127	0.127	0.131	0.141	0.144	0.000

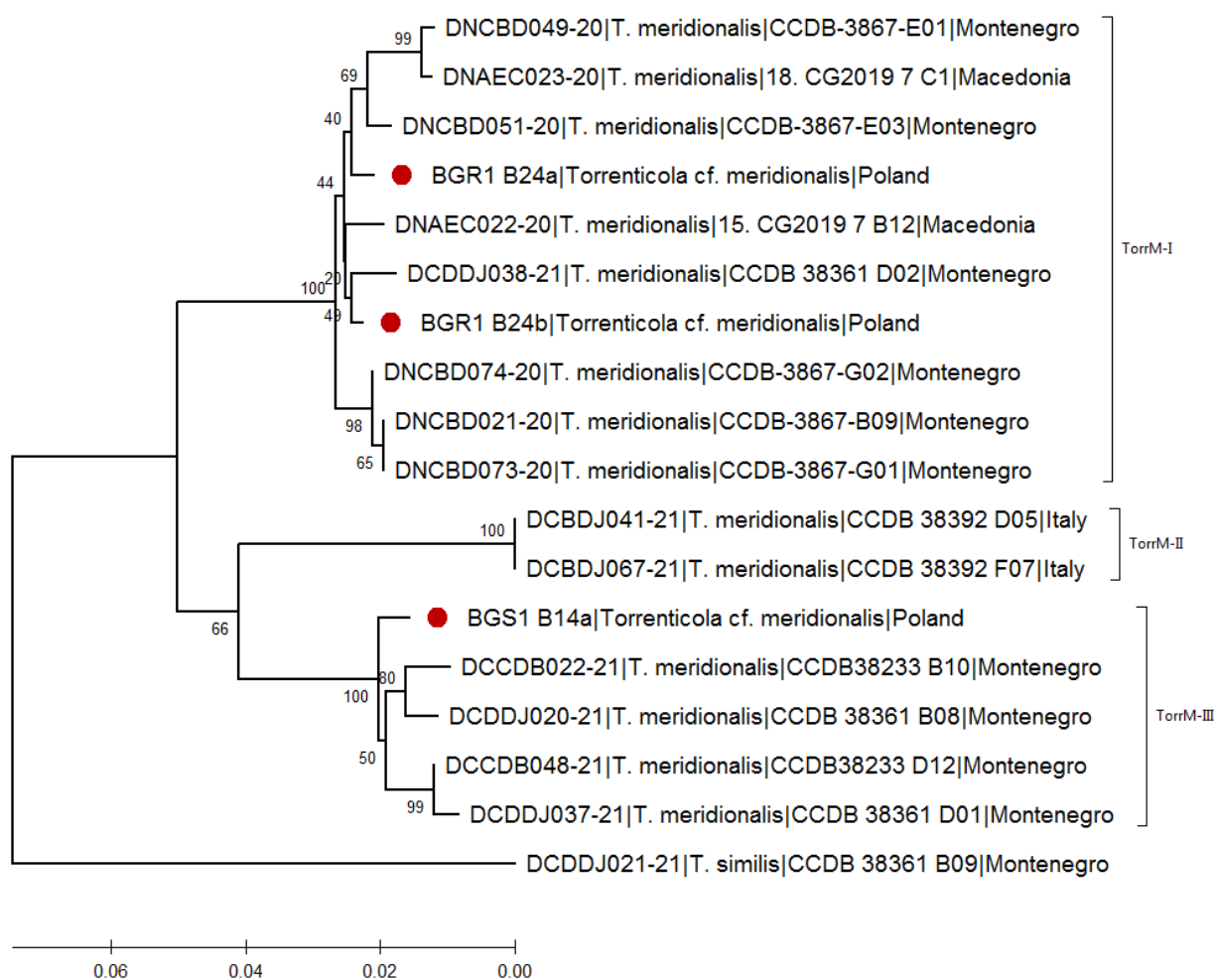


Figure 1. Neighbour-joining (NJ) tree showing the genetic relationships between Polish haplotypes of *Torrenticola* cf. *meridionalis* (were marked as red circles) and the other COI records of *T. meridionalis* (Pešić *et al.* 2021a). Values near branches show bootstrap support (BS).

Morphologically, *T. meridionalis* is very similar to *T. elliptica* Maglio, 1909, from which it can be separated by a relatively wider dorsal shield and genital field, and a higher length ratio coxal plate I/coxal plates II+III. The genetic distance (K2P) between these species (8.6–9.0% K2P) is beyond interspecific level for torrenticolid mites (Pešić *et al.* 2021a, b). Pešić *et al.* (2021a) did not describe the morphology of specimens collected in Montenegro and North Macedonia. Specimens collected

in Poland (Figs. 2, 3), males collected in spring are different from *T. meridionalis* described by Di Sabatino and Cicolani (1990) mainly by location of excretory pore which was fused with the area of primary sclerotization in examined male specimen (Fig. 3).



Figure 2. Female of *Torrenticola* cf. *meridionalis* (BGR1 B24a) from Babia Góra stream – **Right.** Dorsal habitus; **Left.** Ventral habitus.



Figure 3. Male of *Torrenticola* cf. *meridionalis* (BGS1 B14a) from Babia Góra spring – **Right.** Dorsal habitus; **Left.** Ventral habitus.

As emphasized by Pešić *et al.* (2021a) most likely *Torrenticola meridionalis* is a species-complex which have to be divided into separate species. The ABGD analysis singled out three hypothetical species, consistent with the groupings in the NJ tree. The size of the detected barcode gap between the maximum intraspecific and the minimum interspecific genetic distance was 2–6%. The second species delimitation method (ASAP) detected the presence of four OTUs (Operational Taxonomic Units) and indicated a barcode gap range of 0.4–3.49%. ASAP method identified additional OTUs within TorrM-I haplogroup, i.e. for specimens DNCBD049-20 (Montenegro) and DNAEC023-20 (Macedonia). The Polish COI barcodes of *Torrenticola cf. meridionalis* have been assigned to two different haplogroups and can be considered as hypothetically separate species.

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گزارش‌های جدید *Torrenticola cf. meridionalis* از کوه بابیا گورا (لهستان)

مگدالنا شنزگو^{۱*}، آندری زاوال^{۱،۲}، اولینا زاوال^۳، علیرضا صبوری^۲، سوسانا کرزینوک^۴، ماجا کراکویاک^۴، تاپاس تاجرجی^۵ و ولادیمیر پشیچ^۶

۱. مؤسسه علوم دریایی و محیطی، مرکز زیست‌شناسی مولکولی و فناوری زیستی، دانشگاه اشچین، واشکا ۱۳، ۷۱-۴۱۵ اشچین، لهستان؛ رایانامه‌ها: andrzej.zawal@usz.edu.pl Magdalena.szenejko@usz.edu.pl
۲. گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه تهران، کرج، ایران؛ رایانامه: saboori@ut.ac.ir
۳. دبیرستان شماره ۲ در اشچین، مدرسه آی.بی شماره ۳۸۸۵، اشچین، لهستان؛ رایانامه: ewcia.zawal55@gmail.com
۴. مؤسسه زیست‌شناسی، دانشگاه اشچین، واشکا ۱۳، ۷۱-۴۱۵ اشچین، لهستان؛ رایانامه: 224954@stud.usz.edu.pl
۵. نزدیک جاده هاری ماندیر، هیراپور، دانباد ۸۲۶۰۰۱، جارکند، هند؛ رایانامه: drtchatterjee@gmail.com
۶. گروه زیست‌شناسی، دانشگاه مونته‌نگرو، ستینجسکی پوت بی.بی، ۸۱۰۰۰ بودگوریتسا، مونته‌نگرو؛ رایانامه: vladopesic@gmail.com

* نویسنده مسئول

چکیده

گونه *Torrenticola meridionalis* Di Sabatino & Cicolani, 1990، ابتدا از ایتالیا توصیف شد و بعدها در مناطق مختلف بالکان گزارش شد. پژوهش‌های بعدی بر اساس داده‌های مولکولی نشان داده است که کمپلکس-گونه‌ای است. این بررسی داده‌های جدیدی را در مورد *Torrenticola cf. meridionalis* بر اساس بارکد DNA نمونه‌های لهستان ارائه می‌دهد و فرضیات پیشین را در مورد *Torrenticola meridionalis*-complex تأیید می‌کند.

واژگان کلیدی: بارکدینگ، Hydrachnidia، آرایه‌شناسی، Torrenticolidae، کنه‌های آبی.

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