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New records of water mites from Turkey and Iran revealed by DNA barcoding, with the description of a new species (Acari, Hydrachnidia)

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Abstract

New records of water mites from Iran and Turkey revealed by DNA barcoding are presented. DNA barcodes were recovered from 44 water mite specimens morphologically assigned to 18 species. The DNA barcoding data for the twelve species were uploaded into the BOLD database. One species of the *Hygrobates longiporus*-complex, *H. thori* Pešić & Smit **sp. nov.** is described as new for science and *Hydrodroma persica* Pešić & Saboori, 2012 is reported as new for Turkey. We propose to lower the taxonomic status of *Hydrodroma golesanica* to a subspecies of *H. torrenticola*, i.e. *H. torrenticola golesanica* **nov. stat.**

Keywords: Water mites, DNA-barcoding, species delimitation, new species, systematics

Introduction

Over the last decade, research on water mites has been significantly improved by using an integrative approach that combines morphological analysis and DNA barcoding data (Martin *et al.* 2010; Stryjecki *et al.* 2016; Fisher *et al.* 2017; Pešić *et al.* 2017, 2019a, 2020a, b, 2021a; Blattner *et al.* 2019). A large number of similar studies have shown the great potential of DNA barcoding for species identification, allowing the delineation of already described species and the identification of taxa that require further systematic attention (Pešić *et al.* 2017, 2019a, 2020a). In particular, DNA barcoding has proven to be a useful tool in identifying complexes of species with potential cryptic species. This is especially the case in species that were initially considered as a taxa with a wide geographical distribution and / or wide habitats ranges (e.g., Martin *et al.* 2010; Pešić *et al.* 2012, 2017, 2019a; Pešić & Smit 2020). COI data of water mites that have accumulated in recent years in the two main comprehensive reference libraries of DNA barcodes, i.e., the BOLD System (<https://www.boldsystems.org/>) and GenBank (<https://www.ncbi.nlm.nih.gov/>) provide a basis for building DNA barcode libraries at the regional and / or national level (e.g., “DNA Barcode library of water mites of Montenegro”; [dx.doi.org/10.5883/DS-MNEHYD](https://doi.org/10.5883/DS-MNEHYD), Pešić *et al.* 2021c). Establishing such

libraries, and further improving them by expanding their taxonomic and geographical coverage, allows us to assess the molecular diversity of water mite species, and to explore their distribution patterns.

Turkey and Iran have a long tradition in water mite research which has resulted in a large number of identified species that are, however, poorly molecularly analyzed. The most recent check lists of water mites from Turkey and Iran were published by Erman *et al.* (2010, 2019) and Pešić *et al.* (2014), respectively, reporting 335 species for Turkey and 186 species for Iran. In the meantime, this number has been updated with new species that are discovered every year, and some of them have been described using DNA barcodes (e.g., Pešić *et al.* 2017, 2019b, 2020b, 2021a, b). However, the number of publicly available DNA barcodes of water mite species from Iran and Turkey in BOLD and GenBank is still limited, meaning that this study provides the first “glimpse” into the molecular diversity of this group in these two countries.

The main aim of this study is to enrich the existing reference library with new sequences of specimens collected from our sampling campaign in Iran and Turkey. Moreover, one species of the *Hygrobates longiporus*-complex is described as new for science and *Hydrodroma persica* is reported here as new for Turkey.

Material and Methods

Water mites were collected by hand netting, sorted live in the field, and immediately preserved in 96% ethanol for the purpose of the molecular analyses (see below). Water mite specimens used for the molecular study are listed in Table 1. After DNA extraction, the specimen vouchers were stored in 96% EtOH and morphologically examined. Some of these vouchers were dissected and slide mounted in Faure's medium, while the rest was transferred to Koenike's fluid and stored in the collection of the first author. A total of 63 adults based on morphological characters was identified at the species level. DNA sequences prepared in the course of this study were deposited in BOLD (The Barcode of Life Data System) and GenBank. The DNA extracts were archived in -80°C freezers at the Centre for Biodiversity Genomics (CBG; biodiversitygenomics.net).

Morphological nomenclature follows Pešić *et al.* (2017; for explanations concerning morphology and measurements of *Hygrobates* spp. see Figs. 1B–D in Pešić *et al.* 2017). The genital acetabula in both sexes and the genital plate in the female were measured on both sides, therefore their dimensions were given as a range. The holotype and paratypes of the new species are deposited in the Naturalis Biodiversity Center in Leiden (RMNH).

All measurements are given in μm . The photographs of selected structures were made using a camera on Samsung Galaxy smartphone. The following abbreviations are used: Ac-1=most anterior acetabulum; Cx-I–IV=first to fourth coxae; dL=dorsal length; H=height; I-L1–6=first to sixth segments of first leg; L=length; mL=median length; n=number of specimens examined; P-1–P-5 = palp segments 1 to 5; W=width.

Molecular and DNA barcode analyses

The molecular analysis was conducted at the Canadian Centre for DNA Barcoding (Guelph, Ontario, Canada; (CCDB; <http://ccdb.ca/>)). In the latter institution the specimens were sequenced for the barcode region of COI using standard invertebrate DNA extraction (Ivanova *et al.* 2007), amplification (Ivanova & Grainger 2007a) and sequencing protocols (Ivanova & Grainger 2007b).

TABLE 1. Details on barcoded specimens.

Morphological taxonomic unit	Voucher Code	BOLD/GenBank Acc Nos.	Locality	Coordinates
Hydryphantidae Piersig, 1896				
<i>Trichothyas petrophila</i> (Michael, 1895)	CCDB38233 F06	DCCDB066-21/OM321069	Iran, Mazandaran, spring along Karaj-Chalus road	36.1717 N, 51.3153 E
Hydrodromidae K. Viets, 1936				
<i>Hydrodroma persica</i> Pešić & Saboori, 2007	CCDB 38363 G02	SEPTA074-21/OM321105	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
	CCDB 38361 D05	DCDDJ041-21/OM321101	Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E
	CCDB 38361 D06	DCDDJ042-21/OM321075	Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E
	CCDB 38361 D07	DCDDJ043-21/OM321056	Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E
	CCDB 38361 D09	DCDDJ045-21/OM321094	Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E
Sperchontidae Thor, 1900				
<i>Sperchon algeriensis</i> (Lundblad, 1942)	24.IR8_2017_A4	IRANM015-20/OM321052	Iran, Mazandaran, stream in Kandelous	36.3425 N, 51.5491 E
Torrenticolidae Piersig, 1902				
<i>Torrenticola disabatinola</i> Pesic, 2004	CCDB 38363 F12	SEPTA072-21/OM321058	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
	CCDB 38363 G08	SEPTA080-21/OM321083	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
	CCDB 38363 F09	SEPTA069-21/OM321059	Turkey, Burdur, Kuzkoy Village, Celtikci	37.5803 N, 30.4003 E
	CCDB 38363 F10	SEPTA070-21/OM321095	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
	CCDB 38363 F11	SEPTA071-21/OM321047	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
Teutoniidae Koenike, 1910				
<i>Teutonia cometes</i> (Koch, 1837)	CCDB 38363 G09	SEPTA081-21/OM321100	Turkey, Bucak at Kizilli Village	37.3425 N, 30.9256 E
<i>Limnolegeria longiseta</i> (Motaş, 1928)	CCDB 38363 G11	SEPTA083-21/OM321062	Turkey, Bucak at Kizilli Village	37.3425 N, 30.9256 E
Hygrobatidae Koch, 1842				
<i>Atractides inflatus</i> (Walter, 1925)	CCDB 38361 F01	DCDDJ061-21/OM321049	Turkey, Bingol, Capakcur stream	38.8936 N, 40.4772 E
	CCDB 38361 E12	DCDDJ060-21/OM321096	Turkey, Bingol, Capakcur stream	38.8936 N, 40.4772 E
	CCDB 38361 E10	DCDDJ058-21/OM321087	Turkey, Bingol, Capakcur stream	38.8936 N, 40.4772 E
<i>Atractides mossahabii</i> Pesic, 2004	21.IR7_2017_H12	IRANM014-20/OM321057	Iran, Mazandaran, stream along road to Kandelous	36.4264 N, 51.47 E
	20.IR17_2017_H11	IRANM013-20/OM321065	Iran, Mazandaran, stream along road to Kandelous	36.1714 N, 51.3158 E

.....continued on the next page

TABLE 1. (Continued)

Morphological taxonomic unit	Voucher Code	BOLD/GenBank Acc Nos.	Locality	Coordinates
<i>Atractides nahavandii</i> Schwoerbel & Sepasgosarian, 1976	9. IR8_2017_H3	IRANM005-20/OM321102	Iran, Mazandaran, stream in Kandelous	36.3425 N, 51.5491 E
<i>Atractides robustus</i> (Sokolow, 1940)	CCDB 38361 E11	DCDDJ059-21/OM321074	Turkey, Bingol, Capakcur stream	38.8936 N, 40.4772 E
	19. IR17_2017_H10	IRANM012-20/OM321086	Iran, Mazandaran, stream along Karaj-Chalus road	36.1714 N, 51.3158 E
	CCDB38233 F05	DCCDB065-21/OM321078	Iran, Mazandaran, stream along Karaj-Chalus road	36.1714 N, 51.3158 E
<i>Atractides hyrcaniensis</i> Pesic, Jabaleh & Saboori, 2009	11. IR2_2017_H5	IRANM007-20/OM321071	Iran, Golestan, Kaboud-val waterfall	36.8697 N, 54.8892 E
	10. IR2_2017_H4	IRANM006-20/OM321093	Iran, Golestan, Kaboud-val waterfall	36.8697 N, 54.8892 E
	CCDB 38361 F04	DCDDJ064-21/OM321055	Iran, Golestan, Shirabad near Khanbebin	36.9675 N, 55.0278 E
<i>Hygrobatas thori</i> Pešić & Smit sp. nov.	CCDB 38361 F02	DCDDJ062-21/OM321085	Iran, Golestan, Shirabad near Khanbebin	36.9675 N, 55.0278 E
	CCDB 38361 E04	DCDDJ052-21/OM321063	Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E
	CCDB 38361 E06	DCDDJ054-21/OM321082	Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E
	CCDB 38361 E07	DCDDJ055-21/OM321067	Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E
	CCDB 38361 E08	DCDDJ056-21/OM321089	Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E
	CCDB 38361 E09	DCDDJ057-21/OM321050	Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E
<i>Hygrobatas longiporus</i> Thor, 1898 (Balkan/Turkey Clade)	CCDB 38363 G05	SEPTA077-21/OM321060	Turkey, Burdur, Kizilli Village, Bucak	37.3425 N, 30.9256 E
	CCDB 38363 G06	SEPTA078-21/OM321099	Turkey, Burdur, Kizilli Village, Bucak	37.3425 N, 30.9256 E
Pionidae Thor, 1900				
<i>Pionopsis</i> sp.	17. IR16_2017_H9	IRANM011-20/OM321073	Iran, Mazandaran, canal in Nowshahr	36.5594 N, 51.6328 E
<i>Piona alpicola</i> (Neuman, 1880)	16. IR1_2017_H8	IRANM010-20/OM321064	Iran, Tehran, river near Karaj	35.8222 N, 51.0208 E
Anisitsiellidae Koenike, 1910				
<i>Nilotonia iranica</i> Pesic, 2005	12. IR18_2017_H6	IRANM008-20/OM321066	Iran, Mazandaran, spring along Karaj-Chalus road	36.1717 N, 51.3153 E
	13. IR18_2017_H7	IRANM009-20/OM321104	Iran, Mazandaran, spring along Karaj-Chalus road	36.1717 N, 51.3153 E
Mideopsidae Koenike, 1910				
<i>Mideopsis roztoczensis</i> Biesiadka & Kowalik, 1979	CCDB 38361 D10	DCDDJ046-21/OM321051	Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E
	CCDB 38361 E01	DCDDJ049-21/OM321061	Turkey, Bingol, Capakcur stream	38.8936 N, 40.4772 E
	CCDB 38361 E02	DCDDJ050-21/OM321084	Turkey, Bingol, Capakcur stream	38.8936 N, 40.4772 E
	CCDB 38363 F05	SEPTA065-21/OM321088	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
	CCDB 38363 F06	SEPTA066-21/OM321048	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E
	CCDB 38363 F07	SEPTA067-21/OM321070	Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E

Sequence comparisons were performed using MUSCLE alignment (Edgar 2004). Intra- and interspecific genetic distances were calculated based on the Kimura 2-parameter model (K2P; Kimura 1980), using MEGA X (Kumar *et al.* 2018). MEGA X software was used to calculate Neighbour-Joining (NJ) trees 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. All ambiguous positions were removed for each sequence pair.

In order to assess the genetic differentiation of species the online ASAP version (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) was used, with default settings and the K2P distance model. The latter procedure was designated to a list of partitions of species hypotheses using genetic distances, calculated between DNA sequences and ranked by their ASAP-scores: the lower the score, the better the partition (Puillandre *et al.* 2021).

Results and Discussion

DNA barcodes of 44 specimens (29 from Turkey and 15 from Iran), morphologically assigned to 18 species in 13 genera of water mites were newly generated (Table 1). The first DNA barcoding data for twelve species (i.e., *Trichothyas petrophila* (Michael, 1895), *Hydrodroma persica* Pešić & Saboori, 2012, *Hydrodroma golestonica* Pešić, Zawal, Saboori & Smit, 2021, *Sperchon algeriensis* Lundblad, 1942, *Torrenticola disabatinola* Pesic, 2004, *Monatractides aberratus* (Lundblad, 1941), *Limnolegeria longiseta* Motaş, 1928, *Atractides mossahebi* Pesic, 2004, *A. nahavandii* Schwoerbel & Sepasgosarian, 1976, *A. hyrcaniensis* Pesic, Jabaleh & Saboori, 2009, *Pionopsis* sp., and *Nilotonia iranica* Pesic, 2005) were uploaded into the BOLD database. Adding DNA barcodes of these species into BOLD database provides the references for reliable species identification of these taxa in further studies of the mite fauna of Turkey and Iran. Moreover, one species of the *Hygrobat* complex is described as new for science and *Hydrodroma persica* is reported here as new for Turkey.

Nevertheless, the taxonomic status of some taxa merits further attention and requires barcoding material from a wider area, including material from the locus typicus. For example, previously there were no data in the BOLD database for *Trichothyas petrophila* and *Sperchon algeriensis*, two species that are widely distributed in the Western Palaearctic. The barcodes of these two species in our study were recovered from specimens collected in Iran. Future studies may show that they are identical to synonymized species such as *Trichothyas alborzensis* Bader & Sepasgosarian, 1979 and *Sperchon benecke* Bader & Sepasgosarian, 1982.

Trichothyas alborzensis was described by Bader & Sepasgosarian (1979) from Elburs Mountain in North Iran. Later on, Pesic *et al.* (2005) placed the latter species into synonymy with *T. petrophila*, a species widely distributed in the Palaearctic (Di Sabatino *et al.* 2010). The successfully barcoded specimen that matches the original description of *T. alborzensis* was collected distant from the locus typicus of the latter species. *Trichothyas alborzensis* might be resurrected if the barcoding data of *T. petrophila* from its locus typicus reveals significant genetic divergence. *Sperchon benecke* was described by Bader & Sepasgosarian (1982) from River Gele Rud in Tehran Province, North Iran. Later on, Asadi *et al.* (2010) re-examined the holotype of *S. benecke* and placed it into synonymy with *S. algeriensis*, a species originally described from Algeria (Lundblad 1942), but reported from a wide area in the Central and Western Mediterranean (Di Sabatino *et al.* 2010). During our 2017 survey in Northern Iran we collected and successfully barcoded one specimen of *algeriensis*-like mites that matches the original description of *S. benecke*. If the barcoding data of *S. algeriensis* from

its *locus typicus* reveals significant genetic divergence, this could be a reason for resurrecting *S. benecke*.

Family Hydrodromidae K. Viets
***Hydrodroma persica* Pešić & Saboori, 2012**

Material examined—Turkey: Bingöl Province, Cevrimpinar stream, 38.9092 N, 40.4744 E, 15.v.2021, leg. Esen, 1 ♀, sequenced [DCDDJ045-21]; Cicekdere stream, 38.9142 N, 40.4689 E, leg. Esen, 3 ♀♀, sequenced [DCDDJ041-21–23]. Burdur Province, Bucak at Kizilli Village, 37.3425 N, 30.9256 E, leg. Gülle, 1 ♀, sequenced [SEPTA074-21].

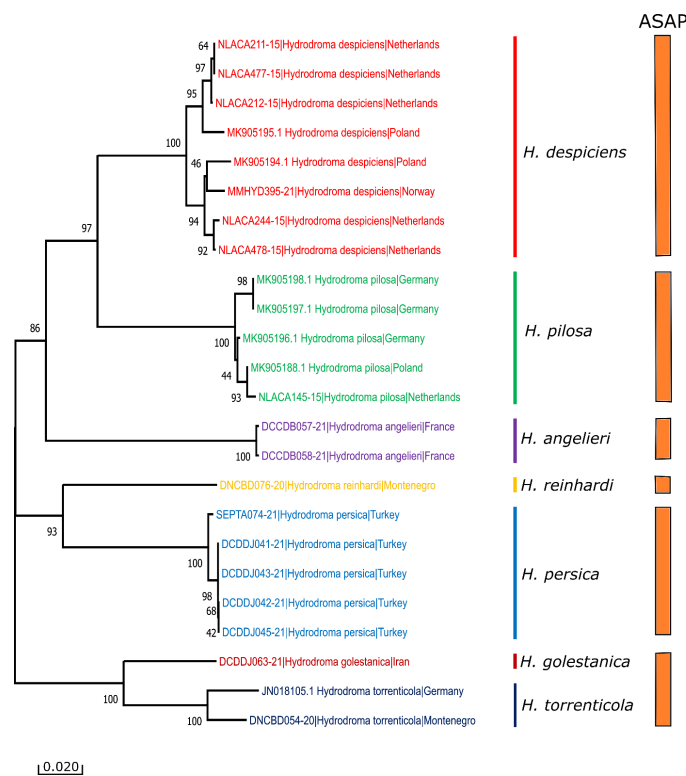


FIGURE 1. Neighbour-Joining tree of the genus *Hydrodroma*, obtained from 24 nucleotide COI sequences. The results of species delimitation by ASAP procedure are indicated by vertical bars.

Remarks—This species was described from a stream in Fars Province of southwest Iran (Pešić and Saboori 2012). The record of this species in the eastern (Bingöl province) and southwestern (Burdur province) parts of Turkey, confirmed by DNA barcode evidence, suggests that this species is more widely distributed than previously thought. The phylogenetic analysis based on COI data placed *H. persica* as a sister clade to *H. reinhardi* (Fig. 1). The average K2P genetic distance between these two species was estimated to be $13.9 \pm 1.7\%$ K2P. The collected specimens from Turkey matches the original description of *H. persica* in regard to swimming setation on the legs (Table 2). Nevertheless, to clarify the taxonomic status of these populations, specimens from the type locality should be barcoded.

The applied ASAP procedure identified 6 MOTUs (hypothetical species) at the threshold distance of 6.08% (K2P), which has the best ASAP-score (2.00) within the available molecular data

(Table 3): *H. despiciens*, *H. pilosa*, *H. angelierii*, *H. reinhardi*, *H. persica*, and *H. torrenticola* (merging *H. golestonica*). The ASAP procedure, grouped together the COI sequence of *H. torrenticola* and *H. golestonica*, the latter recently described from Golestan province of Iran (Pešić *et al.* 2021b). The latter species from Golestan separates the European *H. torrenticola* specimens by $10 \pm 1.3\%$ K2P divergence in COI barcodes indicating their genetic isolation. The results of ASAP delimitation analysis indicate that the status of *H. golestonica* should be lowered to subspecies status as *H. torrenticola golestonica* **nov. stat.**

TABLE 2. Number of swimming setae for *Hydrodroma persica* Pešić & Saboori, 2012.

	CCDB38361D05 ♀	CCDB38361D06 ♀	CCDB38361D09 ♀
II-L-5 posterior	0	0	0
III-L-4 posterior	2	2	2
III-L-5 posterior	4	3	3
IV-L-4 anterior	2	2	1
IV-L-4 posterior	4	3	3
IV-L-5 anterior	0	0	0
IV-L-5 posterior	2	2	3

Family Hygrobatidae Koch, 1842

Hygrobates thori Pešić & Smit **sp. nov.**

Figs. 3, 4A–C, D, 5

Material examined—Holotype ♀ [CCDB 38361 F04], sequenced [DCDDJ064-21], dissected and slide mounted, Iran, IR3 Golestan Province, 36.967499 N, 55.027779 E, Shirabad near Khanbebin, stream, downstream near the bridge, 27.viii.2017 leg. Pešić, Zawal & Saboori. Paratypes: 3♂♂, 4♀♀, same data as holotype; 1♀ sequenced [CCDB 38361 F02]; 1♂, dissected and slide mounted.

Other material—Turkey: Bingöl Province, Cevrimpinar stream, 38.9092 N, 40.4744 E, 15.v.2021, leg. Esen, 1♂, 14♀; *ibid.*, 29.v.2021, leg. Esen, 1♂, 14♀♀; 2♂♂, 2♀♀ sequenced (Table 4), 1♂ [CCDB 38361 E06], dissected and slide mounted.

Species delimitation using DNA-barcodes—The final alignment for species delimitation using COI sequence data comprised of 658 nucleotide positions (nps) for 17 specimens of *H. longiporus*-complex (Table 4) and *H. longipalpis* (Hermann, 1804) and *H. prosiliens* Koenike, 1915 as outgroups. The nucleotide sequences could be translated into amino acid sequences without any stop codons.

Neighbour-Joining (NJ) analysis clustered COI sequences of *H. longiporus*-complex into four strongly supported clades (Fig. 2). Sequences from specimens collected in Turkey were grouped in two different clades. The first highly supported clade contains sequences from the Balkans (Serbia and Greece) and two sequences from southwestern Turkey. This clade is placed as the sister of the clade grouping sequences from the eastern Turkey (Bingöl province) and two sequences from Golestan Province (Northeastern Iran). The two remaining clades corresponded to the geographical origins of mites (Corsica-Clade and Norway-Clade).

The overall mean genetic K2P distance between COI sequence pairs was $14.0 \pm 1.0\%$ and ranged from 10.8 to 24.3%. The minimal mean K2P distance of $10.8 \pm 1.4\%$ was found between Iran/Turkey-Clade and Balkan/Turkey-Clade. The maximum mean distance of $24.3 \pm 2.3\%$ was observed between Norway-Clade and Balkan/Turkey-Clade. The mean intraspecific distance within clades was relatively low, ranging from $2.02 \pm 0.46\%$ K2P in Iran/Turkey-Clade to $0.63 \pm 0.23\%$ K2P in

Balkan/Turkey-Clade. These values were about fivefold lower than the mean genetic distance among groups representing species-specific clades. The species status of the clades was supported by results of the ASAP analysis. The latter method found in the genetic distances a barcoding gap between 4 and 18% pairwise distances. The applied ASAP procedure identified 4 MOTUs (hypothetical species) and two outgroups at the threshold distance of 6.08% (K2P) which has the best ASAP-score (2.50) within the available molecular data: Iran/Turkey-Clade, Balkan/Turkey-Clade, Corsica-Clade and Norway-Clade.

TABLE 3. List of specimens of *Hydrodroma* used in this study.

Locality (country, name)	Lat/Long	Voucher code	BOLD Acc. Nos.
<i>Hydrodroma pilosa</i> Besseling, 1940			
Schluensee, Schleswig-Holstein, Germany	54.186799 N 10.462404 E	AMUeu335	MK905197
Schluensee, Schleswig-Holstein, Germany	54.186799 N 10.462404 E	AMUeu336	MK905196
Schluensee, Schleswig-Holstein, Germany	54.186799 N 10.462404 E	AMUeu337	MK905198
Morasko ponds, Poznan, Poland	52.467828 N 16.933358 E	AMUeu319	MK905188
the Netherlands, Alkmaar: Oudorpolder, ditch	52.63911 N, 4.76657 E	RMNH.ACA.383	NLACA145-15
<i>Hydrodroma despicens</i> (Müller, 1776)			
Brachowo Lake, Borne Sulinowo, Poland	53.550826 N 16.469663 E	AMUeu317	MK905195
the Netherlands, Gelderland, Tongerense heide	52.34095 N, 5.90389 E	RMNH.ACA.473	NLACA210-15
the Netherlands, Gelderland, Tongerense heide	52.34095 N, 5.90389 E	RMNH.ACA.475	NLACA212-15
the Netherlands, Gelderland, Tongerense heide	52.34095 N, 5.90389 E	RMNH.ACA.474	NLACA211-15
the Netherlands, Limburg, Epen: Pool Klitserbeek valley	50.78250 N, 5.92873 E	RMNH.ACA.919	NLACA477-15
the Netherlands, Limburg, Epen: Pool Klitserbeek valley	50.78250 N, 5.92873 E	RMNH.ACA.920	NLACA478-15
the Netherlands, Overijssel, Weerribben, ditch	52.80024 N, 5.93571 E	RMNH.ACA.512	NLACA244-15
<i>Hydrodroma goleanica</i> Pešić, Zawal, Saboori & Smit, 2021			
Iran, Golestan, Khanbebin, stream	36.9675 N, 55.0278 E	CCDB 38361 F03	DCDDJ063-21
<i>Hydrodroma torrenticola</i> (Walter, 1908)			
The Neckar River, Baden-Württemberg, Tübingen, Germany	-	MNHN-JAC68	JN018105
Montenegro, Bar, Međurječka river	42.0363 N, 19.2179 E	CCDB-3867-E06	DNCBD054-20
<i>Hydrodroma reinhardi</i> Pešić, 2002			
Montenegro, Podgorica, Cijevna river	42.4057 N, 19.3569 E	CCDB-3867-G04	DNCBD076-20
<i>Hydrodroma angelieri</i> Pešić & Smit, 2022			
France, Corsica, tributary of Ruisseau de Canne	42.38307 N, 8.69903 E	CCDB38233 E09	DCCDB057-21
France, Corsica, tributary of Ruisseau de Canne	42.38307 N, 8.69903 E	CCDB38233 E10	DCCDB058-21
<i>Hydrodroma persica</i> Pešić & Saboori, 2012			
Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E	CCDB 38361 D05	DCDDJ041-21
Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E	CCDB 38361 D06	DCDDJ042-21
Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E	CCDB 38361 D07	DCDDJ043-21
Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E	CCDB 38361 D09	DCDDJ045-21
Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E	CCDB 38363 G02	SEPTA074-21

Discussion—Based on our molecular analyses, the specimens from northeastern Iran here used as the type material of the new species, *Hygrobatas thori* sp. nov., belongs to the Iran/Turkey clade (Fig. 2). The latter clade was placed as a sister of the clade grouping sequences from the Balkan and southwestern Turkey (Balkan/Turkey-Clade). The level of COI differentiation between these two clades was estimated to 10.1% K2P divergence. According to Pešić *et al.* (2021d) the taxonomic

status of the Balkan/Turkey-Clade should be clarified by resolving the taxonomic status of *H. falcilaminatus* Walter, 1926, a species originally described from Azrou, Morocco (Walter 1926) and reported from a wide area of the Western Mediterranean (Spain and France). It was synonymized with *H. longiporus* by Lundblad (1956) (see also Gerecke 1991 for a discussion about this species). Moreover, we cannot exclude the possibility that the *H. falcilaminatus*, is in fact conspecific with the Corsican clade. Anyhow, for now it seems most appropriate to leave unnamed both Mediterranean clades (Corsica-Clade and Balkan/Turkey-Clade) until the sequences of *H. falcilaminatus* are available. On the other hand, it is unlikely that the clade that comprises of individuals from Eastern Turkey and Northeastern Iran belong to *H. falcilaminatus*. Therefore, we describe it as a new species, *H. thori* **sp. nov.** (see below).

TABLE 4. List of specimens of *Hygrobatas longiporus-complex* used in this study. BINs are based on the barcode analysis from 14-1-2022.

Locality (country, name)	Lat/Long	Voucher code	BOLD Acc. Nos.	BIN
Norway – Clade				
Norway, upstream of the bridge at old Soegne church	58.0895 N, 7.83998 E	HYDCA284	NTNU-VM 227724	BOLD:AEB8359
Norway, Ved Blakstad, upstream of the bridge Frolandsveien	58.51 N, 8.648 E	HYDCA518	NTNU-VM 227721	BOLD:AEB8359
Norway, Ved Blakstad, upstream of the bridge Frolandsveien	58.51 N, 8.648 E	HYDCA519	NTNU-VM 227722	BOLD:AEB8359
Balkan/Turkey - Clade				
Serbia, Crni Rzav near Vodice	43.6559 N, 19.704 E	CCDB 38363 D11	SEPTA047-21	-
Turkey, Kizilli Village, Bucak	37.3425 N, 30.9256 E	CCDB 38363 G05	SEPTA077-21	-
Turkey, Kizilli Village, Bucak	37.3425 N, 30.9256 E	CCDB 38363 G06	SEPTA078-21	-
Greece, Amygdalorema stream	41.1374 N, 25.5358 E	CCDB 38362 B01	SEPTB013-21	-
Iran/Turkey - Clade				
Iran, Golestan, Shirabad near Khanbebin	36.9675 N, 55.0278 E	CCDB 38361 F04	DCDDJ064-21	BOLD:AEK7910
Iran, Golestan, Shirabad near Khanbebin	36.9675 N, 55.0278 E	CCDB 38361 F02	DCDDJ062-21	BOLD:AEK7910
Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E	CCDB 38361 E04	DCDDJ052-21	BOLD:AEL4319
Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E	CCDB 38361 E06	DCDDJ054-21	BOLD:AEL4319
Turkey, Bingol, Cevrimpinar stream	38.9092 N, 40.4744 E	CCDB 38361 E07	DCDDJ055-21	BOLD:AEL4319
Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E	CCDB 38361 E08	DCDDJ056-21	BOLD:AEL4320
Turkey, Bingol, Cicekdere stream	38.9142 N, 40.4689 E	CCDB 38361 E09	DCDDJ057-21	BOLD:AEK7910
Corsica-Clade				
France, Corsica, Ruisseau de Campaghiu	42.2413 N, 8.7324 E	CCDB 38559 B02	NOVMB014-21	-
France, Corsica, Ruisseau d' Enova	42.2659 N, 8.71405 E	CCDB 38559 G05	NOVMB077-21	-
France, Corsica, Ruisseau d' Enova	42.2659 N, 8.71405 E	CCDB 38559 G06	NOVMB078-21	-
Outgroup: <i>Hygrobatas longipalpis</i> (Hermann, 1804)				
Netherlands, Vaassen, Rode Beek	52.291 N, 5.956 E	RMNH.ACA.568	NLACA286-15	BOLD:ACR9783
Outgroup: <i>Hygrobatas prosiliens</i> Koenike, 1915				
Netherlands, Utrecht, Maarseveense Plas	52.144 N, 5.084 E	RMNH.ACA.436	NLACA180-15	BOLD:AAX2654

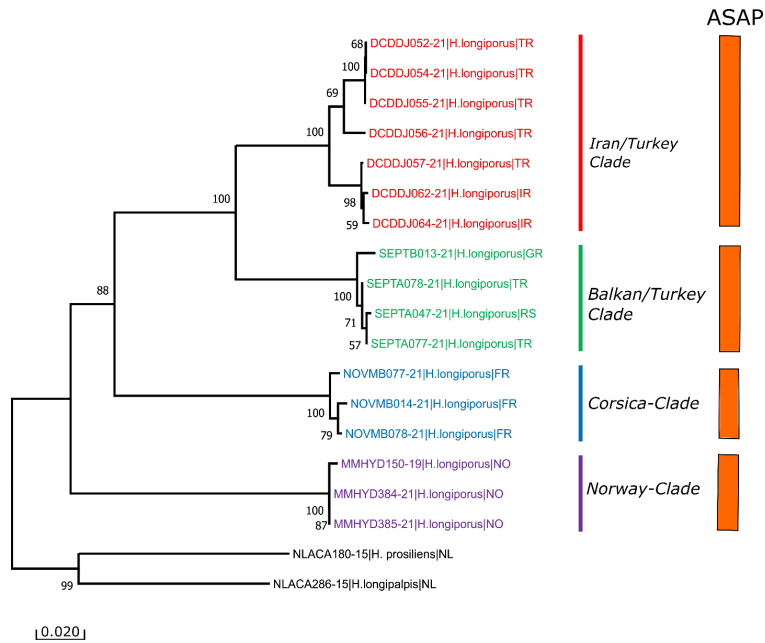


FIGURE 2. Neighbour-Joining tree of *Hygrobates longiporus*-complex, obtained from 17 nucleotide COI sequences. The results of species delimitation by ASAP procedure are indicated by vertical bars. Country codes (alpha-2 code): GR=Greece, FR=France, IR=Iran, NO=Norway, NL=the Netherlands, RS=Serbia, TR=Turkey.

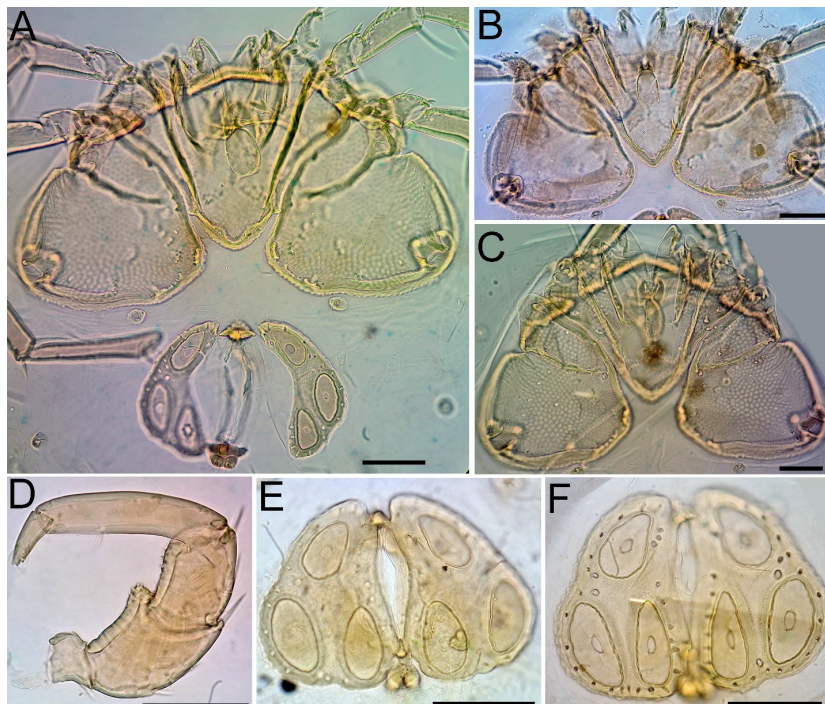


FIGURE 3. Photographs of selected structures of *Hygrobates thori* sp. nov. (A=holotype ♀, Iran, Shirabad; B, D–E, paratype ♂, Iran, Shirabad; E, F=♂, Turkey, Cevrîmpinar [38361 E06]). A=coxal and genital field; B–C=coxal field; D=palp, medial view; E–F=genital field. Scale bar=100 µm.

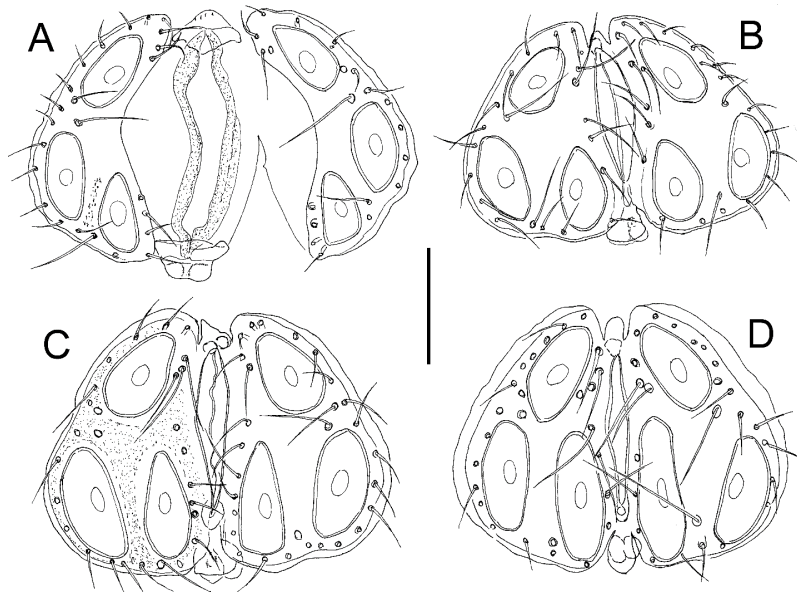


FIGURE 4. A–C: *Hygrobates thori* sp. nov., genital field (A, ♀; B–D, ♂). A=holotype, Shirabad, Iran; B=paratype, Shirabad, Iran; C=Cevrimpinar, Turkey [38361 E06]. D: *H. longiporus* Balkan/Turkey-Clade; D=Pusta Reka river, Serbia. Scale bar=100 µm.

TABLE 5. Estimates of average genetic distance (K2P) (given as distance ± standard error) between each clades (lower diagonal) and within clade (diagonal) of examined *H. longiporus*-complex sequence pairs.

Haplogrups	(1)	(2)	(3)	(4)
(1). <i>H. longiporus</i> Corsica-Clade	0.0075±0.0028			
(2). <i>H. longiporus</i> Norway-Clade	0.224±0.022	0.002±0.0014		
(3). <i>H. longiporus</i> Iran/Turkey-Clade	0.196±0.020	0.222±0.021	0.0202±0.0046	
(4). <i>H. longiporus</i> Balkan/Turkey- Clade	0.205±0.020	0.243±0.023	0.108±0.014	0.0063±0.0023

The ASAP analysis supported the species status of the above-mentioned clades with a high COI divergence (ranging from 22.4±2.2 to 24.3±2.3% K2P) of the *H. longiporus* Norway-Clade. Pešić *et al.* (2021d) suggested that the Norway-Clade likely corresponds to the nominal species as it was originally described from Norway (Thor 1898).

From a morphological point of view, *H. longiporus* can be separated from *H. thori* sp. nov. in the shape and size of the male Ac-3 (Ac-3 elongated, drop-shaped, L Ac-3 > Ac-2, compare Figs. 4B–D).

Diagnosis—Posterior margin of Cx-I pointed. Male: Ac-3 subtriangular, not drop-shaped, Ac-3 equal in length or shorter than Ac-2. Female: pregenital sclerite between anterior genital plate margins.

Description: General features—Colour yellowish to brown. Integument finely striated. Posteromedial margin of Cx-I triangular (Figs. 3A–C); Cx-IV with a distinct nose-like protruding medial margin, often with a border of secondary sclerotization. Acetabula in an obtuse triangle (Figs. 3A, E–F, 4A–C). Chelicera basal segment dorsally with a small hyaline pointed extension (Fig. 5C). P-2 ventral margin distally forming a blunt, nose-shaped projection, denticles covering distal half of ventral margins of P-2 and P-3 (Figs. 5A–B, E); P-4 ventral setae in distal part, separated by 6–9 µm. **Male**—Anterior margin of genital field convex with a small triangular median tip projecting in a deep

indentation, posterior margin indented, with a knob-shaped posteromedial protrusion (Fig. 4B–C). *Female*—Genital plates sickle-shaped, with concave medial margins, pregenital sclerite between anterior genital plate margins (Fig. 4A).

Measurements: Female (Holotype)—Idiosoma L 920; coxal field: L 394; Cx-III W 534; mL of Cx-I + gnathosoma L 353; distance between lateralmost ends of Cx-II apodemes, 156; genital field L/W 238/338, pregenital sclerite W 62; genital plate L 222–225; gonopore L 203; L gonopore/genital plate ratio 0.90–0.91; L Ac 1–3: 72–78, 78–80, 64–69. Egg (n=1) maximum diameter 198.

Chelicera total L 391; L basal segment 259, claw 128; L basal segment/claw ratio 2.02. Palp: total L 534; dL/H, dL/H ratio: P-1, 40/52, 0.77; P-2, 148/92, 1.61; P-3, 98/76, 1.3; P-4, 189/49, 3.84; P-5, 59/24, 2.46; P-2/P-4 ratio 0.79; distance between P-4 setae, 9.0.

Legs: dL of I-L-1–6: 69, 100, 141, 188, 209, 203. dL of IV-L-1–6: 144, 159, 225, 322, 328, 281.

Male (paratype from IR3; in parentheses specimen from Turkey [CCDB 38361 E06])—Idiosoma L 900 (1190); coxal field: L 419 (519); Cx-III W 530 (589); mL of Cx-I + gnathosoma L 347 (400); distance between lateralmost ends of Cx-II apodemes, 134 (166); genital field L/W 192 (225)/273 (297), ratio 0.7 (0.76); L Ac 1–3: 66–72 (81–84), 76–78 (100–101), 75–76 (97–101).

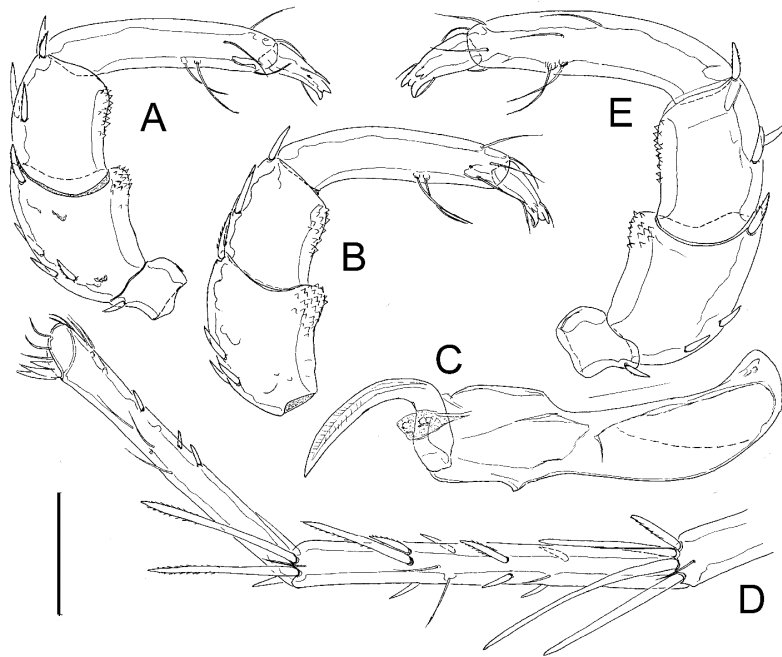


FIGURE 5. *Hygrobatés thori* sp. nov. (A–D=holotype ♀, Iran, Shirabad; E=♂, Turkey, Cevrimpinar [38361 E06]). A, E=palp, lateral view; B, palp, medial view (P-1 lacking); C=chelicera; D=IV-L-5 and -6. Scale bar=100 µm.

Chelicera total L 366 (428); L basal segment 244 (281), claw 128 (138); L basal segment/claw ratio 1.91 (2.04). Palp: total L 513 (626); dL/H, dL/H ratio: P-1, 39/47, 0.83 (48/61, 0.79); P-2, 140/89, 1.58 (170/108, 1.58); P-3, 94/70, 1.33 (119/89, 1.34); P-4, 181/45, 4.0 (219/53, 4.1); P-5, 59/23, 2.54 (70/28, 2.5); P-2/P-4 ratio 0.77 (0.78); distance between P-4 setae, 6.0 (8.0).

Legs: dL of I-L-1–6: 66 (78), 100 (122), 131 (159), 184 (228), 198 (241), 191 (234). dL of IV-L-1–6: 141 (169), 138 (184), 214 (272), 306 (397), - (388), - (322).

Etymology—Named after Sig Thor (1856–1937), who described the first species of the *longiporus* complex.

Distribution—Iran, East Turkey.

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