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Water mites of Corsica: DNA barcode and morphological evidences

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ABSTRACT

Water mites are a significant component of freshwater ecosystems. On Corsica (France) they are inhabiting mainly running waters. DNA barcodes of 84 specimens morphologically assigned to 27 water mite species were newly generated for this study. The first DNA barcoding data for six species were uploaded into the BOLD database, three of these, *Protzia pycnica* Gerecke, 1996, *Atractides corsicus* E. Angelier, 1954, *A. giustinii* Gerecke & Di Sabatino, 2013, are endemics for Corsica and Sardinia. Comparison with publicly available DNA barcodes from BOLD and GenBank allowed us on one side to link the Corsican clades with their sister-clades and assess which clades may represent putative species, and on the other hand to recognize several species complexes that needs additional studies. Based on the results of the molecular analysis, we propose to resurrect *Sperchon meridianus* Angelier, 1954, a species previously synonymized with *S. setiger* Thor, 1898.

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DNA barcoding; COI; water mites; France; species delimitation

Introduction

Corsica is fourth-largest islands in the Mediterranean Sea, located approximately 170 km from southern France. The island belongs to the Tyrrhenian region, together with Sardinia, of which it is separated by a strait of only 11 km wide. The separation of both islands from the European mainland was completed about 9 Ma ago (Alvarez 1972). Due to the desiccation of the Mediterranean basin the connections between the Corsica – Sardinia archipelago and Europe and North Africa) have been re-established during the Messinian Salinity Crisis (MSC; 5.96–5.33 Ma) (Duggen et al. 2003). The last time both islands were temporarily connected was during the Last Glacial Maximum (LGM) as a result of changes in sea level during the glacial periods in the Pleistocene (Grill et al. 2007).

In regard to water mites, a cosmopolitan group of freshwater organisms, Corsica is one of the best studied part of the France (Smit and Gerecke 2010). The systematic work of water mites of Corsica began in 1954 when the famous French acarologist Eugenius Angelier (1925–2011) published three papers on the water mites (Angelier 1954a, 1954b, 1954c). Since then, a large number of papers on the fauna of Corsican water mites have been published by Santucci (1970, 1971, 1975, 1977), Gerecke (1994, 1996; 2009, 2014), Di Sabatino and Gerecke (1996), Gerecke and Di Sabatino (1996, 2013), Pešić (2002), Pešić and Gerecke (2003), Pešić et al. (2007, 2017), Di Sabatino et al. (2009), Smit and Gerecke (2010), Gerecke et al. (2014), Pešić and Smit (2022a, 2022b). The latest checklist of the water mites of France published by Smit and Gerecke (2010) listed 121 species of water mites from Corsica. This number makes up about 50% of the French water mite fauna which is estimated to 420 species and subspecies (Smit and Gerecke 2010).

The main aim of this study is to develop the first barcode reference library for the molecular identification of water mites of Corsica based on specimens collected the junior author. Establishing such an publish database will allow us, (i) to assess molecular diversity of water mite species inhabiting Corsica, (ii) explore their distribution patterns in Europe, (iii) to test DNA barcoding efficiency for identification of water mite species taking advantage of publicly available DNA barcode reference libraries, such as the BOLD System (<https://www.boldsystems.org/>) and GenBank (<https://www.ncbi.nlm.nih.gov/>), and (iv), to recognize species groups problematic both for traditional taxonomy and

for DNA barcoding, with potential cryptic and/or pseudocryptic species that require an additional effort aimed to resolve their taxonomy.

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. Water mites were collected from 16 locations on Corsica by the junior author during his collecting trip in 2015. Photos from each studied specimen were taken before molecular work started.

Molecular analyses were conducted in the Canadian Centre for DNA Barcoding (Guelph, Ontario, Canada; (CCDB; <http://ccdb.ca/>)). In CCDB the specimens were sequenced for the barcode region of COI using standard invertebrate DNA extraction (Ivanova et al. 2007), amplification (Ivanova and Grainger 2007a) and sequencing protocols (Ivanova and Grainger 2007b). Primers LCO1490 and HCO2198 were used to sequence the COI barcode fragment (Folmer et al. 1994).

DNA sequences prepared in the course of his study were deposited in BOLD (Dataset “DS-CORHYD DNA barcode library for water mites of Corsica”) and GenBank (BankIt2546772 gnl, Accession numbers OM502265 to OM502278; BankIt2562203 gnl, Accession numbers ON002547 to ON002621). The DNA extracts were archived in –80°C freezers at the Centre for Biodiversity Genomics (CBG; biodiversitygenomics.net). After DNA extraction, the specimen vouchers were stored in 96%EtOH and returned to the first author for morphological examination. Some of these vouchers were dissected and slide mounted in Faure’s medium, while the rest was transferred to Koenike’s fluid. The photographs of selected structures were made using a camera on Samsung Galaxy smartphone. The voucher material will be deposited in Naturalis Biodiversity Center in Leiden (RMNH).

DNA barcode analysis

In CCDB the chromatograms were assembled into consensus sequences for each specimen and uploaded to BOLD. Detailed voucher information, taxonomic classifications, photos, DNA barcode sequences, primer pairs used and trace files (including their quality) were uploaded on the Barcode of Life Data Systems (BOLD).

Table 1. Details on barcoded specimens from Corsica, France.

Morphospecies	Sex	Locality	Coordinates	Voucher code	BOLD/GenBank Acc. Nos.
Hydryphantidae					
<i>Protzia lata</i> (Walter, 1906)	F	Corsica, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB38233 E01	DCCDB049-21/ OM502278
	F	Corsica, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB38233 E02	DCCDB050-21/ OM502277
	F	Corsica, Ruisseau de Campaghiu, Porto	42.2413 N, 8.7324 E	CCDB38233 E04	DCCDB052-21/ OM502273
	F	Corsica, Ruisseau de Campaghiu, Porto	42.2413 N, 8.7324 E	CCDB38233 E05	DCCDB053-21/ OM502275
	F	Corsica, Ruisseau de Casa Infurcata	42.256 N, 8.76513 E	CCDB38233 E06	DCCDB054-21/ OM502274
	F	Corsica, Ruisseau de Casa Infurcata	42.256 N, 8.76513 E	CCDB38233 E07	DCCDB055-21/ OM502266
	F	Corsica, Ruisseau de Casa Infurcata	42.256 N, 8.76513 E	CCDB38233 E08	DCCDB056-21/ OM502276
	F	Corsica, Rivière la Figurella	42.4873 N, 8.80532 E	CCDB38233 E11	DCCDB059-21/ OM502271
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB38233 E12	DCCDB060-21/ OM502265
	M	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB38233 F01	DCCDB061-21/ OM502270
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB38233 F02	DCCDB062-21/ OM502269
<i>Protzia pycnica</i> Gerecke, 1996	F	Corsica, Ruisseau de Battesta	41.5449 N, 9.02292 E	CCDB38233 E03	DCCDB051-21/ OM502267
Lebertiidae					
<i>Lebertia fimbriata</i> Thor, 1899	F	Corsica, Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D06	NOVMB042-21/ ON002572
	F	Corsica, Ruisseau d'Aitone at crossing with road D84	42.2636 N, 8.84597 E	CCDB 38559 H06	NOVMB090-21/ ON002573
	F	Corsica, Ruisseau d'Enova, at crossing with road D124	42.2659 N, 8.71405 E	CCDB 38559 G03	NOVMB075-21/ ON002574
<i>Lebertia maglioi</i> Thor, 1907	F	Corsica, Ruisseau Regolo at crossing with road D69	42.1181 N, 9.18393 E	CCDB 38559 B08	NOVMB020-21/ ON002601
	F	Corsica, Ruisseau Regolo at crossing with road D69	42.1181 N, 9.18393 E	CCDB 38559 B09	NOVMB021-21/ ON002570
	M	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C06	NOVMB030-21/ ON002579
	M	Corsica, Rivière la Figurella	42.4873 N, 8.80532 E	CCDB 38559 C12	NOVMB036-21/ ON002581
	F	Corsica, Rivière Casaluna near San Lorenzo	42.3822 N, 9.28863 E	CCDB 38559 E11	NOVMB059-21/ ON002580
	M	Corsica, Ruisseau de Casa Infurcata, at crossing with road D124	42.256 N, 8.76513 E	CCDB 38559 H04	NOVMB088-21/ ON002571
<i>Lebertia porosa</i> Thor, 1900	F	Ruisseau de San Petru, N of Quenza	41.7801 N, 9.13483 E	CCDB 38559 A08	NOVMB008-21/ ON002577
	F	Corsica, Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D05	NOVMB041-21/ ON002575
	F	Corsica, Rivière de Tavulella, at crossing with road D84	42.2419 N, 8.81322 E	CCDB 38559 G01	NOVMB073-21/ ON002576
	F	Corsica, Ufium Orbu near outflow Ruisseau de Quella	42.1043 N, 9.24482 E	CCDB 38559 G07	NOVMB079-21/ ON002578
Sperchontidae					
<i>Sperchon clupeiifer</i> Piersig, 1896	F	Corsica, Ruisseau de Battesta at crossing with road D50	41.5449 N, 9.02292 E	CCDB 38559 F02	NOVMB062-21/ ON002604
	F	Corsica, Ruisseau de Battesta at crossing with road D50	41.5449 N, 9.02292 E	CCDB 38559 F03	NOVMB063-21/ ON002602
<i>Sperchon denticulatus</i> gr.	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C02	NOVMB026-21/ ON002600
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C10	NOVMB034-21/ ON002599
	F	Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D10	NOVMB046-21/ ON002606
<i>Sperchon meridianus</i> Angelier, 1954	F	Corsica, Ruisseau de l'Umbert acciu, at Pont Chiuni/D81	42.1838 N, 8.61712 E	CCDB 38559 A04	NOVMB004-21/ ON002603
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C01	NOVMB025-21/ ON002605
	F	Corsica, Ruisseau de Casa Infurcata, at crossing with road D124	42.256 N, 8.76513 E	CCDB 38559 H02	NOVMB086-21/ ON002597
	F	Corsica, Ufium Orbu near outflow Ruisseau de Quella	42.1043 N, 9.24482 E	CCDB 38559 G08	NOVMB080-21/ ON002607
<i>Sperchon thienemanni</i> Koenike, 1907	F	Corsica, Ruisseau de Arja Alta at crossing with road D83	41.8973 N, 9.08583 E	CCDB 38559 H10	NOVMB094-21/ ON002598
	M	Corsica, Ruisseau de Arja Alta at crossing with road D83	41.8973 N, 9.08583 E	CCDB 38559 H11	NOVMB095-21/ ON002596

(Continued)

Table 1. (Continued).

Morphospecies	Sex	Locality	Coordinates	Voucher code	BOLD/GenBank Acc. Nos.
Torrenticolidae					
<i>Monatractides stadleri</i> (Walter, 1924)	F	Corsica, Ruisseau de San Petru, N of Quenza	41.7801 N, 9.13483 E	CCDB 38559 A12	NOVMB012-21/ ON002590
	F	Corsica, Porto, Ruisseau de Campaghiu	42.2413 N, 8.7324 E	CCDB 38559 B04	NOVMB016-21/ ON002587
	M	Corsica, Rivière La Figurella	42.4873 N, 8.80532 E	CCDB 38559 D03	NOVMB039-21/ ON002588
	F	Corsica, Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D09	NOVMB045-21/ ON002591
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C05	NOVMB029-21/ ON002589
	F	Corsica, Riviere Casaluna near San Lorenzo	42.3822 N, 9.28863 E	CCDB 38559 E12	NOVMB060-21/ ON002592
<i>Torrenticola laskai</i> Di Sabatino, 2009	F	Corsica, Ruisseau de Battesta at crossing with road D50	41.5449 N, 9.02292 E	CCDB 38559 F07	NOVMB067-21/ ON002613
	M	Corsica, unnamed stream N of Col de Bavella	41.8165 N, 9.25267 E	CCDB 38559 G11	NOVMB083-21/ ON002610
	M	Corsica, Rivière Casaluna near San Lorenzo	42.3822 N, 9.28863 E	CCDB 38559 F01	NOVMB061-21/ ON002616
	F	Corsica, Rivière de Tavulella, at crossing with road D84	42.2419 N, 8.81322 E	CCDB 38559 F12	NOVMB072-21/ ON002619
	F	Corsica, Ruisseau d Aitone at crossing with road D84	42.2636 N, 8.84597 E	CCDB 38559 H05	NOVMB089-21/ ON002612
<i>Torrenticola barsica</i> (Szalay, 1933)	F	Corsica, Ruisseau de l Umbert acciu, at Pont Chiuni/D81	42.1838 N, 8.61712 E	CCDB 38559 A05	NOVMB005-21/ ON002617
	M	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C03	NOVMB027-21/ ON002611
	M	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C04	NOVMB028-21/ ON002609
	F	Corsica, Rivière La Figurella	42.4873 N, 8.80532 E	CCDB 38559 D01	NOVMB037-21/ ON002618
	M	Corsica, Rivière La Solenzara	41.8383 N, 9.33055 E	CCDB 38559 E02	NOVMB050-21/ ON002614
	M	Corsica, Ruisseau de Casa Infurcata, at crossing with road D124	42.256 N, 8.76513 E	CCDB 38559 H01	NOVMB085-21/ ON002608
<i>Torrenticola anomala</i> (Koch, 1837)	F	Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D11	NOVMB047-21/ ON002615
<i>Pseudotorrenticola rhynchota</i> Walter, 1906	F	Corsica, Rivière La Solenzara	41.8383 N, 9.33055 E	CCDB 38559 E06	NOVMB054-21/ ON002594
	F	Corsica, Ruisseau d Aitone at crossing with road D84	42.2636 N, 8.84597 E	CCDB 38559 H08	NOVMB092-21/ ON002595
Limnesiidae					
<i>Limnesia marmorata</i> Neuman, 1870	F	Corsica, Barrage de Codole	42.5817 N, 8.94238 E	CCDB 38559 E09	NOVMB057-21/ ON002582
Hygrobatidae					
<i>Atractides allgaier</i> Gerecke, 2003	F	Ruisseau de Arja Alta at crossing with road D83	41.8973 N, 9.08583 E	CCDB 38559 H09	NOVMB093-21/ ON002554
<i>Atractides corsicus</i> E. Angelier, 1954	F	Porto, Ruisseau de Campaghiu	42.2413 N, 8.7324 E	CCDB 38559 B03	NOVMB015-21/ ON002557
	F	Ruisseau de Casa Infurcata, at crossing with road D124	42.256 N, 8.76513 E	CCDB 38559 H03	NOVMB087-21/ ON002549
<i>Atractides giustinii</i> Gerecke & Di Sabatino, 2013	F	Corsica, Ruisseau de San Petru, N of Quenza	41.7801 N, 9.13483 E	CCDB 38559 A09	NOVMB009-21/ ON002561
<i>Atractides gibberipalpis</i> Piersig, 1898	F	Corsica, Ruisseau de San Petru, N of Quenza	41.7801 N, 9.13483 E	CCDB 38559 A10	NOVMB010-21/ ON002552
	F	Corsica, Ruisseau Regolo at crossing with road D69	42.1181 N, 9.18393 E	CCDB 38559 B07	NOVMB019-21/ ON002548
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 C08	NOVMB032-21/ ON002553
	F	Corsica, Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D08	NOVMB044-21/ ON002551
	F	Corsica, Rivière de Tavulella, at crossing with road D84	42.2419 N, 8.81322 E	CCDB 38559 F10	NOVMB070-21/ ON002556
	F	Corsica, Rivière de Tavulella, at crossing with road D84	42.2419 N, 8.81322 E	CCDB 38559 F11	NOVMB071-21/ ON002547
	F	Corsica, Ufium Orbu near outflow Ruisseau de Quella	42.1043 N, 9.24482 E	CCDB 38559 G09	NOVMB081-21/ ON002560
	F	Corsica, Unnamed stream N of Col de Bavella	41.8165 N, 9.25267 E	CCDB 38559 G10	NOVMB082-21/ ON002555
	F	Corsica, Ruisseau d Aitone at crossing with road D84	42.2636 N, 8.84597 E	CCDB 38559 H07	NOVMB091-21/ ON002550
<i>Atractides inflatus</i> (Walter, 1925)	F	Corsica, Rivière Le Fango, crossing road D81	42.415 N, 8.683 E	CCDB 38559 B01	NOVMB013-21/ ON002562
<i>Atractides pumilus</i> (Szalay, 1946)	F	Corsica, Ruisseau de Battesta at crossing with road D50	41.5449 N, 9.02292 E	CCDB 38559 F05	NOVMB065-21/ ON002559

(Continued)

Table 1. (Continued).

Morphospecies	Sex	Locality	Coordinates	Voucher code	BOLD/GenBank Acc. Nos.
<i>Hygrobatas calliger</i> Piersig, 1896	F	Corsica, Ruisseau de l Umbert acciu, at Pont Chiuni/D81	42.1838 N, 8.61712 E	CCDB 38559 A07	NOVMB007-21/ON002565
	F	Corsica, R Rivière La Solenzara	41.8383 N, 9.33055 E	CCDB 38559 E08	NOVMB056-21/ON002563
<i>Hygrobatas corsicus</i> Pešić & Smit, 2017	M	Corsica, Ruisseau de San Petru, N of Quenza	41.7801 N, 9.13483 E	CCDB 38559 A11	NOVMB011-21/ON002566
<i>Hygrobatas longiporus</i> Thor, 1898	F	Corsica, Porto, Ruisseau de Campaghiu	42.2413 N, 8.7324 E	CCDB 38559 B02	NOVMB014-21/ON002569
	F	Corsica, Ruisseau d Enova, at crossing with road D124	42.2659 N, 8.71405 E	CCDB 38559 G05	NOVMB077-21/ON002568
	F	Corsica, Ruisseau d Enova, at crossing with road D124	42.2659 N, 8.71405 E	CCDB 38559 G06	NOVMB078-21/ON002567
Pionidae					
<i>Piona imminuta</i> (Piersig, 1897)	F	Corsica, Barrage de Codole	42.5817 N, 8.94238 E	CCDB 38559 E10	NOVMB058-21/ON002593
Aturidae					
<i>Ljania bipapillata</i> Thor, 1898	F	Corsica, Unnamed stream N of Col de Bavella	41.8165 N, 9.25267 E	CCDB 38559 G12	NOVMB084-21/ON002621
Mideopsidae					
<i>Mideopsis roztoczensis</i> gr.	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 B10	NOVMB022-21/ON002586
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 B11	NOVMB023-21/ON002584
	F	Corsica, Ruisseau de Sattu	41.6907 N, 9.1525 E	CCDB 38559 B12	NOVMB024-21/ON002583
	F	Corsica, Gorge de Spelunca, Ruisseau Tavulella	42.255 N, 8.76658 E	CCDB 38559 D07	NOVMB043-21/ON002585

Table 2. Estimates of genetic distance (K2P) between studied *Sperchon* spp. The number of base substitutions per site from averaging over all sequence pairs between species are shown. Standard error estimate(s) are shown above the diagonal. This analysis involved 41 nucleotide sequences. There were a total of 669 positions in the final dataset.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) <i>S. violaceus</i>									
(2) <i>S. vaginosus</i>	0,151	0,017							
(3) <i>S. denticulatus</i> _Montenegro	0,140	0,150	0,016						
(4) <i>S. hibernicus</i>	0,159	0,151	0,017	0,017					
(5) <i>S. denticulatus</i> gr._Corsica	0,178	0,177	0,146	0,154	0,018				
(6) <i>S. meridianus</i> _Corsica	0,192	0,201	0,191	0,196	0,018	0,212			
(7) <i>S. setiger</i> _Norway	0,212	0,214	0,208	0,216	0,232	0,103	0,020		
(8) <i>S. setiger</i> /insignis	0,178	0,206	0,190	0,208	0,211	0,108	0,021	0,013	
(9) <i>S. setiger</i> _Netherlands	0,194	0,195	0,202	0,209	0,207	0,122	0,135	0,013	0,109

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 metric for computing these distances in barcoding studies, Ward 2009) 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 84 specimens morphologically assigned to 27 species from 12 genera and 9 families of water mites from Corsica were newly generated for this study. Fragment lengths of the analysed DNA barcode fragments ranged from 322 to 658 base pairs, including no stop codons, insertions or deletions. Full barcodes of 658 bp were recovered for 58 specimens; for a further 26 specimens a sequence

ranging between 322 and 646 bp was recovered. Sequences less than 500 bp were obtained for only 4 specimens. Following BOLD standard, 79 sequences were considered to be barcode compliant.

The families Hygrobatidae and Torrenticolidae are represented by the highest number of sequences (21 and 20, respectively). The opposite, the families Aturidae, Limnesiidae and Pionidae by the lowest number of sequences (each with one sequence). BOLD and GenBank ID and accession numbers for all specimens included in final data set are given in Table 1. The first DNA barcoding data were uploaded into the BOLD database for six species i.e. *Protzia lata* (Walter, 1906), *P. pycnica* Gerecke, 1996, *Atractides corsicus* E. Angelier, 1954, *A. allgaier* Gerecke, 2003, *A. giustinii* Gerecke & Di Sabatino, 2013, and *A. pumilus* (Szalay, 1946). All species recorded in this study have been previously reported from Corsica, and three of these species, i.e. *Protzia pycnica* Gerecke, 1996, *Atractides corsicus* E. Angelier, 1954, *A. giustinii* Gerecke & Di Sabatino, 2013, are endemic for Corsica and Sardinia.

Most of the morphologically-identified species show an intraspecific variation of less than 2%; only two morphospecies, *Torrenticola laskai* Di Sabatino, 2009 and *T. barsica* (Szalay, 1933) showed a maximum interspecific divergence larger than 5% divergence. Moreover, the comparison of the newly generated sequences with publicly available DNA barcodes from BOLD and GenBank allowed us recognize several problematic complexes of species that need further study, to link the Corsican clades with their sister-clades, to calculate genetic divergence between them, and assess which clades may represent putative species.

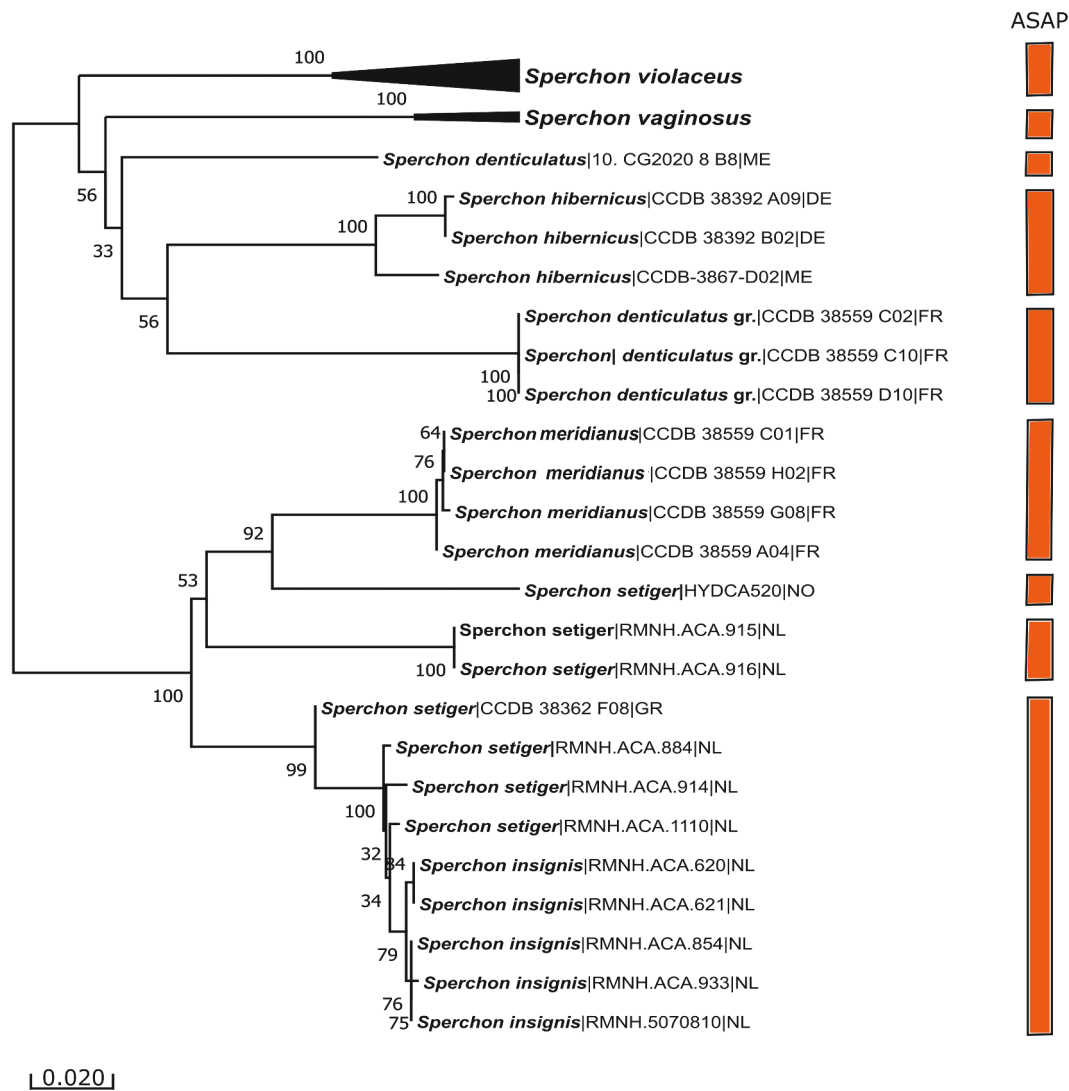


Figure 1. Neighbour-Joining tree of the genus *Sperchon* obtained from 41 nucleotide COI sequences. The results of species delimitation by ASAP procedure are indicated by vertical bars. The applied ASAP procedure identified 9 MOTUs (hypothetical species) at the threshold distance of 6.24% (K2P) which has the best ASAP-score (2.00) within the available molecular data. Country codes (alpha-2 code): DE = Germany, GR = Greece, FR = France, IR = Iran, ME = Montenegro, NL = the Netherlands, NO = Norway, RS = Serbia.

Sperchon meridianus Angelier, 1954

In regard to the shape of the integument formed exclusively by fine denticles and P-3 with three or four ventral setae, the bar-coded specimens from Corsica match the morphology of *S. setiger* Thor, 1898, a species widely distributed in Europe (Di Sabatino et al. 2010).

In our COI tree, the sequences of specimens from Corsica form a highly supported clade which is placed (albeit with a low support) as a sister clade that include sequences of *S. setiger* from Norway, from where the latter species was originally described (Thor 1898). The results of our study reveals a high interspecific distance between the Corsican clade and its European mainland sister clades grouping sequences retrieved from the specimens morphologically assigned to *Sperchon setiger* Thor, 1898 and/or *S. insignis* Walter, 1906 (Table 2). The level of COI differentiation between the Corsican clade and the *S. setiger*-clade from Norway was estimated to be $10.3 \pm 1.3\%$ K2P, respectively, indicating its possible species status. Moreover, the results of ASAP delimitation analysis supported the species status of population from Corsica.

In our opinion, the *Sperchon setiger*-like population from Corsica should be assigned to *S. meridianus* Angelier, 1954, a species originally described by Angelier (1954b) from Corsica on the basis of a single male. Later on, Gerecke (1991) re-examined the holotype of *S. meridianus* and additional material

from its locus typicus and placed this species into synonymy with *S. setiger*. Morphologically, *S. meridianus* can be separated by the slender P-3 (stout in *S. setiger*) bearing 2–3 thicker ventral setae on the medial surface and a single, hair-like ventral seta on the lateral side of P-3 (see Figure 3a-b). The results of our study indicate that *S. meridianus* Angelier, 1954 should be resurrected as a valid species distinct from *S. setiger*.

Sperchon denticulatus gr.

All bar-coded specimens of *Sperchon denticulatus* group in our study were females not allowing morphological identification at the species level. The clade grouping the COI sequences retrieved from the specimens of *S. denticulatus* gr. collected on Corsica was placed as a sister clade to a clade containing specimens of *S. hibernicus* Halbert, 1944 from Germany and Montenegro. The level of COI differentiation between these two clades was estimated to be 15.4% K2P divergence, thus confirming the validity of the species from Corsica, proved also by the results of the ASAP analysis (Figure 1).

Gerecke and Di Sabatino (2013) who re-examined the collection of Daniele Benfatti (stored in the Museo Civico di Storia Naturale Verona) mentioned that in regard to the morphology of palp and ejaculatory complex, populations of *S. denticulatus* group from

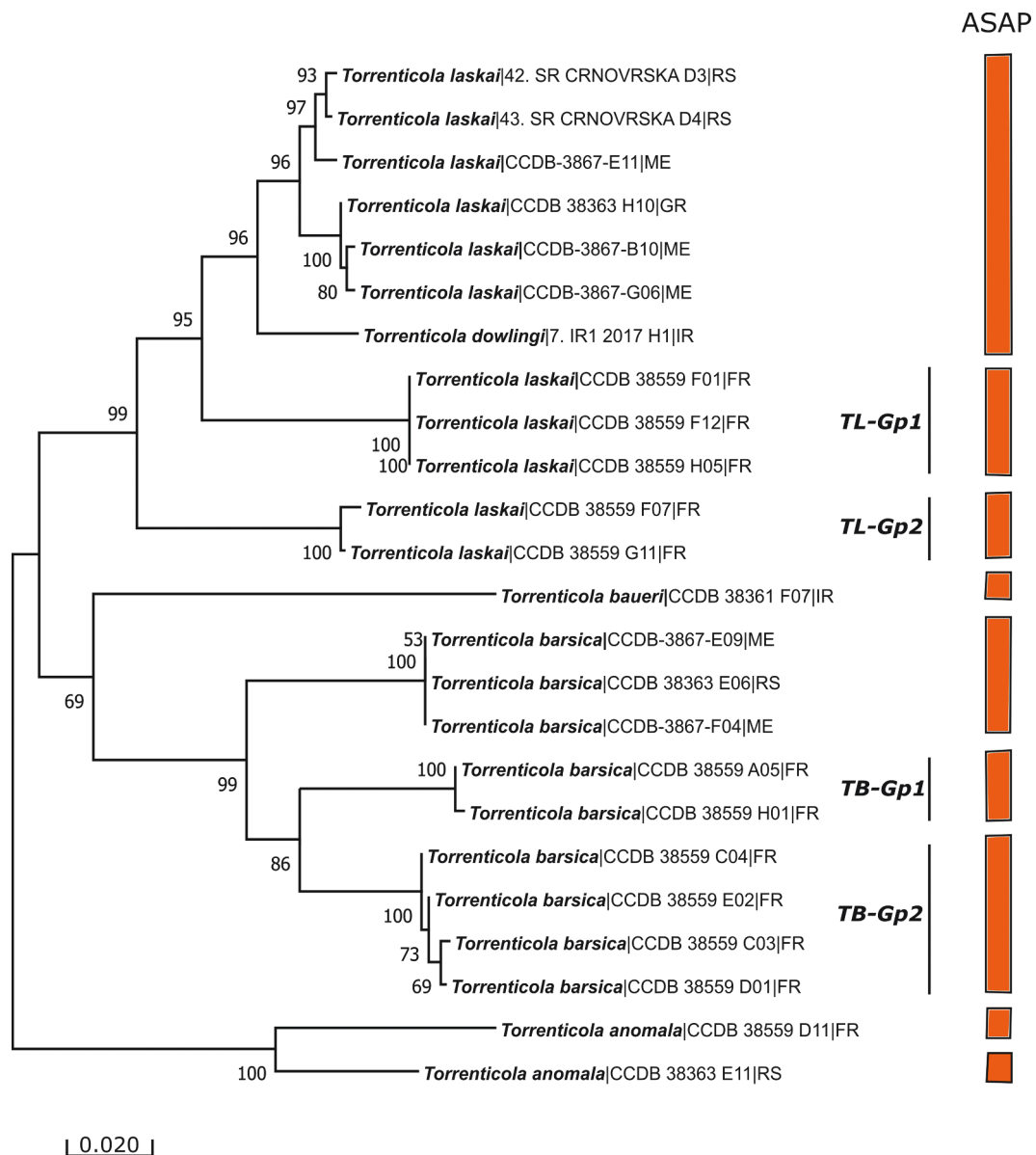


Figure 2. Neighbour-Joining tree of the genus *Torrenticola* obtained from 24 nucleotide COI sequences. The results of species delimitation by ASAP procedure are indicated by vertical bars. The applied ASAP procedure identified 9 MOTUs (hypothetical species) at the threshold distance of 4.94% (K2P) which has the best ASAP-score (1.00) within the available molecular data. Country codes (alpha-2 code): GR = Greece, FR = France, IR = Iran, ME = Montenegro, RS = Serbia.

Corsica resemble to *S. denticulatus* but differing from the latter species in more slender and projecting tips of the ejaculatory complex and the postgenital plate being oval in shape, without straight posterior margin. According to the latter authors, populations of *S. denticulatus* group from Corsica “could represent a distinct taxonomic unit at species or subspecies level.” At the moment, this populations should be left unnamed until males are available.

***Torrenticola laskai* gr.**

The specimens from Corsica used in this study for molecular analysis match the description of *Torrenticola laskai* Di Sabatino, 2009. The latter species was described from populations formerly attributed to *T. lativalvata* K. Viets, 1952, a species described from Algeria (see Gerecke and Di Sabatino 1996 for a discussion). The type locality of *T. laskai* is located on Corsica (F. Porto, Ponte Vecchio), but the species is considered to be more widely distributed with known records from the Czech Republic, Bulgaria, Bosnia and Herzegovina, Italy, Montenegro and Greece (Di Sabatino et al. 2009). In Montenegro it is one of the most common and abundant species at low to middle elevation (Pešić et al. 2010, 2021).

In our study, we obtained two highly-supported clades (TL-Gp1 and TL-Gp-2) among the *T. laskai* specimens. The only difference between the examined males of two Corsican clades refers to the shape of the ejaculatory complex (proximal chamber more enlarged, ejaculatory complex large, L 288 µm) in one male (CCDB 38559 F01) belonging to the TL-Gp1 clade. On the other hand, one male (CCDB 38559 G11) matches the original description of *T. laskai*, in regard to size (L 234 µm) and shape of the ejaculatory complex (proximal chamber comparatively small, see Figure 3d), suggesting that TL-Gp2 clade likely corresponds to the nominal species. The level of COI differentiation between these two clades was estimated to be $11.04 \pm 1.46\%$ K2P divergence, indicating genetical separation between these two clades. Nonetheless, to resolve the taxonomic status of these clades, additional genetic marker (e.g. 28S or 18S rDNA) are needed to be included in further studies.

It is worth mentioning here that the applied ASAP procedure proved species status of the Corsican clades at the threshold distance of 6.08% (K2P) which has the best ASAP-score (2.00). On the other hand, the ASAP procedure, grouped together the COI sequences of *T. laskai* from Serbia, Macedonia and Greece and *T. dowlingi* Pešić, 2020, a species

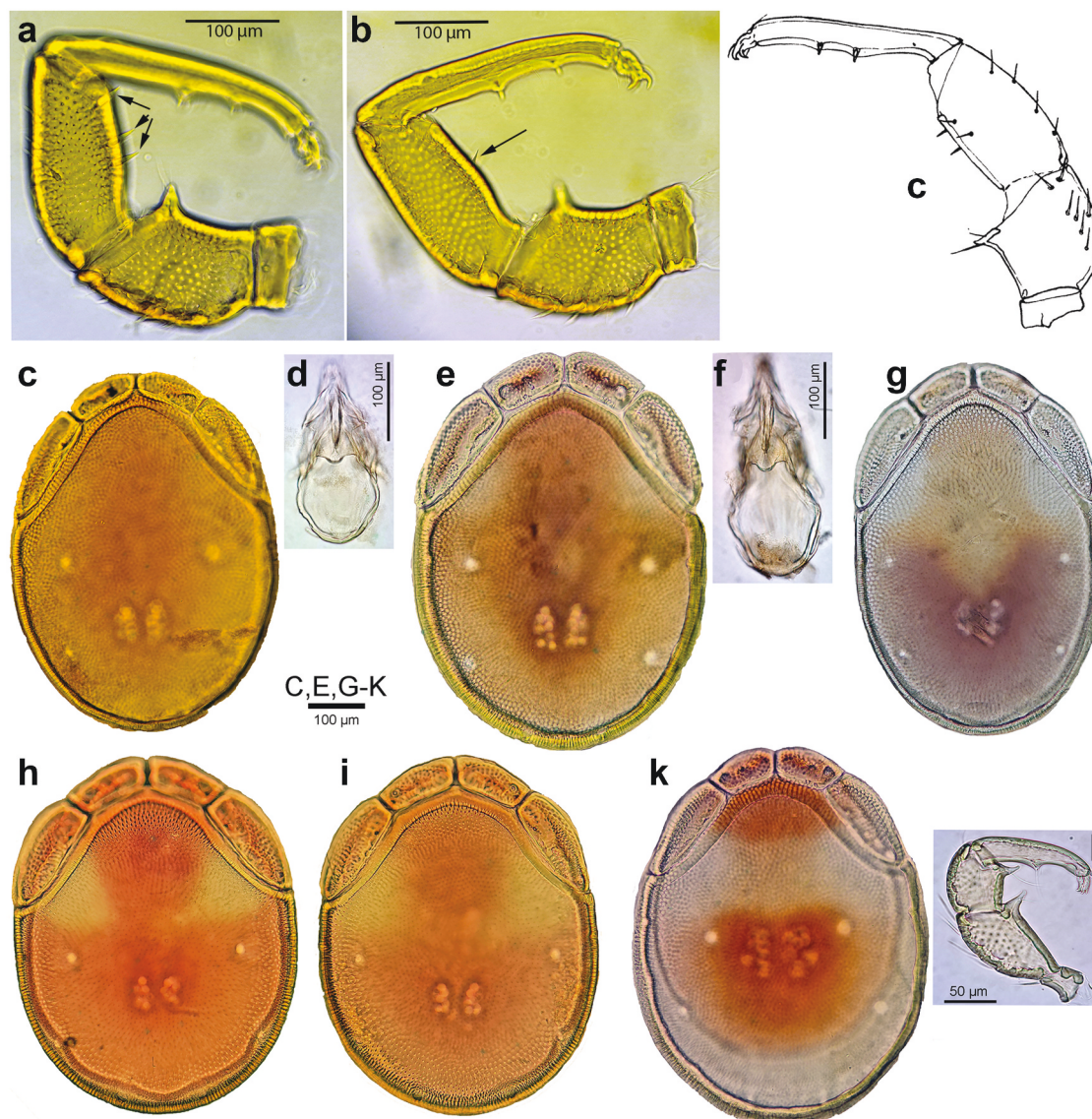


Figure 3. Photographs of selected structures (a-b, l – palp; C, E, H-K – dorsal shield; d, f – ejaculatory complex): a-b *Sperchon meridianus* ♀ [CCDB 38559 C01], Ruisseau de Sattu, Corsica. a – palp, medial view (arrows indicate setae on ventral margin of p-3); b – palp, lateral view (arrow indicate a single, hair-lake seta on ventral margin of P-3). c – *S. setiger*, cotype from Norway; from K. Viets 1936, fig. 148a. c-d – *Torrenticola laskai* ♂ [CCDB 38559 F01], Riviere Casaluna, Corsica. e-f – *T. laskai* ♂ [CCDB 38559 G11], stream N of Col de Bavella, Corsica; g – *T. laskai* ♂, Crnovrška River, Serbia. h – *T. barsica* ♂ [CCDB 38559 C03]. i – *T. barsica* ♂ [CCDB 38559 H01], Ruisseau de Casa Infurcata, Corsica. k-l – *T. anomala* ♀ [CCDB 38559 D11], Ruisseau Tavulella, Corsica.

recently described from Mazandaran province of Iran (Pešić et al. 2020). The latter species and populations of *T. laskai* from the Balkans share the characteristic colour pattern on the dorsal shield (Figure 3g), which clearly separates them from the two Corsican clades. It is possible, that *T. laskai* as well the second unidentified clade represent local endemics of Corsica, while populations from the Balkans (or SE Europe) should be assigned to *T. dowlingi*. However, more material is needed to clarify the status of the Corsican clades.

Torrenticola barsica gr.

The sequenced specimens from Corsica match morphologically the description of *Torrenticola barsica* (Szalay, 1933), a species widely distributed in the Mediterranean. The analysis based on COI data reveals the presence of two haplogroups (TB-Gp-1 and TB-Gp-2 in Figure 2) within the successfully barcoded *T. barsica* specimens from Corsica. However, we did not find differences between the studied specimens. Both haplogroups are highly supported as sister clades, and placed as a sister clade grouping sequences of *T. barsica* from Montenegro and Serbia (Figure 2).

The level of COI differentiation between two clades from Corsica was estimated to be $6.7 \pm 1.09\%$ K2P divergence, while the divergence with the *T. barsica* clade from the Balkans was about 8.7% K2P (see Table 3).

The ASAP analysis supported the species status of the above-mentioned clades. Nevertheless, to clarify the taxonomic status of the above described lineages, more specimens from Europe need to be molecularly analysed, including specimens from the type locality of *T. barsica*, possible by including an additional genetic marker.

Torrenticola anomala gr.

Torrenticola anomala (Koch, 1837) is species supposed to have a Holarctic distribution (Di Sabatino et al. 2010). We successfully barcoded a single female from Corsica. In regard to the gnathosoma with an almost straight ventral margin and long rostrum and apically serrate surface of the ventrodistal projection of P-2 (Figure 3l), the examined female matches the description of *T. anomala*. In our COI tree, the specimen from Corsica appeared as sister clade of *T. anomala* from Serbia. The average intraspecific

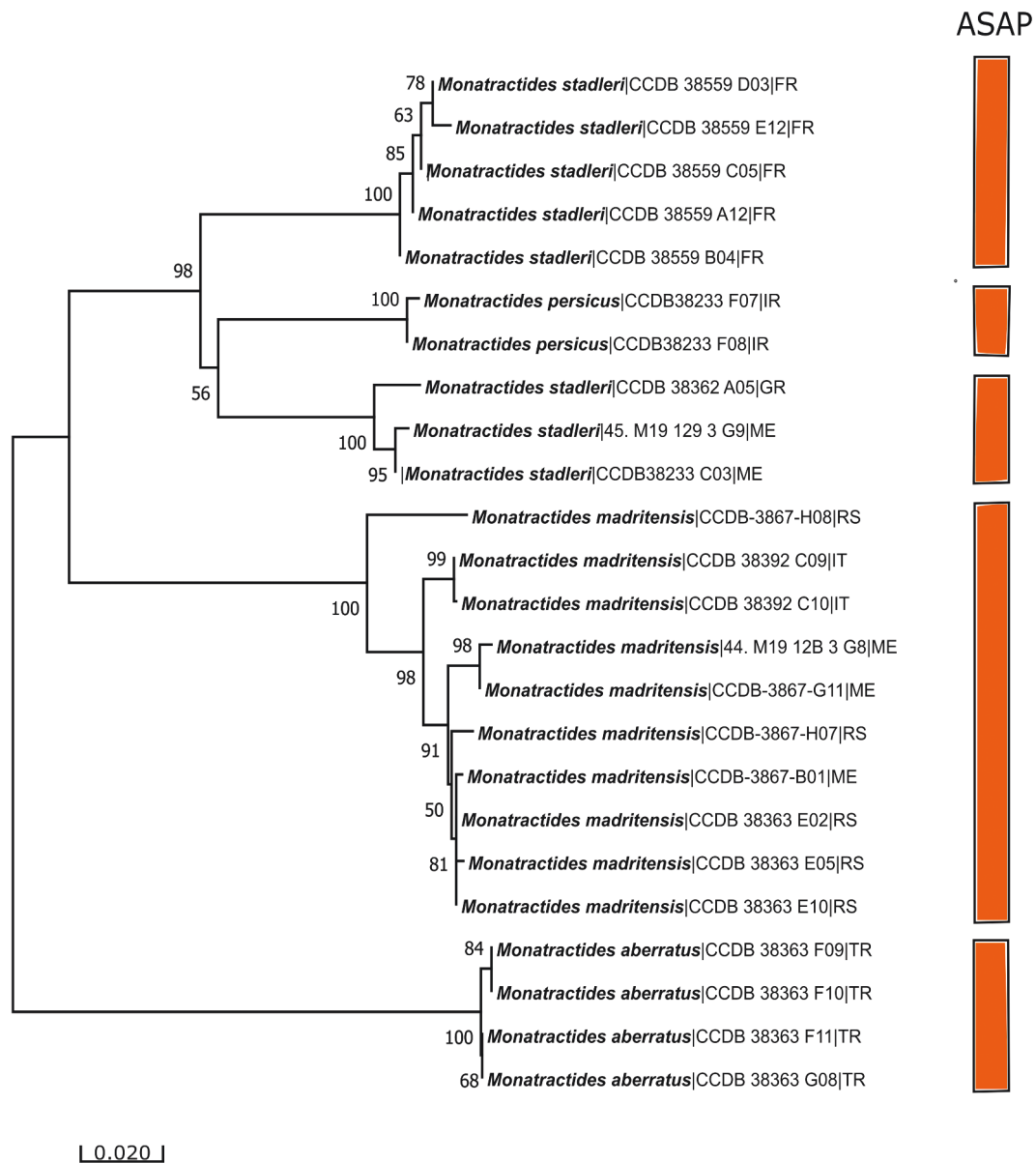


Figure 4. Neighbour-Joining tree of the genus *Monatractides*, obtained from 24 nucleotide COI sequences. The results of species delimitation by ASAP procedure are indicated by vertical bars. The applied ASAP procedure identified 5 MOTUs (hypothetical species) at the threshold distance of 6.37% (K2P) which has the best ASAP-score (2.00) within the available molecular data. Country codes (alpha-2 code): GR = Greece, FR = France, IR = Iran, IT = Italy, ME = Montenegro, RS = Serbia, TR = Turkey.

Table 3. Estimates of genetic distance (K2P) between studied *Torrenticola* spp. The number of base substitutions per site from averaging over all sequence pairs between species are shown. Standard error estimate(s) are shown above the diagonal. This analysis involved 24 nucleotide sequences. There were a total of 669 positions in the final dataset.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) <i>T. laskai</i> _TL-Gp1		0,0146	0,0119	0,0135	0,0205	0,0201	0,0181	0,0277	0,0201	0,0193
(2) <i>T. laskai</i> _TL-Gp2	0,1104		0,0129	0,0146	0,0186	0,0188	0,0182	0,0256	0,0192	0,0176
(3) <i>T. laskai</i> _Balkans	0,0810	0,0981		0,0078	0,0177	0,0171	0,0169	0,0253	0,0185	0,0171
(4) <i>T. dowlingi</i> _Iran	0,0867	0,1029	0,0431		0,0201	0,0193	0,0185	0,0249	0,0193	0,0185
(5) <i>T. barsica</i> _TB-Gp1	0,1923	0,1728	0,1668	0,1813		0,0109	0,0125	0,0245	0,0213	0,0196
(6) <i>T. barsica</i> _TB-Gp2	0,1868	0,1651	0,1582	0,1672	0,0674		0,0127	0,0268	0,0214	0,0192
(7) <i>T. barsica</i> _Balkans	0,1641	0,1698	0,1621	0,1671	0,0876	0,0874		0,0262	0,0206	0,0198
(8) <i>T. baueri</i> _Iran	0,2009	0,1781	0,1781	0,1654	0,1621	0,1831	0,1741		0,0265	0,0274
(9) <i>T. anomala</i> _Corsica	0,2051	0,1934	0,1907	0,1846	0,2101	0,2199	0,2268	0,2059		0,0120
(10) <i>T. anomala</i> _Serbia	0,1951	0,1701	0,1708	0,1766	0,1892	0,1939	0,2106	0,2097	0,0847	

K2P distances between these two *T. anomala* specimens was estimated to $8.47 \pm 1.2\%$, suggesting their possible species status as proved by results of ASAP delimitation procedure. To clarify the

taxonomic status of the Corsican clade, additional specimens from Corsica and Europe should be barcoded including specimens from the type locality of *T. anomala*.

Table 4. Estimates of genetic distance (K2P) between studied *Monatractides* spp. The number of base substitutions per site from averaging over all sequence pairs between species are shown. Standard error estimate(s) are shown above the diagonal. This analysis involved 24 nucleotide sequences. There were a total of 669 positions in the final dataset.

	(1)	(2)	(3)	(4)	(5)
(1) <i>M. stadleri</i> _Corsica Clade		0,0134	0,0144	0,0187	0,0214
(2) <i>M. stadleri</i> _Balkans Clade	0,1029		0,0128	0,0176	0,0192
(3) <i>M. persicus</i>	0,1020	0,0929		0,0188	0,0212
(4) <i>M. madritensis</i>	0,1803	0,1800	0,1795		0,0205
(5) <i>M. aberratus</i> _Turkey	0,2253	0,1964	0,2046	0,2257	

Table 5. List of specimens used in this study. For specimens from Corsica see Table 1.

Locality (country, name)	Lat/Long	BOLD Acc. Nos	Voucher code
Sperchontidae			
<i>Sperchon denticulatus</i> Koenike, 1895			
Montenegro, Berane spring near Monastery	42.8527 N, 19.862 E	DNAEC019-20	10. CG2020_8 B8
<i>Sperchon hibernicus</i> Halbert, 1944			
Germany, Ammer bei Aeule-Brucke, Lustnau	48.5268 N, 9.0794 E	DCBDJ009-21	CCDB 38392 A09
Germany, Ammer bei Aeule-Brucke, Lustnau	48.5268 N, 9.0794 E	DCBDJ014-21	CCDB 38392 B02
Montenegro, Rikavac stream above Old Bar	42.1001 N, 19.1432 E	DNCBD038-20	CCDB-3867-D02
<i>Sperchon setiger</i> Thor, 1898			
Netherlands, Overijssel, Ootmarsum	52.431 N, 6.904 E	NLACA079-15	RMNH.ACA.1110
Netherlands, Limburg, Mechelen	50.796 N, 5.941 E	NLACA455-15	RMNH.ACA.884
Netherlands, Limburg, Nuth: Platsbeek	50.916 N, 5.87 E	NLACA474-15	RMNH.ACA.914
Netherlands, Limburg, Nuth: Platsbeek	50.916 N, 5.87 E	NLACA475-15	RMNH.ACA.915
Netherlands, Limburg, Nuth: Platsbeek	50.916 N, 5.87 E	NLACA476-15	RMNH.ACA.916
Norway, Badstudalen Naturresevat, bekk i Egedalen	58.205 N, 8.236 E	MMHYD386-21	HYDCA520
<i>Sperchon insignis</i> Walter, 1906			
Netherlands, Limburg, Epen: Terziet	50.755, 5.904 E	NLACA326-15	RMNH.ACA.620
Netherlands, Limburg, Epen: Terziet	50.755, 5.904 E	NLACA327-15	RMNH.ACA.621
Netherlands, Limburg, Epen: Nutbron	50.784, 5.912 E	NLACA429-15	RMNH.ACA.854
Netherlands, Limburg, Geulle: Molenbeek	50.912, 5.741 E	NLACA489-15	RMNH.ACA.933
Netherlands, Limburg, Cottessen: Belleterbeek	50.762, 5.931 E	NLACA1052-17	RMNH.5070810
<i>Sperchon vaginosus</i> Thor, 1902			
Netherlands, Limburg, Mechelen	50.796 N, 5.941 E	NLACA416-15	RMNH.ACA.840
Netherlands, Limburg, Mechelen	50.796 N, 5.941 E	NLACA417-15	RMNH.ACA.841
Netherlands, Limburg, Mechelen	50.796 N, 5.941 E	NLACA418-15	RMNH.ACA.842
Netherlands, Heythuizen: Hulsbergerbeek bij Heythuizen	50.888 N, 5.879 E	NLACA944-17	RMNH.5070702
<i>Sperchon violaceus</i> Walter, 1944			
Switzerland, Val Mustair, La Posa	46.6419 N, 10.3559 E	LBCWS035-19	CrenoBarcode_H112
Austria, NP Gesaeuse, Rohrbach	47.5977 N, 14.6351 E	LBCWS160-19	CrenoBarcode_H388
Austria, NP Gesaeuse, Rohrbach	47.5977 N, 14.6351 E	LBCWS161-19	CrenoBarcode_H389
Austria, NP Gesaeuse, Rohrbach	47.5977 N, 14.6351 E	LBCWS162-19	CrenoBarcode_H390
Montenegro, Bijelo Polje, Lještanica stream	43.0631 N, 19.5809 E	DNAEC010-20	56. CG2020_1
Montenegro, Bijelo Polje, Lještanica stream	43.0631 N, 19.5809 E	DNAEC011-20	57. CG2020_8
Montenegro, Mojkovac, Bistrica stream	42.9871 N, 19.4338 E	DCCDB042-21	CCDB38233 D06
Montenegro, Bijelo Polje, Lještanica stream	43.0631 N, 19.5809 E	DNAEC012-20	58. CG2020
Montenegro, Žabljak, Mliniski potok	43.1494 N, 19.0898 E	DNAEC088-20	Hyd_MN_VP8
Montenegro, Kolašin, Biogradska river	42.8968 N, 19.6047 E	DNAEC061-20	26. M19_16A_3_F4
Montenegro, Kolašin, Biogradska river	42.8968 N, 19.6047 E	DNAEC062-20	27. M19_16A_3_F5
Montenegro, Kolašin, Biogradska river	42.8968 N, 19.6047 E	DNAEC063-20	28. M19_16A_3_F6
Montenegro, Mojkovac, Bistrica spring	42.9862 N, 19.4349 E	DCCDB094-21	CCDB38233 H10
Montenegro, Mojkovac, Bistrica spring	42.9862 N, 19.4349 E	DCCDB095-21	CCDB38233 H11
Torrenticolidae			
<i>Torrenticola anomala</i> (Koch, 1837)			
Serbia, Zlatibor, Katušnica river	43.6576 N, 19.8392 E	SEPTA059-21	CCDB-3863-E11
<i>Torrenticola barsica</i> (Szalay, 1933)			
Montenegro, Bar, Međurječka river	42.0363 N, 19.2179 E	DNCBD057-20	CCDB-3867-E09
Montenegro, Bar, Međurječka river	42.0363 N, 19.2179 E	DNCBD064-20	CCDB-3867-F04
Serbia, Zlatibor, Crni Rzav river	43.6559 N, 19.704 E	SEPTA054-21	CCDB-3863-E06
<i>Torrenticola baueri</i> Bader & Sepasgozarian, 1987			
Iran, Shirabad near Khanbebin, stream	36.9675 N, 55.0278 E	DCDDJ067-21	CCDB 38361 F07
<i>Torrenticola dowlingi</i> Pešić, 2020			
Iran, river near Karaj	35.8222 N, 51.0208 E	IRANM003-20	7. IR1_2017_H1
<i>Torrenticola laskai</i> Di Sabatino, 2009			
Montenegro, Tara River near Mateševo	42.7898 N, 19.5374 E	DNCBD022-20	CCDB-3867-B10
Montenegro, Cijevna river near Dinoša	42.4057 N, 19.3569 E	DNCBD078-20	CCDB-3867-G06
Montenegro, Bar, Međurječka river	42.0363 N, 19.2179 E	DNCBD059-20	CCDB-3867-E11
Serbia, Stara Planina, Crnovrška river	43.4045 N, 22.5131 E	DNAEC036-20	42.SR_CRNOVRSKA_D3
Serbia, Stara Planina, Crnovrška river	43.4045 N, 22.5131 E	DNAEC037-20	43.SR_CRNOVRSKA_D4
Greece, Glaukos river at Souli	38.2008 N, 21.7908 E	SEPTA094-21	CCDB-3863-H10
<i>Monatractides aberratus</i> (Lundblad, 1941)			
Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E	SEPTA072-21	CCDB 38363 F12
Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E	SEPTA080-21	CCDB 38363 G08

(Continued)

Table 5. (Continued).

Locality (country, name)	Lat/Long	BOLD Acc. Nos	Voucher code
Turkey, Burdur, Kuzkoy Village, Celtikci	37.5803 N, 30.4003 E	SEPTA069-21	CCDB 38363 F09
Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E	SEPTA070-21	CCDB 38363 F10
Turkey, Burdur, Bucak at Kizilli Village	37.3425 N, 30.9256 E	SEPTA071-21	CCDB 38363 F11
<i>Monatractides madritensis</i> (K. Viets, 1930)			
Montenegro, Lastva Grbaljska	42.3103 N, 18.8138 E	DNAEC075-20	44. M19_12B_3_G8
Montenegro, Podgorica, Cijevna river	42.4057 N, 19.3569 E	DNCBD083-20	CCDB-3867-G11
Montenegro, Godinje, stream	42.2206 N, 19.1118 E	DNCBD013-20	CCDB-3867-B01
Serbia, Crnovrska river	43.4045 N, 22.5131 E	DNCBD091-20	CCDB-3867-H07
Serbia, Crnovrska river	43.4045 N, 22.5131 E	DNCBD092-20	CCDB-3867-H08
Serbia, Zlatibor, Crni Rzav river	43.6559 N, 19.704 E	SEPTA050-21	CCDB-3863-E02
Serbia, Zlatibor, Crni Rzav river	43.6559 N, 19.704 E	SEPTA053-21	CCDB-3863-E05
Serbia, Zlatibor, Katusnica river	43.6576 N, 19.8392 E	SEPTA058-21	CCDB-3863-E10
Italy, Calabria, Serra San Bruno	38.5542 N, 16.3096 E	DCBDJ033-21	CCDB-3892-C09
Italy, Calabria, Serra San Bruno	38.5542 N, 16.3096 E	DCBDJ034-21	CCDB-3892-C10
<i>Monatractides persicus</i> Pešić, 2004			
Iran, Ramsar, small waterfall	36.8897 N, 50.5681 E	DCCDB067-21	CCDB38233 F07
Iran, Ramsar, small waterfall	36.8897 N, 50.5681 E	DCCDB068-21	CCDB38233 F08
<i>Monatractides stadleri</i> (Walter, 1924)			
Montenegro, Lastva Grbaljska	42.3103 N, 18.8138 E	DNAEC076-20	45. M19_129_3_G9
Montenegro, Bar, Rikavac stream	42.1001 N, 19.1432 E	DCCDB027-21	CCDB38233 C03
Greece, Peloponnese, Selinountas River	38.228 N, 22.1064 E	SEPTB005-21	CCDB-3862-A05

Monatractides stadleri gr.

Monatractides stadleri (Walter, 1944) is a species widely distributed in the Mediterranean region, often very frequent in lowland running waters (Di Sabatino et al. 2010). The specimens from Corsica used in this study for molecular analysis perfectly matches the description of *M. stadleri* from Southern Italy (see Di Sabatino et al. 1991). The sequences of specimens from Corsica form a highly supported clade which is placed as a sister to two other clades corresponding to *M. stadleri* from the Balkans and *M. persicus* Pešić, 2004 from Iran, respectively (Figure 4). The average K2P genetic distance between the Corsican clade and the Balkans clade of *M. stadleri* was estimated to be $10.29 \pm 1.34\%$ (Tables 4–5). The applied ASAP procedure proved the species status of the Corsican clade at the threshold distance of 6.37% (K2P) which has the best ASAP-score (2.00). Nevertheless, the taxonomic status of Corsican clade should be left unnamed until the sequences of *M. stadleri* from its locus typicus are available.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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