Integrative taxonomy reveals a new species of the water mite genus Monatractides K. Viets, 1926 from Corsica (Acari, Hydrachnidia: Torrenticolidae)

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ABSTRACT

In the present study, we use morphological data and DNA barcodes to describe a new species, i.e. *Monatractides corsicus* **sp. nov**. from Corsica, France. The new species belongs to the *M. stadleri*-complex which includes at least four distinct genetic lineages in the Western Palaearctic. Morphologically, the new species can be identified on the basis of a comparatively large palp, thick chelicera and the colour pattern of the dorsal shield. Moreover, we present the first DNA barcoding data for *M. latissimus* (K. Viets, 1936) and *M. lusitanicus* (Lundblad, 1941).

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New species; DNA barcoding; species delimitation; France

Introduction

The genus *Monatractides* K. Viets, 1926 consists of three subgenera (Smit 2020), i.e. the nominate subgenus *Monatractides* known from the Afrotropical and Nearctic regions; the monotypic *Pinguicola* Gerecke & Di Sabatino, 1996, represented by *M. adoratus* Gerecke & Di Sabatino, 1996, recorded from the Mediterranean; and the widely distributed subgenus *Rusetriella* K. Viets, 1931, recorded from all continents except Antarctica. According to Wiles (1997) *Monatractides* may be considered an ancient clade from which other torrenticolid genera and subgenera evolved following the break-up of Pangea, or a recent clade which has subsequently spread to Australia. Most representatives of the genus inhabit streams, preferably low-order streams, with pools having accumulations of leaf litter.

At the present level of morphological knowledge, nine species of the genus *Monatractides* are present in Europe (Lundblad 1956; Di Sabatino et al. 1991, 2003, 2010), i.e. *Monatractides aberratus* (Lundblad, 1941) (Mediterranean), *M. adoratus* Gerecke & Di Sabatino, 1996 (Corsica, Sardinia, and Greece), *M. algeriensis* (Lundblad, 1941) (North Africa, South Italy), *M. fridericianus* Di Sabatino & Gerecke, 2003 (Greece), *M. latissimus* (K. Viets, 1936) (Croatia, Bosnia, SW-Germany), *M. lusitanicus* (Lundblad, 1941) (Iberian Peninsula, Corsica), *M. madritensis* (K. Viets, 1930) (Western Palaearctic), *M. stadleri* (Walter, 1924) (Central, Western, and Southern Europe), and *M. stenostomus* (K. Viets, 1936) (Portugal, Spain, France). Nevertheless, recent studies by applying an integrative approach based on the DNA barcode of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence challenged the status of some of species suggesting possible presence of cryptic species within known *Monatractides* morphospecies. Pešić and Smit (2022) and Pešić et al. (2023) revealed that European populations of *Monatractides stadleri*, a species considered to have a Western Palearctic distribution, likely include several distinct lineages with a genetic distance which are beyond interspecific level.

So far, three species of the genus *Monatractides*, i.e. *M. adoratus, M. lusitanicus* and *M. stadleri* have been reported from Corsica (Gerecke and Di Sabatino 1996; Di Sabatino and Gerecke, 1996; Di Sabatino et al. 2003). In this paper, we use morphological data and COI barcodes to describe one new species of the genus *Monatractides* from Corsica.

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. After DNA extraction, the holotype specimen was dissected and slide mounted in Faure's medium. Holotype and paratype of the new species are deposited in Naturalis Biodiversity Center in Leiden (RMNH). DNA sequences prepared in the course of this study were deposited in BOLD (The Barcode of Life Data System) and GenBank (see Table 1). The DNA extracts were archived in –80°C freezers at the Centre for Biodiversity Genomics in Guelph, Canada (CBG; biodiversitygenomics.net).

Morphological nomenclature follows Gerecke et al. (2016). All measurements are in µm. The photographs of were made using the camera of a Samsung Galaxy smartphone. The following abbreviations are used: Ac = acetabula; asl = above sea level; Cx-I = first coxae; dL = dorsal length; H = height; I-L-1-6 = first-sixth segment of first leg; L = length; P-1 to P-5 = palp segment 1-5; RMNH = Naturalis Biodiversity Center, Leiden; W = width.

DNA barcode analyses

Molecular analyses were conducted at the Canadian Centre for DNA Barcoding (Guelph, Ontario, Canada; (CCDB; http://ccdb.ca/)). In the later institution, 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). DNA sequences prepared in the course of his study were deposited in BOLD and GenBank with accession numbers indicated in Table 1.

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 software (Kumar et al. 2018). The latter software was used to calculate Maximum Likelihood (ML) trees (model selected by the BIC (Bayesian Information Criterion) implemented in MEGA X: GTR+G+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 by the nonparametric bootstrap method (Felsenstein 1985) with 500 replicates and shown next to the branches. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions

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Table 1. List of sequenced *Monatractides* specimens used in this study.

Locality (country, name)	Lat/Long	Voucher code	BOLD/Genbank Acc. nos.	BOLD BIN	Reference
***Monatractides aberratus* (Lundblad, 1941)**					
Turkey, Burdur, Kuzkoy, Celtikci	37.5803°N, 30.4003°E	CCDB 38363 F09	SEPTA069-21/OM321059	BOLD:AEM6344	Pešić et al. (2022)
Turkey, Burdur, Kizilli, Bucak	37.3425°N, 30.9256°E	CCDB 38363 F10	SEPTA070-21/OM321095		
		CCDB 38363 F11	SEPTA071-21/OM321047		
		CCDB 38363 G08	SEPTA080-21/OM321083		
Turkey, Isparta, Aksu Stream-Koprucay River	37.7454°N, 31.0275°E	CCDB_44300_F01	HYDOC061-22/OQ413521		This study
		CCDB_44300_F02	HYDOC062-22/OQ413520		This study
		CCDB_44300_F03	HYDOC063-22/OQ413519		This study
***Monatractides corsicus* sp. nov.**					
France, Corsica, Ruisseau de San Petru, N of Quenza	41.7801°N, 9.13483°E	CCDB 38559 A12	NOVMB012-21/ON002590	BOLD:AEN9161	Pešić and Smit (2022) as *M. stadleri*
France, Corsica, Ruisseau de Campaghiu	42.2413°N, 8.7324°E	CCDB 38559 B04	NOVMB016-21/ON002587		
France, Corsica, Ruisseau de Sattu	41.6907°N, 9.1525°E	CCDB 38559 C05	NOVMB029-21/ON002589		
France, Corsica, Rivière La Figurella	42.4873°N, 8.80532°E	CCDB 38559 D03	NOVMB039-21/ON002588		
France, Corsica, Rivière Casaluna	42.3822°N, 9.28863°E	CCDB 38559 E12	NOVMB060-21/ON002592		
***Monatractides latissimus* (K. Viets, 1936)**					
Croatia, Požega, Veličanka	45.4597°N, 17.6586°E	CCDB_44300_G10	HYDOC082-22/OQ507480	BOLD:AEY8127	This study
Monatractides lusitanicus					
France, Corsica, Ruisseau Tavulella	42.255°N, 8.76658°E	CCDB 38559 D09	NOVMB045-21/ON002591	BOLD:AEN9160	This study
***Monatractides madritensis* (K. Viets, 1930)**					
Montenegro, Budva, Lastva Grbaljska	42.3103°N, 18.8138°E	44. M19_12B_3_G8	DNAEC075-20/OL870248	BOLD:AED3803	Pešić et al. (2021a, 2021b)
Montenegro, Bar, stream in Godinje vill.	42.2206°N, 19.1118°E	CCDB-3867-B01	DNCBD013-20/OL870106		
Monteengro, Podgorica, Cijevna river	42.4057°N, 19.3569°E	CCDB-3867-G11	DNCBD083-20/OL870125		
Serbia, Stara Planina, Crnovrska reka	43.4045°N, 22.5131°E	CCDB-3867-H07	DNCBD091-20/OL874900		Pešić et al. (2021b, 2021c)
Italy, Calabria, Serra San Bruno, stream	38.5542°N, 16.3096°E	CCDB 38392 C09	DCBDJ033-21/OM502292		This study
		CCDB 38392 C10	DCBDJ034-21/OM502288		This study
Serbia, Zlatibor, Crni Rzav	43.6559°N, 19.704°E	CCDB 38363 E02	SEPTA050-21/OL874861		Pešić et al. (2021c)
		CCDB 38363 E05	SEPTA053-21/OL874875		
Serbia, Zlatibor, Katušnica river	43.6576°N, 19.8392°E	CCDB 38363 E10	SEPTA058-21/OL874870		
Serbia, Stara Planina, Crnovrska reka	43.4045°N, 22.5131°E	CCDB-3867-H08	DNCBD092-20/OL874892	BOLD:AEF2866	
***Monatractides persicus* Pešić, 2004**					
Iran, Mazandaran, Ramsar	36.8897°N, 50.5681°E	CCDB38233 F07	DCCDB067-21/OM321053	BOLD:AEI6459	Pešić et al. (2021b)
		CCDB38233 F08	DCCDB068-21/OM321097		
***Monatractides stadleri* (Walter, 1924)**					
Belgium, Rur N of Bosfagne	50.5032°N, 6.18713°E	CCDB_44300_H10	HYDOC094-22/OQ507477	BOLD:AEU1504	This study
		CCDB_44300_H11	HYDOC095-22/OQ507476		
Portugal, Corgo da Ponte Quebrada	37.6961°N, 8.71219°E	CCDB_39397_B05	HYDAS017-22/OQ211649		Pešić et al. (2023)
Monatractides stadleri Balkan-Clade					
Montenegro, Budva, Lastva Grbaljska	42.3103°N, 18.8138°E	45. M19_129_3_G9	DNAEC076-20/OL870104	BOLD:AED3802	Pešić et al. (2021a, 2021b)
Montenegro, Bar, Rikavac stream	42.1001°N, 19.1432°E	CCDB38233 C03	DCCDB027-21/OL870076		
Greece, Peloponnese, Selinountas River	38.228°N, 22.1064°E	CCDB 38362 A05	SEPTB005-21/OL874899		Pešić et al. (2021c)

were removed for each sequence pair. Additionally, the sequence data were analysed using the Assemble Species by Automatic Partitioning (ASAP), a method designed to species partitioning using a hierarchical clustering algorithm based on the pairwise distance distribution (Puillandre et al. 2021). We used the online ASAP version (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) with default settings and K2P distance model.

Results and discussion

Species delimitation using DNA-barcodes

The final alignment for species delimitation using COI sequence data included 32 *Monatractides* specimens and *Torrenticola amplexa* (Koenike, 1908) (SEPTAB011-21) from Croatia as outgroup (Table 1). The final alignment consisted of 669 nucleotide positions. The ML tree is presented in Figure 1.

The sequences retrieved from *Monatractides* specimens from Corsica, here described as *M. corsicus* **sp. nov.**, appeared as a sister to clade grouping sequences of *M. stadleri* from Belgium and Portugal (Figure 1). The mean genetic distance between COI sequences ranged from 10.38±1.38% between *M. corsicus* **sp. nov**. and *M. persicus* to 23.40±2.19% K2P between *M. madritensis* and *M. aberratus*. These genetic distances were higher than the barcoding gap found by the ASAP method (6% to 9%) in the genetic distances among all studied *Monatractides* (Fig. 2), which additionally supported the species-status of the new species. The mean intraspecific distance within clades was relatively low, ranging from 1.92±0.32% in *M. madritensis* to 0.11 ±0.11% K2P in *M. stadleri*. The intraspecific distance of *M. corsicus* **sp. nov.**, was 0.24±0.11% K20 whereas *M. persicus* showed no intraspecific variation (Table 2).

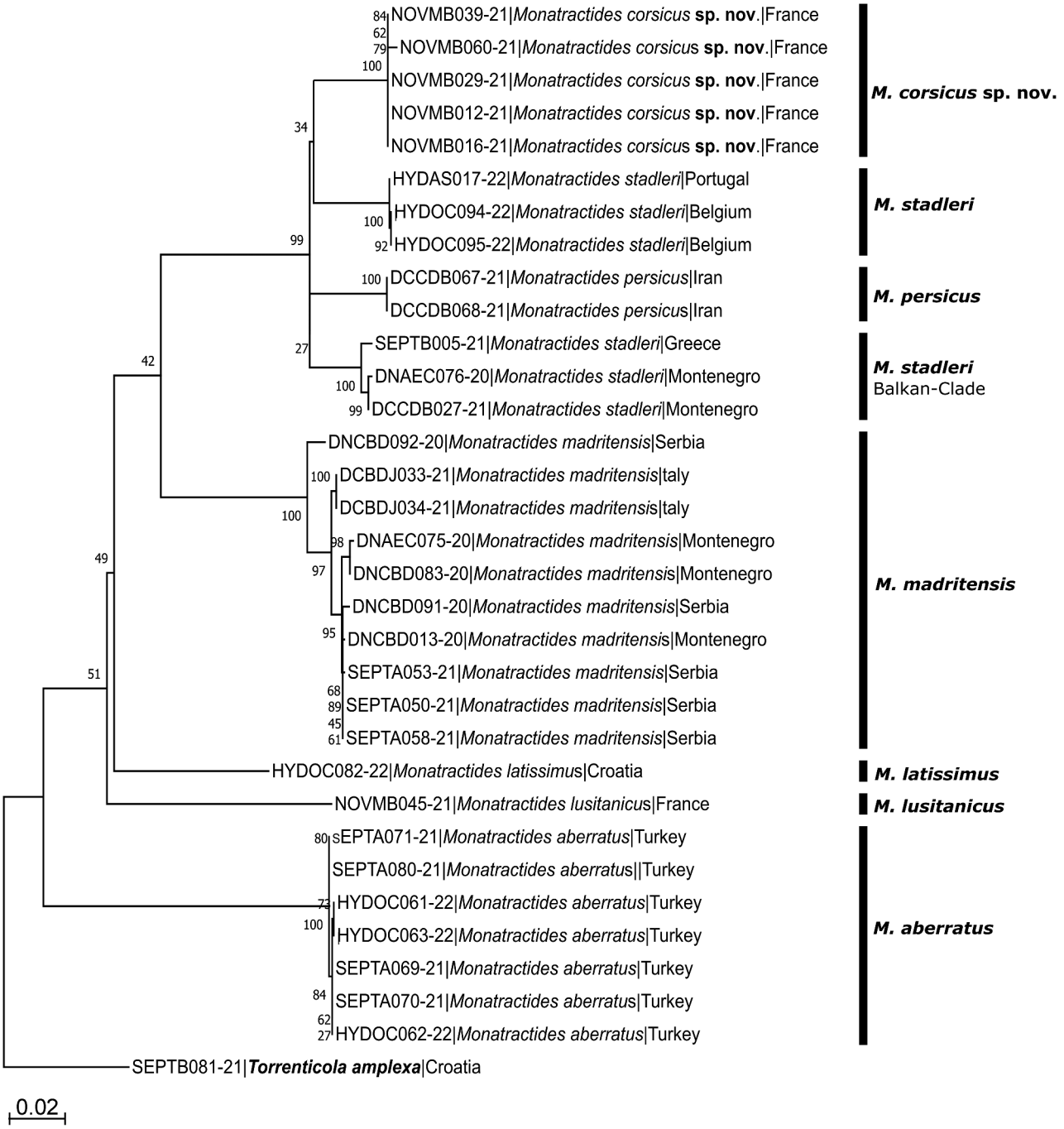

Figure 1. Maximum Likelihood tree (GTR+G + I model) of the genus *Monatractides* obtained from 32 nucleotide COI sequences. The results of species delimitation by ASAP procedure are indicated by vertical bars.

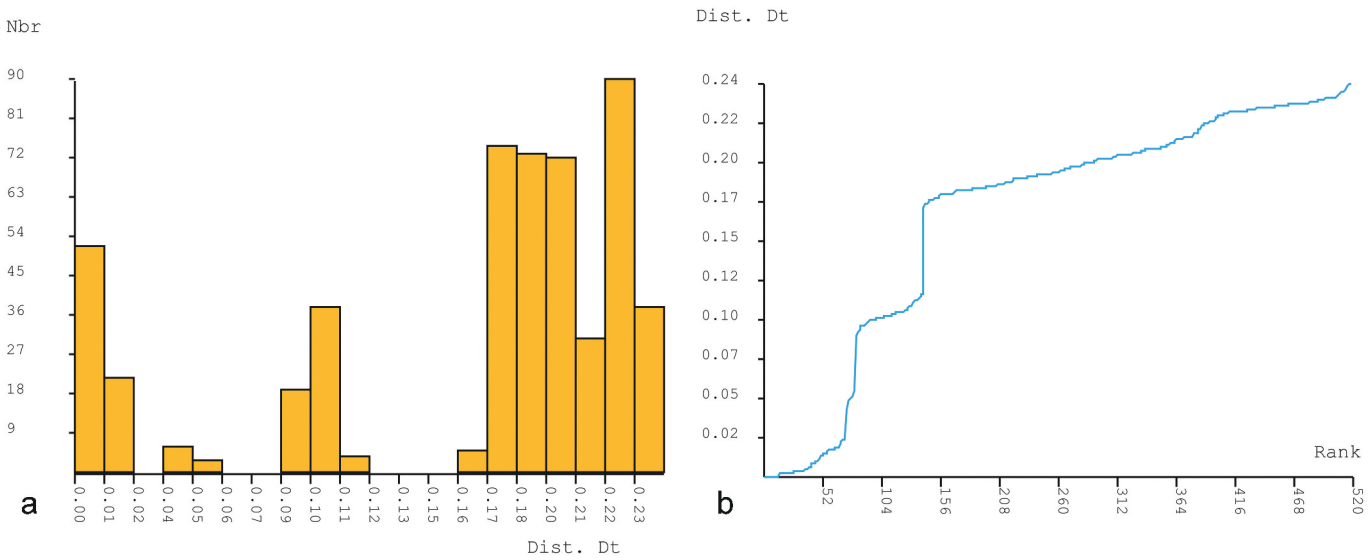

Figure 2. Results of ASAP analysis for COI sequences of the genus *Monatractides*. (A) Distribution of pairwise differences, (B) Ranked pairwise differences.

Systematics

Family Torrenticolidae

Genus *Monatractides* K. Viets, 1926

Diagnosis — Di Sabatino *et al.* 2010: 179.

Subgenus *Rusetriella* K. Viets, 1931

Diagnosis — Smit 2020: 278.

Monatractides corsicus Pešić & Smit sp. nov.

Figs. 3, 4 A-C, 5 , 6D-F

Material examined

Holotype ♂, France, Corsica, Rivière La Figurella, at crossing with road D151, 42.4873° N, 8.80532° E, 147 m asl., 15 April 2015, leg. Smit, sequenced (NOVMB039-21/ON002588), dissected and slide mounted (RMNH). Paratypes: France, Corsica: 1♀, Ruisseau de San Petru, N of Quenza, 41.7801° N, 9.13483° E, 897 m asl., 10 April 2015 leg. Smit, sequenced; 1♀ Porto, Ruisseau de Campaghiu, 42.2413° N, 8.7324° E, 188 m asl., 11 April 2015 leg. Smit, sequenced (NOVMB016-21), dissected (gnathosoma, palps and I-legs slide mounted, dorsal and ventral shields in Koenike fluid); 1♀, Ruisseau de Sattu, 41.6907° N, 9.1525° E, 370 m asl., 10 April 2015 Smit sequenced; 1♂, Rivière Casaluna near San Lorenzo, 42.3822° N, 9.28863° E, 612 m asl., 19 April 2015 leg. Smit, sequenced, dissected (gnathosoma and palps slide mounted, dorsal and ventral shields in Koenike fluid).

Compared material

M. stadleri: Belgium, Rur N of Bosfagne, 50.5032° N, 6.18713° E, 522 m asl., 28 August 2022, leg. Smit, 1♂, 1♀ (juven.), sequenced (Table 1), 1♂ dissected (gnathosoma and palps slide mounted, dorsal and ventral shields in Koenike fluid); Portugal, Corgo da Ponte Quebrada, 37.6961° N, 8.71219° E, 23 May 2022, leg. Jovanović, dissected (gnathosoma, palps and I-legs slide mounted, dorsal and ventral shields in Koenike fluid).

Diagnosis

Idiosoma large, >1000 µm in both sexes; dorsal shield with colour pattern as illustrated in Figures 6(d,f); three pairs of knob-shaped protrusions at the margin of the gnathosomal bay; medial suture line of Cx-II+III in male relatively short, 80-100 µm; palp large and robust, P-2 > 90, P-4 > 80 µm, distal margins of P-3 and P-4 medially and laterally with several pointed extensions; P-4 ventral setae short (< 30µm); chelicera L/W ratio 5.5-5.9.

Description

General features — Colour brownish to dark–yellow; idiosoma roundish; dorsal shield with a colour pattern as illustrated in Figure 6(d,f); area of primary sclerotization of the dorsal plate with two dorsoglandularia; gnathosomal bay deep U-shaped, proximally rounded, three pairs of knob-shaped protrusions at the distal margin of the gnathosomal bay; Cxgl-4 apical; suture lines of Cx-IV well evident, distinctly extending posteriorly beyond posterior margin of genital field; line of primary sclerotization close to posterior margin of genital field; area of secondary sclerotization extensive. Excretory pore and Vgl-2 distant from the line of primary sclerotization, Vgl-2 posterior to excretory pore (Fig. 3). Palp with thick, pennate setae on P-2 and P-3; P-2 longer than P-4; distal margins of P-3 and P-4 medially and laterally with several pointed extensions; P-4 with short, pennate ventral setae, located near distal edge (Figures 4 (c) and 5(d)). *Male* — Medial suture line of Cx-II+III relatively short; genital field large, anterior margin forming an obtuse

Table 2. Estimates of average genetic distance (K2P) within and between clades examined species of the *Monatarctides* sequence pairs in and between COI haplogroups are shown. Standard error estimates are shown above the diagonal.

		Intergroup							
	Intragroup	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) *Monatractides corsicus* **sp. nov.**	0.0024 ± 0,0011		0.0150	0.0138	0.0149	0.0196	0.0202	0.0195	0.0228
(2) *Monatractides stadleri*	0.0011 ± 0,0011	0.1071		0.0139	0.0153	0.0201	0.0234	0.0197	0.0217
(3) *Monatractides stadleri* Balkan-Clade	0.013 ± 0,0039	0.1047	0.1059		0,0132	0.0185	0,0200	0.0184	0.0204
(4) *Monatractides persicus*	0 ± 0	0.1038	0.1117	0.0944		0.0198	0,0240	0.0215	0.0226
(5) *Monatractides madritensis*	0.0192 ± 0,0032	0.1849	0.2049	0.1847	0.1842		0,0199	0.0188	0.0219
(6) *Monatractides lusitanicus*	n/c	0.1854	0.2337	0.1990	0.2240	0.1987		0.0195	0.0211
(7) *Monatractides latissimus*	n/c	0.1763	0.1928	0.1816	0.1951	0.1808	0.1874		0.0213
(8) *Monatractides aberratus*	0.0022 ± 0,0013	0.2330	0.2281	0.2033	0.2119	0.2340	0.2052	0.2155	

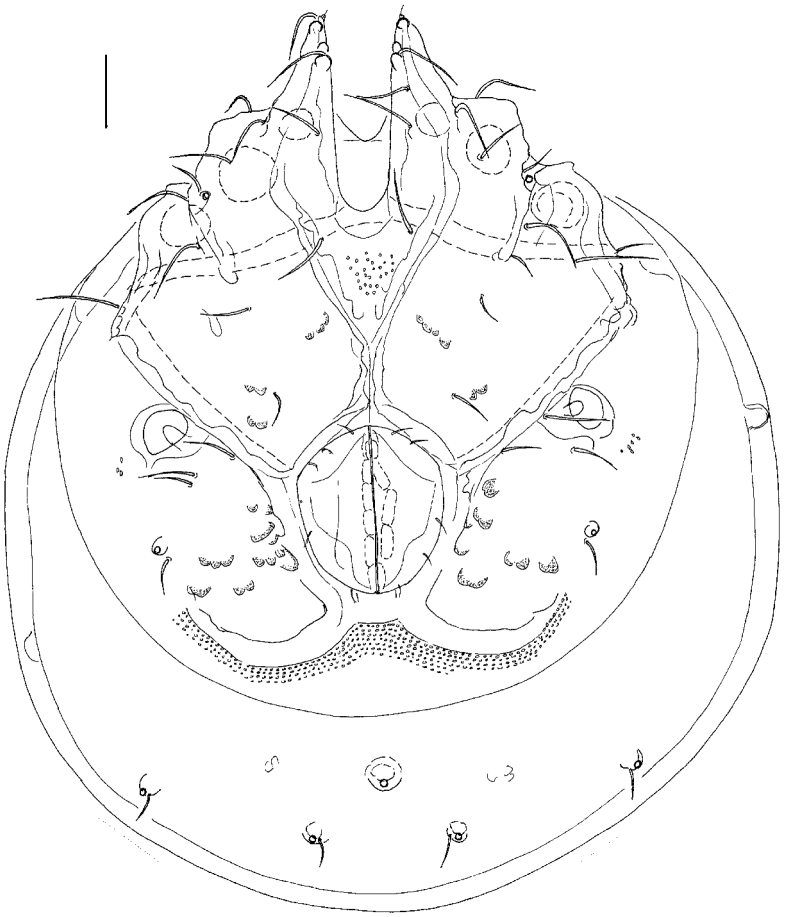

Figure 3. *Monatractides corsicus* **sp. nov.**, holotype ♂ [CCDB 38559 D03]: ventral shield. Scale bar = 100 µm.

angle; ejaculatory complex conventional in shape (with well-developed anterior keel and proximal arms; Figure 4(b)). *Female* — Genital field pentagonal (Figure 5(c)).

Measurements. *Male* (holotype [CCDB 38559 D03]; in parentheses some measurements of paratype [CCDB 38559 E12]) — Idiosoma L 1256 (1238), W 1088 (994); dorsal shield L 1103 (1019), W 913 (825), L/W ratio 1.21 (1.24); dorsal platelet L 1019 (944); frontal plate L 197-200 (177), W 130-131 (99), L/W ratio 1.5-1.54 (1.78); shoulder platelet L 306-309 (278), W 138 (119), L/W ratio 2.23-2.25 (2.34); L shoulder/frontal platelet ratio 1.53-1.57 (1.57). Gnathosomal bay L 263, Cx-I total L 450 (421), Cx-I mL 188 (178), Cx-II+III mL 100 (87); ratio Cx-I L/Cx-II+III mL 4.5 (4.8); Cx-I mL/Cx-II+III mL 1.88 (2.05). Genital field L/W 234 (238)/197 (192), L/W ratio 1.19 (1.24); distance genital field-excretory pore 269 (231), genital field-caudal idiosoma margin 446 (478). Ejaculatory complex L 328. Gnathosoma vL 258 (252), chelicera L (325), H (55), L/H ratio (5.9), L basal segment (269), claw (55), L basal segment/claw ratio (4.9); palp total L 325 (320), dL/H, dL/H ratio: P-1, 40/38, 1.05 (34/38, 0.92); P-2, 101/64, 1.58 (99/64, 1.55); P-3, 66/57, 1.15 (64/50, 1.28); P-4, 84/38, 2.25 (86/37, 2.34); P-5, 34/20, 1.72 (37/19, 1.95); L ratio P-2/P-4, 1.2 (1.16). dL of I-L-1 to −6: 63, 106, 134, 163, 156, 148; I-L-6 H 52; dL/H I-L-6 ratio 2.9; dL of IV-L: 163, 159, 214, 283, 297, 291.

Female (paratype [CCDB 38559 B04], n = 1) — Idiosoma L 1468, W 1131; dorsal shield L 1213, W 1018, L/W ratio 1.19; dorsal plate L 1113; frontal platelet L 206-217, W 119-128, L/W ratio 1.7-1.73; shoulder platelet L 316-327, W 130-131, L/W ratio 2.41-2.59; L shoulder/frontal platelet ratio 1.46-1.59. Gnathosomal bay L 288, Cx-I total L 488, Cx-I mL 200, Cx-II+III mL 84; ratio Cx-I L/Cx-II+III mL 5.8; Cx-I mL/Cx-II+III mL 2.38. Genital field L/W 272/258, L/W ratio 1.05; distance genital field-excretory pore 331, genital field-caudal idiosoma margin 603. Gnathosoma vL 284; chelicera total L 341, H 63, L/H ratio 5.45, L basal segment 276, claw 64, L basal segment/claw ratio 4.31; palp total L 347, dL/H, dL/H ratio: P-1, 41/45, 0.92; P-2, 108/69, 1.57; P-3, 66/59, 1.1; P-4, 91/39, 2.34; P-5, 41/20, 2.0; L ratio P-2/P-4, 1.19. dL of I-L-2-6: 113, 144, 182, 178, 161; I-L-6 H 58; dL/H I-L-6 ratio 2.8; dL of IV-L: 175, 178, 234, 302, 331, 296.

Etymology

Named after the island where the new species was discovered.

Discussion

Based on our molecular analyses, the clade which groups studied *Monatractides stadleri*-like specimens from Corsica here used as the type material of the new species, *M. corsicus* **sp. nov.**, was placed as a sister of the clade grouping sequences of *M. stadleri* from Belgium and Portugal. The latter specimens clusters within BOLD:AEU1504 which include also one private sequence of a specimen from Spain (see Pešić et al. 2023) suggesting that this lineage is widely distributed from Belgium to Portugal. *Monatractides stadleri* was originally described by Walter (1924) from Lower Franconia (Unterfranken), Germany. Therefore, it is reasonable to assume that the lineage which includes the examined specimens from Belgium and Portugal, molecularly corresponds to *M. stadleri*. Examined specimens of *M. stadleri* from Belgium and Portugal differ in having comparably smaller palps (P-2 < 90, P-4 < 80 µm; compare Figures 4(c) and (d)), more slender chelicera (L/H ratio 6.1-6.6 *vs*. 5.5-5.9) than M. *corsicus* **sp. nov**. They differ as well in the colour pattern of the dorsal shield (see Figure 6(a and b)).

Monatractides persicus Pešić, 2004, a species originally described from a stream in the Elburs mountains of North Iran resembles the new species in having relatively thick chelicera (ratio

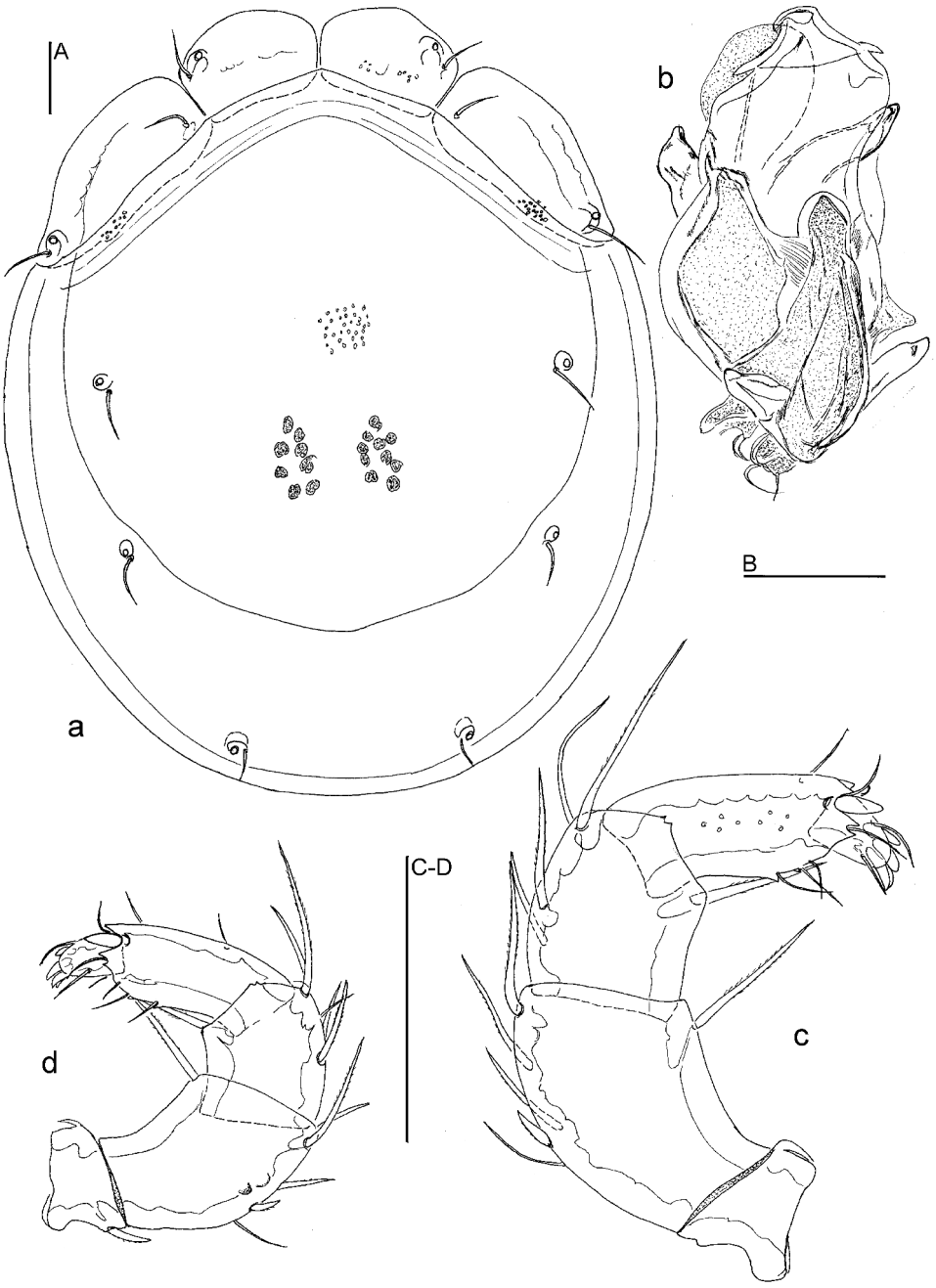

Figure 4. A-C *Monatractides corsicus* **sp. nov**., holotype ♂ [CCDB 38559 D03]. A – dorsal shield; B – ejaculatory complex; C – palp, medial view. D *M. stadleri* (Walter, 1944), ♂ [CCDB_39397_B05]: palp, medial view. Scale bars = 100 µm.

L/H 5.6-5.9), but differs in a smaller size of idiosoma (L < 1000 µm in ♂) and palps (P-2 < 90, P-4 < 80 µm; in parentheses data taken from Pešić and Saboori 2004). *Monatractides stadleri* specimens from Montenegro and Greece, which form a separate clade in the COI tree, can be distinguished by the colour pattern limited to the area of secondary sclerotization of the dorsal shield (see Figure 6(c)). The status of the latter clade should be clarified by collecting additional material from a wider area, which will be the subject of future studies.

Remarks — The former records of *M. stadleri* from Corsica (Angelier 1954; Santucci 1971; Gerecke and Di Sabatino 1996) likely should be assigned to the new species.

Distribution

France (Corsica).

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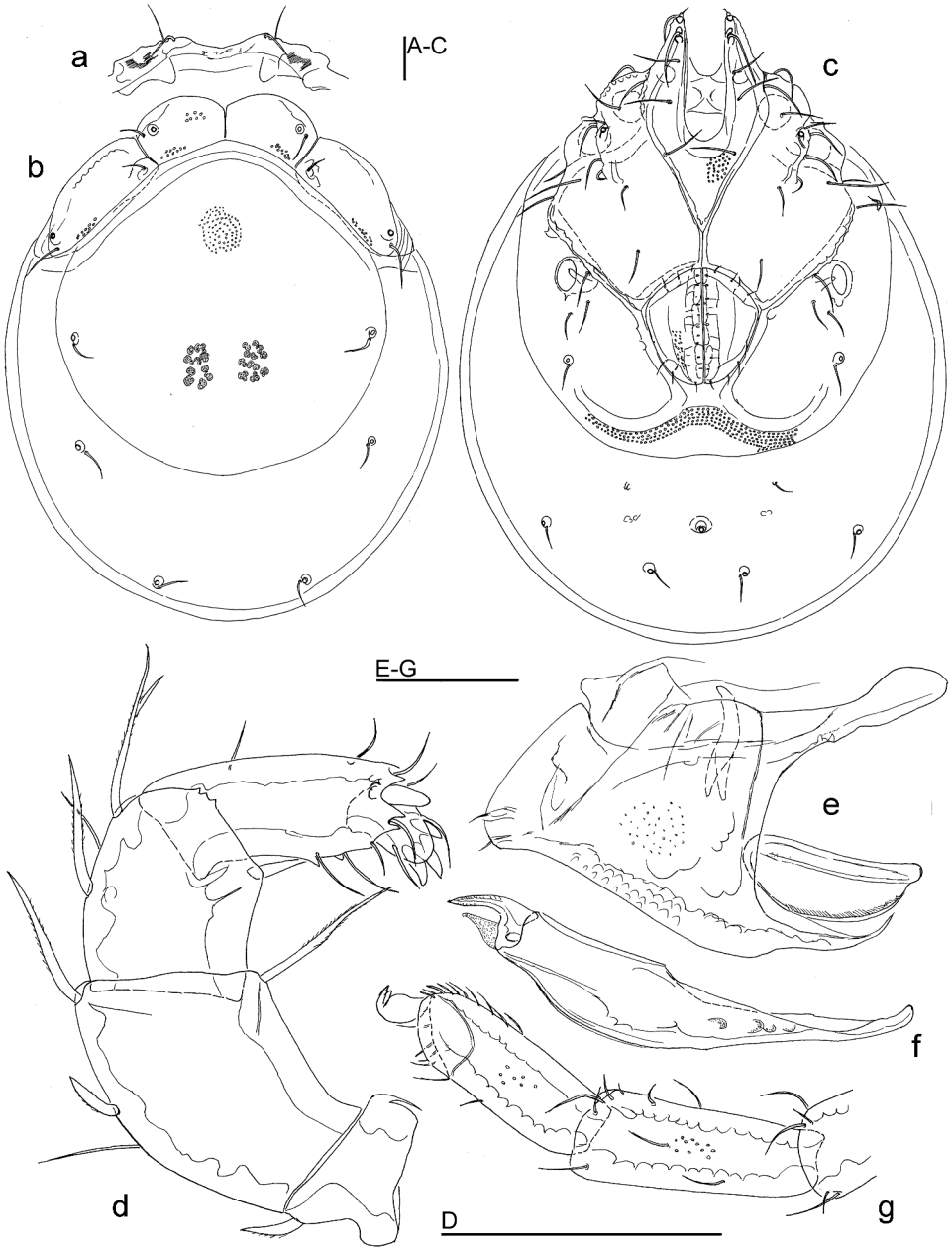

Figure 5. *Monatractides corsicus* **sp. nov.**, paratype ♀ [CCDB 38559 B04]. A – frontal margin of idiosoma, dorsal view; B – dorsal shield; C – ventral shield; D – palp, medial view; E – gnathosoma; F – chelicera; G – I-L-5 and 6. Scale bars = 100 µm.

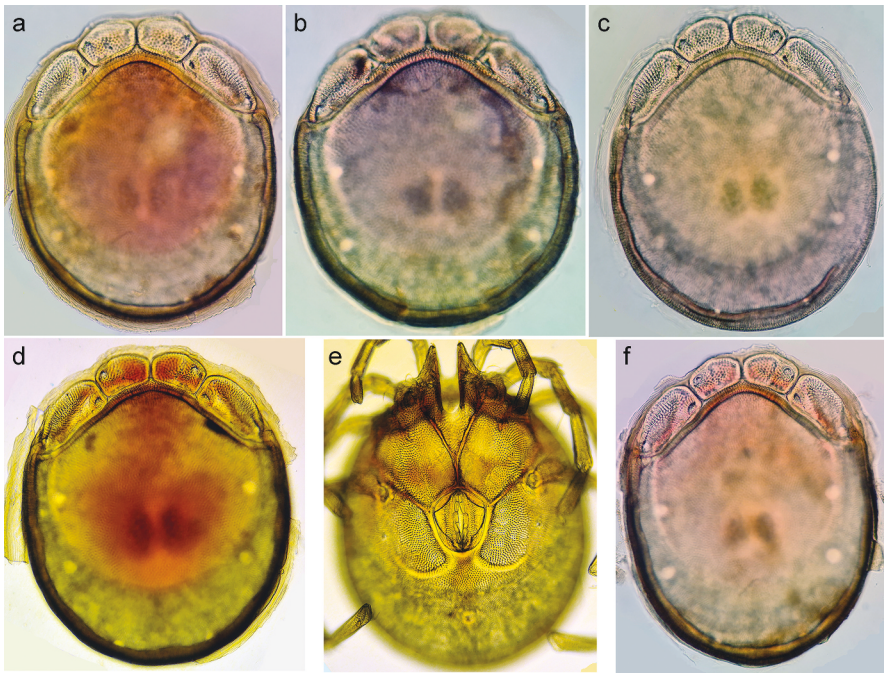

Figure 6. Photographs of dorsal (A-D, F) and ventral (E) shields. A-B *Monatractides stadleri* (Walter, 1924), ♂. A – [CCDB_44300_H10]; B – [CCDB_39397_B05]. C *M. stadleri* Balkan-Clade, ♂ [CCDB_38233_C03]. D-F *M. corsicus* **sp. nov.**, D-E – holotype ♂ [CCDB 38559 D03]; F – paratype ♂ [CCDB 38559 E12].

Disclosure statement

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

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