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A new species of the genus *Atractides* Koch, 1837 from Montenegro (Acari: Hydrachnidia: Hygrobatidae), separated from *A. nodipalpis* (Thor, 1899), one of the most common water mite species in running waters in Europe

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Abstract

A new species of water mite from Montenegro, i.e. *Atractides milosevici* **sp. nov**., is described, using morphological data and DNA barcodes. Morphologically, the new species is closely related to *A. nodipalpis* sensu Gerecke, 2003 but differs in its distinct COI barcode sequence, as well as details of the terminal segments of the first legs. Morphological examination of specimens of *A. nodipalpis* from the Netherlands which belong to the same BIN (BOLD:ACA0209) as the specimens of the nominal species collected near its type locality in Norway reveals that the latter, one of the most common water mite species in running waters in Europe, can be identified by the shape of male genital plate with a distinct anteromedial peg-like fissure. A redescription is given of *Atractides nodipalpis* (Thor, 1899) based on material from the Netherlands.

Keywords: Water mites, DNA-barcoding, new species, taxonomy

Introduction

The water mite genus *Atractides* Koch, 1837 has been found in all biogeographical regions except Antarctica and Australasia (Gerecke 2003; Pešić & Smit 2011). The name *Atractides* was coined in 1837 by C.L. Koch, and five years later, in 1842 the same author designated *spinipes* Koch, 1837, as *typus generi*s. In the Palaearctic, the genus comprises of three subgenera (Gerecke 2003), i.e., *Atractides* s. s., *Tympanomegapus* Thor, 1923, and *Polymegapus* K. Viets, 1926.

Atractides nodipalpis, a species originally described by Thor (1899) from Norway, was often considered in the water mite literature as the most frequently found species of the genus in Europe (see Gerecke 2003 for a discussion). Over the last hundred years, this species has been recorded under numerous different names, which resulted in a large number of partly uncertain subspecies (Gerecke 2003). Reinhard Gerecke (2003) published a comprehensive revision of the genus in the Western Palaearctic providing more light on the taxonomic status of *A. nodipalpis*. The latter author redescribed *A. nodipalpis* and selected a neotype from Gudbrandsdalen in Norway, although this site was not included in the list of sampling sites by Thor. Gerecke (2003) made an extensive study on the variability of the latter species based on population from the stream Goldersbach in southern

Germany. As a result of this revision (Gerecke 2003), a number of taxa previously considered as subspecies were considered as separate species, and eight species i.e., *A. tenuitarsus* (Komárek, 1921), *A. tivdiae* (Sokolow, 1926), *A. parviporus* (Besseling, 1933), *A. semidistans* (Husiatinschi, 1937), *A. moravicus* Làska, 1952, *A. limnicola* Schwoerbel, 1959, *A. microcavaticus* Schwoerbel, 1961, and *A. schlienzi* Schwoerbel, 1961, were proposed as junior synonyms of *A. nodipalpis*.

More recently, based on the available DNA barcodes, Gerecke *et al*. (2022) stated that his Norwegian specimens keying out as *A. nodipalpis* belong to two different genetical lineages, both widely distributed in Norway. They found that specimens of *A. nodipalpis* collected near the type locality in Norway belong to cluster BOLD:ACA0209 (Gerecke pers. communication), a lineage that has a wide distribution from SE Europe over Fennoscandia up to Greenland. On the other hand, the population from southern Germany which was the basis for defining *A. nodipalpis* in Gerecke (2003) belongs to a different molecular lineage (Gerecke *et al*. 2022). The new molecular evidence emphasized the need for redefining the diagnostic morphological characters of these two lineages.

In this paper we used morphological data and COI barcodes to describe one new species of the *Atractides nodipalpis*-complex from Montenegro. Moreover, we give a redescription of *A. nodipalpis* based on material from the Netherlands.

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). 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-fluid and stored in the collection of the first author. The DNA extracts were archived in −80 °C freezers at the Centre for Biodiversity Genomics (CBG; biodiversitygenomics.net) while the DNA sequences prepared in the course of this study were deposited in BOLD and GenBank.

Morphological nomenclature follows Gerecke *et al*. (2016). The genital acetabula in both sexes were measured on both sides, and their dimensions are, therefore, given as a range of values, rather than a single number. The holotype and paratype of the new species are deposited in the Naturalis Biodiversity Center in Leiden (RMNH).

All measurements are given in µm. The following abbreviations are used: Ac-1=first acetabulum; Cx-I=first coxae; Dgl-4=dorsoglandularia 4; dL=dorsal length; H=height; HB=medial height; I-L-4-6=fourth-sixth segments of first leg; L=length; lL=lateral length; mL=medial length; P-1-P-5=palp segment 1-5; S-1=proximal large ventral seta at I-L-5; RMNH=Naturalis Biodiversity Center, Leiden; S-2=distal large ventral seta at I-L-5; Vgl-1=ventroglandularia 1; 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/)). 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 (Ivanova & Grainger 2007b) protocols.

Data related to each BIN, including the minimum p-distance to the nearest neighbouring BIN, was estimated through BOLD. DNA barcode sequences were aligned using MUSCLE alignment (Edgar 2004). None of the DNA sequences showed evidence of pseudogenes. The final dataset includes 180 COI sequences of the genus *Atractides* downloaded from the respective sequence data archives; 175 sequences were taken from Pešić *et al.* 2023 (the complete list available at https://doi.org/10.3897/zookeys.1151.100766.suppl1) and five private sequences representing *A. spinipes*

(HYDAS082-22, HYDAS162-22) from Austria, *A. pumilus* (NOVMB065-21) from France, and *A. glandulosus* (HYDOC007-22) from Serbia were taken directly from the BOLD.

MEGA X software (Kumar et al. 2018) 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 $1^{st}+2^{nd}+3^{rd}$+Noncoding.

Systematics

Family Hygrobatidae

Genus *Atractides* Koch, 1837
Subgenus *Atractides s.s.*

Atractides nodipalpis Thor, 1899
Fig. 2A, C, E–F, Table 1

Material examined—the Netherlands: Geul, Valkenburg, Prov. Limburg, 50.86724° N, 5.81398° E, 18 April 2012, leg. B. van Maanen & M. Korsten, 1♂, dissected and slide mounted (RMNH.ACA.1533), sequenced (NLACA1118-17); Molenbroekerbeek, Wolfhaag, Vaals, Prov. Limburg, 50.76182° N, 5.99580° E, 28 April 2012, leg. Smit, 1♂, dissected and slide mounted (RMNH.ACA.829), sequenced (NLACA408-15); Mechelderbeek near crossing with Voortweg, Mechelen, Prov. Limburg, 50.79697° N, 5.94112° E, 27 April 2012, leg. Smit, 1♂, dissected and slide mounted (RMNH.ACA.878), sequenced (NLACA452-15).

Diagnosis (Male)—Genital field anteriorly and posteriorly indented, anteromedial indentation with a peg-shaped fissure, Ac in triangular arrangement. P-2 with a strongly developed distal extension consisting of a bluntly pointed medial hump and an equally convex lateral thickening, P-4 sword seta between ventral setae; separation S-1-2, 24-31, dL/HB ratio I-L-5 3.4-3.6, I-L-6 5.9-7.2; distomedial margins of IV-L-4/5 equally convex, only slightly extending beyond the base of subsequent segments.

Remarks—Molecular data revealed that examined specimens of *A. nodipalpis* from the Netherlands belong to the same BIN (BOLD: ACA0209) as the specimens of the nominal species collected near its type locality in Norway. As underlined in the Introduction, recent genetical data showed that populations from southern Germany, which were used for diagnosing *A. nodipalpis* by Gerecke (2003), belong to a different molecular lineage (BOLD:ADP2485). In comparison to the description of *A. nodipalpis* given by Gerecke (2003), the examined males from the Netherlands differ in the shape of the male genital field (anteromedial indentation with a peg-shaped fissure *vs*. anterior indentation V-shaped, often with a small projection in the centre of the indentation in *A. nodipalpis* sensu Gerecke, 2003).

Atractides concavus Pešić & Smit, 2021 a species recently described from Türkiye (Pešić & Smit 2021) resembles the examined specimens of *A. nodipalpis* from the Netherlands (BOLD: ACA0209) in a similar shape of the male genital plate and a more slender I-L-6. Morphological evidences provides sufficient evidence for the synonymization of *A. concavus* with *A. nodipalpis*, which, as shown by molecular data, has a wide distribution from SE Europe through Fennoscandia to Greenland. However, a final decision on the synonymization of these two species must be postponed until barcodes of *A. concavus* from Türkiye become available.

***Atractides milosevici* sp. nov.**
Figs. 1, 2B, D

Type material—Holotype ♂, Montenegro, Danilovgrad, Zeta river near Spuž, 42.5113° N, 19.1982° E, 29 June 2019 leg. Pešić & Zawal, dissected and slide mounted (RMNH), sequenced (Voucher ID: 40. M19_29A_1_G4; BOLD/GenBank ID: DNAEC071-20/OL870145). Paratype: 1 ♂, same site and data as the holotype, dissected and slide mounted (RMNH), sequenced (Voucher ID: CCDB-3867-F05; BOLD/GenBank ID: DNCBD065-20/OL870182).

Diagnosis (Male)—Characters of the *A. nodipalpis*-complex (integument finely striated, muscle insertions unsclerotized; males with anteriorly indented genital field, P-2 with distoventral projection and ventral margin of P-4 projecting); anterior and posterior margin of genital field indented, anteriorly with a small projection in the centre of indentation; I-L-5 slender, separation S-1–2, 30–34 µm; I-L-6 slender, ratio dL/HB 8.2–8.6.

Description. *Male*—Integument striated, muscle insertions unsclerotized. Glandularia enlarged (Dgl-3/-4 diameter 30 µm). Mediocaudal margin of Cx-1 slightly convex, apodemes of Cx-II in an obtuse angle. Genital plate with both anterior and posterior margin indented, anteriorly with a small projection in the centre of indentation (Figs. 1A); acetabula relatively large, subtriangular; Ac-3 rather long and with their anterior margin approaching the posterior margin of Ac-1. Acetabula in a triangular arrangement. Excretory pore smooth; Vgl-1 not fused to Vgl-2. Palp with a strong ventrodistal extension on P-2 consisting of a bluntly pointed medial hump and an equally convex lateral thickening; P-4 stout, with a dense cover of fine dorsal setae, maximum H on the level of the proximoventral seta; sword seta inserting slightly anterior to proximoventral seta (Figs. 1C, 2D). I-L-5 long and slender, equally enlarged from the base to the insertion of S-1-2 setae, S-1 slender, bluntly pointed; S-2 shorter and distinctly thicker; I-L-6 slender (L/HB ratio 6.0–6.8), slightly inflated proximally, equally curved and narrowed from base to tip (Figs. 1B, 2B); distomedial margins of IV-L-4/5 equally convex, only slightly extending beyond the base of subsequent segments.

Measurements. Male (holotype, in parentheses measurements of paratype)—Idiosoma L 856 (838), W 719 (666); maximum diameter Dgl-3, (30). Coxal shield L 481 (469); Cx-III W 566 (525); Cx-I+II mL 172 (169), Cx-I+II lL 356 (325). Genital field L/W 164 (148)/210 (205), ratio 0.78 (0.72), L Ac-1-3: 63–64 (63), 76–77 (64), 88–89 (70–72).

Palp—total L 442 (418); dL/H, dL/H ratio: P-1, 47/45, 1.05 (45/43, 1.05); P-2, 107/81, 1.32 (104/72, 1.44); P-3, 109/63, 1.73 (97/59, 1.64); P-4, 132/53, 2.49 (125/48, 2.58); P-5, 47/19, 2.5 (47/19, 2.5); length ratio P-2/P-4 0.81 (0.83). Gnathosoma vL 148; chelicera total L 328.

Legs—I-L-5 dL 309 (302), vL 210 (208), dL/vL ratio 1.47 (1.45), HB 81 (80), dL/HB 3.8 (3.77), S-1 L 131 (130), L/W ratio 10.5 (10.4), S-2 L 95 (94), L/W ratio 5.6 (5.45), distance S-1-2, 34 (30), dL ratio S-1/2 1.38 (1.39); I-L-6 dL 191 (189), HB 23 (22), dL/HB ratio 8.2 (8.6); L I-L-5/6 ratio 1.6 (1.6).

Female—Unknown.

Etymology—The new species is named after Prof Đurađ Milošević (University of Niš) in recognition of his enthusiasm and work on freshwater ecosystems.

Species delimitation using DNA-barcodes—The final alignment for species delimitation using COI sequence data comprised 686 nucleotide positions (nps) of the 180 *Atractides* specimens, and one outgroup, *Mixobates processifer* (Thor, 1905) from Norway to root the tree. The NJ tree is presented in Fig. 3. The COI tree sequences retrieved from two specimens of *A. milosevici* **sp. nov**. form a separate clade (BIN AED3547) that is solely composed of the specimens from Montenegro. The closest neighbouring BIN (BOLD: AAM4306) is that of an unidentified *Atractides* sp. from the United States, with the *p*-distance estimated at 13.3% (Table 1).

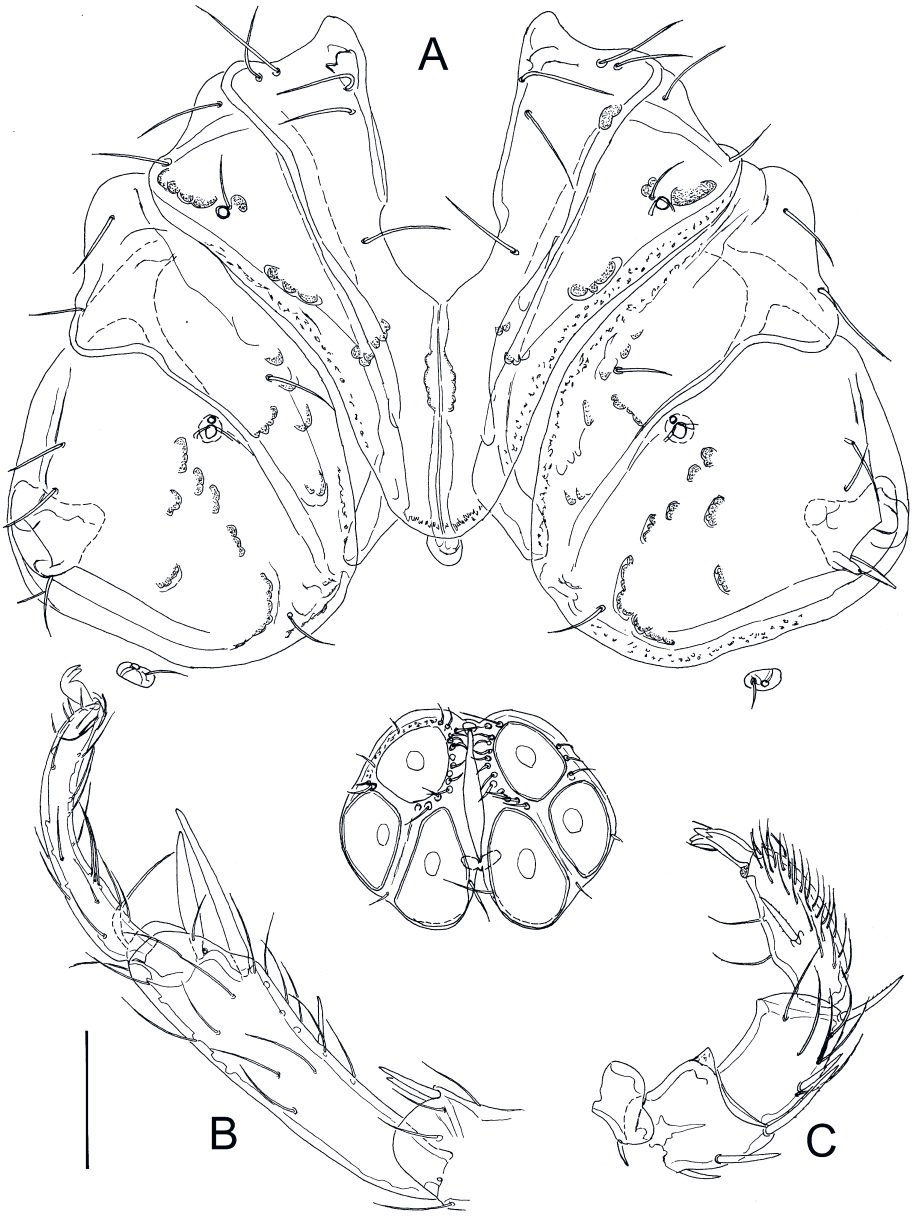

FIGURE 1. *Atractides milosevici* **sp. nov**., ♂ holotype. A—coxal and genital field; B—I-L-5 and -6; C—palp, medial view. Scale bar=100 μm.

Discussion—The new species from Montenegro belongs morphologically to the *A. nodipalpis*-complex (for diagnostic features of the group see under diagnosis of the new species). The latter group includes a number of species known from Europe, which can be separated in two (sub)groups based on the shape of the anterior margin of the male genital field. *Atractides nodipalpis*, as defined in this study by examining specimens from The Netherlands molecularly assigned to BIN ACA0209, differ from the new species from Montenegro in the shape of the male genital field (see Figs. 2E–F).

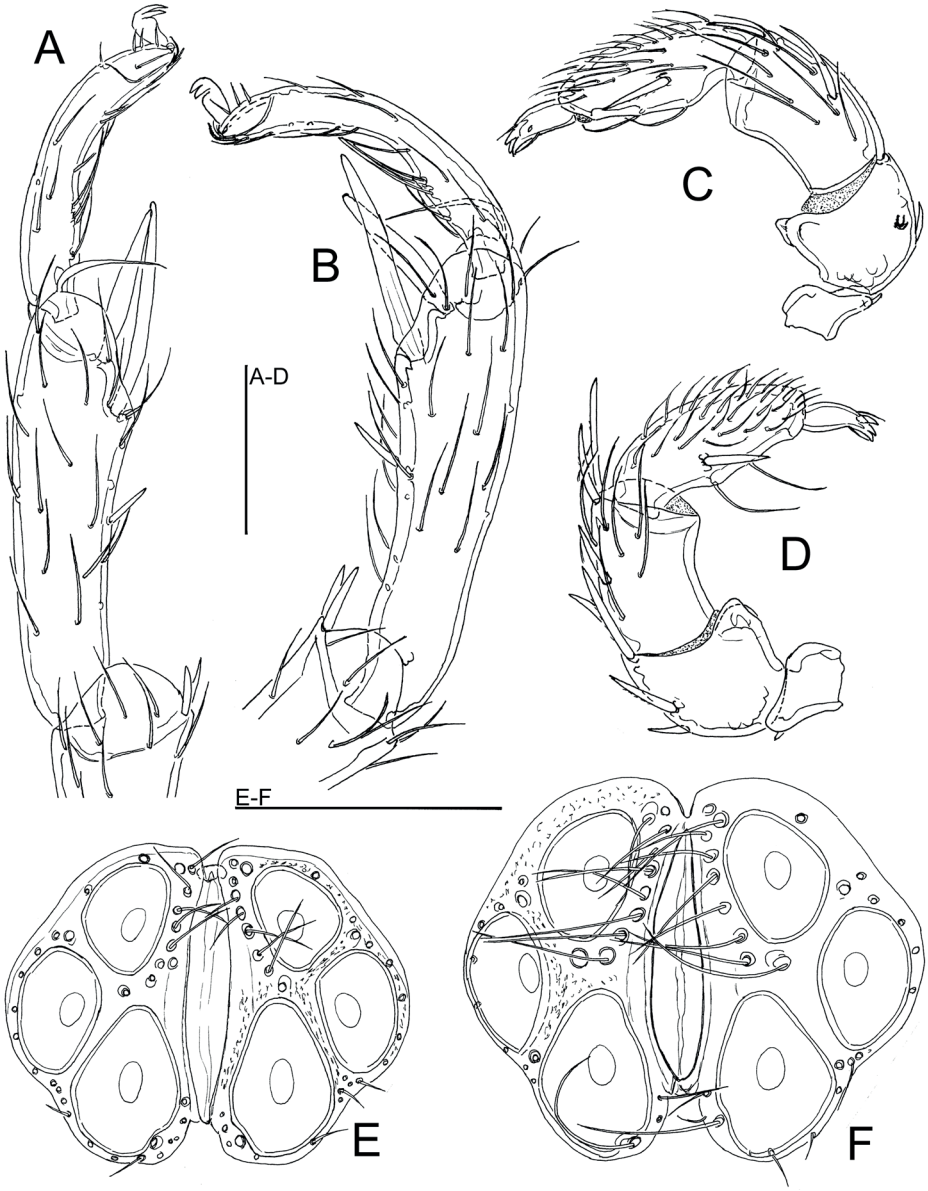

FIGURE 2. **A, C, E–F** *Atractides nodipalpis* Thor, 1899 (BIN BOLD:ACR209), ♂ (A, C, F—Geul, Valkenburg, RMNH.ACA.1533; E—Mechelderbeek, RMNH.ACA.878). **B, D** *A. milosevici* **sp. nov**., ♂ paratype. A–B—I-L-5 and -6; C–D—palp, medial view; E–F—genital field; Scale bars=100 μm.

Males of *Atractides nodipalpis* sensu Gerecke, 2003 (in parentheses data taken from Gerecke 2003 for a population from Goldersbach), a species resembling *A. milosevici* **sp. nov**., are characterized by the anterior genital plate having a deep indentation and with a small central projection. The new species differ in a more slender I-L-6 (dL/HB ratio 8.2–8.6 *vs*. 5.63–8.79, mean 7.07 in *A. nodipalpis* sensu Gerecke 2003) and generally a larger S-1/-2 separation (30–34 *vs*. 9–31, mean 23 μm).

Distribution—Montenegro; known only from the *locus typicus* (Fig. 4).

TABLE 1. Measurements of *Atractides nodipalpis* (Thor, 1899) (BIN ACR2029).

	Netherlands, RMNH.ACA.1533	Netherlands, RMNH.ACA.829
Idiosoma L	738	694
Idiosoma W	687	606
Coxal field L	444	387
Cx-I+II mL	166	142
Cx-I+II lL	325	284
Cx-III W	522	450
Genital field L	146	122
Genital field W	175	153
Ac-1 L	52–53	41–44
Ac-2 L	55	47–48
Ac-3 L	63–64	58–61
Palp, total L	406	343
P-1 dL/H	39/36	33/33
P-2 dL/H	91/74	81/69
P-3 dL/H	101/58	83/48
P-4 dL/H	128/52	106/41
P-5 dL/H	47/17	40/14
P-1 dL/H ratio	1.09	1.0
P-2 dL/H ratio	1.22	1.27
P-3 dL/H ratio	1.75	1.71
P-4 dL/H ratio	2.48	2.59
P-5 dL/H ratio	2.73	2.84
P-2/P-4 ratio	0.71	0.77
Gnathosoma vL	-	164
Chelicera total L	291	272
I-L-5 dL	266	226
I-L-5 vL	180	155
I-L-5 dL/vL ratio	1.48	1.46
I-L-5 HB	73	66
I-L-5 dL/HB ratio	3.62	3.44
S-1 L	131	111
S-1 L/W ratio	15.0	12.9
S-2 L	98	90
S-2 L/W ratio	7.0	7.4
S-1/2 L ratio	1.33	1.23
S-1-2 separation	31	24
I-L-6 dL	172	147
I-L-6 HB	24	25
I-L-6 dL/HB ratio	7.2	5.95
I-L-5/6 L ratio	1.54	1.54

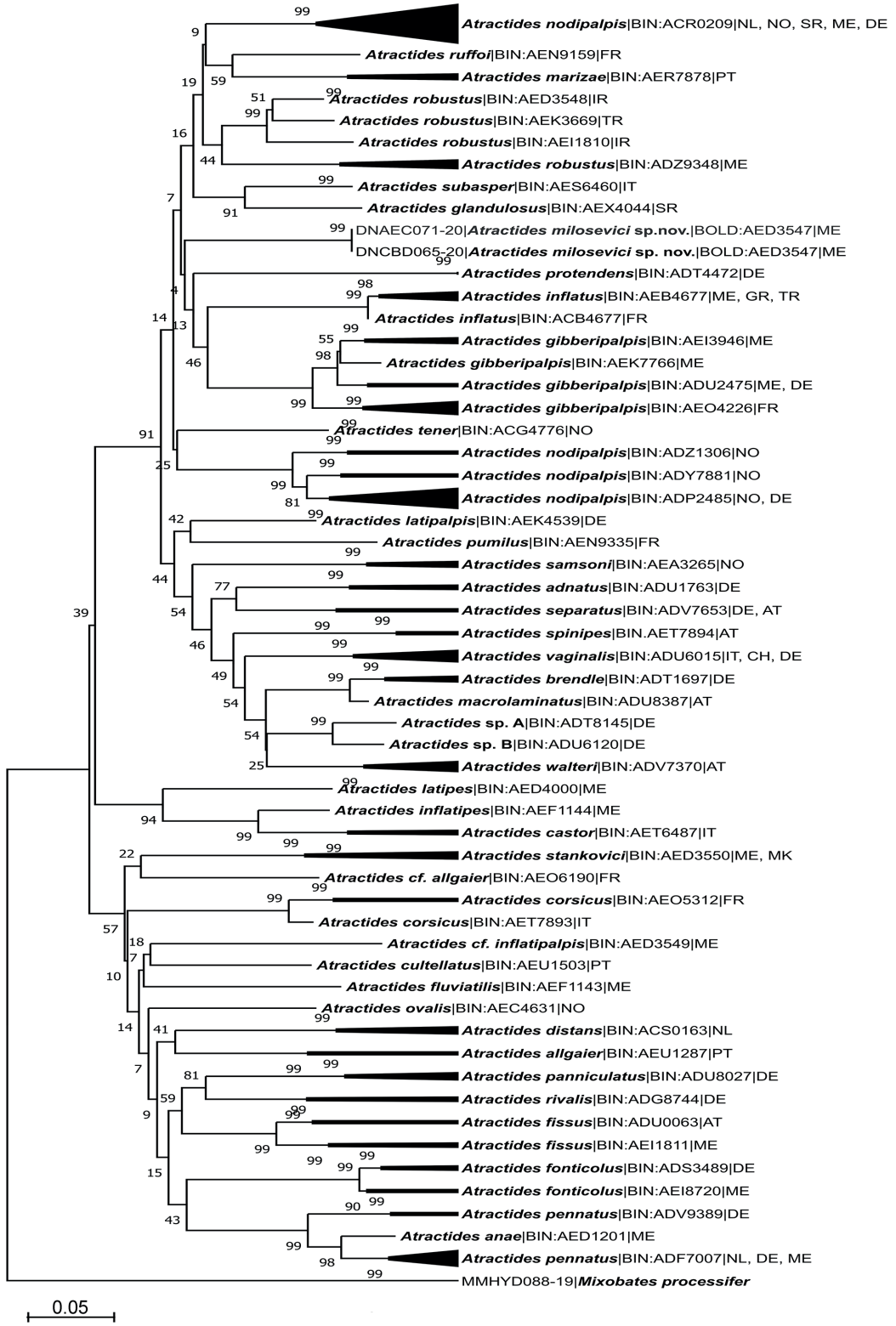

FIGURE 3. Neighbour-joining tree of the genus *Atractides* obtained from 180 nucleotide COI sequences. For country code abbreviations see Table 2.

Discussion

Over the past 100 years, a large number of poorly defined subspecies of *A. nodipalpis* have been described. By defining a neotype and providing an amended morphological diagnosis, the revision of Gerecke (2003) provided, for the first time, a solid basis for a stable taxonomy of *A. nodipalpis* and similar (sub)species (the so-called *A. nodipalpis*-complex). This enables the testing of the traditional taxonomic delimitation with that of molecular data.

During the last two decades, a large number of *Atractides* species that were described on the basis of morphological characters (Gerecke & Di Sabatino 2013; Pešić *et al*. 2012, 2016; Pešić & Smit 2021) were often assigned to the so-called *A. nodipalpis*-complex. The latter complex is characterized primarily by the following features: integument finely striated, muscle insertions unsclerotized; males with anteriorly indented genital field, P-2 with distoventral projection and ventral margin of P-4 projecting. Molecular analysis of our study based on the COI marker showed that the species of the *A. nodipalpis*-complex (e.g., *A. nodipalpis*, *A. robustus*) do not represent a monophyletic group (see Fig. 3), and that some diagnostic characters such as the anteriorly indented male genital field, and male P-2 distoventral projection probably evolved separately in different complexes.

For example, our COI phylogenetic tree revealed that the *A. nodipalpis* lineage (BIN ACA0209), which also includes individuals collected near the *locus typicus*, appeared as sister to the clade comprising *A. ruffoi* Gerecke & Di Sabatino, 2013 (Corsica; Gerecke & Di Sabatino 2013) and *A. marizae* Pešić, 2023 (Portugal; Pešić *et al*. 2023). All three species are primarily defined by a similar shape of the male genital plate with a peg- or drop-shaped anteromedial fissure. On the other hand, the lineage of *A. nodipalpis* (BIN ADP2485), which includes populations from S Germany and Norway, forms a monophyletic group with two other closest BIN neighbours (ADZ1306, ADY7881) from Norway, seems to be more related to *A. tener* Thor, 1899, a species differing in males by lacking the distoventral projection of P-2 and anteriorly indented margin of genital plate.

FIGURE 4. River Zeta near Spuž town in central Montenegro, the *locus typicus* of *A. milosevici* **sp. nov**. Photo by V. Pešić.

TABLE 2. Details on barcoded specimens, including data and coordinates of sampling sites, the barcode index number (^{N} indicates a new BIN that contains only current sequences) and associated data obtained from BOLD. DNN=distance to nearest neighbor; NN BIN=nearest neighbor BIN; NN taxonomy=species assigned to nearest neighbor BIN. BOLD data presented here was last accessed on 10th January 2023. Country codes (alpha-2 code): AT=Austria, BA=Bosnia and Herzegovina, CH=Switzerland, DE=Germany, FR=France, GL=Greenland, GR=Greece, IT=Italy, IR=Iran, MA=Morocco, ME=Montenegro, MK=North Macedonia, NO=Norway, NL=Netherlands, PT=Portugal, RO=Romania, RS=Serbia, SK=Slovakia, TR=Türkiye.

Species	**BIN**	**Member Count**	**Country**	**Average Distance % (p-dist)**	**NN BIN BOLD**	**DNN (%)** (p-dist)	**NN taxonomy**
Atractides adnatus	BOLD:ADU1763	6 [3 Public]	DE	0.09	BOLD:ADV7653	10.67	*A. separatus*
Atractides allgaier	BOLD:AEU1287	2 [2 Public]	PT	1.12	BOLD:ACS0163	14.58	*A. distans*
Atractides cf. allgaier	BOLD:AEO6190	1 [1 Public]	FR	N/A	BOLD:AED3550	16.51	*A. stankovici*
Atractides anae	BOLD:AED1201	5 [1 Public]	MN, MK	0.14	BOLD:ADF7007	5.66	*A. pennatus*
Atractides brendle	BOLD:ADT1697	8 [4 Public]	DE	0.07	BOLD:AES2192	2.09	*Atractides* sp.
Atractides castor	BOLD:AET6487	3 [0 Public]	IT	0	BOLD:AEF1144	8.7	*A. inflatipes*
Atractides corsicus	BOLD:AEO5312	2 [2 Public]	FR	0.33	BOLD:AET7893	4.17	*A. corsicus*
Atractides corsicus	BOLD:AET7893	1 [0 Public]	IT	N/A	BOLD:AEO5312	4.17	*A. corsicus*
Atractides cultelatus	BOLD:AEU1503	1 [1 Public]	PT	N/A	BOLD:ADG8744	16.01	*A. rivalis*
Atractides fissus	BOLD:ADU0063	4 [2 Public]	AT	0	BOLD:AEI1811	5.17	*A. fissus*
Atractides fissus	BOLD:AEI1811	3 [2 Public]	ME	0.21	BOLD:ADU0063	5.17	*A. fissus*
Atractides fluviatiilis	BOLD:AEF1143	4 [1 Public]	ME, CR	0.16	BOLD:AEI1811	16.35	*A. fissus*
Atractides fonticolus	BOLD:ADS3489	6 [3 Public]	DE	0	BOLD:AEI8720	1.67	*A. fonticolus*
Atractides fonticolus	BOLD:AEI8720	2 [2 Public]	ME	0	BOLD:ADS3489	1.67	*A. fonticolus*
Atractides gibberipalpis	BOLD:ADU2475	4 [1 Public]	DE, RO, ME	1.03	BOLD:AEI3946	4.33	*A. gibberipalpis*
Atractides gibberipalpis	BOLD:AEI3946	3 [1 Public]	ME	1.72	BOLD:AES8583	3.69	*A. gibberipalpis*
Atractides gibberipalpis	BOLD:AEK7766	1 [1 Public]	ME	N/A	BOLD:AES8583	2.72	*A. gibberipalpis*
Atractides gibberipalpis	BOLD:AEO4226	9 [9 Public]	FR	1.04	BOLD:AEI3946	5.61	*A. gibberipalpis*
Atractides glandulosus	BOLD:AEX4044	1 [0 Public]	RS	N/A	BOLD:AES6460	12.02	*A. subasper*
Atractides inflatus	BOLD:AEO3635	2 [2 Public]	ME, GR	0.85	BOLD:ACB4677	0.99	*A. inflatus*
Atractides inflatus	BOLD:ACB4677	8 [8 Public]	IR, MA, TR, GR, FR	0.43	BOLD:AEO3635	0.99	*A. inflatus*
Atractides inflatipalpis	BOLD:AED3549	1 [1 Public]	ME	N/A	BOLD:AED7022	5.27	*A. hyrcaniensis*
Atractides inflatipes	BOLD:AEF1144	1 [1 Public]	ME	N/A	BOLD:AET6487	8.7	*A. castor*
Atractides latipes	BOLD:AED4000	1 [1 Public]	ME	N/A	BOLD:AEF1144	16.58	*A. inflatipes*
Atractides nodipalpis	BOLD:ACR0209	49 [22 Public]	NO, NL, DE, ME, RO, RS, GL	0.66	BOLD:AED3548	12.6	*A. cf. robustus*
Atractides nodipalpis	BOLD:ADY7881	3 [4 Public]	NO, CA	0.64	BOLD:ADP2485	3.21	*A. nodipalpis*
Atractides nodipalpis	BOLD:ADP2485	16 [16 Public	NO, DE	0.45	BOLD:ADY7881	3.21	*A. nodipalpis*
Atractides nodipalpis	BOLD:ADZ1306	2 [2 Public]	NO	0	BOLD:ADP2485	5.17	*A. nodipalpis*
Atractides macrolaminatus	BOLD:ADU8387	3 [1 Public]	AT	0	BOLD:AES9346	1.72	*Atractides* sp.
Atractides marizae	BOLD:AER7878	4 [4 Public]	PT	1.15	BOLD:AEN9154	12.98	*A. ruffoi*
Atractides milosevici **sp. nov.**	BOLD:AED3547	2 [2 Public]	ME	0	BOLD:AAM4306	13.3	*Atractides* sp.
Atractides distans	BOLD:ACS0163	5 [0 Public]	NL	0	BOLD:AEU1287	14.58	*A. allgaier*
Atractides ovalis	BOLD:AEC4631	1 [1 Public]	NO	N/A	BOLD:AEU1287	16.03	*A. allgaier*
Atractides panniculatus	BOLD:ADU8027	10 [5 Public]	DE	0.12	BOLD:ADG8744	11.3	*A. rivalis*
Atractides pennatus	BOLD:ADF7007	13 [10 Public]	NL, DE, ME, MK	0.78	BOLD:AED1201	5.66	*A. anae*

.....continued on the next page

TABLE 2. (Continued)

Species	BIN	Member Count	Country	Average Distance % (p-dist)	NN BIN BOLD	DNN (%) (p-dist)	NN taxonomy
Atractides pennatus	BOLD:ADV9389	2 [0 Public]	DE, AT	0	BOLD:AES4768	1.97	*A. cf. nodipalpis*
Atractides protendens	BOLD:ADT4472	4 [2 Public]	DE	0	BOLD:ACW7095	19.12	*Atractides* sp.
Atractides rivalis	BOLD:ADG8744	6 [1 Public]	AT, SL, DE	0.23	BOLD:ADU8027	11.3	*A. panniculatus*
Atractides robustus	BOLD:AED3548	1 [1 Public]	IR	N/A	BOLD:AEK3669	7.18	*A. robustus*
Atractides robustus	BOLD:ADZ9348	13 [5 Public]	DE, ME, RO, BA, AT	0.31	BOLD:AEK3669	11.38	*A. robustus*
Atractides robustus	BOLD:AEI1810	1 [1 Public]	IR	N/A	BOLD:AEK3669	7.85	*A. robustus*
Atractides robustus	BOLD:AEK3669	1 [1 Public]	TR	N/A	BOLD:AED3548	7.18	*A. robustus*
Atractides ruffoi	BOLD:AEN9154	1 [1 Public]	FR	N/A	BOLD:AER7878	12.98	*A. marizae*
Atractides separatus	BOLD:ADV7653	4 [2 Public]	DE, AT	0	BOLD:ADU1763	10.67	*A. adnatus*
Atractides spinipes	BOLD:AET7894	2 [0 Public] 2 [0 Public]	AT	0	BOLD:ADT8145	12.94	*Atractides* sp. A
Atractides stankovici	BOLD:AED3550	5 [5 Public]	ME, MK	0.58	BOLD:AEO6190	16.51	*A. cf. allgaier*
Atractides subasper	BOLD:AES6460	1 [0 Public]	IT	N/A	BOLD:AEX4044	12.02	*A. glandulosus*
Atractides tener	BOLD:ACG4776	20 [6 Public]	DE, NO, RO	0.99	BOLD:ACY6768	13.59	*Atractides* sp.
Atractides vaginalis	BOLD:ADU6015	21 [8 Public]	CH, IT, DE, AT	1.24	BOLD:ADT8145	11.67	*Atractides* sp. A
Atractides walteri	BOLD:ADV7370	14 [7 Public]	AT	0.37	BOLD:AES9346	10.46	*Atractides* sp.
Atractides sp. A	BOLD:ADT8145	5 [1 Public]	DE, AT	0.43	BOLD:ADU6120	6.17	*Atractides* sp. B
Atractides sp. B	BOLD:ADU6120	2 [1 Public]	DE	0	BOLD:ADT8145	6.17	*Atractides* sp. A

As shown in this study by morphological analysis of specimens from the Netherlands belonging to BIN ACA0209, *A. nodipalpis* can be defined primarily by the shape of the male genital plate with a distinct anteromedial peg-like fissure. The presence of this last character allows a morphological separation of the latter species with certainty from other *A. nodipalpis*-like specimens. On the other hand, as emphasized by Gerecke *et al*. (2022) resolving the taxonomy of *A. nodipalpis* lineage (BIN ADP2485) which encompasses specimens from southern Germany and Norway requires collecting and barcoding taxa that in the past were considered junior synonyms of *A. nodipalpis*.

Another species that requires particular attention is *Atractides robustus* (Sokolow, 1940), which was originally described as a subspecies of *A. nodipalpis*. From the latter species, *A. robustus* can be separated with certainty only in males (IV-L-4/5 with pointed lateral sheets covering the articulation of the next segments, P-4 sword seta proximal to proximoventral seta; Gerecke 2003). *Atractides robustus* was originally described by Sokolow (1940) from the affluents of Kuban river in the Caucasus. It is likely that BINs of specimens from Eastern Türkiye and Northern Iran which form the nearest-neighbour clusters (and belong to the same species as indicated by the results of the ASAP delineation procedure) are conspecific with *A. robustus*. In this concept, populations from Central Europe and the Balkans would represent a new sister species. Anyhow, this should be proved by barcoding material of *A. robustus* from the Caucasus.

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