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New records of aturid water mites from Portugal, with the integrative description of one new species (Acari, Hydrachnidia, Aturidae)

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Abstract

In this paper, three species of the water mite family Aturidae collected in Portugal were identified using an integrative approach that combines morphology and DNA barcodes. *Parabrachypoda lusitanica* **sp. nov.** is described as new for science. Two species, *Hexaxonopsis romijni* (K. Viets, 1923) and *Woolastookia rotundifrons* (K. Viets, 1922) are reported for the first time for the water mite fauna of Portugal.

Key words DNA-barcoding, cytochrome c oxidase subunit I (COI), taxonomy, systematics.

Introduction

The water mite family Aturidae is a polyphyletic taxon including a high number of genera widely distributed on all continents except Antarctica (Gerecke *et al.* 2016; Smit 2020). In the Western Palearctic, the family comprises of three subfamilies, Albiinae K. Viets, 1925, Aturinae Thor, 1900

and Axonopsinae K. Viets, 1929. At present, six species of the family are known from Portugal (Cantallo *et al.* 2022, Pešić *et al.* 2024), one species of Aturinae (*Aturus scaber* Kramer, 1875) and five species of Axonopsinae (*Axonopsalbia mediterranea* Lundblad, 1954, *Barbaxonella ovalis* Lundblad, 1954, *Brachypodopsis gracilis* Piersig, 1903, *Hexaxonopsis serrata* (Walter, 1928) and *Ljania bipapillata* Thor, 1898). Two of these, *Axonopsalbia mediterranea* and *Barbaxonella ovalis*, to date are only known from Portugal.

Lately, as the results of recent DNA barcoding initiatives conducted within the framework of the project Biodiversity Genomics Europe (BGE, <https://biodiversitygenomics.eu/>), three species of the family Aturidae were collected, one of which is new to science and two species are reported for the first time for the water mite fauna of Portugal.

Material and Methods

Water mites were collected by hand netting and preserved in 96% ethanol for the purpose of the molecular analyses. The 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.

Morphological nomenclature follows Gerecke *et al.* (2016). 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 photograph of ejaculatory complex was made using a camera on Samsung Galaxy smartphone. The following abbreviations are used: Ac-1 = first acetabulum; Cx-I = first coxae; I-L-4-6 = fourth-sixth segments of first leg; P-1-P-5 = palp segments 1-5; RMNH = Naturalis Biodiversity Center, Leiden.

Table 1. Details on DNA barcoded specimens, including coordinates of sampling sites, the barcode index number (^N indicates a new BIN that contains only current sequences) and associated data obtained from BOLD. BOLD data presented here was last accessed on 10th December 2024.

Locality	Coordinates	Sample ID	Process ID	BIN
Family Aturidae				
<i>Parabrachypoda lusitanica</i> Pešić & Smit sp. nov.				
Beja, Mértola, São João dos Caldeireiros	37.626°N, 7.810°W	BGE_00110_B01	BSNTN488-23	^N BOLD:AFN8479
		BGE_00228_H08	BSNTN947-23	
<i>Hexaxonopsis (Hexaxonopsis) romijni</i> (K. Viets, 1923)				
Bragança, Mirandela, Torre de Dona Chama	41.665°N, 7.146°W	BGE_00109_B07	BBIOP019-24	^N BOLD:AFW0017
<i>Woolastookia rotundifrons</i> (K. Viets, 1922)				
Guarda, Seia, Rio Alva, Praia Fluvial de Sabugueiro	40.401°N, 7.640°W	BGE_00227_A01	BSNTN951-23	
		BGE_00108_H06	BBIOP185-24	
		BGE_00108_H08	BBIOP187-24	
Guarda, Manteigas, Poio do Leão	40.399°N, 7.541°W	BGE_00108_D05	BBIOP136-24	^N BOLD:AFV8119
Guarda, Manteigas, Ponte dos Frades	40.403°N, 7.526°W	BGE_00108_D07	BBIOP138-24	
Guarda, Manteigas, Covão da Ponte	40.443°N, 7.514°W	BGE_00108_G02	BBIOP169-24	

Molecular and DNA barcode analyses

The molecular analysis was conducted at the University of Florence (Florence, Italy). DNA was extracted using a non-destructive protocol. For the methods used for cytochrome *c* oxidase subunit I (COI) gene amplification and sequencing see Pešić *et al.* (2024). Raw reads were demultiplexed using

the Pacific Biosciences SMRT Link software. Consensus sequences were generated with the PacBio Amplicon Analysis (pbaa) tool. Primer trimming, translation and stop codon checking were performed using Geneious Prime 2024.0.1.

Consensus sequences were made available in the Barcode of Life Data Systems (BOLD). Relevant voucher information, photos, and recently generated DNA barcodes are publicly accessible through the Dataset - DS-BGEPL03 BGE (available at https://v4.boldsystems.org/index.php/MAS_Management_DataConsole?codes=DS-BGEPL03) in BOLD.

In this study DNA was extracted from 9 specimens of the family Aturidae from Portugal listed in Table 1. For the other species used in the analysis, COI sequence data were downloaded from the respective sequence data archives. In total we used an COI dataset with 62 sequences of aturid water mites for phylogenetic analyses. Representative of *Hungarohydracarus subterraneus* Szalay, 1943 of the family Hungarohydracaridae was used as outgroup.

Sequence comparisons were performed using MUSCLE alignment (Edgar 2004). Intra and interspecific genetic distances were calculated based on the Kimura 2-parameter (K2P) model (Kimura 1980), using MEGA 11 software (Tamura *et al.* 2021). The latter software was used to calculate Neighbor-Joining (NJ) trees based on K2P distances (standard for barcoding studies) using pairwise deletion for missing data. Branch support was calculated using nonparametric bootstrap (Felsenstein 1985) with 1000 replicates and shown next to the branches. All codon positions were considered in the analyses.

Results

Systematics

Family Aturidae Thor, 1900

Genus *Parabrachypoda* K. Viets, 1926

***Parabrachypoda lusitanica* Pešić & Smit sp. nov.**

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Figs 1-4

Material examined — Holotype ♂, dissected and slide mounted (RMNH), Portugal, **Beja**, Mértola, São João dos Caldeireiros, 37.626°N, 7.810°W, 17 May 2023, leg. Ferreira, Benitez-Bosco, Ekrem, Stur & Turaccio, sequenced (Voucher ID: BGE_00110_B01; BOLD ID: [BSNTN488-23](#)). Paratype: 1♂ (RMNH) same data as the holotype, preserved in Koenike-fluid (sequenced; BOLD ID: [BSNTN947-23](#)).

Diagnosis (Female unknown) — Dorsal shield nearly as wide as long, posterior margin medially projecting; a pair of posteriorly directed, pointed sheaths located laterally near the posterior end of ventral shield; gonopore anteriorly surrounded by well sclerotized lips, Ac-1 located on the surface of these sclerotized lips, numerous setae anteriorly in transverse line, and posteriorly from the level of Ac-3; IV-L-4 distally with one curved, spatula-shaped seta, IV-L-4 digitiform ventral extension apically with three different setae (one longer flanked by two stout setae, one thicker, peg-like, the other thinner).

Description — Male (holotype) — Idiosoma dorsally 470 long and 493 wide, ventrally 500 long. Colour yellowish with one unpaired anterior and a pair of posterolateral violet marks (see Fig. 1A). Dorsal shield nearly as wide as long, posterior margin medially projecting and with excretory pore placed terminally dorsal on a projection (Fig. 1A). Anterior margins of Cx-I extending slightly beyond frontal margin, gnathosomal bay 128 deep; distance between IV-L insertion 271. A pair of posteriorly directed pointed sheaths located laterally near the posterior end of ventral shield (Figs. 1A, 2). Posterior margin of the venter forming a rounded concave ridge. Gonopore in distal part flanked by well-developed sclerotized lips, Ac-1 located on the surface of these sclerotized lips, acetabula arranged in an obtuse triangle, numerous fine setae, anteriorly from the level of Ac-2 longer, and posteriorly from the level of Ac-3 shorter (Fig. 2). Ejaculatory complex 116 long.

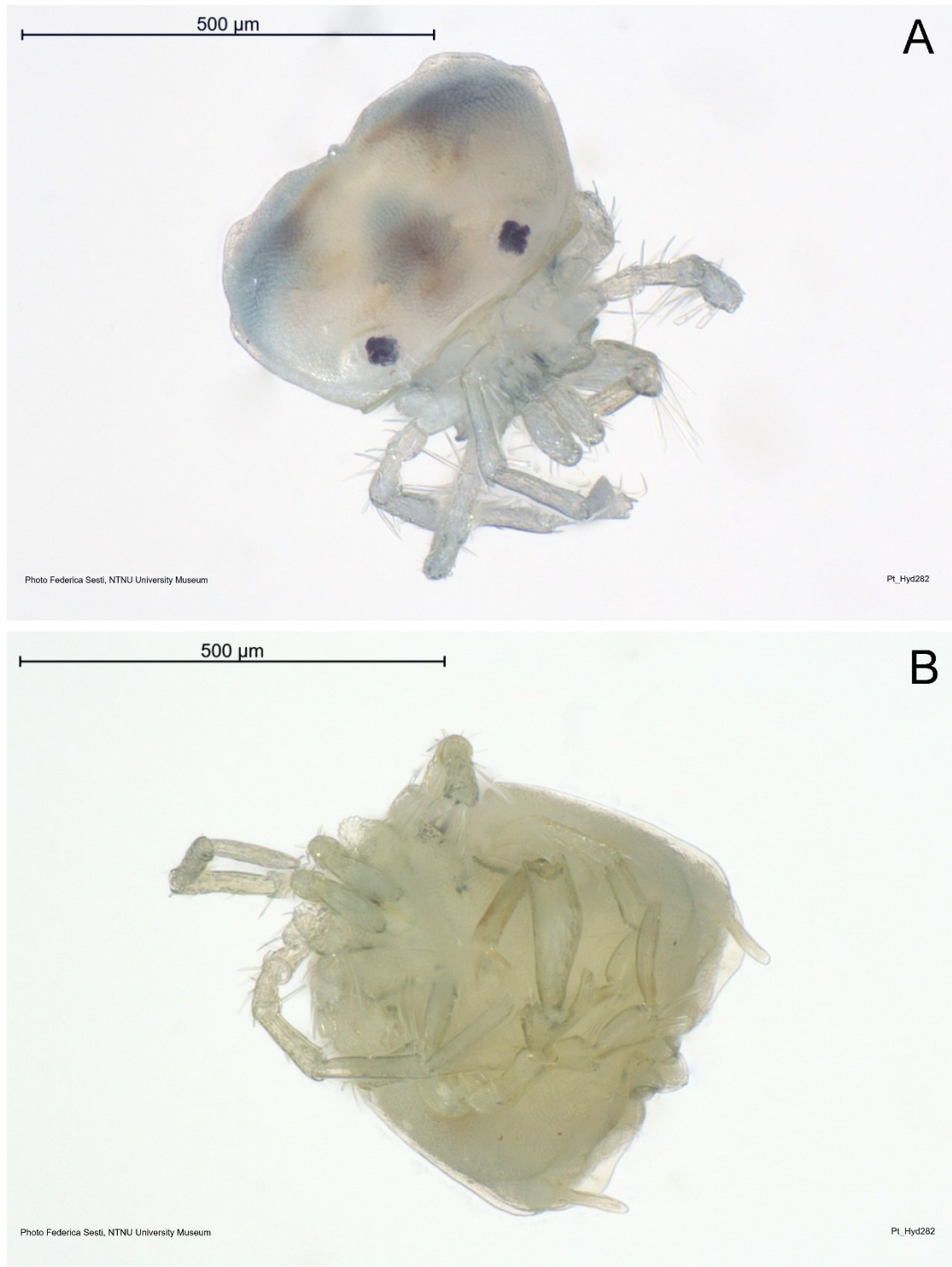


Figure 1. Photographs of *Parabrachypoda lusitanica* Pešić & Smit **sp. nov.**, male paratype (BGE_00110_B01): A – idiosoma, frontal view; B – A – idiosoma, ventral view. Photographs by F. Sesti.

Palp as illustrated in Figs. 2B-C: length/height: P-1, 49/19; P-2, 73/52; P-3, 36/33; P-4, 113/36; P-5, 36/20; palpal segment relatively stout (length/height ratio P-2, 1.4; P-3, 1.1; P-4, 3.1); P-1 with one dorsal seta; P-2 ventral margin nearly straight, distally forming a short blunt projection; P-3 ventral margin concave; P-4 proximally narrow, in the centre, near the insertion of two ventral setae slightly elevated to form an obtuse projection near the insertion of the stronger ventral seta, a slightly thickened seta and a finer seta laterally on a further blunt elevation, dorsal surface (medially and ventrally) of P-4 with several fine setae. Capitulum ventral length 102; chelicera 134 long.

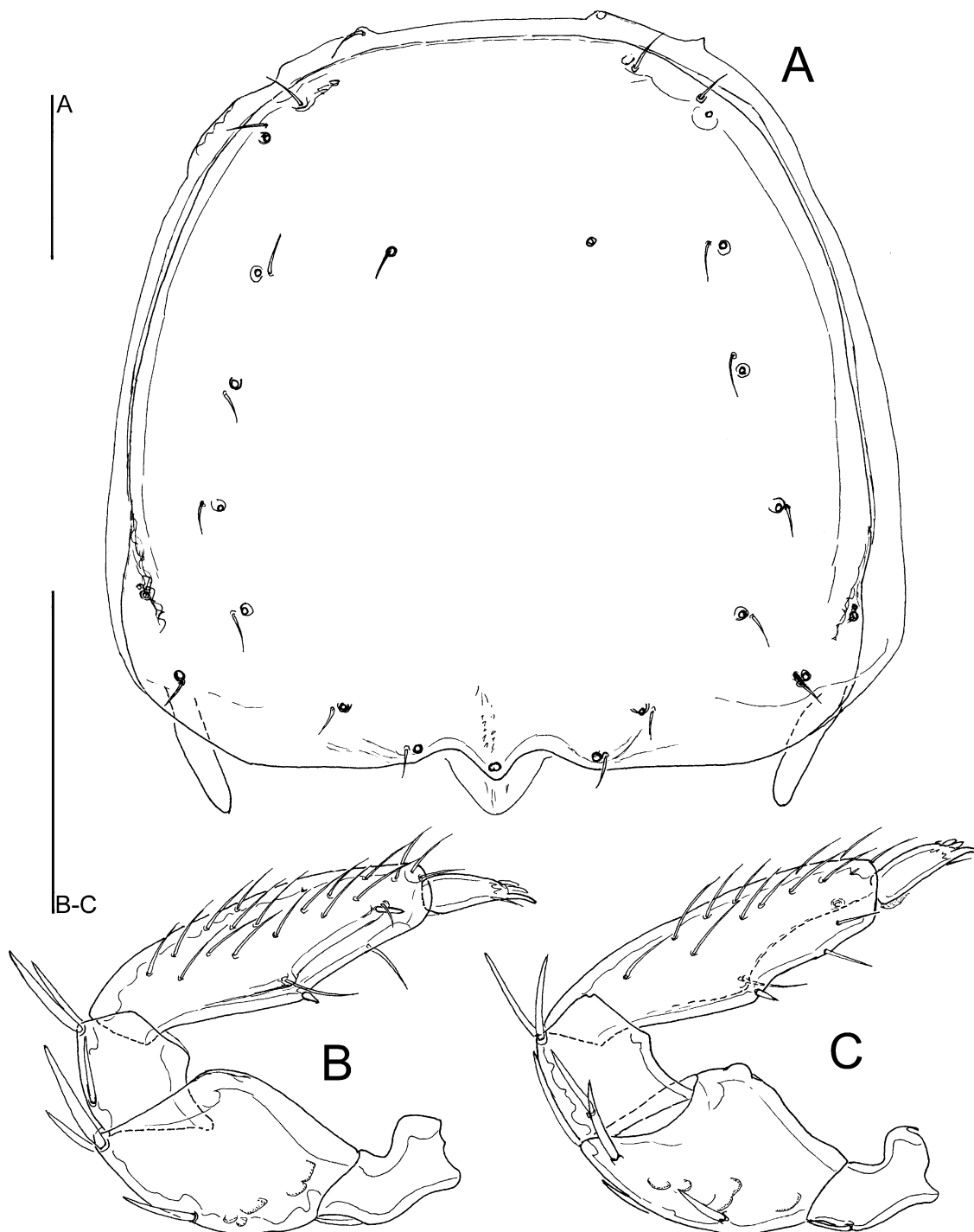


Figure 2. *Parabrachypoda lusitanica* Pešić & Smit **sp. nov.**, male holotype (BGE_00110_B01): A – idiosoma, dorsal view; B – palp, lateral view; C – palp, medial view. Scale bars = 100 μ m.

Legs: I-L and II-L as shown in Figs. 4A-B, surface of I-L-6 and II-L-6 with numerous fine setae; III-L as illustrated in Fig. 4C, III-L-5 distally enlarged, here with four dorsal club-shaped setae, one of them longer than the other three (Fig. 4C inset); IV-L-4-6 as shown in Figs. 4D-E; IV-L-4 distally enlarged, here bearing six to seven setae, two of them heavy (one curved, spatula-shaped seta, shifted more to ventrodistal margin, the other straight and longer), the remaining slender and pointed, and forming a prominent digitiform ventral extension apically with three different setae (one longer flanked by two stout setae, one thicker, peg-like, the other thinner); IV-L-5 thickened, length/height ratio 3.3, dorsal margin curved, ventral margin, with scattered setae, in proximal part a peg-like setae; IV-L-6 slightly

curved, length/height ratio 5.3, ventrally with a row of longer setae; claws modified as given in Figs. 3A(inset), anterior claw longer than posterior claw.

Number of swimming setae: II-L-4: 2, II-L-5: 3; III-L-4: 5; IV-L-5: 6. Dorsal length: I-L-2-6: 55, 81, 122, 139, 131; II-L-1-6: 72, 63, 91, 134, 144, 128; III-L-1-6: 66, 69, 94, 103. 98, 134; IV-L-1-6: 116, 88, 80, 69, 188, 166.

Female: unknown.



Figure 3. *Parabrachypoda lusitanica* Pešić & Smit **sp. nov.**, male holotype (BGE_00110_B01): ventral shield. Scale bar = 100 μ m.

Etymology — The name refers to the geographical provenance (from Latin *lusitanicus*, Lusitanian, Portuguese) from where the new species is described.

Species delimitation using DNA barcodes — We used a *COI* dataset with 79 sequences of water mite specimens of the family Aturidae listed in Table 1 and Supplement material 1 for phylogenetic analyses. *Hungarohydracarus subterraneus* from Bosnia and Herzegovina was used to root the tree. The NJ tree is presented in Fig. 5. The *COI* tree sequences retrieved from two specimens from Portugal, which form a unique cluster (**BOLD:AFN8479**), appeared as a sister group (albeit with a low support) to the sequence belonging to *Parabrachypoda montii*, a rhitrobiontic species widely distributed in Europe. The mean K2P genetic distance between *COI* sequences of *Parabrachypoda lusitanica* **sp. nov.** and *P. montii* was estimated at 26.3%.

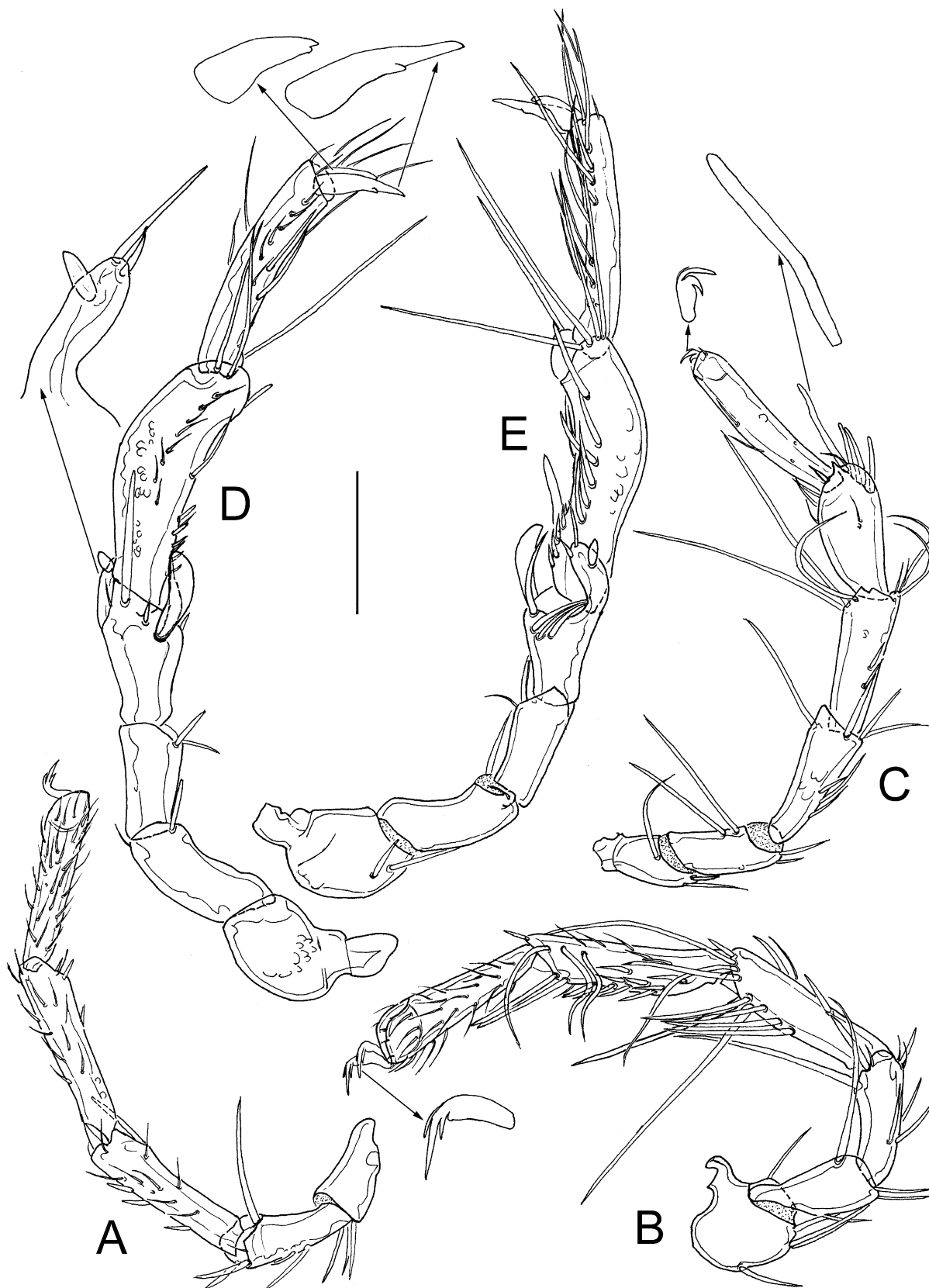


Figure 4. *Parabrachypoda lusitanica* Pešić & Smit *sp. nov.*, male holotype (BGE_00110_B01): A – I-L-2-6; B – II-L (in inset: claw, enlarged 2x); C – III-L (in inset: claw and a club-shaped seta, enlarged 2x); D – IV-L anterior (in inset: IV-L-4 digitiform ventral extension, and claws, enlarged 2x); E – IV-L posterior. Scale bar = 100 μ m.

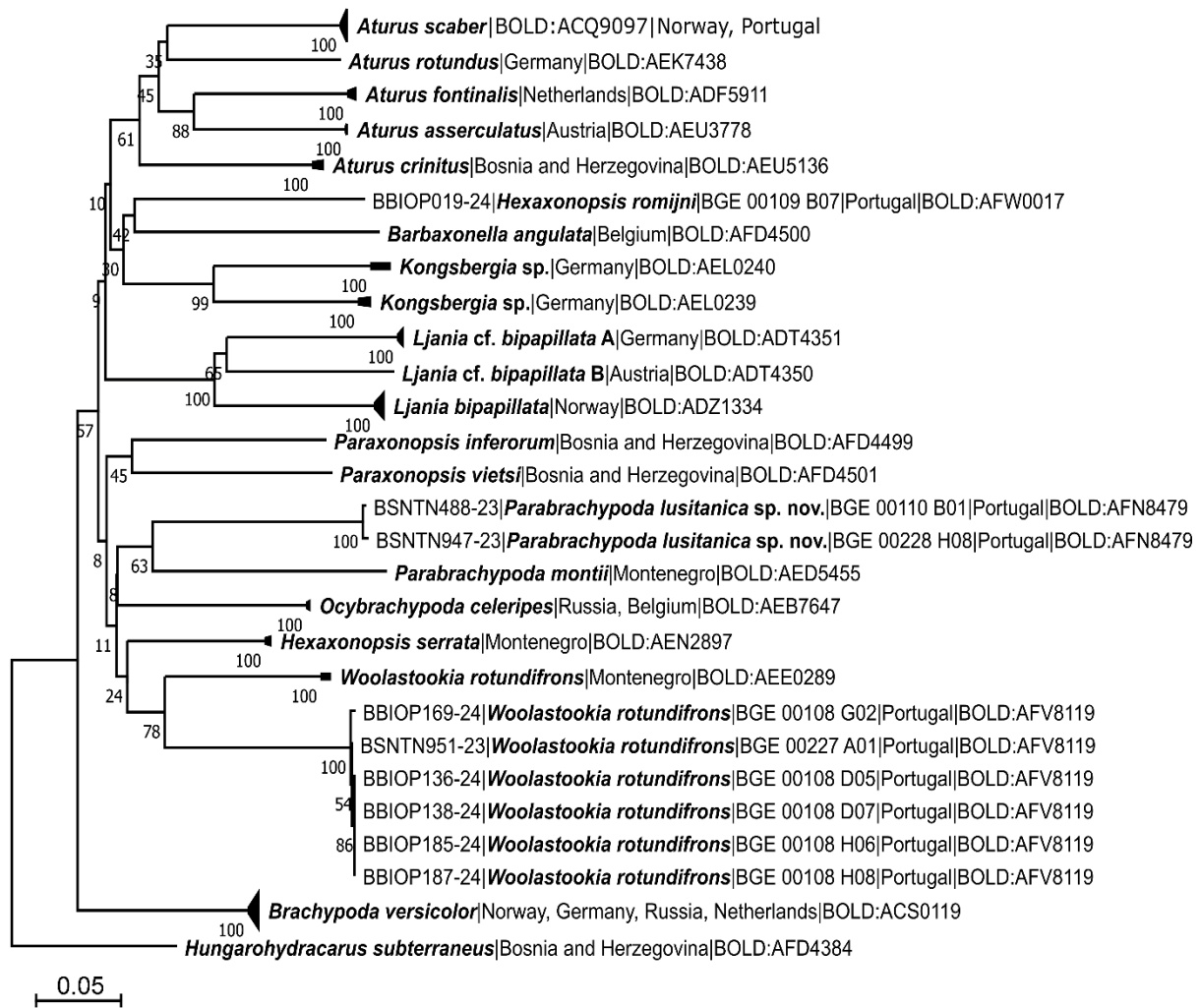


Figure 5. Neighbour-Joining tree of the family Aturidae, obtained from 79 nucleotide COI sequences listed in Table 1 and Suppl. material 1. *Hungarohydracarus subterraneus* Szalay, 1943 was used to root the tree. BINs are based on the barcode analysis from 15 December 2024.

Remarks — In regard to the position of the genital field (located close to the posterior margin of the ventral shield) and similar shape of palp (P-2 with a very short, rounded distoventral projection) and setation of IV-L, the new species from Portugal is placed in genus *Parabrachypoda*. Currently, the latter genus, includes five species known from the Western Palaearctic, i.e. *P. modesta* (Koenike, 1911), known from Central and eastern Europe, *P. baderi* Di Sabatino & Cicolani, 1991, known from central Mediterranean (Italy: Abruzzo, Sicily, Sardinia; France: Corsica; Sabatino & Cicolani, 1991, Gerecke *et al.* 2014) and Turkey (Esen *et al.* 2014), *Parabrachypoda mutila* (Walter, 1928), known from the West Mediterranean area (Algeria, Tyrrhenian islands - Gerecke *et al.* 2014), *P. montii* (Maglio, 1924) known from central and southern Europe and *P. orientalis* Pešić & Esen, 2013. The latter species was originally described from Turkey (Esen *et al.* 2014), and includes also populations under the (erroneous) name of *P. mutila* from Crimea (Tuzovskij 1978, including also the reared larval stage) and Turkey (Pešić *et al.* 2006). From all these species, the new species from Portugal can be separated by the unique shape of dorsal shield (nearly as wide as long, posterior margin medially projecting) and genital field (gonopore anteriorly surrounded by well sclerotized lips, Ac-1 located on surface of these sclerotized lips, numerous setae around the gonopore, anteriorly in transverse line, and in a field postero-medially of Ac-3. Moreover, the presence of a pair of posteriorly directed pointed sheaths located laterally near the posterior end of ventral shield is a unique feature and not present in other species of the genus.

In regard to IV-L setation, males of *Parabrachypoda mutila* resemble the new species in the presence of three different apical setae on the ventral digitiform extension of IV-L-4 (one fine, longer

seta flanked by two stout setae, one of them thicker than the other). However, in the latter species IV-L-5 is much slenderer, with subparallel dorsal and ventral margins, bearing numerous longer ventral setae. In *P. lusitanica* IV-L-5 is thickened and with more curved dorsal margin, more similar to *B. orientalis*, ventral setae in proximal part peg-like. Moreover, the distal margin of IV-L-4 of *P. mutila* lacks the characteristic spatula-like seta present in the new species.

Distribution — Portugal; known only from the type locality (Fig. 6) in the Alentejo region (Southern Portugal).



Figure 6. Photograph of the type locality (São João dos Caldeireiros) of *Parabrachypoda lusitanica* sp. nov. Photograph by P. Gadawski.

Genus *Hexaxonopsis* K. Viets, 1926

***Hexaxonopsis (Hexaxonopsis) romijni* (K. Viets, 1923)**

Material examined — Portugal, **Bragança**, Mirandela, Torre de Dona Chama, 41.665°N, 7.146°W, 256 m asl., 13 Jul. 2023 leg. Ferreira & Padilha, 1♂ (sequenced), dissected and slide mounted (RMNH).

Remarks — The Portuguese specimen molecularly analyzed in this study keys out as *Hexaxonopsis romijni* with the key of Gerecke *et al.* (2016). This specimen forms a unique BIN ([BOLD:AFW0017](#)). In the phylogenetic tree, the BIN is positioned as a sister clade of *H. serrata* from Montenegro.

Distribution — Western and central Europe (Pešić *et al.* 2010; Gerecke *et al.* 2016); Turkey (Esen *et al.* 2011). New for Portugal.

Genus *Woolastookia* Habeeb, 1954

***Woolastookia rotundifrons* (K. Viets, 1922)**

Material examined — Portugal, **Guarda**: Seia, Rio Alva, Praia Fluvial de Sabugueiro, 40.401°N, 7.64°W, 1021 m a.s.l., 24 Aug. 2023, leg. Ferreira, Benitez-Bosco & Padilha, 2♂, 1♀

(BGE_00227_A01, BGE_00108_H06, BGE_00108_H08); Manteigas, Poio do Leão, 40.399°N, 7.541°W, 734 m a.s.l., 22 Aug. 2023, leg. Ferreira, Benitez-Bosco & Padilha, 1♀ (BGE_00108_D05); Manteigas, Zêzere, Ponte dos Frades, 40.403°N, 7.526°W, 672 m a.s.l., 22 Aug. 2023, leg. Ferreira, Benitez-Bosco, Padilha & Stur 1♂ (BGE_00108_D07), dissected and slide mounted (RMNH); Manteigas, Mondego, Covão da Ponte, 40.443°N, 7.514°W, 999 m a.s.l., 24 Aug. 2023, leg. Ferreira, Benitez-Bosco & Padilha, 1♀ (BGE_00108_G02).

Remarks — The specimens from Portugal morphologically match the description of *Woolastookia rotundifrons* following Pešić *et al.* (2010). The sequenced specimens from Portugal form a unique BIN: [BOLD:AFV8119](https://doi.org/10.26434/chemrxiv-2023-afv81), with the nearest neighbouring BIN being [BOLD:AEE0289](https://doi.org/10.26434/chemrxiv-2023-afv81) (*p*-distance 17.39%), which includes specimens from Montenegro morphologically as well assigned to *W. rotundifrons*, indicating the need for taxonomic revision of this species from a wider geographical area.

Distribution — Central, southern and eastern Europe (Pešić *et al.* 2010; Gerecke *et al.* 2016). New for Portugal.

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Supplementary material 1

List of Aturidae specimens

Data type: doc

Explanation note: List of water mite specimens of family Aturidae, used for building the Neighbour-Joining (NJ) tree (Fig. 5). Details on the DNA barcoded specimens from Portugal are given in Table 1. GenBank numbers are indicated by *. BINs are based on the barcode analysis from 15 December 2024.

Link: <https://www.biotaxa.org/em/article/view/86633/81339>