Zoological Studies **65:**20 (2026)
doi:10.6620/ZS.2026.65-20

Zoological Studies

Open Access

New and Known Species of Tardigrada from the Coast of Vietnam

Anastasiia A. Saulenko[1,2,*], Anastassya S. Maiorova[1], Viktoriia D. Yagodina[1,2], Viet Dung Vu[3], Thi-Lan Nguyen[3], Trong Huan Phan[3], and Vladimir V. Mordukhovich[1,2,*]

[1]*A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, Paltchevsky Street 17, Vladivostok, 690041, Russia. *Correspondence: E-mail: saulenko.aa@mail.ru (Saulenko); vvmora@mail.ru (Mordukhovich) E-mail: anastasia.mayorova@gmail.com (Maiorova); iagodinavd@gmail.com (Yagodina)*
[2]*Far Eastern Federal University, Ajax Bay 10, Vladivostok, 690922, Russia*
[3]*Coastal Branch of Joint Vietnam-Russia Tropical Science and Technology Research Center (Tropical Center), 30 Nguyen Thien Thuat, Khanh Hoa, Nha Trang, Vietnam. E-mail: vuvietdung01031982@gmail.com (Vu); nguyenlanst1805@gmail.com (Nguyen); tronghuan1369@yahoo.com (Phan)*

urn:lsid:zoobank.org:pub:115153BE-7141-4B57-8A11-78D523B5C2D0
Received 26 June 2025 / Accepted 7 March 2026 / Published 27 May 2026
Communicated by Daniel Stec

Four species of tardigrades have been found in the meiofauna communities of the seagrass meadows and adjacent areas off the coast of Vietnam. For two of them, *Batillipes binhdinhicus* sp. nov. and *Batillipes ampullus* sp. nov., integrative descriptions are presented. *Batillipes binhdinhicus* sp. nov. belongs to the B1 subgroup of species by having toe 3 shorter than toe 4 on legs IV, while *Batillipes ampullus* sp. nov. with toes 3 and 4 on legs IV of equal length belongs to the A group of species. *Batillipes binhdinhicus* sp. nov. can be distinguished from all previously described species by a set of features: very long cephalic cirri and leg sensory spines, both with swollen tips, presence of a single pair of conical lateral body projections between all pairs of legs and simple triangular caudal appendage. The distinctive features for *Batillipes ampullus* sp. nov. are short sensory organs on the fourth pair of legs, which consists of two parts and have swollen tips with a tuft of additional sensory filaments that are structurally similar to those found on the cephalic appendages. In addition, *Batillipes ampullus* sp. nov. does not have noticeable lateral body projections between the legs, and the body ends in a semi-circular tail appendage. Based on the obtained data on the nucleotide sequences of 18S rDNA and 28S rDNA, the distinction between the new species was confirmed.

Keywords: *Batillipes*, *Halechiniscus*, Integrate taxonomy, Meiofauna, Morphology, *Orzeliscus*, Tardigrade, Vietnam

BACKGROUND

Seagrass meadows are considered one of the most productive ecosystems, capable of providing a significant proportion of the primary productivity for associated habitats (Unsworth et al. 2014; Baldrighi et al. 2021). Seagrasses can be considered as ecosystem engineers because of their impact on granulometric composition via particle trapping and stabilization, as well as on geochemical properties and the structure of associated biotic communities (Bos et al. 2007; Baldrighi et al. 2021; Vieira et al. 2024). They provide a habitat for a variety of organisms, including meiofauna, and research suggests a positive correlation between seagrass presence and species richness and abundance of benthic fauna (Liao et al. 2016; Baldrighi et al. 2021).

Thorough studies of meiobenthic communities of seagrass biotopes were carried out in various region of the ocean (*e.g.*, Abe et al. 2012; Alongi 1987; De Troch et al. 2001; Fonseca et al. 2011; Leduc and

Citation: Saulenko AA, Maiorova AS, Yagodina VD, Dung Vu V, Nguyen T-L, Phan TH, Mordukhovich VV. 2026. New and known species of Tardigrada from the coast of Vietnam. Zool Stud **65:**20. doi:10.6620/ZS.2026.65-20.

Probert 2011; Liao et al. 2015 2016; Min et al. 2006; Monthum and Aryuthaka 2006; Ndaro and Olafsson 1999; etc.). Tardigrades were rarely observed in these studies, but occasionally with high abundance, up to tens of specimens per 10 cm^2 (Min et al. 2006). Despite the absence of the studies specifically focused on the tardigrade fauna of the seagrass meadows, according to available data at least 43 species from 23 genera have been recorded from these biotopes (Biserov 1998; Kaczmarek et al. 2015).

In Vietnam, seagrass meadows are present along the entire coastline, and the total area of these meadows is around 14,200 ha, according to the latest estimate (Veettil et al. 2022). The diversity and distribution of most meiofaunal taxa in seagrass meadows and coast ecosystems of Vietnam in general remain largely unstudied. Only two species of marine tardigrades have been identified from the coast of Vietnam to date: *Halechiniscus jejuensis* Chang & Rho 2002, described from a mangrove biotope (Tchesunov 2011) and the recent discovery of *Florarctus hulingsi* Renaud-Mornant, 1976, founded in a plantation of mangrove trees *Rhizophora stylosa* (Tchesunov 2024). Unidentified *Florarctus* and *Batillipes* species were also reported from Vietnam, discovered together with *Hal. jejuensis* by Tchesunov (2011). Despite the absence of other described species of marine tardigrades in the study region, literature data show that this type of invertebrate occurs in meiobenthic communities both on the coast of Vietnam (Ngo et al. 2013) and in the South China Sea (Renaud-Mornant 1967; Renaud-Mornant and Serène 1967; Chang and No 1997; Lee et al. 2017; Bai et al. 2022; Wang et al. 2023). Apart from *Hal. jejuensis* and *Flo. hulingsi*, six other species of marine tardigrades are known from the South China Sea: *Parastygarctus higginsi* Renaud-Debyser, 1965, *Batillipes mirus* Richters, 1909, *Batillipes philippinensis* Chang & Rho 1997, *Florarctus kwoni* Chang & Rho 1997, *Orzeliscus asiaticus* Lee, Rho & Chang, 2017 and *Halechiniscus janus* Bai et al. 2022 (Renaud-Mornant 1967; Renaud-Mornant and Serène 1967; Chang and No 1997; Lee et al. 2017; Bai et al. 2022).

A recent study of the meiofauna in the seagrass meadows and adjacent waters off the coast of Vietnam discovered several species of marine tardigrades. In the present article we provide integrative descriptions of two new species, *Batillipes binhdinhicus* sp. nov. and *Batillipes ampullus* sp. nov., as well as data on the new records of *Halechiniscus* and *Orzeliscus* species.

MATERIALS AND METHODS

Sample processing

Sediment samples were collected during a low tide in March 2021, in October 2022, and in April 2023 at the seagrass bed and adjacent area in Van Phong Bay (with dominance of *Enhalus acaroides*) and in Thi Nai Lagoon (with dominance of *Zostera japonica*). During the sampling, the water depth ranged from 0 to 0.2 meters below sea level. In each season, samples were taken from 6 to 9 stations at each area using a hand sampler (3 cm diameter) in three repetitions per 1 m^2. The depth of the sampler into the sediment was 5 cm or more. Therefore, the volume of sediment in quantitative samples was a minimum of 35 ml, the number of samples for each location was at least 54, and the total number of samples was 117. The distance between each sampler is approximately 10 cm. The distance between stations in each area ranged from about 0.5 m to 200 m. The examined material is summarized in table 1. Tardigrades were found in Van Phong Bay at stations with coarse sand and gravel, and in Thi Nai Lagoon at stations with medium sand.

For morphological studies, all samples were preserved in 5% buffered formaldehyde. To extract meiofauna, sediments were washed in the laboratory

Table 1. Information about stations where tardigrades were found

Species	Location	Latitude, °N	Longitude, °E	Year	Season	Number of stations with tardigrades / total number of stations
Halechiniscus sp.	Van Phong Bay, Khanh Hoa province, Vietnam	12.6445	109.2045	2021	Spring	2/9
				2022	Autumn	0/6
				2023	Spring	0/6
Orzeliscus asiaticus	Thi Nai Lagoon, Binh Dinh province, Vietnam	13.8107	109.2276	2021	Spring	2/6
				2022	Autumn	0/6
				2023	Spring	0/6
Batillipes binhdinhicus sp. nov., *Batillipes ampullus* sp. nov.	Thi Nai Lagoon, Binh Dinh province, Vietnam	13.8107	109.2276	2021	Spring	0/6
				2022	Autumn	0/6
				2023	Spring	2/6

with tap water, passed through a 32 µm sieve, and centrifuged in a solution of kaolin/colloidal silica three times (4000 rpm for 6 min) (McIntyre and Warwick 1984). Afterwards, the meiofauna samples were refixed in formaldehyde and stained with rose Bengal. Tardigrades were sorted and collected using stereomicroscopes, transferred by a fine tungsten wire needle and Irwin loop. Later, specimens were placed in a small drop of VECTASHIELD® mounting medium on Higgins-Shirayama plastic slides (H-S slides) between two coverslips (22 and 18 mm in length), which enabled viewing from both sides (Shirayama et al. 1993). The slides were sealed with transparent nail polish.

Microscopy and imaging

Drawings and photographs were made on an Olympus BX 53 optical microscope using differential interference contrast (DIC) microscopy. The morphological structures were measured only if they were undamaged and if their orientation was suitable. Several specimens were prepared for scanning electron microscopy (SEM) and processed according to the standard protocol (Sørensen et al. 2018): selected specimens were transferred from 5% formaldehyde to distilled water and washed using a detergent to clean the body surface. The cleaned specimens were dehydrated through a graded series of ethanol (30–50–70–90–96% (I)–96% (II) with 15' in each solution), transferred to acetone (10' in each solution), and critical-point-dried. The dried specimens were mounted on aluminum stubs and sputter-coated with gold. The SEM imaging was conducted using a Zeiss® Sigma 300 VP.

DNA sequencing

Specimens for DNA analysis were preserved in 96% ethanol. After photo-vouchering, total DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol (elution with 40 µl AE buffer instead 100 µl to increase the final DNA concentration). Fragments of 18S rDNA and 28S rDNA were amplified. For 18S rDNA the set of primers 1F, 4R, 3F, 9R (Giribet et al. 1996), and 18Sbi, 18Sa2.0 (Whiting et al. 1997) were used. Amplifications were performed in a 12 µl volume of a solution containing 6 µl of ddH_2O, 4 µl Go Taq Green Master Mix (Promega corp, USA), 0.5 µl of each primer, and 1 µl of genomic DNA. The PCR protocol developed to amplify the 18S rRNA fragments consisted of an initial denaturing step at 95°C for 1 min, 40 amplification cycles (95°C for 10 s, 55°C for 30 s and 72°C for 50 s), and a final elongation step of 10 min at 72°C. Unfortunately, we were able to get amplicons of the 18S rDNA fragments only for the pair of primers 1F and 4R, so sequences obtained using them were used in further analysis. A fragment of the nuclear ribosomal 28S rRNA was amplified using the pair of primers 28Sa (Whiting et al. 1997) and 28S rd5b (Schwendinger and Giribet 2005). PCR conditions for this gene fragment were the same as for 18S rDNA. All obtained amplicons were visualized in 1.5% agarose gel, then they were purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA).

Sequencing of PCR products was performed with the BigDye Terminator v3.1 cycle kit v3.1 (Applied Biosystems, USA) following the manufacturer's protocol. Sequence products were cleaned with sodium acetate and ethanol and directly sequenced with an ABI 3130xl (Applied Biosystems, USA). Contigs were trimmed and assembled with Chromas 2.6.6 program (http://technelysium.com.au). The final sequence length for the 18S rRNA gene fragment was 537 bp, for 28S rDNA—from 489 to 494 bp depending on the individual. Obtained sequences were deposited to GenBank, their accession numbers are described below.

28S rDNA fragments of the new species were aligned together with *Batillipes mirus*, *Batillipes similis* Schulz, 1955, *Batillipes pennaki* Marcus, 1946 (Jørgensen et al. 2010) and two sequences of *Batillipes* sp. (Fujimoto et al. 2017). *Cornechiniscus lobatus* (Jørgensen et al. 2011), *Pseudechiniscus facettalis* (Guil and Giribet 2012) and *Echiniscoides* sp. (Møbjerg et al. 2016) were used as an outgroup due to its suitable nucleotide sequence overlap and belonging to another family. For the 18S rRNA gene fragment, there are two records in the GenBank for *Batillipes*: for *Bat. mirus* (Jørgensen et al. 2010) and for *Bat.* sp. (Fujimoto et al. 2017). However, we could not use these sequences, since for 18S rDNA we only obtained an amplicon for the primer pair 1F and 4R. Thus, only the sequences from GenBank that were most similar to ours were added and used as an outgroup: *Cornechiniscus lobatus* Ramazzotti, 1943 (Guidetti et al. 2009), *Co. madagascariensis* Maucci, 1993 (Gasiorek et al. 2022), *Echiniscoides sigismundi* M. Schultze, 1865 (Jørgensen et al. 2010) and *Bryodelphax maculatus* Gasiorek, Stec, Morek, Marnissi and Michalczyk, 2017 (Gasiorek et al. 2017). All sequences were aligned in the MEGA 11 program (Tamura et al. 2021) using the MUSCLE algorithm (Edgar 2004). Obtained sequences were deposited to GenBank, their accession numbers and accession numbers of used from other sources sequences are described in Supplementary Materials (Table S1). Additionally, files in FASTA format with the sequences used in the analysis are presented in Supplementary materials (SM S1 and S2). *P*-distances

(Nei and Kumar 2000) were calculated in MEGA 11 (Tamura et al. 2021).

We carried out a maximum likelihood trees in IQ-TREE 3.0.1 (Wong et al. 2025) with 500,000 bootstrap replicates using ultrafast bootstrapping (Hoang et al. 2018) after choosing the optimal models of molecular evolution with ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE. TN+G4 (Tamura and Nei 1993) and TVM+F+G4 were the best models for both 18S and 28S rRNA genes, respectively.

Bayesian phylogenetic analysis was conducted in MrBayes v3.2.7 (Ronquist et al. 2012). PartitionFinder 2.1.1 (Lanfear et al. 2012) was used to select the best-fit partitioning scheme and models for ribosomal genes using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion as the optimality criterion for model selection. K80+G (Kimura 1980) was the best model for both genes. Bayesian Inference was performed with two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses, with each run comprising one cold chain and three heated chains at a default temperature of 0.1. The chains were run for 10 million generations and sampled every 100 generations. A burn-in of 2.5 million generations (or 25% of the sampled trees) was used. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut et al. 2018). Analysis in the Tracer 1.7.1 show the convergence of the -LnL values of both runs immediately after the starting generations. The average -LnL values for both runs were -1647 with an effective sample size of 31327.

Tardigrade taxonomy followed Guil et al. (2019), and Degma et al. (2009–2025). Abbreviations for the names of genera are given according to Perry et al. (2019).

RESULTS

MORPHOLOGICAL ANALYSIS

Phylum Tardigrada Doyère, 1840
Class Heterotardigrada Marcus, 1927
Family Batillipedidae Ramazzotti, 1962

Genus *Batillipes* Richters, 1909
Batillipes binhdinhicus sp. nov.

(Figs. 1–6, Tables 2, S4)
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Type locality: Seagrass bed of *Zostera japonica*, Thi Nai Lagoon, Binh Dinh province, Vietnam (13°48'38"N, 109°13'40"E), medium sand.

Type material: The female holotype (No. MIMB51986); three female paratypes (No. MIMB51987, MIMB51990 and MIMB51991), and four male paratypes (No. MIMB51992–MIMB51995), collected from Thi Nai Lagoon, Binh Dinh province, Vietnam. All material deposited in the Museum of the A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Vladivostok, Russia).

Other material: three SEM specimens (two male and one of unknown sex).

Etymology: The species name "binhdinhicus" refers to the Binh Dinh province where Thi Nai Lagoon is located.

Diagnosis: All cephalic cirri and leg sensory spines with swollen tips. Tips of cephalic cirri have a tuft of additional sensory filaments. The scapular region is well-developed, projecting laterally at the level of the first pair of legs. The presence of a single pair of conical lateral body projections is observed between all pairs of legs. A simple triangular appendage attached directly to the caudal base. Each leg bears six toes of varying length, ending in oval suction discs. On the fourth pair of legs, toe 3 is shorter than toe 4.

Description: The holotype is an adult female (Fig. 2) measuring 137.3 µm in length, including the caudal apparatus, and 73 µm in width between the second and third pairs of legs. Typical trapezoidal head (38.8 µm in width) bearing eleven cephalic appendages: unpaired median cirrus, paired internal and external cirri, paired lateral cirrus and primary clavae (Figs. 1, 4B, 4C). The median cirrus is 20.6 µm in length and is located at the large cirrophore in the anterior-mid cephalic region. The internal cirri are inserted on the frontal edge of the head and measure 25.9 µm in length. The external cirri are 16.6 µm long, situated near the lateral cirri and the primary clavae. The internal and external cirri also have distinct cirrophores. The primary clavae are tubular (14.8 µm long), situated ventrally to the very long lateral cirrus (32.8 µm) on a shared pedestal. All cephalic cirri have swollen tips, accompanied by a tuft of additional sensory filaments (Figs. 1, 4B, 4C). Secondary clavae, eye spots, stylet and stylet support are absent. The ovoid pharyngeal bulb measures 23.7 µm in length and 20.6 µm in width.

Sensory spines are present on each of the telescopic legs. The spine on the first pair of legs is the shortest (10.2 µm), while the spine on the fourth pair is the longest (20.8 µm). The spines on the second and third pairs are of medium length, 13.3 and 12.8 µm long respectively. All the sensory spines on legs have swollen tips at their distal end (Figs. 4C, 6A, 6D) and sensory organs on legs IV have a van der Land's organ in the middle part (Fig. 6A, 6D). The cirrus *E*, not measured in the holotype, has a sharp tip, cirrophore and annulated

Table 2. Body measurements (in µm) of *Batillipes binhdinhicus* sp. nov. type series. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information

Character	Holotype, Female MIMB51986	Paratype, Female MIMB51987	Paratype, Female MIMB51990	Paratype, Female MIMB51991	Paratype, Male MIMB51992	Paratype, Male MIMB51993	Paratype, Male MIMB51994	Paratype, Male MIMB51995
Body length	137.3	124.9	126.3	145.3	175.9	177.9	156.9	168.7
Body width	73.0	58.8	62.8	68.2	65.3	56.4	53.6	?
Head width	38.8	40.3	35.9	35.4	37.6	35.9	34.6	?
Pharyngeal bulb (length x width)	23.7 × 20.6	19.8 × 18.6	23.0 × 17.1	25.7 × 22.5	24.2 × 18.5	22.2 × 17.3	18.8 × 17.1	?
Median cirrus	20.6	21.0	?	?	?	?	16.5	19.8
Internal cirri	25.9	22.2	22.7	25.1	27.6	24.4	17.6	19.5
External cirri	16.6	16.0	14.9	14.5	15.9	16.8	11.4	12.7
Lateral cirrus *A*	32.8	36.1	?	34.2	33.3	31.2	?	31.1
Primary clavae	14.8	14.0	14.6	16.8	15.2	14.7	?	13.6
Cirrus *E*	?	15.6	16.2	?	13.4	16.3	?	13.1
Legs I spine	10.2	8.7	9.0	9.9	9.5	9.8	9.0	8.2
Legs II spine	13.3	11.7	11.3	?	11.2	10.0	12.0	10.6
Legs III spine	12.8	12.3	11.0	?	15.5	11.7	13.8	10.5
Legs IV spine	20.8	17.3	15.8	?	19.1	18.2	?	15.6
Caudal apparatus (length x width)	12.4 × 15.7	9 × 13.8	10.7 × 14.4	18.5 × 19.1	16.8 × 19.6	15.6 × 13.1	11.7 × 13.2	?
Gonopore–anus (distance)	12.4	11.4	13.6	11.7	8.1	?	?	?
Legs I toe 1	15.4	?	?	10.4	12.6	10.5	5.6	15.4
toe 2	9.6	7.1	?	6.1	7.6	7.0	?	9.3
toe 3	19.8	12.5	?	14.4	16.1	15.8	12.8	18.0
toe 4	7.1	?	?	9.1	9.3	9.3	6.8	8.5
toe 5	12.0	17.5	?	18.3	22.2	18.3	13.4	?
toe 6	8.3	12.4	?	11.2	10.5	11.1	8.3	?
Legs IV toe 1	14.7	12.8	14.0	?	12.7	14.8	13.4	12.3
toe 2	20.0	17.9	18.6	?	18.7	19.6	17.6	19.5
toe 3	9.2	8.7	9.9	9.4	8.0	9.0	8.3	8.3
toe 4	13.2	12.4	12.7	13.6	10.8	12.5	11.6	10.7
toe 5	25.1	22.8	?	?	24.6	22.8	23.6	21.4
toe 6	14.2	15.1	14.9	?	13.3	12.8	12.6	?

basal portion (Fig. 5C).

All legs have unequal-length toes, possess oval adhesive discs. The first three pairs of legs have toes 3 and 5 that are significantly longer; toes 1 and 6 are of medium length; toes 2 and 4 are the shortest. On the fourth pair of legs the toes conform to the pattern of the B1 group of species proposed by Santos et al. (2018). Specifically, toe 3 (9.2 µm) is shorter than toe 4 (13.2 µm), while toes 2 (20 µm) and 5 (25.1 µm) are the longest (Fig. 6A). Toes 1 and 6 are of intermediate length, 14.7 and 14.2 µm long respectively.

Well-developed scapular region protrudes laterally at the level of the first pair of legs. A conical lateral projection is present between all pairs of legs, slight and blunt between each of the first three pairs of legs, and very prominent longer sharp conical projections

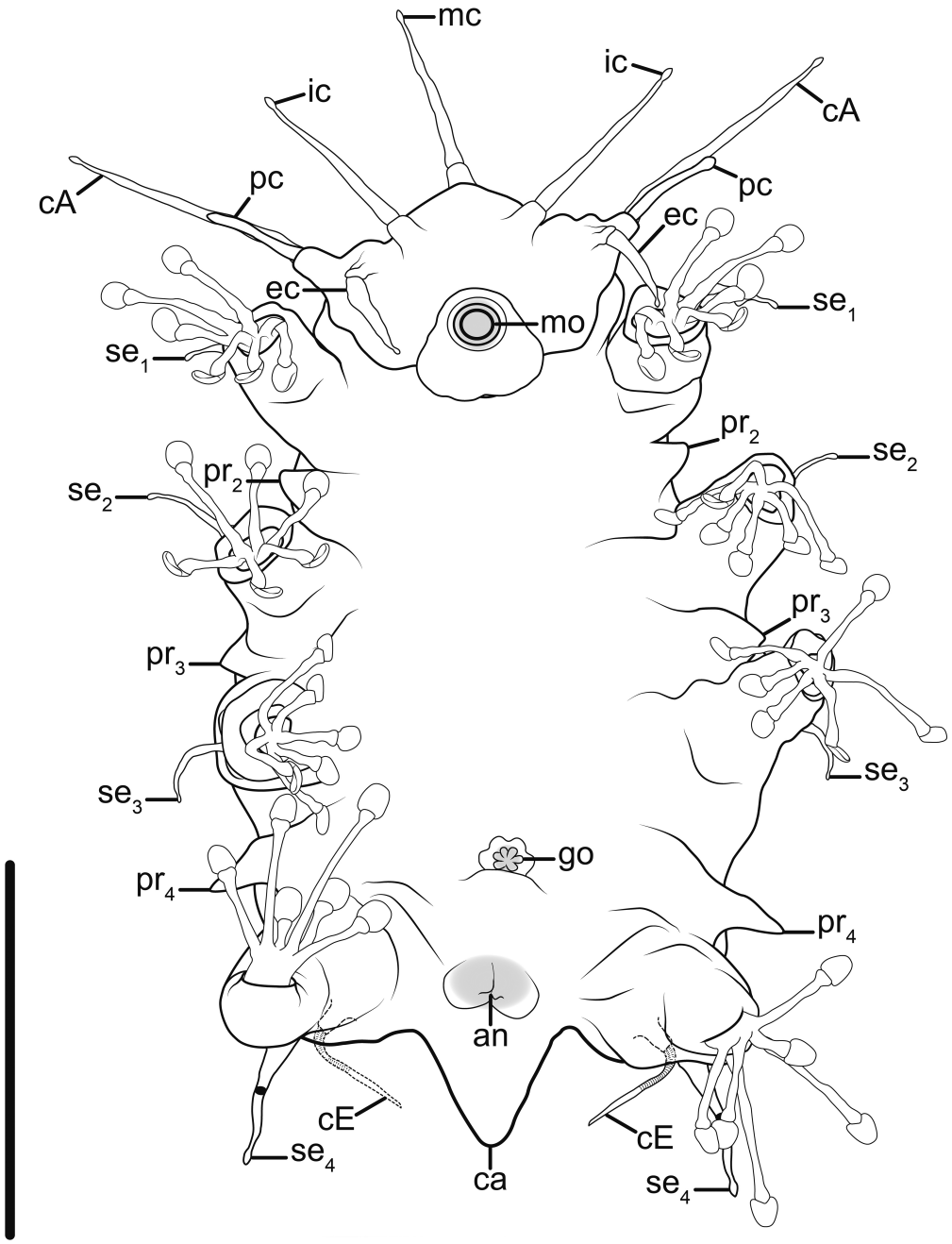

Fig. 1. Drawing of *Batillipes binhdinhicus* sp. nov. based on the holotype. The letter designations are as follows: an, anus; ca, caudal appendage; c*A*, lateral cirrus *A*; c*E*, cirrus *E*; ec, external cirrus; go, gonopore; ic, internal cirrus; mc, median cirrus; mo, mouth opening; pc, primary clava; $pr_{2–4}$, lateral projections; $se_{1–4}$, sensory organ on legs I–IV. Scale bar = 50 µm.

between the third and fourth pairs of legs (Figs. 1, 6B, 6C). A simple triangular appendage, 12.4 µm long and 15.7 µm wide at its widest point, is attached to the caudal base.

The female gonopore is rosette-shaped, composed of six segments, encircled by a fold of cuticle (Fig. 6B, C). The anus is formed by two enlarged cuticular lobes (Fig. 6A), positioned 12.4 µm from the gonopore. The male gonopore is circular with a thick crescent-shaped fold (Fig. 6D).

SEM specimens: All external features are visible in both DIC and SEM (Figs. 3, 4A, 5). SEM allowed to see the structure and details of the organs more precisely; in particular, additional sensory filaments at the swollen tips of the cephalic cirri are clearly visible (Fig. 4A).

Differential diagnosis: *Batillipes binhdinhicus* sp. nov. belongs to subgroup B1 (proposed by Santos et al. 2018), which is distinguished by the specific toe patterns on leg IV (middle toe 3 shorter than middle toe 4). This subgroup contains 12 described species: *Bat. africanus* Morone De Lucia, D'Addabbo Gallo & Grimaldi de Zio, 1988, *Bat. brasiliensis* Santos, Rocha, Gomes Jr & Fontoura, 2017, *Bat. chandrayaani* Vishnudattan, Rubal & Bijoy Nandan, 2024, *Bat. dandarae* Santos, Rocha, Gomes Jr & Fontoura, 2017, *Bat. friaufi* Riggin, 1962, *Bat. homocercus* Bartels & Fontoura, 2021, *Bat. ichthyocercus* Bartels & Fontoura, 2021, *Bat. malaysianus* Chen, Ng, Teng & Kajita, 2025, *Bat. lesteri* Kristensen & Mackness, 2000, *Bat. littoralis* Renaud-Debyser, 1959, *Bat. similis* Schulz, 1955 and *Bat. wyedeleinorum* Bartels, Fontoura, Nelson & Kaczmarek, 2024 (Schulz 1955; Renaud-debyser 1959;

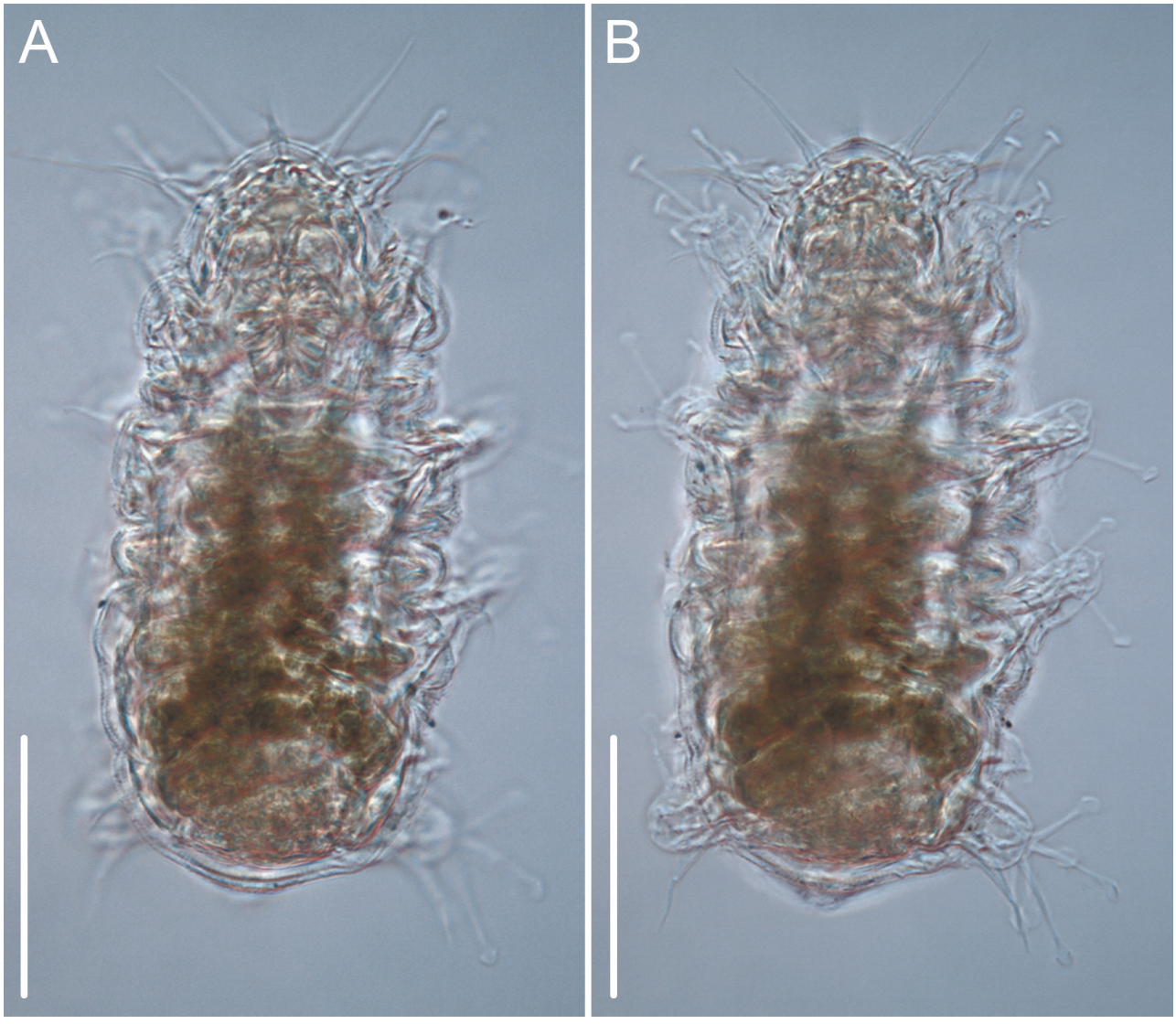

Fig. 2. *Batillipes binhdinhicus* sp. nov. Habitus of female holotype. Scale bar = 50 µm.

Riggin 1962; Morone de Lucia et al. 1988; Kristensen and Mackness 2000; Santos et al. 2017; Bartels et al. 2021 2024; Vishnudattan et al. 2024; Chen et al. 2025). However, *Batillipes binhdinhicus* can be easily distinguished from *Bat. brasiliensis*, *Bat. friaufi*, *Bat. homocercus*, *Bat. ichthyocercus*, *Bat. malaysianus*, *Bat. lesteri* and *Bat. littoralis* by having a simple triangular caudal appendage.

Batillipes binhdinhicus sp. nov. also can be distinguished from other species in subgroup B1

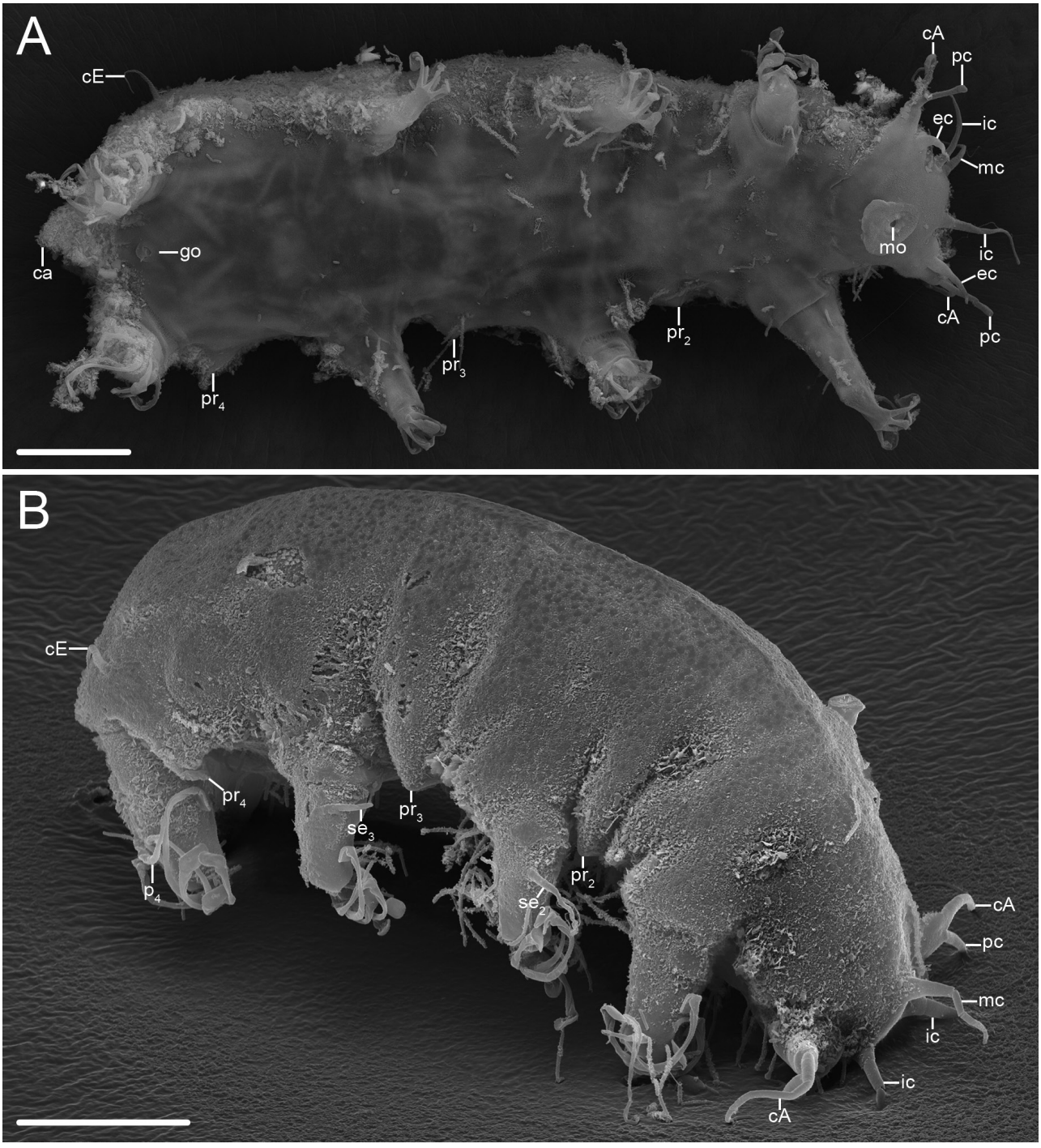

Fig. 3. *Batillipes binhdinhicus* sp. nov. Scanning electron microscopy. A, habitus, ventral view; B, habitus, dorsolateral view. The letter designations are as follows: ca, caudal appendage; c*A*, lateral cirrus *A*; c*E*, cirrus *E*; ec, external cirrus; go, gonopore; ic, internal cirrus; mc, median cirrus; mo, mouth opening; pc, primary clava; pr_{2-4}, lateral projections; se_{1-4}, sensory organ on legs I–IV. Scale bar = 20 μm.

because:

- *Bat. africanus* lacks lateral body projections, especially between legs III–IV, has wedge-shaped caudal appendage with a wide hemispherical base and has shorter legs III and legs IV spine.
- *Bat. chandrayaani* has all cephalic cirri and legs I–III spines sharp tipped, has longer and hook-shaped posteriorly oriented body projection between the legs III and IV, has shorter median cirrus, external cirri and lateral cirrus.
- *Bat. dandarae* lacks all lateral body projections, has caudal apparatus more conical than triangular in shape and has shorter leg IV spine.
- *Bat. similis* has long hook-shaped posteriorly oriented body projection between the legs III and IV, has much longer spine on legs IV (reach up to the tip of the longest toe of leg IV) and has drum-stick like primary clavae.
- *Bat. wyedeleinorum* has much smaller caudal apparatus with S-shaped median thickening, has blunt lateral body projection between the legs

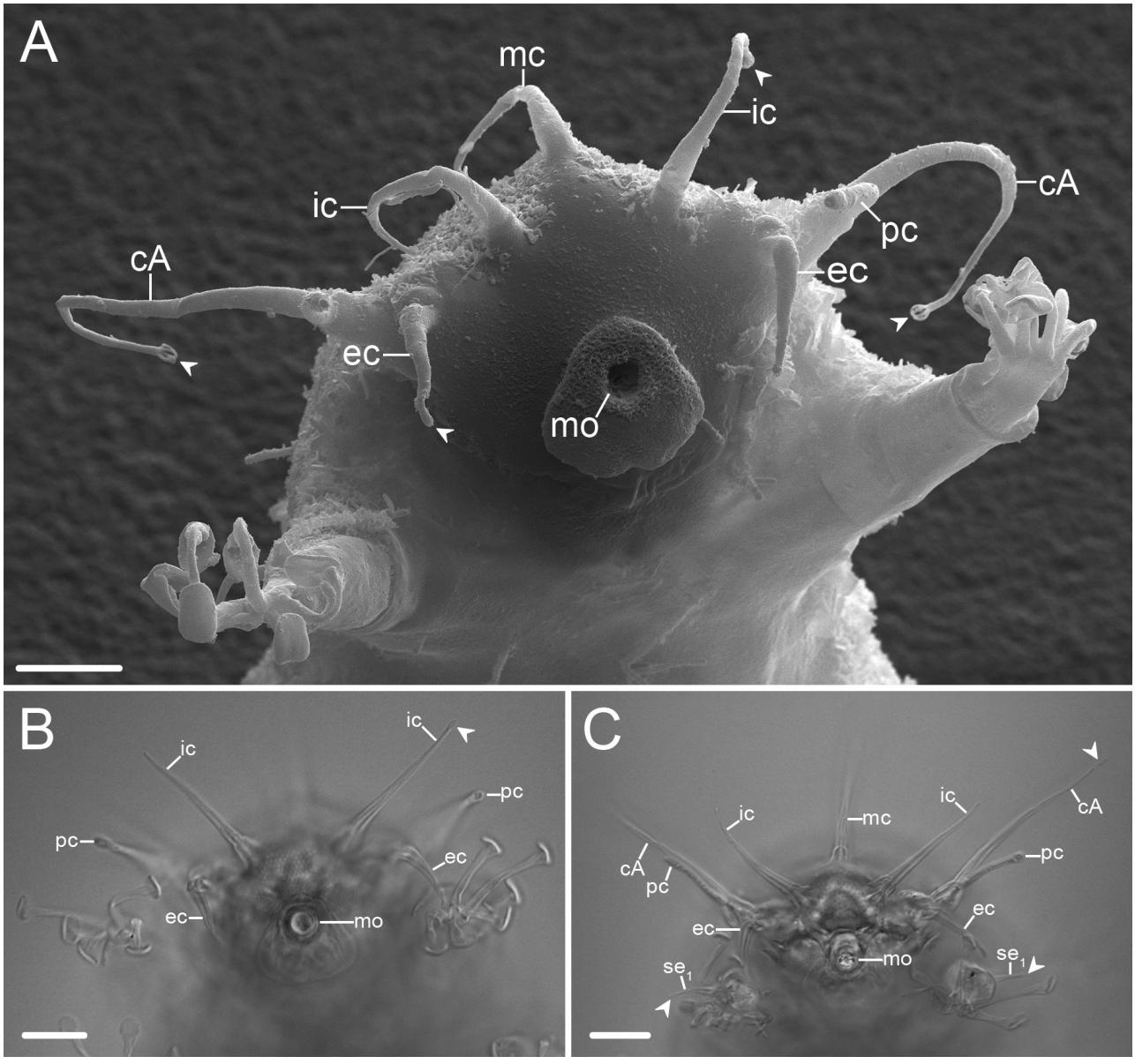

Fig. 4. *Batillipes binhdinhicus* sp. nov. Cephalic region with sensory organs. A, scanning electron microscopy; B, female holotype, ventral view; C, female paratype, ventral view. The letter designations are as follows: c*A*, lateral cirrus *A*; ec, external cirrus; ic, internal cirrus; mc, median cirrus; mo, mouth opening; pc, primary clava; se_1, sensory organ on legs I. Arrows indicate swollen tips with a tuft of additional sensory filaments on cephalic cirri and sensory spine on legs I. Scale bars: A = 5 µm; B, C = 10 µm.

III–IV, has shorter spine on all legs.

Remarks: The high variability in the morphology of the caudal appendage and possibility of using this feature as viable diagnostic character has been discussed by various authors (Morone de Lucia et al. 1988; Villora-Moreno and Grimaldi De Zio 1993; Gallo D'Addabbo et al. 2000; Kristensen and Mackness 2000; Santos et al. 2018). Some authors have noted a significant intraspecific variation in the morphology and size of the caudal appendage, which evidences both between adult individuals as sexual dimorphism and across different life stages. One of the most frequently observed traits is the difference in the number of spines on the caudal appendage. For example, adult individuals of *Bat. noerrevangi* Kristensen, 1978 have two spines, whereas juveniles possess only one (Kristensen 1978; Zawierucha et al. 2015). Similar morphological changes related have been described for *Bat. africanus* (Morone de Lucia et al. 1988). Variability in the morphology of the caudal apparatus also been described for *Bat. dandarae*, *Bat. lesteri*, *Bat. lusitanus* Santos, Rubal, Veiga, da Rocha & Fontoura, 2018, *Bat. pennaki*, *Bat. phreaticus* Renaud-Debyser, 1959 and *Bat. tridentatus* Pollock, 1989 (Pollock 1989; Villora-Moreno and Grimaldi de Zio 1993; Kristensen and Mackness 2000; Santos et al. 2017 2018 2019). Meanwhile, variations in the morphology of the caudal apparatus have not been observed in some other species, and it remains

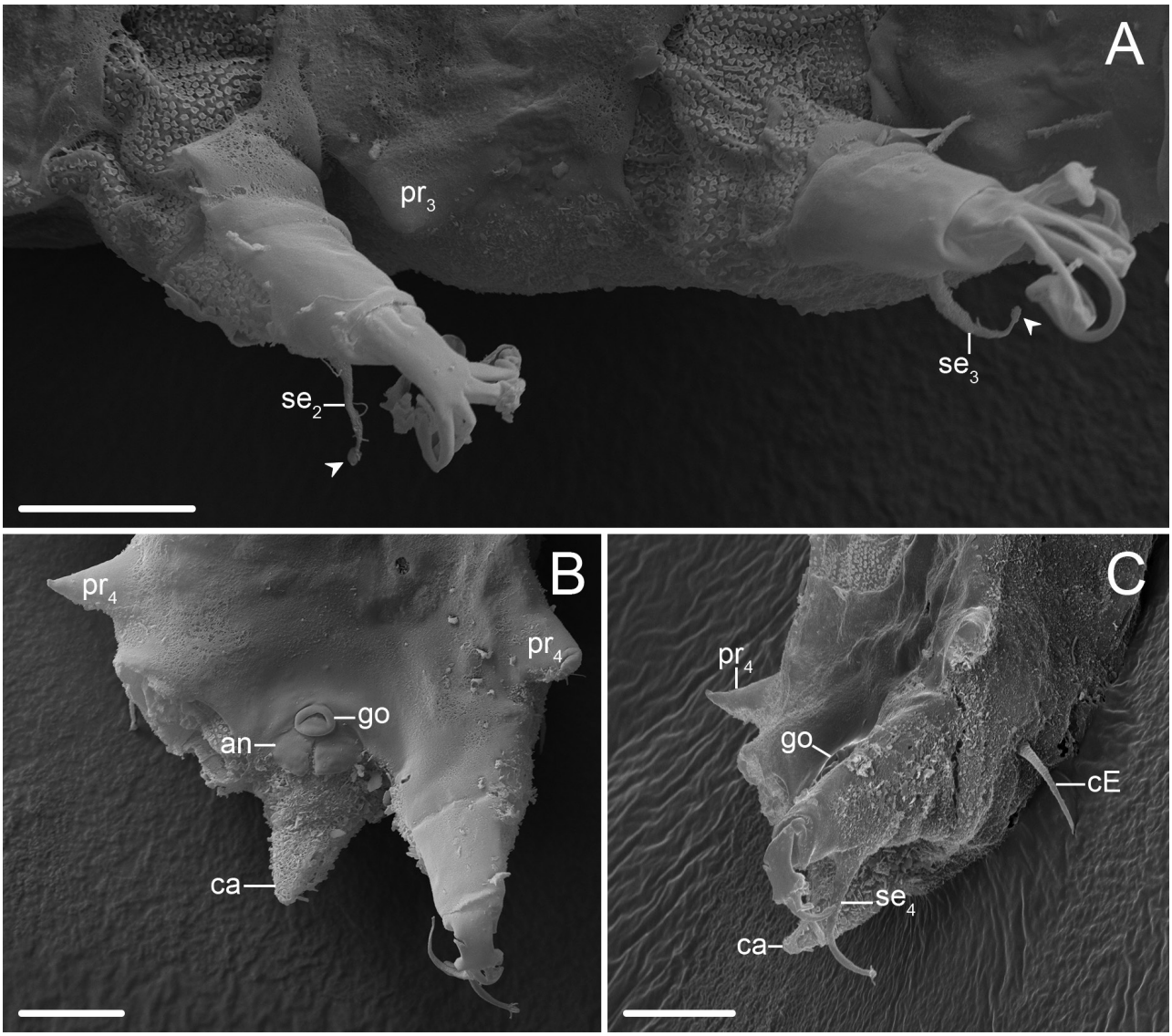

Fig. 5. *Batillipes binhdinhicus* sp. nov. Scanning electron microscopy. A, leg II and III; B, C, caudal region. The letter designations are as follows: an, anus; ca, caudal appendage; c*E*, cirrus *E*; go, gonopore; pr_4, lateral projections; se_4, sensory organ on legs IV. Arrows indicate swollen tips with a tuft of additional sensory filaments on cephalic cirri and sensory spine on legs. Scale bar = 10 μm.

stable for adult specimens (*e.g.*, *Bat. amblypyge* Menechella, Bulnes & Cazzaniga, 2017, *Bat. adriaticus* Grimaldi de Zio, Morone de Lucia, D'Addabbo Gallo & Grimaldi, 1979, *Bat. chandrayaani*, *Bat. crassipes* Tchesunov & Mokievsky, 1995, *Bat. homocercus*, *Bat. lingularum* Menechella, Bulnes & Cazzaniga, 2017, *Bat. minius* Rubal, Veiga, Fontoura & Sousa-Pinto, 2016, *Bat. philippinensis*, *Bat. potiguarensis* Santos, Rocha, Gomes Jr & Fontoura, 2017, *Bat. solitarius* Jørgensen, Boesgaard, Møbjerg & Kristensen, 2014, *Bat. spinicauda* D'Addabbo Gallo, Sandulli & Grimaldi de Zio, 2005) (Grimaldi de Zio et al. 1978; Tchesunov and Mokievsky 1995; Chang and No 1997; D'Addabbo et al. 2005; Jørgensen et al. 2014; Rubal et al. 2017; Santos et al. 2017; Menechella et al. 2018; Bartels et al. 2021; Vishnudattan et al. 2024). In this study, we did not observe significant morphological intraspecific variation of the caudal appendage among individuals of *Batillipes binhdinhicus* sp. nov., except for differences in length. Based on this, we suggest considering it as one of the distinguishing features for differential diagnosis for our species.

Nucleotide sequences

GenBank accession numbers: PQ778920–PQ778923, PQ778925, PQ778927–PQ778928 (for 28S rDNA); PQ778911–PQ778914, PQ778916, PQ778918–PQ778919 (for 18S rDNA).

***Batillipes ampullus* sp. nov.**

(Figs. 7–12; Tables 3, S5)
urn:lsid:zoobank.org:act:31CC2274-2770-4407-8ED1-283760BF28FF

Type locality: Seagrass bed of *Zostera japonica*, Thi Nai Lagoon, Binh Dinh province, Vietnam (13°48'38"N, 109°13'40"E), medium sand.

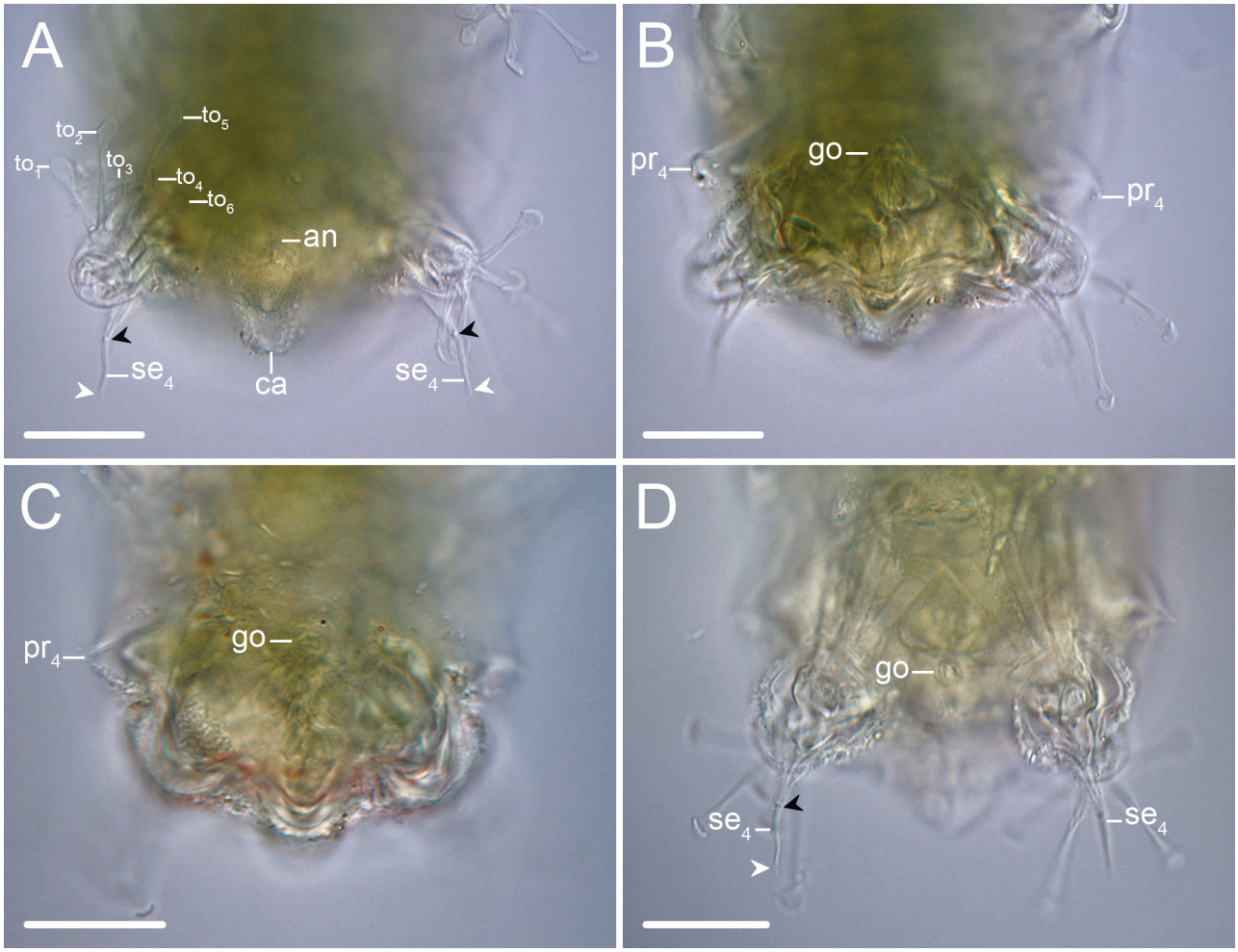

Fig. 6. *Batillipes binhdinhicus* sp. nov. A–C, caudal region of female holotype; D, caudal region of male paratype. The letter designations are as follows: an, anus; ca, caudal appendage; go, gonopore; pr_4, lateral projections; se_4, sensory organ on legs IV; $to_{1–6}$, toe 1 to toe 6. White arrows indicate swollen tips on legs IV spine, black arrows showing van der Land's organs. Scale bar = 20 µm.

Type material: The female holotype (No. MIMB51988); two female paratypes (No. MIMB51989 and MIMB51996), and four male paratypes (No. MIMB51997–MIMB52000), collected from Thi Nai Lagoon, Binh Dinh province, Vietnam. All material deposited in the Museum of the A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS (Vladivostok, Russia).

Other material: two SEM specimens (one male and one of unknown sex).

Etymology: The species name "ampullus" refers to the bottle-like shape of the sensory organ on legs IV.

Diagnosis: All cephalic cirri have swollen tips with a tuft of additional sensory filaments. The scapular region is well-developed, projecting laterally at the level of the first pair of legs. There are no discernible lateral body projections between the legs, particularly between the third and fourth pairs of legs. All legs have sensory spines, but the bottle-shaped sensory organs on the fourth pair of legs are shorter and consist of two parts: a broad basal and a slender distal one. The distal end of the leg IV spine is notable for possessing a tuft of additional sensory filaments that are structurally similar to those found on the cephalic appendages. Each leg bears six toes of varying length, ending in oval adhesive pads. On the fourth pair of legs, the middle toes (3 and 4) are of equal length. The body ends in a semicircular caudal appendage.

Description: The holotype is an adult female (Fig. 8) with body 165.6 μm long, including the caudal apparatus, and 64.4 μm wide between the second and third pair of legs. The head is typically trapezoidal in shape, measuring 48.1 μm in width, and has eleven cephalic appendages (Figs. 7, 9B, 9C). The unpaired median cirrus is 15.2 μm in length and is positioned at the large cirrophore. Paired internal cirri are inserted on the frontal edge of the head and are 18.2 μm long, also with large cirrophores. Paired external cirri are 11 μm in length, exhibit indistinct cirrophores and are inserted close to the lateral cirri and primary clavae. Tubular primary clavae are 11.6 μm long, with a constriction in front of a round tip, situated ventrally to the long lateral cirrus (28.4 μm) on a shared pedestal. All cephalic cirri with swollen tips with a tuft of additional sensory

Table 3. Body measurements (in μm) of *Batillipes ampullus* sp. nov. type series. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information

Character	Holotype, Female MIMB51988	Paratype, Female MIMB51989	Paratype, Female MIMB519996	Paratype, Male MIMB51997	Paratype, Male MIMB519998	Paratype, Male MIMB51999	Paratype, Male MIMB52000
Body length	165.6	212.9	218.0	178.0	167.9	149.4	121.7
Body width	64.4	53.0	?	49.9	?	65.7	43.9
Head width	48.1	39.0	?	40.6	?	41.6	37.9
Pharyngeal bulb (length × width)	19.4 × 16.6	20.2 × 17.1	?	19.0 × 15.6	?	18.0 × 17.2	14.5 × 14.2
Median cirrus	15.2	16.2	13.9	12.6	17.8	14.9	11.8
Internal cirri	18.2	22.7	17.6	21.0	18.6	19.0	17.7
External cirri	11.0	12.2	14.5	11.6	10.7	10.9	12.1
Lateral cirrus *A*	28.4	27.6	29.2	27.7	22.7	29.6	21.0
Primary clavae	11.6	15.3	13.4	12.9	9.4	12.9	12.0
Cirrus *E*	18.1	16.1	?	24.4	18.6	21.9	18.2
Legs I spine	6.4	6.1	7.5	7.2	6.5	5.2	6.0
Legs II spine	8.7	7.2	8.1	8.3	?	6.6	8.3
Legs III spine	8.3	7.7	7.6	8.1	7.3	6.3	6.5
Legs IV spine	4.2	6.7	6.8	6.0	5.4	5.9	5.3
Caudal apparatus (length × width)	11.6 × 31.4	13.4 × 31.3	?	12.7 × 25.7	?	12.7 × 23.1	?
Gonopore-anus (distance)	8.3	?	?	8.8	?	12.7	?
Legs I toe 1	16.2	23.2	21.2	16.5	18.9	22.6	10.8
toe 2	11.0	16.1	15.2	9.3	12.9	13.8	7.7
toe 3	18.1	20.7	23.4	?	?	?	18.1
toe 4	10.1	17.7	10.8	?	8.5	12.3	9.8
toe 5	12.7	14.2	16.7	12.0	15.2	21.2	19.5
toe 6	10.2	9.8	11.1	10.0	11.4	15.1	12.6
Legs IV toe 1	14.9	14.3	18.7	11.6	9.8	18.8	14.3
toe 2	22.4	22.9	24.2	18.1	13.0	22.8	19.7
toe 3	13.8	13.7	15.1	8.1	7.3	9.9	12.7
toe 4	14.1	13.8	15.8	8.6	7.6	10.1	12.4
toe 5	22.3	25.6	28.4	?	14.7	23.6	23.3
toe 6	15.3	16.8	?	15.4	?	17.4	16.3

filaments. Secondary clavae, eye spots, stylets and stylets support are not visible. The ovoid pharyngeal bulb is 19.4 µm long and 16.6 µm wide.

Each of the telescopic legs has a sensory spine. The sensory spines on legs I, II and III are simple spines, without clear cirrophores, measure 6.4 µm, 8.7 µm and 8.3 µm in length, respectively, and have swollen tips. The sensory organ on leg IV is shorter (4.2 µm), also without clear cirrophores, bottle-like in shape and divided into two segments: a broad basal and a slender distal segment. The distal end of leg IV's sensory spine is distinguished by a tuft of additional sensory filaments that bear a structural resemblance to those found on the cephalic appendages (Fig. 12A, 12B).

Toes are unequal in length, with oval adhesive discs. The first three pairs of legs have toes 1 and 3 that

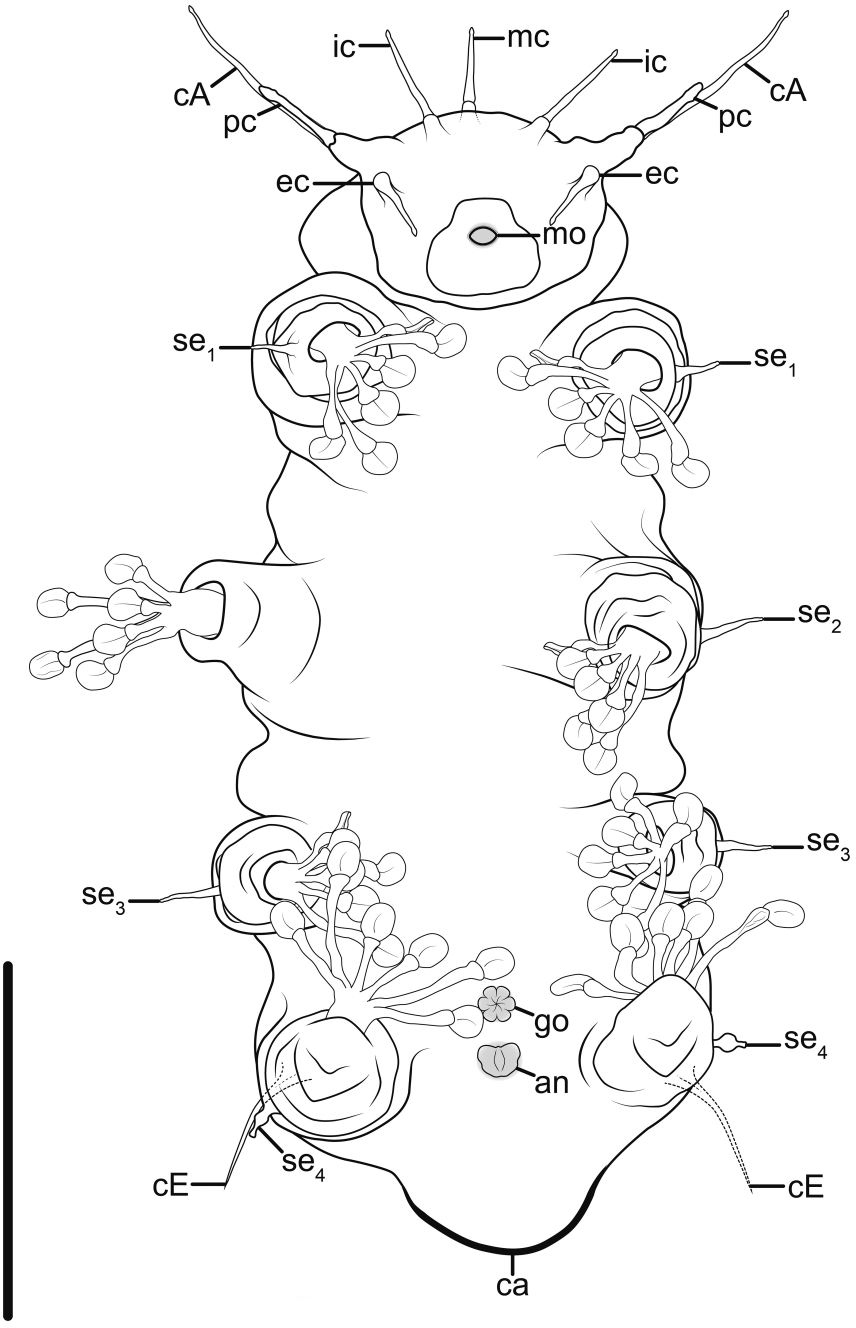

Fig. 7. Drawing of *Batillipes ampullus* sp. nov. based on the holotype. The letter designations are as follows: an, anus; ca, caudal appendage; c*A*, lateral cirrus *A*; c*E*, cirrus *E*; ec, external cirrus; go, gonopore; ic, internal cirrus; mc, median cirrus; mo, mouth opening; pc, primary clava; $se_{1–4}$, sensory organ on legs I–IV. Scale bar = 50 µm.

are significantly longer; toes 2 and 5 are of medium length; toes 4 and 6 are the shortest. On the fourth pair of legs the toes conform to the pattern of the A group of species proposed by Kristensen and Mackness (2000). Specifically, toe 3 and toe 4 exhibit approximate equivalency in length (13.8 and 14.1 µm, respectively), while toes 2 and 5 are longer (22.4 and 22.3 µm long respectively). Toes 1 and 6 are 14.9 and 15.3 µm long respectively, with toe 6 being slightly shorter than toe 1.

The cirrus *E* is sharply pointed and medium in length (18.1 µm long) with small cirrophore.

Well-developed scapular region protrudes laterally at the level of the first pair of legs. There are no obvious lateral body projections between the legs, especially between the third and fourth pairs of legs. The caudal appendage is semi-circular, protruding ca. 11.6 µm from the body.

The female gonopore is rosette-shaped with six segments (Fig. 12B). The anus is constituted by two enlarged cuticular lobes, situated 8.3 µm from the gonopore (Fig. 12B). The male gonopore is simply circular with thick folds (Fig. 12A).

SEM specimens: All external features are observable in both DIC and SEM (Figs. 9A, 10, 11). In some cases, SEM allows for more precise structure and detail of organs; in particular, additional sensory filaments at the swollen tips of the cephalic cirri are clearly visible (Fig. 9A).

Differential diagnosis: The newly discovered species, *Batillipes ampullus* sp. nov., is classified within the A group of species (Kristensen and Mackness 2000) based on the toe pattern of leg IV. Specifically, the middle toes 3 and 4 are similar in length and are less than or equal to toes 1 and 6, while toes 2 and 5 are the longest. There are 27 known species included in this group. However, similar to the new species, only four other species have a blunt or semi-circular caudal protrusion: *Bat. lusitanus*, *Bat. potiguarensis*, *Bat. rotundiculus* Rho & Chang, 1999 and *Bat. solitarius* (Rho et al. 1999; Jørgensen et al. 2014; Santos et al. 2017 2018). *Batillipes ampullus* sp. nov. exhibits significant distinguishing characteristics compared with

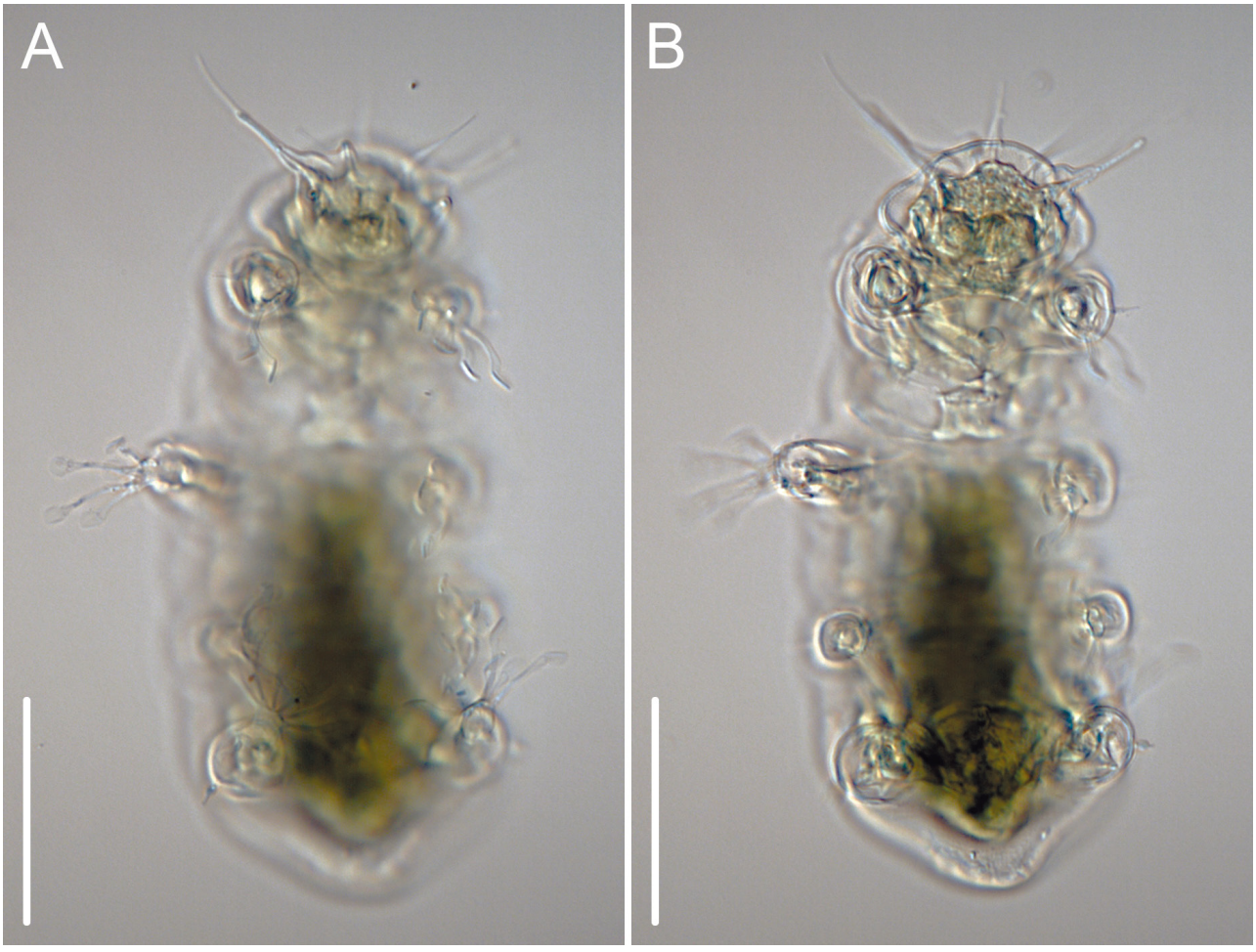

Fig. 8. *Batillipes ampullus* sp. nov. Habitus of female holotype. Scale bar = 50 µm.

all these species. These include the length and structure of the spine on legs IV, and the absence of pronounced lateral body projections, especially between the third and fourth pairs of legs. The spine organ on legs IV in *Batillipes ampullus* sp. nov. is rather short compared to other species, bottle-like in shape and divided into two parts: a broad basal and a slender distal. The distal tips of legs IV spine possess a tuft of additional sensory filaments, which bear a structural resemblance to those observed on the cephalic appendages.

Additionally, *Batillipes ampullus* sp. nov. differs because:

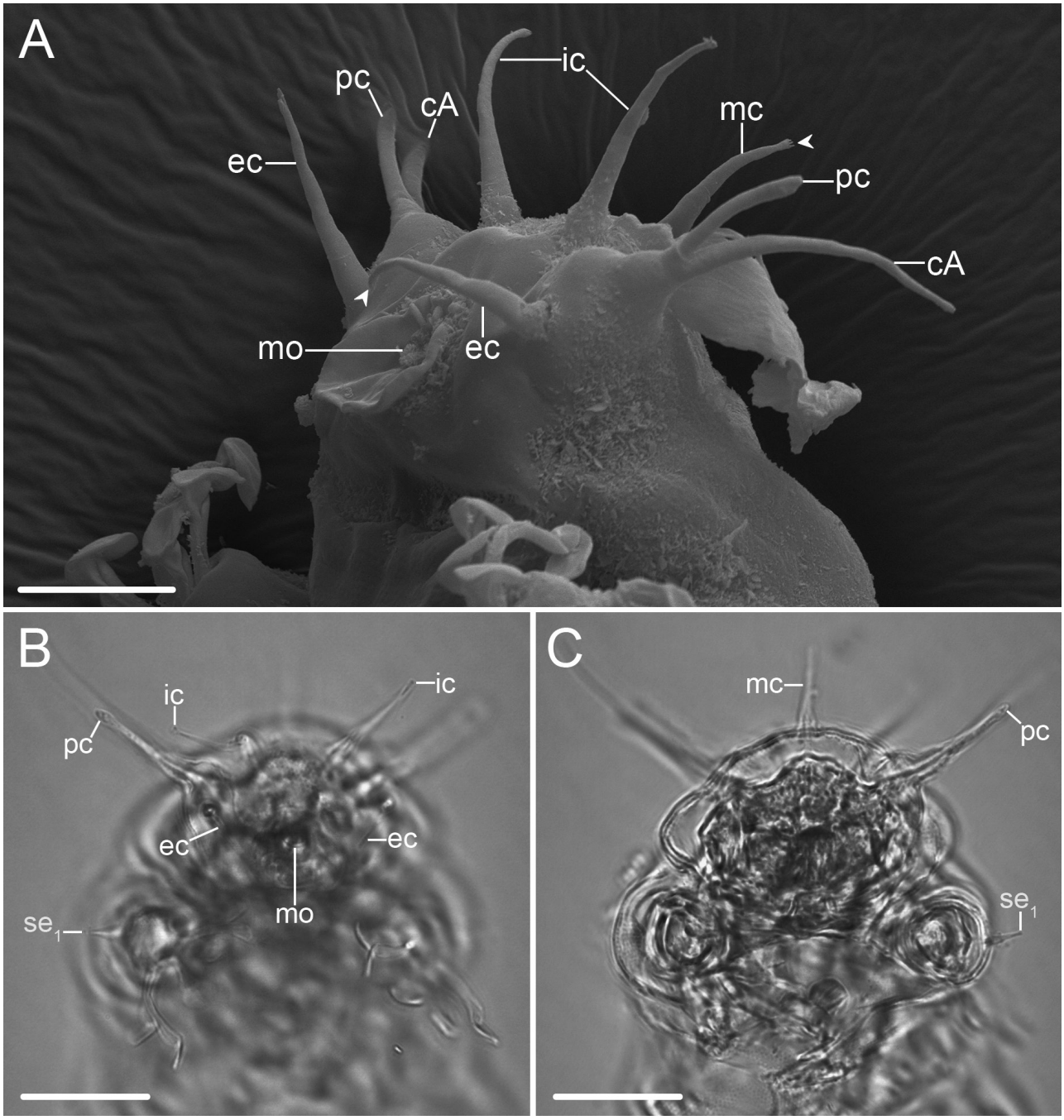

Fig. 9. *Batillipes ampullus* sp. nov. Cephalic region with sensory organs. A, scanning electron microscopy; B, C, Female holotype, ventral view. The letter designations are as follows: c*A*, lateral cirrus *A*; ec, external cirrus; ic, internal cirrus; mc, median cirrus; mo, mouth opening; pc, primary clava; se_1, sensory organ on legs I. Arrows indicate swollen tips with a tuft of additional sensory filaments on cephalic cirri. Scale bars: A = 10 μm; B, C = 20 μm.

- *Bat. lusitanus* has primary clavae with a wrinkled surface, has a small rigid process in the distal extreme of legs IV, has very long cephalic appendages and long legs spines.
- *Bat. potiguarensis* has sense organ on legs I divided into a basal and a distal portion.
- *Bat. rotundiculus* has a short spiky spine on legs IV femur, has sharply pointed cephalic cirri.
- *Bat. solitarius* has very long cephalic appendages, has internal cirri shorter than external cirri.

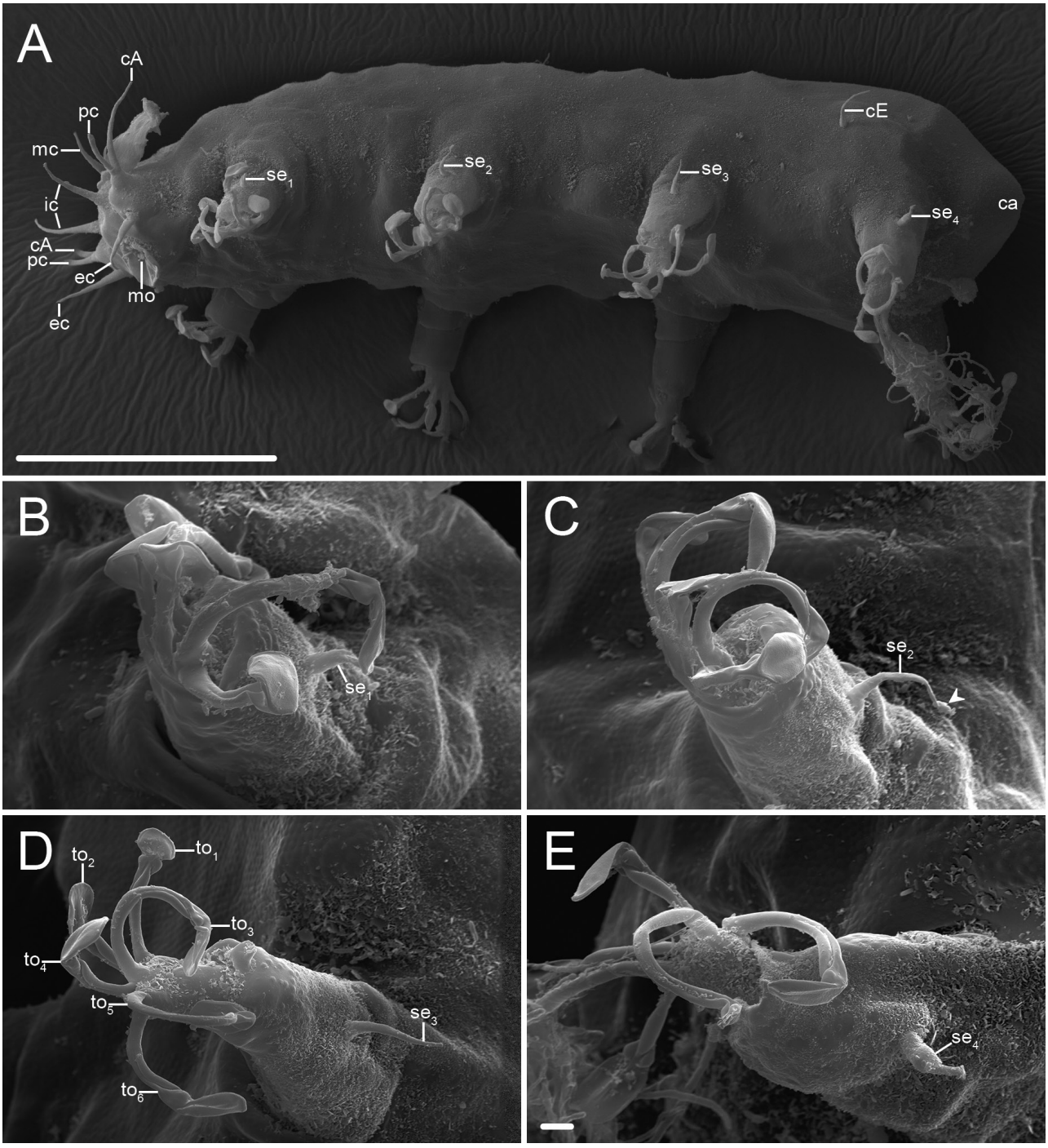

Fig. 10. *Batillipes ampullus* sp. nov. Scanning electron microscopy. A, habitus, ventrolateral view; B, leg I with spine; C, leg II with spine that exhibits swollen tip (arrow); D, leg III with spine; E, leg IV with spine. The letter designations are as follows: ca, caudal appendage; c*A*, lateral cirrus *A*; c*E*, cirrus *E*; ec, external cirrus; ic, internal cirrus; mc, median cirrus; mo, mouth opening; pc, primary clava; $se_{1–4}$, sensory organ on legs I–IV; $to_{1–6}$, toe 1 to toe 6. Scale bars: A = 50 µm; B–E = 2 µm.

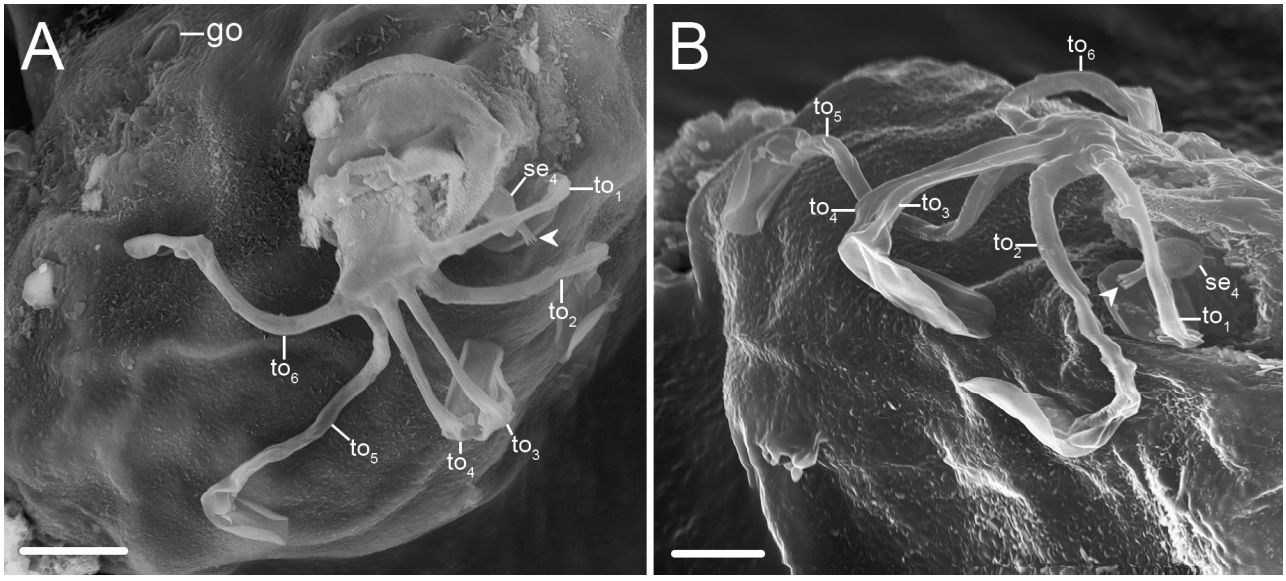

Fig. 11. *Batillipes ampullus* sp. nov. Scanning electron microscopy. Caudal region, showing legs IV with toes and sensory spine, and male gonopore. The letter designations are as follows: go, gonopore; se_4, sensory organ legs IV; $to_{1–6}$, toe 1 to toe 6. Arrows indicate a tuft of additional sensory filaments on legs IV spine. Scale bars: A = 5 µm; B = 3 µm.

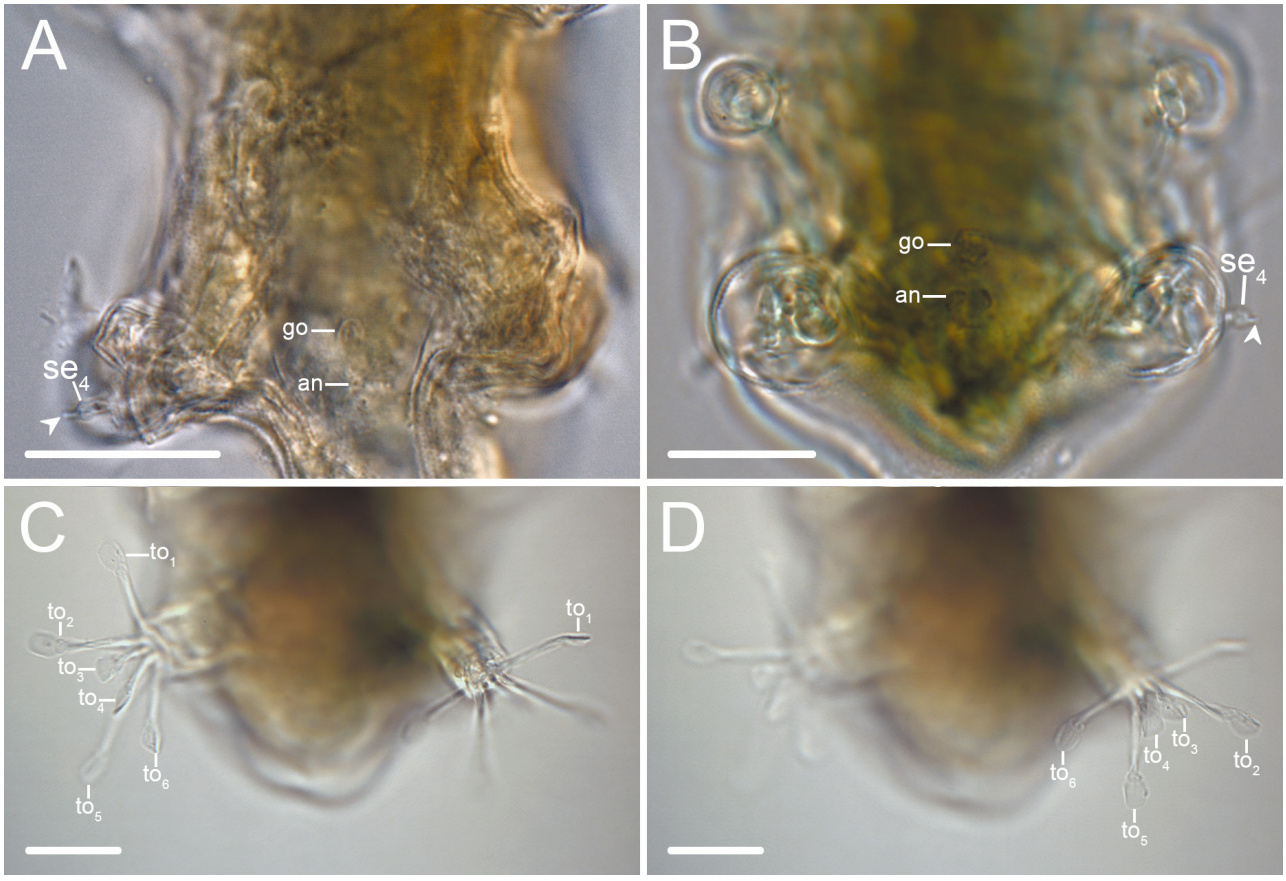

Fig. 12. *Batillipes ampullus* sp. nov. A, caudal region of male paratype, showing male gonopore, anus and sensory spine on legs IV with slender distal part (arrows); B, caudal region of female holotype, showing female gonopore, anus and sensory spine on legs IV with slender distal part (arrows); C, D, toes of female paratype. The letter designations are as follows: an, anus; go, gonopore; se_4, sensory organ on legs IV; $to_{1–6}$, toe 1 to toe 6. Scale bar = 20 µm.

Remarks: As for *Batillipes binhdinhicus* sp. nov., there was no intraspecific variation in the number and shape of the caudal appendage among the individuals of *Batillipes ampullus* sp. nov. Based on this, we suggest considering it as one of the distinguishing features for differential diagnosis for our species

Nucleotide sequences

GenBank accession numbers: PQ778924, PQ778926 (for 28S rDNA); PQ778915, PQ778915 (for 18S rDNA).

Family Halechiniscidae Thulin, 1928
Subfamily Orzeliscinae Schulz, 1963

Genus *Orzeliscus* du Bois-Reymond Marcus, 1952
Orzeliscus asiaticus Lee, Rho & Chang, 2017
(Fig. 13, Tables 4, S6)

Material examined: Two female specimens collected from seagrass bed of *Zostera japonica*, Thi Nai Lagoon, Binh Dinh province, Vietnam (13°48'39"N, 109°13'41"E), medium sand.

Morphological comments: Specimens with stocky body, 180.1–193.7 µm long and 104.4 µm width. Head had neck constriction and bears eleven cephalic appendages: unpaired median cirrus (16.5 µm), paired internal cirri (18.6–19.2 µm), paired external cirri (14.1–14.7 µm), paired lateral cirrus *A* (25.3–29.7 µm) and paired primary clavae (12.4–15.7 µm). All cephalic cirri exhibit a consistent pattern: well-developed scapus at the base, followed proximally by a flagellum, and terminating distally in a sensillum. Slender primary clavae situated with lateral cirrus on a shared pedestal. Eye spots were not observed. Legs with fourth blade-shaped digits with suction pads. Legs I–III have sensory spines, 8.1 µm, 12.4 µm and 16.9 µm respectively. Sensory spine on legs IV was not observed. Cirrus *E* 23.6 µm long, situated dorsally at the lateral corner of body. The female gonopore consists of six rosette-like cells, located at 13 µm from the anus. Both of our specimens generally match the description of *Orzeliscus asiaticus* Lee, Rho & Chang, 2017 (Lee et al. 2017).

Subfamily: Halechiniscinae Thulin, 1928
Genus *Halechiniscus* Richters, 1908

Halechiniscus sp.
(Fig. 14, Tables 5, S7)

Material examined: Three specimens of unknown sex collected from Van Phong Bay, Khanh Hoa province, Vietnam (12°38'38"N, 109°12'18"E), coarse sand with gravel.

Morphological comments: Short elongated body, 119.7–187.4 µm long and 39.4–73.8 µm width. Head with long paired cephalic appendages: internal cirri (21.1–28.4 µm), external cirri (14.8–18.7 µm), lateral cirrus *A* (24.6–35.8 µm) and primary clavae (13–21.7 µm). Median cirrus was not observed. Legs

Table 4. Body measurements (in µm) of *Orzeliscus asiaticus*. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information

Character	Specimen 1, Female	Specimen 2, Female
Body length	193.7	180.1
Body width	104.4	?
Median cirrus	16.5	?
Internal cirri	19.2	18.6
External cirri	14.1	14.7
Lateral cirrus *A*	29.7	25.3
Primary clavae	15.7	12.4
Cirrus *E*	23.6	?
Legs I spine	8.1	?
Legs II spine	12.4	?
Legs III spine	16.9	?
Legs IV spine	?	?
Gonopore-anus (distance)	13.0	?

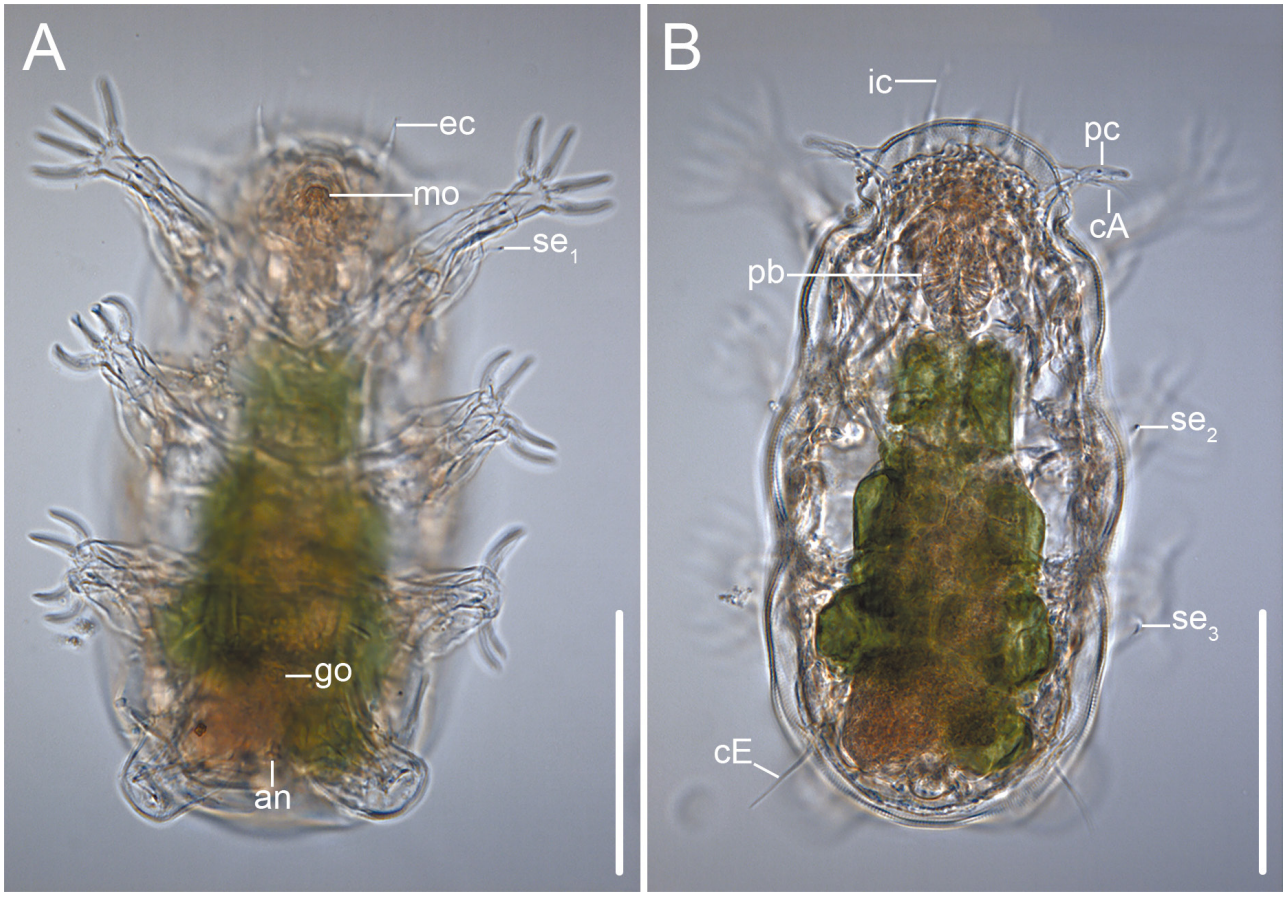

Fig. 13. *Orzeliscus asiaticus*. A, ventral view; B, dorsal view. The letter designations are as follows: an, anus; c*A*, lateral cirrus *A*; c*E*, cirrus *E*; ec, external cirrus; go, gonopore; ic, internal cirrus; mo, mouth opening; pb, pharyngeal bulb; pc, primary clava; se_{2-3}, sensory organ on legs II–III. Scale bar = 50 µm.

Table 5. Body measurements (in µm) of *Halechiniscus* sp. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information

Character	Specimen 1	Specimen 2	Specimen 3
Body length	187.4	138.8	119.7
Body width	73.8	54.2	39.4
Internal cirri	28.4	25.9	21.1
External cirri	14.8	15.3	18.7
Lateral cirrus *A*	33.8	34.8	24.6
Primary clavae	21.7	13.0	?
Cirrus *E*	43.1	?	29.7
Legs I spine	?	?	?
Legs II spine	18.3	?	12.9
Legs III spine	22.7	?	15.4
Legs IV spine	21.0	21.9	17.5

bear four digits, each with simple crescent-shaped claws. Each leg has a sensory spine. The first legs spine was not measurable, legs II–IV spines are 12.9–18.3 µm, 15.4–22.7 µm and 17.5–21.9 µm respectively. Long cirrus *E* 29.7–43.1 µm. Gonopore and anus were not recognized. Unfortunately, due to the poor condition of the specimens, we were unable to identify strong distinguishing features. Therefore, we identified the *Halechiniscus* specimens only at the genus level.

Molecular analyis

A total of 9 *Batillipes* specimens were sequenced for two gene fragments (voucher numbers BT1, BT2, BT5, BT6, BT7, TB4, VT2, VT3, VT24). According to the morphological analysis (see above), the obtained samples belong to 2 species: *Batillipes ampullus* sp. nov. – BT2, BT6, *Batillipes binhdinhicus* sp. nov. – BT1, BT5, BT7, TB4, VT2, VT3, VT24. Based on the 28S rRNA gene fragments, a phylogenetic tree was constructed (Fig. 15) including *Batillipes* species from GenBank. Members of the genus *Batillipes* form a robust clade (Bootstrap value, BS = 100, Bayesian posterior probability, PP = 1.00), within which *Bat. binhdinhicus* sp. nov. and *Bat. ampullus* sp. nov. occupy phylogenetically distinct positions, localizing to different subclades. *P*-distances within studied species were 0% and *p*-distance between *Batillipes binhdinhicus* sp. nov. and *Batillipes ampullus* sp. nov. was 8.81% (Table S2). The distances between species of the genus *Batillipes* ranged from 1.54% to 9.91%.

Based on a fragment of the 18S rRNA gene, a phylogenetic tree was also constructed (Fig. 16). The genus *Batillipes* forms a well-supported monophyletic group (BS = 100, PP = 1.0) consisting of two distinct subclades, comprising *Bat. binhdinhicus* sp. nov. and *Bat. ampullus* sp. nov., respectively. *P*-distance between species *Batillipes binhdinhicus* sp. nov. and *Batillipes ampullus* sp. nov. was 3.32% (Table S3). *P*-distances between species of the genus *Batillipes* and other species ranged from 14.14% to 16.65%.

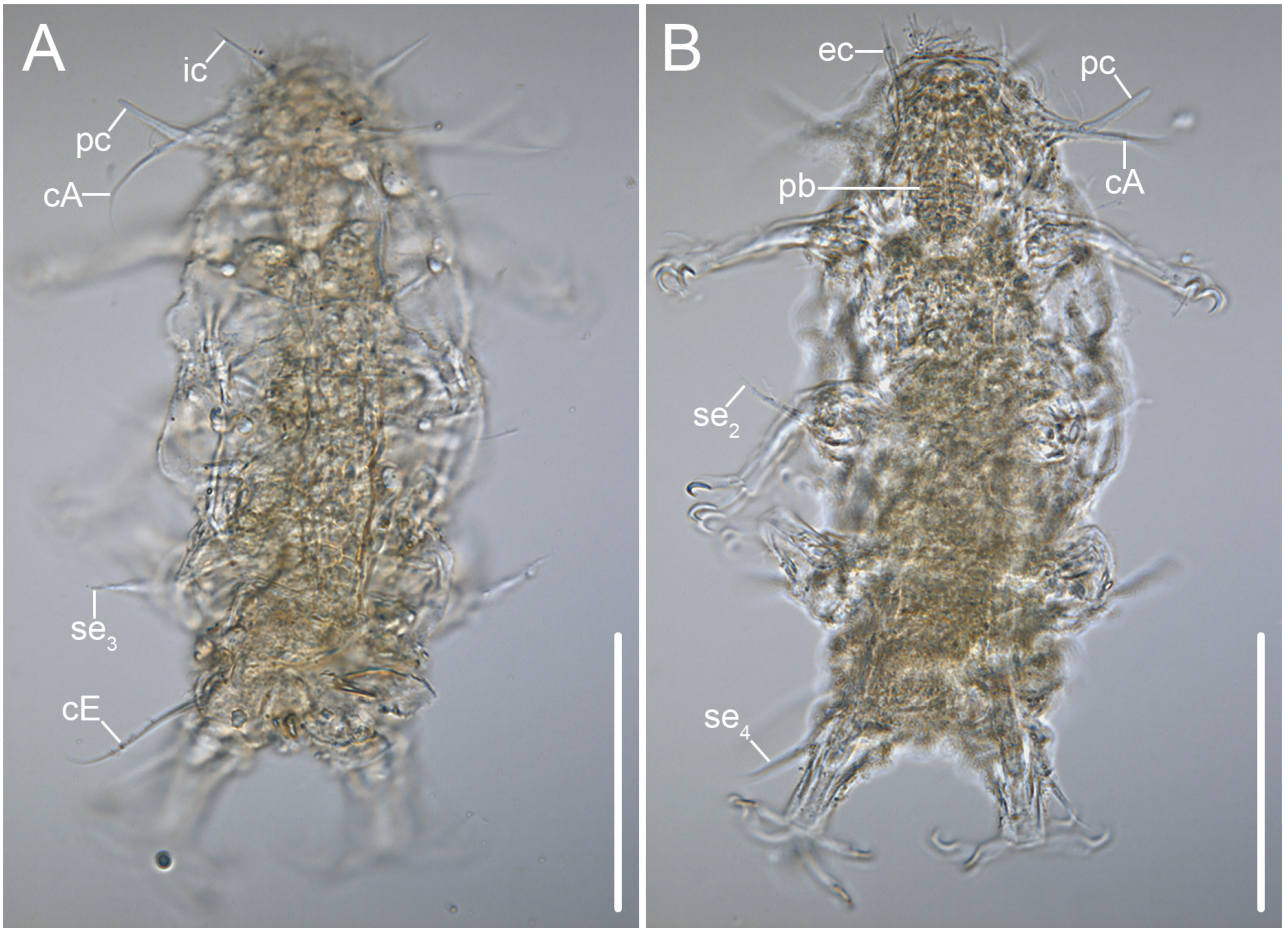

Fig. 14. *Halechiniscus* sp. A, dorsal view; B, ventral view. The letter designations are as follows: c*A*, lateral cirrus *A*; c*E*, cirrus *E*; ec, external cirrus; ic, internal cirrus; pb, pharyngeal bulb; pc, primary clava; se_{2-4}, sensory organ on legs II–IV. Scale bar = 50 µm.

DISCUSSION

A comparative molecular genetic analysis of *Batillipes ampullus* sp. nov. BT2+BT6 and *Batillipes binhdinhicus* sp. nov. BT1+BT5+BT7+TB4+VT2+VT3 +VT24 samples revealed that the genetic distances for genes coding 18S rDNA and 28S rDNA correspond to interspecific differentiation among tardigrades (Gąsiorek et al. 2017; Sugiura et al. 2020; Kayastha et al. 2021; Tumanov et al. 2023). For example, the *p*-distances between 28S rDNA sequences among different species of the genus *Mesobiotis*, ranged from 5% to 13% (Kayastha et al. 2021). Recently, Tumanov et al. (2023), found that *p*-distances according to 28S rDNA data between individuals of the same species were less than 2%, while those between different species averaged 18.29%. In the genus *Ramazzottius*, the *p*-distances ranged from 2.74% to 6.72%, and in the genus *Pilatobius* they reached up to 9.77%. The average intraspecific *p*-distances from the 18S rRNA gene fragment data for individuals of the same species, *Milnesium pacificum* Sugiura, Minato, Matsumoto & Suzuki, 2020, were found to be 0.3% according to Sugiura et al. (2020). The uncorrected genetic *p*-distances between the *Astatumen tenue* and other *Hypsibioidea* species ranged from 3.52% to 14.24% (Tumanov et al. 2023). The average genetic distance between species of *Pilatobius* based on 18S rDNA sequences was 1.7% (Gąsiorek et al. 2017). Therefore, based on the analysis of the 18S rRNA gene fragment, the tardigrade individuals presented in this study likely do not belong to the same species but may belong to the same genus. Furthermore, the validity of the studied species of *Batillipes* is supported by the distribution of sequences into differentiated (distinct) subclades within a common genus, supported by strong nodal statistical

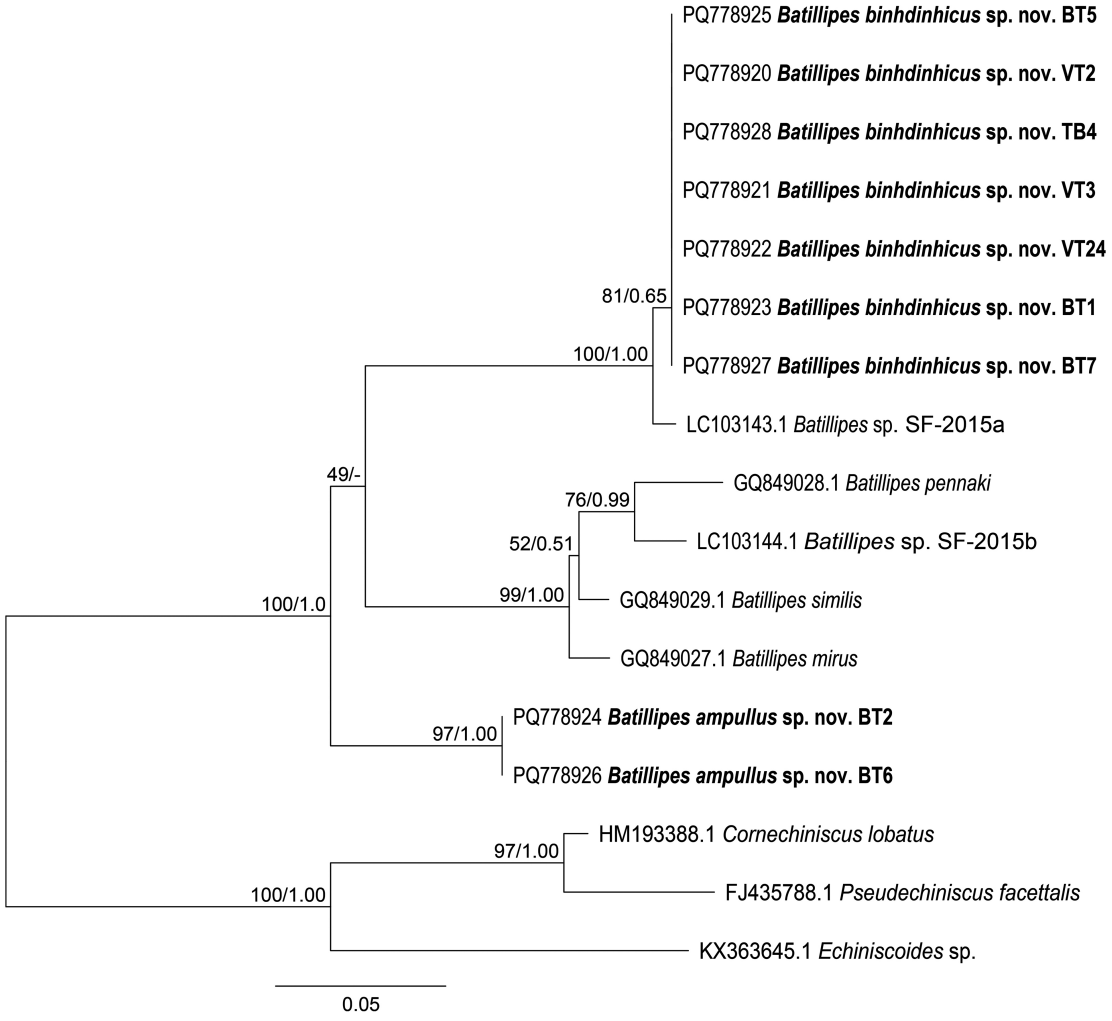

Fig. 15. A Bayesian phylogenetic tree based on 28S rDNA sequences, using K2+G model of nucleotide substitution. Numbers at nodes indicate bootstrap values after IQ-Tree3 (BS, first values) and Bayesian posterior probability values (PP, second values).

evidence.

Both new species possess cephalic cirri with tips that feature a tuft of additional sensory filaments. This identical character was originally outlined by McKirdy (1975) as a common but at the same time difficult-to-observe structure. Gallo D'Addabbo et al. (2000) provide a summary of these observations. The filamentous part of the sensory organs has also been illustrated in *Bat. orientalis* (Chang and Rho 1997) and described by Jørgensen et al. (2014) in *Bat. solitarius* and recently mentioned by Bartels et al. (2024). We propose that this element of the cephalic cirri is more widespread, although it may be overlooked using optical microscopy.

Global assessments of marine tardigrade diversity indicate that the phylum remains poorly studied at present (Bartels et al. 2016). Many areas and habitats of the World Ocean are waiting to be investigated for their tardigrade fauna. Based on our data regarding two new taxa, the total species richness of tardigrades for the entire South China Sea stands at 10 species. In contrast to the Tyrrhenian Sea, which has an area more than an order of magnitude smaller, 55 species are known (Kaczmarek et al. 2015). A significant increase in species numbers can be anticipated with further research (Bartels et al. 2016). Marine meiofauna are known to have a heterogeneous and patchy distribution (Fleeger and Decho 1987; Giere 2009). The spatial and temporal distribution of tardigrades in the areas we studied showed some heterogeneity (Table 1). Our results are based on a rather limited sample size, number of stations and replicates and should be taken with caution against strong conclusions. However, our findings align well with those obtained previously on the occurrence and distribution of tardigrades in marine coastal habitats. Changes in species abundance have been previously recorded over the course of the year (Pollock 1970; Gallo D'Addabbo et al. 1999), several weeks (Renaud-Debyser 1956; Schmidt 1969) or even days (Grimaldi de Zio and Grimaldi 1966). We can speculate that similar patterns may be observed in seagrass meadow communities, where tardigrades may

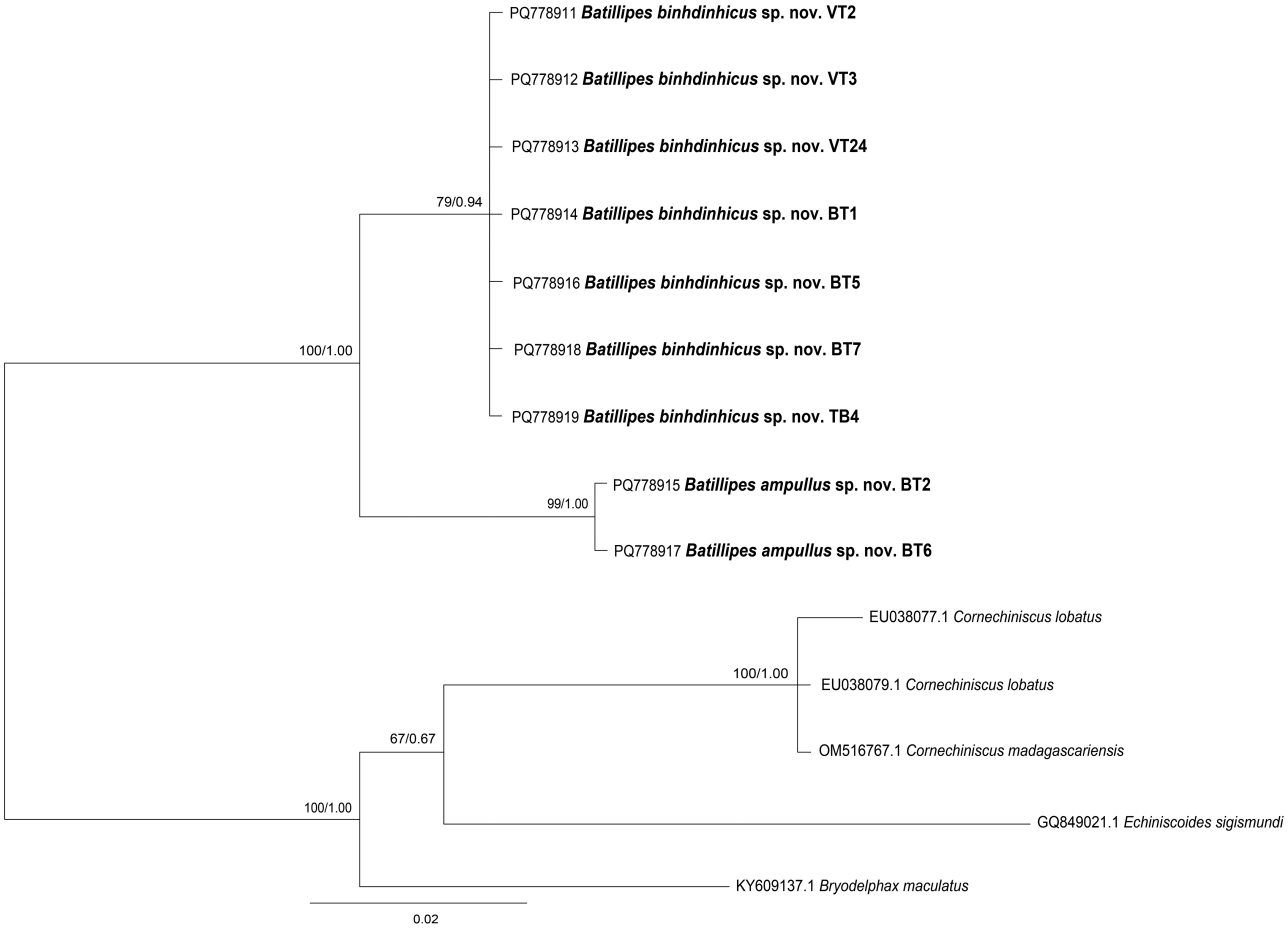

Fig. 16. A Bayesian phylogenetic tree based on 18S rDNA sequences, using K2+G model of nucleotide substitution. Numbers at nodes indicate bootstrap values after IQ-Tree3 (BS, first values) and Bayesian posterior probability values (PP, second values).

also exhibit a mosaic distribution along with seasonal and interannual variability in the composition and abundance of fauna. Further research involving larger sampling effort is required to accurately assess the dynamics of marine tardigrade populations in seagrass meadow communities.

Acknowledgments: The scanning electron microscopy investigations were done in the A.V. Zhirmunsky National Scientific Centre of Marine Biology FEB RAS. The light microscopy investigations were done in the Laboratory of Ecology and Evolutionary Biology of Aquatic Organisms of the Far Eastern Federal University. This research was support by the Program of Scientific Research and Applied Works of the Joint Russian-Vietnamese Tropical Research and Technology Center "Ecolan E-3.5".

Authors' contributions: Conceptualization: AAS, VVM; Methodology: AAS, ASM, VDY, VVM; Investigation: AAS, ASM, VDY, VDV, TLN, THP, VVM; Data Curation: AAS, VVM; Writing - Original Draft: AAS, VDY, VVM; Writing - Review & Editing: AAS, ASM, VDY, VDV, TLN, THP, VVM; Project administration: TLN, VVM; Funding acquisition: TLN, VVM.

Competing interests: The authors declare that there are no competing interests.

Availability of data and materials: The authors confirm that the data supporting the findings of this study are available in the article and its supplementary materials. The new collected specimens from the type locality are deposited at the Museum of the A.V. Zhirmunsky National Scientific Center of Marine Biology FarEastern Branch of the Russian Academy of Sciences, Palchevskogo str. 17, Vladivostok, Russia. The DNA sequences obtained in this study are deposited in GenBank with respective accession numbers.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Supplementary materials

Table S1. GenBank accession numbers for the 18S and 28S rDNA sequences used for analysis. (download)

Table S2. *P*-distances for the studied species of tardigrades according to the 28S rRNA gene fragment data. (download)

Table S3. *P*-distances for the studied species of tardigrades according to the 18S rRNA gene fragment data. (download)

Table S4. Body measurements (in µm) of *Batillipes binhdinhicus* sp. nov. type series. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information. (download)

Table S5. Body measurements (in µm) of *Batillipes ampullus* sp. nov. type series. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information. (download)

Table S6. Body measurements (in µm) of *Orzeliscus asiaticus*. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information. (download)

Table S7. Body measurements (in µm) of *Halechiniscus* sp. Body width was measured between legs III and IV; ?: trait not measurable due to the position of the specimen and the inability to obtain reliable information. (download)

SM S1. Files in FASTA format with the sequences used in the analysis for 18S_tardigrade. (download)

SM S2. Files in FASTA format with the sequences used in the analysis for 28S_tardigrade. (download)