



## Research article

urn:lsid:zoobank.org:pub:A09EB44B-286F-439A-A970-48F09416584A

# Integrative taxonomy supports two new species of *Macrobiotus* (Tardigrada: Eutardigrada: Macrobiotidae) allowing further discussion on the genus phylogeny

Daniel STEC<sup>1B</sup>

Institute of Systematics and Evolution of Animals, Polish Academy of Sciences,  
Sławkowska 17, 31-016 Kraków, Poland.

E-mail: [daniel.stec@isez.pan.krakow.pl](mailto:daniel.stec@isez.pan.krakow.pl), [daniel\\_stec@interia.eu](mailto:daniel_stec@interia.eu)

urn:lsid:zoobank.org:author:13C435F8-25AB-47DE-B5BB-8CE788E92CF6

**Abstract.** In this study, I describe two new species of *Macrobiotus* based on morphological data collected through light and scanning electron microscopy. Both species are accompanied by DNA sequences from four commonly used molecular markers (18S rDNA, 28S rDNA, ITS-2, and COI). *Macrobiotus ovovittatus* sp. nov. was discovered in Greenland and can be distinguished from similar taxa of *Macrobiotus* by its continuous, solid, and clearly wrinkled egg surface, adorned with sparse, very small and irregularly spaced pores. Additionally, the terminal discs of egg processes are covered in multiple light-refracting dots, resembling crocheted napkins. *Macrobiotus mileri* sp. nov. was found in Israel and is characterized by unique pore arrangements in its body cuticle, expressed in two distinct animal forms: (i) forma porata with large pores arranged in five distinct patches and (ii) forma aporata with single, almost undetectable pores. It also features weakly defined convex terminal discs with smooth edges. Furthermore, the phylogenetic analyses conducted in this study offer the most updated phylogeny of superclade I within the family Macrobiotidae. This facilitates additional discussion concerning the interrelationships among species within the genus *Macrobiotus* and the circumscription of species groups within it.

**Keywords.** Egg ornamentation, morphology, *Macrobiotus polonicus-persimilis* species complex, new species, water bears.

Stec D. 2024. Integrative taxonomy supports two new species of *Macrobiotus* (Tardigrada: Eutardigrada: Macrobiotidae) allowing further discussion on the genus phylogeny. *European Journal of Taxonomy* 930: 79–123. <https://doi.org/10.5852/ejt.2024.930.2481>

## Introduction

Tardigrades are a phylum of small invertebrates, with a body size rarely exceeding 1 mm. They are considered part of the meiofauna because they require at least a thin film of water surrounding their bodies to carry out all life activities. Currently, nearly 1500 recognized species have been discovered

worldwide in various habitats. However, the majority of these species have been found in limno-terrestrial environments, inhabiting mosses and lichens. (Nelson *et al.* 2019; Degma & Guidetti 2023).

One of the most speciose and diverse limno-terrestrial cosmopolitan tardigrade groups is the family Macrobiotidae, which comprises approximately 340 nominal species and subspecies classified within 14 genera (Stec *et al.* 2021a; Degma & Guidetti 2023). Importantly, among them, the genus *Macrobiotus* Schultze, 1834 seems to have the most complicated revisional history. This tardigrade group exhibits extreme phenotypic diversity, particularly in terms of egg ornamentation morphologies (Guidetti *et al.* 2013; Kaczmarek & Michalczyk 2017; Stec *et al.* 2021a; Kaczmarek *et al.* 2023). Therefore, it is not surprising that several recent studies have sparked lively discussions and efforts to obtain a more uniform definition for the genus, as well as smaller species groups within it (Kaczmarek & Michalczyk 2017; Massa *et al.* 2021; Stec *et al.* 2021a, 2021b, 2022; Vecchi & Stec 2021; Kaczmarek *et al.* 2023; Bertolani *et al.* 2023). Nevertheless, due to the exceptionally vast morphological diversity that cannot be easily accommodated within clear and strict man-made definitions while also considering phylogenetic relationships, a satisfactory consensus has not yet been reached. For now, only the fact of the internal division of *Macrobiotus* into three distinct and deeply divergent phylogenetic lineages has been well supported by several studies (clades A, B, and C, respectively; Stec *et al.* 2021a, 2022; Vecchi & Stec 2021; Bertolani *et al.* 2023). Several smaller groups (morphologically uniform) within the genus have also been supported as monophyletic, and as such, they were confirmed as species complexes: *M. pallarii* complex (Stec *et al.* 2021a, 2021b), *M. pseudohufelandi* complex (Stec *et al.* 2021a), *M. ariekammensis* complex (Stec *et al.* 2022), and *M. polonicus* complex (Stec *et al.* 2021a; Vecchi & Stec 2021). The latter was recently reinvestigated and redefined as the *M. polonicus-persimilis* complex by Bertolani *et al.* (2023). All remaining taxa of *Macrobiotus* have been accommodated into informal species morpho-groups that are not monophyletic: *M. hufelandi* morpho-group (Stec *et al.* 2021a), *M. nelsonae* complex (Stec *et al.* 2021a; redefined into morpho-group by Kaczmarek *et al.* 2023), and *M. persimilis* morpho-group (Bertolani *et al.* 2023). These grouping strategies allowed all taxa of *Macrobiotus* to be assigned to one of these groups, especially thanks to the very general definition of the *M. hufelandi* morpho-group, which now serves also as a basket for undecided species. However, these subdivisions might have been considered still too complicated or not sufficient for taxonomists and other name users. Therefore, Kaczmarek *et al.* (2023) proposed a solution that neglects phylogenetic relationships, providing an artificial division of all taxa of *Macrobiotus* into 12 morphological groups based on eggshell morphology. Theoretically, such action might be helpful in facilitating taxonomic or faunistic research and better navigation between taxa within the tardigrade group, but time will show if the community will acknowledge and utilize this approach.

In this paper, I propose integrative descriptions of two new species of *Macrobiotus*. For both of them, I provide detailed morphological and morphometric data obtained through light and scanning electron microscopy, along with a set of DNA sequences from the four molecular markers commonly used in tardigrade taxonomy. Additionally, I present an updated molecular phylogeny of superclade I of the family Macrobiotidae, which includes the genera *Macrobiotus*, *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016, and *Sisubiotus* Stec, Vecchi, Calhim & Michalczyk, 2021. These results allow for a discussion of the phylogeny of the genus *Macrobiotus* and the species composition of the groups distinguished within it.

## Material and methods

### Sample processing

A moss sample (GL.001) containing one new species was collected in Greenland near Zackenberg Valley by Michał Kolasa in July 2021. Another moss sample (IL.001), containing a second new species, was collected in Tel-Aviv, Israel, by Krzysztof Miler in November 2019. Samples were examined for

terrestrial tardigrades using standard methods as described in Stec *et al.* (2015). In order to perform integrative taxonomic descriptions, isolated animals and eggs extracted from both samples were split into three groups for specific analyses: morphological analysis with phase contrast light microscopy, morphological analysis with scanning electron microscopy, and DNA sequencing (for details please see sections “Material examined” provided below for each species description). Additionally, 10 females with oocytes on different developmental stages and 5 smaller animals without visible oocytes from the sample GL.001 were stained with aceto-orcein staining to check the presence of sperm (Bertolani 1971).

### Microscopy and imaging

Specimens for light microscopy were mounted on microscope slides in a small drop of Hoyer’s medium and secured with a cover slip, following the protocol by Morek *et al.* (2016). Slides were then dried for five to seven days at 60°C. Dried slides were sealed with a transparent nail polish and examined under a Leica DMLB light microscope with phase contrast (PCM), associated with an digital camera (DLT-Cam PRO). Immediately after mounting the specimens in the medium, slides were also checked under PCM for the presence of males and females in the studied population, as the spermatozoa in testis and vas deferens are visible only for several hours after mounting (Coughlan *et al.* 2019; Coughlan & Stec 2019). In order to obtain well-extended animals and clean eggs for SEM, the specimens were processed according to the protocol by Stec *et al.* (2015). In short, eggs were first subjected to a water/ethanol and an ethanol/acetone series, then to CO<sub>2</sub> critical point drying and finally sputter coated with a thin layer of gold. Specimens were examined under high vacuum in a Versa 3D Dual Beam Scanning Electron Microscope at the ATOMIN facility of the Jagiellonian University, Kraków, Poland. All figures were assembled in Corel Photo-Paint X6. For structures that could not be satisfactorily focused in a single photograph, a stack of 2–6 images was taken with an equidistance of ca 0.2 µm and assembled manually into a single deep-focus image.

### Morphometrics and morphological nomenclature

All measurements are given in micrometres (µm). Sample size was adjusted following recommendations by Stec *et al.* (2016a). Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. The buccal apparatus and claws were classified according to Pilato & Binda (2010). The terminology used to describe oral cavity armature and egg shell morphology follows Kaczmarek & Michalczyk (2017). Macroplacoid length sequence is given according to Kaczmarek *et al.* (2014) whereas morphological states of cuticular bars on legs follow Kiosya *et al.* (2021). Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). The pt index is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981). All other measurements and nomenclature follow Kaczmarek & Michalczyk (2017). Buccal tube width was measured as the external and internal diameter at the level of the stylet support insertion point. Lengths of the claw branches were measured from the base of the claw (i.e., excluding the lunula) to the top of the branch, including accessory points. Distance between egg processes was measured as the shortest distance between the base edges of the two closest processes. Morphometric data were handled using the “Parachela” ver. 1.8 template available from the Tardigrada Register (Michalczyk & Kaczmarek 2013) and are given in Supplementary files (Supp. file 1, Supp. file 2). Tardigrade taxonomy follows Bertolani *et al.* (2014) and Stec *et al.* (2021a).

### DNA sequencing

The DNA was extracted from individual animals following a Chelex® 100 resin (Bio-Rad) extraction method by Casquet *et al.* (2012) with modifications described in detail in Stec *et al.* (2020a). Before the DNA extraction, each specimen was mounted on slides with water and checked under a light microscope. Four DNA fragments differing in mutation rates were sequenced. Namely: the small ribosome subunit

**Table 1.** Primers with their original references used for amplification of the four DNA fragments sequenced in the study.

| DNA marker | Primer name   | Primer direction | Primer sequence (5'-3')   | Primer source                              |
|------------|---------------|------------------|---------------------------|--------------------------------------------|
| 18S rRNA   | 18S_Tar_Ff1   | forward          | AGGCGAAACCGCGAATGGCTC     | Stec <i>et al.</i> (2017a)                 |
|            | 18S_Tar_Rr1   | reverse          | GCCGCAGGCTCCACTCCTGG      |                                            |
| 28S rRNA   | 28SF0002      | forward          | GRCRAGAKTACCCGCTGAAC      | Mironov <i>et al.</i> (2012); Stec (2022a) |
|            | 28SR0990      | reverse          | CCTTGGTCCGTGTTTCAAGAC     |                                            |
| ITS-2      | ITS2_Eutar_Ff | forward          | CGTAACGTGAATTGCAGGAC      | Stec <i>et al.</i> (2018a)                 |
|            | ITS2_Eutar_Rr | reverse          | TCCTCCGCTTATTGATATGC      |                                            |
| COI        | LCO1490-JJ    | forward          | CHACWAAYCATAAAGATATYGG    | Astrin & Stüben (2008)                     |
|            | HCO2198-JJ    | reverse          | AWACTTCVGGRTGVCCAAARAATCA |                                            |

(18S rRNA, nDNA), the large ribosome subunit (28S rRNA, nDNA), the internal transcribed spacer (ITS-2, nDNA), and the cytochrome oxidase subunit I (COI, mtDNA). All fragments were amplified and sequenced according to the protocols described in Stec *et al.* (2020a); primers are listed in Table 1. Sequencing products were read with the ABI 3130xl sequencer at the Genomed company (Warsaw, Poland). Sequences were processed in BioEdit ver. 7.2.5 (Hall 1999) and submitted to GenBank. Prior submission all obtained COI sequences were translated into protein sequences in MEGA11 (Tamura *et al.* 2021) to check against pseudogenes.

### Phylogenetic analysis and molecular species delimitation

To establish phyletic positions of both new species, a phylogenetic tree was constructed. For this purpose, a data set was compiled from taxa for which DNA sequences of at least two (out of all four analyzed in this study) molecular sequences are available and suitable for concatenation (Appendix 1). The only exception were sequences of *M. cf. nelsonae* from Bertolani *et al.* (2014) and *Macrobiotus muralis* Bertolani, Cesari, Giovannini, Rebecchi, Guidetti, Kaczmarek & Pilato, 2022 from Bertolani *et al.* (2023) for which only 18S rDNA sequences are currently available. DNA sequences of three species that represents Macrobiotidae super clade II sensu Stec *et al.* (2021a) were used as outgroup (Appendix 1). The sequences were aligned using the AUTO method (for COI and ITS-2) and the Q-INS-I method (for ribosomal markers: 18S rRNA and 28S rRNA) of MAFFT ver. 7 (Katoh *et al.* 2002; Katoh & Toh 2008) and manually checked against non-conservative alignments in BioEdit. The aligned sequences were trimmed to: 1064 (18S rRNA), 858 (28S rRNA), 658 (COI) bp. The ITS-2 alignment was processed using GBlocks ver. 0.91b (Castresana 2000; <https://www.biologiaevolutiva.org/jcastresana/Gblocks.html>) to remove ambiguously aligned regions, using the default ‘less stringent’ settings, and its final length was 236 bp. The sequences were then concatenated using SequenceMatrix (Vaidya *et al.* 2011). Before partitioning, the concatenated alignment was divided into 6 data blocks constituting three separate blocks of ribosomal markers and three separate blocks of three codon positions in the COI data set. Using PartitionFinder (Lanfear *et al.* 2016) under the Akaike Information Criterion (AIC), the best scheme of partitioning and substitution models was chosen for a Bayesian phylogenetic analysis. Bayesian inference (BI) marginal posterior probabilities were calculated for the concatenated (18S rRNA+28S rRNA+ITS-2+COI) data set using MrBayes ver. 3.2 (Ronquist & Huelsenbeck 2003). Random starting trees were used and the analysis was run for fifteen million generations, sampling the Markov chain every 1000 generations. An average standard deviation of split frequencies of < 0.01 was used as a guide to ensure the two independent analyses had converged. The program Tracer ver. 1.6 (Rambaut *et al.* 2014) was then used to ensure Markov chains had reached stationarity, and to determine the correct ‘burn-in’ for the analysis which was the first 10% of generations. The ESS values were greater than 200 and the consensus tree was obtained after summarising the resulting topologies and discarding the ‘burn-in’. ModelFinder (Kalyaanamoorthy *et al.* 2017) was used to choose the best-fit models according



to the AIC for Maximum Likelihood (ML) analysis. Then, a ML reconstruction was conducted using W-IQ-TREE (Nguyen *et al.* 2015; Trifinopoulos *et al.* 2016). One thousand ultrafast bootstrap (UFBoot) replicates were applied to provide support values for branches (Hoang *et al.* 2018). The consensus trees was viewed and visualized by FigTree ver. 1.4.3 available from <http://tree.bio.ed.ac.uk/software/figtree>. The best evolutionary models of sequence evolution selected for BI and ML analyses, as well as respective raw trees, are given in Supplementary files (Supp. file 3). Additionally, the COI data set was used for genetic species delimitation with ASAP analysis, after removing the outgroups. Two analyses have been conducted: (i) one for the whole COI data set representing Macrobiotidae super clade I and (ii) the second one for taxa of *Macrobiotus* only. Both analyses were run on the respective server (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) with default settings. The outputs from the ASAP analyses are given in Supp. file 4.

### Abbreviations

ISEA PAS = Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland

PCM = phase contrast light microscopy

*pt* = index showing ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage

SEM = scanning electron microscopy

## Results

### Taxonomic treatment

Phylum Tardigrada Doyère, 1840

Class Eutardigrada Richters, 1926

Order Parachela Schuster, Nelson, Grigarick & Christenberry, 1980

Superfamily Macrobitoidea Thulin, 1928 (in Marley *et al.* 2011)

Family Macrobiotidae Thulin, 1928

Genus *Macrobiotus* Schultze, 1834

*Macrobiotus ovovittatus* sp. nov.

urn:lsid:zoobank.org:act:9C2931D3-8EBE-47AB-A3B0-991AB28F03A2

Figs 1–8, Tables 2–3

### Etymology

The species name refers to the terminal discs of the egg processes which resemble crocheted napkins. From the Latin “egg” = “ovo” and “chaplet” = “vittatus”.

### Material examined

32 animals, 57 eggs mounted on microscope slides in Hoyer’s medium, 15 animals and 15 eggs examined under SEM, 15 animals stained with orcein and two animals processed for DNA sequencing.

### Type material

#### Holotype

GREENLAND • near Zackenberg Valley; 74°29′0.766″ N, 20°32′18.308″ W; 77 m a.s.l.; Jul. 2021; M. Kolasa leg.; mixed sample of moss and lichen collected from the rock in arctic tundra; ISEA PAS, slide GL.001.01.

### Paratypes

GREENLAND • 46 animals; same collection data as for the holotype; ISEA PAS, slides GL.001.01 to GL.001.03, SEM stub TAR.015 • 72 eggs; same collection data as for the holotype; ISEA PAS, slides GL.001.04 to GL.001.09, SEM stub TAR.015.

### Description

#### Animals

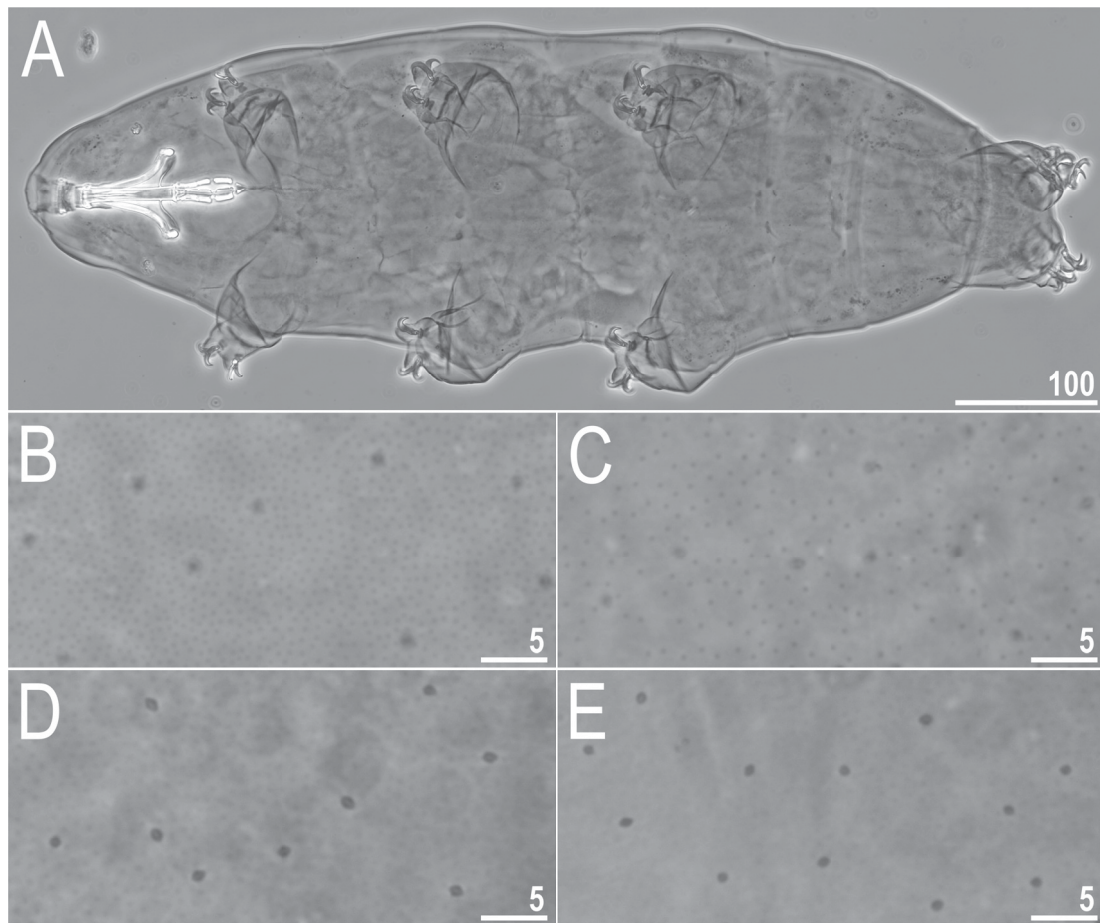
Body transparent in juveniles and white in adults, after fixation in Hoyer's medium transparent (Fig. 1A). Eyes present. Round and oval pores (0.4–0.6 µm in diameter), scattered randomly throughout the cuticle (distributed more sparsely on the ventral side of the body) (Figs 1B–E, 2A–B), including the external and internal surface of all legs (Fig. 3A–F). Granulation is present on the entire body cuticle and clearly visible under PCM and SEM, with granulation on the ventral side of the body being less dense (Figs 1B–E, 2A–B). Moreover, evident dense granulation patches on the external and internal surface of all legs I–III are visible under PCM and SEM (Fig. 3A–D). This dense granulation is also present on the lateral and dorsal surfaces of legs IV (Fig. 3E–F). A pulvinus-shaped cuticular bulge is centrally present on the internal surface of all legs I–III (Fig. 3C–D). This structure is visible only if the legs are fully extended and well oriented.

Claws small and slender, of the *hufelandi* type (Fig. 4A–F). Primary branches with distinct accessory points, a long common tract, and an evident stalk connecting the claw to the lunula (Fig. 4A–F). The lunulae on legs I–III are smooth (Figs 4A, D, E), while there is a dentation in the lunulae on legs IV (Fig. 4B, C, F). A single continuous cuticular bar and double muscle attachments are present above claws I–III (Figs 3C–D, 4A, D, E). Shadowed extensions that extend from the lunulae of the claws on legs I–III are present and visible only under PCM (Figs 3C, 4A). A horseshoe-shaped structure connects the anterior and posterior lunules on leg IV (Fig. 4B).

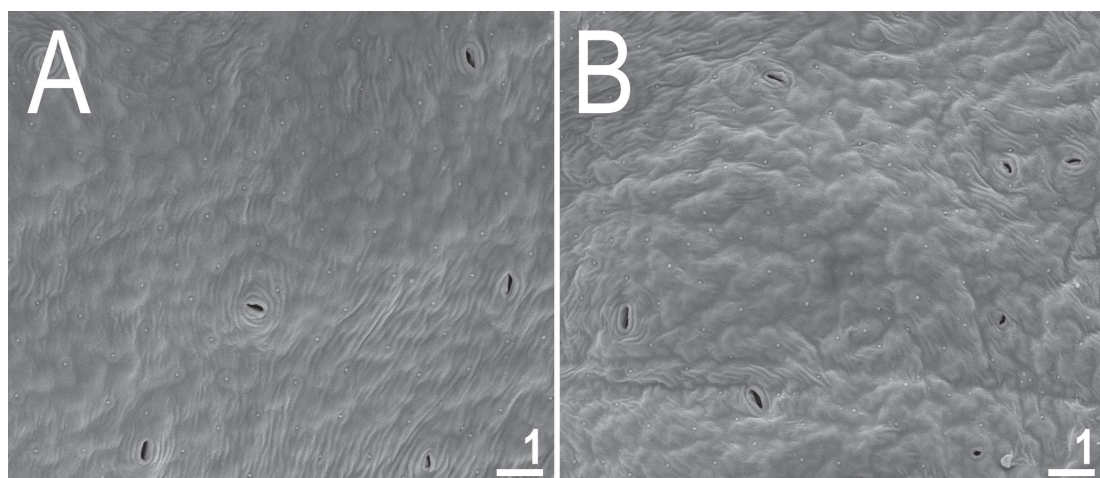
Mouth antero-ventral. Bucco-pharyngeal apparatus of *Macrobiotus* type, with ventral lamina and ten small peribuccal lamellae followed by six buccal sensory lobes (Figs 5A, 6A–B). Under PCM, the oral cavity armature is of *hufelandi* type – three bands of teeth are always visible (Fig. 5B–C). The first band of teeth is composed of numerous extremely small cones arranged in four to six rows located anteriorly in the oral cavity, at the bases of the peribuccal lamellae and just behind them (Figs 5B–C, 6A–B). The second band of teeth is located between the ring fold and the third band of teeth and comprises 4–6 rows of small cones, larger than those of the first band (Figs 5B–C, 6A–B). The teeth of the third band are located within the posterior portion of the oral cavity, between the second band of teeth and the opening of the buccal tube (Figs 5B–C, 6A–B). The third band of teeth is discontinuous and divided into the dorsal and ventral portions. Under PCM, the dorsal teeth are seen as three distinct transverse ridges, whereas the ventral teeth appear as two separate lateral transverse ridges between which a round median tooth is visible (Fig. 5B–C). Under SEM, both dorsal and ventral teeth are also clearly distinct (Fig. 6A–B). Under SEM, the margins of the dorsal teeth are clearly serrated (Fig. 6A) whereas the margins of the ventral teeth are less serrated (Fig. 6B). Pharyngeal bulb spherical, with triangular apophyses, two rod-shaped macroplacoids and a large triangular microplacoid (Fig. 5A). The macroplacoid length sequence being  $2 < 1$ . The first and the second macroplacoid are constricted centrally and subterminally, respectively (Fig. 5D–E). The animals' measurements and statistics are given in Table 2.

#### Eggs

Laid freely, white, spherical and ornamented (Figs 7A–H, 8A–F). The surface between processes is of intermediate state between the *maculatus* and the *persimilis* type, that is, the surface is continuous/solid and clearly wrinkled with sparse, very small and irregularly spaced pores (Figs 7B, D, F, 8C–D). These pores are faintly visible under PCM but clearly visible under SEM (0.3–0.5 µm in diameter; Figs 7B, D, F, 8C–D). Under PCM the wrinkles in egg surface are visible as dark dots/comas/bars making the



**Fig. 1.** *Macrobiotus ovovittatus* sp. nov., PCM images of habitus, body granulation and cuticular pores of the holotype (GL.001.01, ISEA PAS). **A.** Habitus, dorso-ventral projection. **B.** Granulation in the dorsal cuticle. **C.** Granulation in the ventral body cuticle. **D.** Pores in the dorsal cuticle. **E.** Pores in the ventral cuticle. Scale bars in µm.



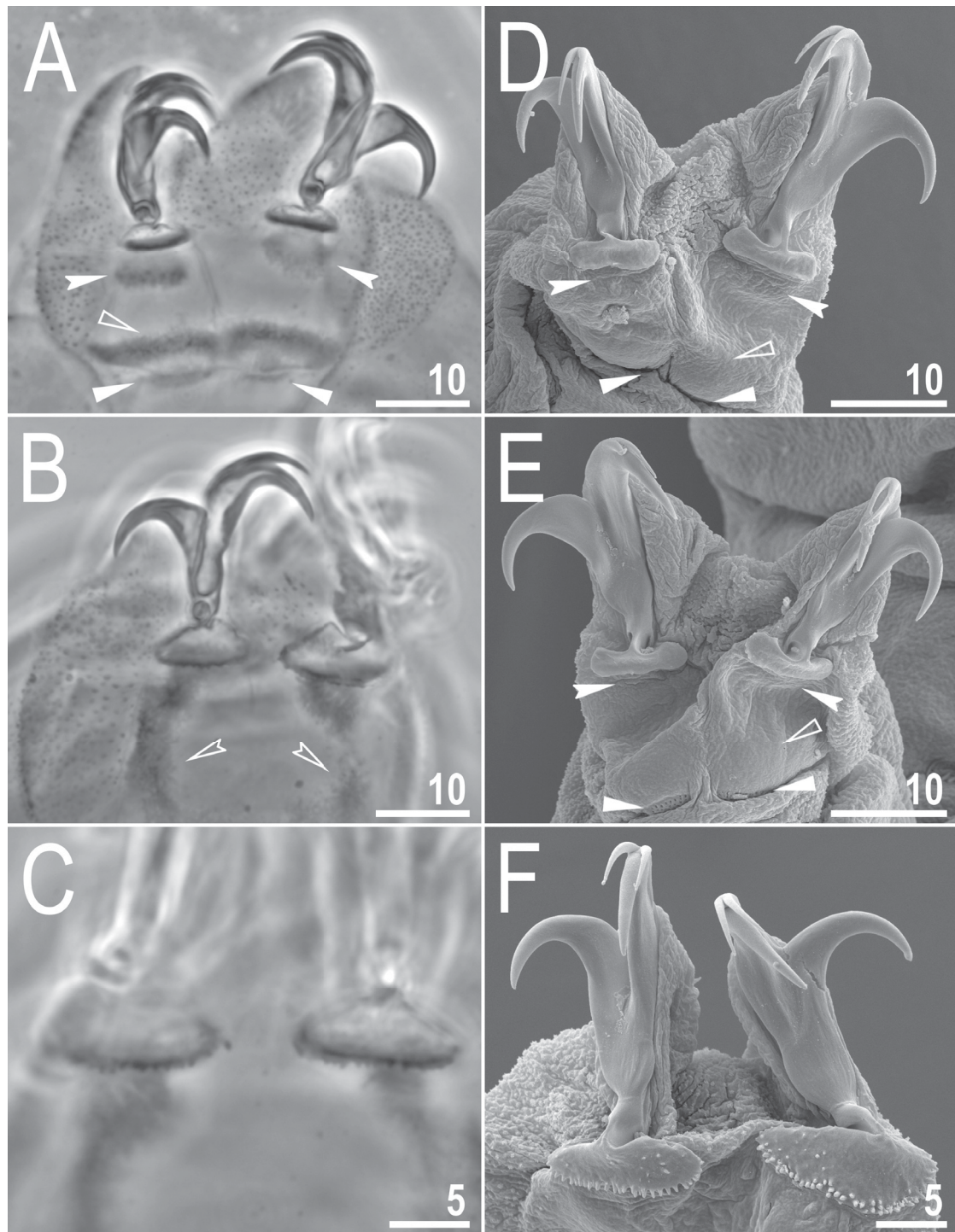
**Fig. 2.** *Macrobiotus ovovittatus* sp. nov., SEM images of body granulation and cuticular pores of a paratype (ISEA PAS). **A.** Body granulation and pores in the dorsal cuticle. **B.** Body granulation and pores in the ventral cuticle. Scale bars in µm.





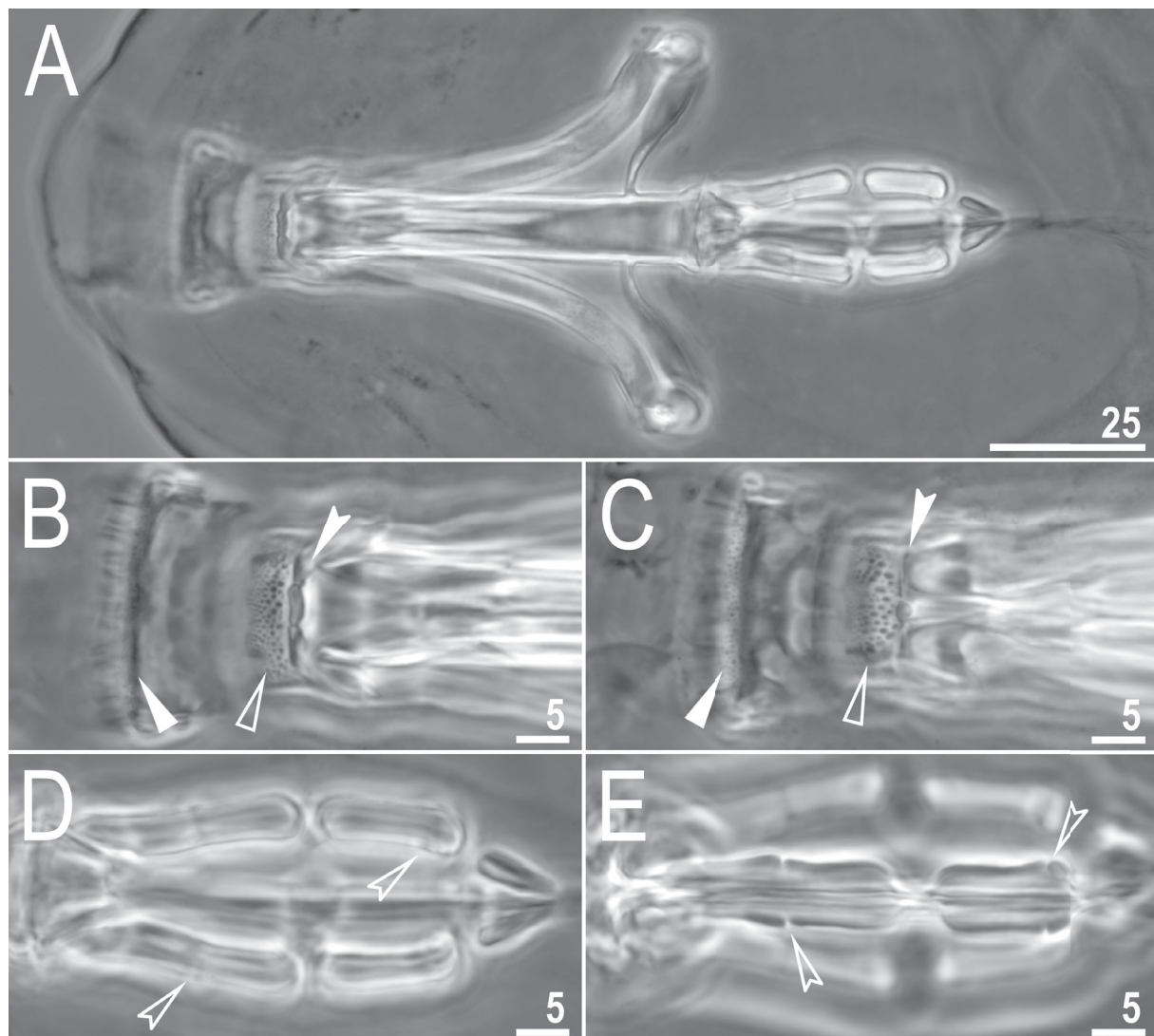
**Fig. 3.** *Macrobiotus ovovittatus* sp. nov., PCM (A, C, E) and SEM (B, D, F) images of dense granulation patches on legs of paratypes (ISEA PAS). A–B. Granulation on the external surface of leg III. C–D. Granulation on the internal surface of leg III and II, respectively. E–F. Granulation on the hind legs. The empty flat arrowheads indicate a single continuous cuticular bar above the claws, the filled flat arrowheads indicate a pulvinus-shaped cuticular bulge, and the filled indented arrowheads indicate shadowed extensions extending from the lunulae. Scale bars in μm.





**Figure 4.** *Macrobiotus ovovittatus* sp. nov., images of claws. **A–C.** PCM, holotype (GL.001.01, ISEA PAS). **D–F.** SEM, paratype (ISEA PAS). **A.** Claws II with smooth lunulae. **B.** Claws IV with dentate lunulae. **C.** Dentate lunulae. **D–E.** Claws II and III with smooth lunulae, respectively. **F.** Claws IV with dentate lunulae. The empty flat arrowheads indicate a single continuous cuticular bar above the claws, the filled indented arrowheads indicate shadowed extensions extending from the lunulae (under PCM) or the places where they should be expected (SEM), the filled flat arrowheads indicate paired muscles attachments, and the empty indented arrowheads indicate the horseshoe structure connecting the anterior and the posterior claw. Scale bars in  $\mu\text{m}$ .

impression of incomplete reticulation (Fig. 7B, D, F). The processes are in the shape of inverted goblets with concave conical trunks and well-defined terminal discs (Figs 7A–H, 8A–F). Faint annulations are visible on the trunk of the process, especially in its distal portion, which is also covered by fine granulation (characters visible only under SEM; Fig. 8C–D). A crown of gently marked thickenings is visible around the bases of the processes as darker dots under PCM (Fig. 7B, D, F) and as thicker wrinkles at the processes bases under SEM (Fig. 8C–D). The terminal discs are cog-shaped, with a concave central area and 10–18 distinct teeth (Figs 7A–H, 8A–F). Terminal discs under PCM are covered by multiple light-refracting dots, and as such resemble crocheted napkins (Fig. 7A, C, E). These light refracting dots, when viewing the egg process laterally, give the impression that the terminal discs are rough and ragged (visible under PCM; Fig. 7 B, D, F–H). However, the terminal discs under SEM are



**Fig. 5.** *Macrobiotus ovovittatus* sp. nov., PCM images of the buccal apparatus, A–D. Holotype (GL.001.01, ISEA PAS). E. Paratype (ISEA PAS). A. An entire buccal apparatus. B–C. The oral cavity armature, dorsal and ventral teeth, respectively. D–E. Placoid morphology, dorsal and ventral placoids respectively. The filled flat arrowheads indicate the first band of teeth, the empty flat arrowheads indicate the second band of teeth, the filled indented arrowheads indicate the third band of teeth, and the empty indented arrowheads indicate central and subterminal constrictions in the first and second macroplacoid, respectively. Scale bars in  $\mu\text{m}$ .

**Table 2.** Measurements [in  $\mu\text{m}$ ] and *pt* values of selected morphological structures of animals of *Macrobiotus ovovittatus* sp. nov.; specimens mounted in Hoyer's medium; N: number of specimen/structures measured; range: refers to the smallest and the largest structure among all measured specimens; SD: standard deviation.

| Character                      | N  | Range         |           | Mean          |           | SD            |           | Holotype      |           |
|--------------------------------|----|---------------|-----------|---------------|-----------|---------------|-----------|---------------|-----------|
|                                |    | $\mu\text{m}$ | <i>pt</i> | $\mu\text{m}$ | <i>pt</i> | $\mu\text{m}$ | <i>pt</i> | $\mu\text{m}$ | <i>pt</i> |
| Body length                    | 20 | 570–879       | 981–1249  | 726           | 1120      | 95            | 67        | 757           | 1083      |
| Buccal tube                    |    |               |           |               |           |               |           |               |           |
| Buccal tube length             | 20 | 54.5–75.3     | –         | 64.6          | –         | 5.6           | –         | 69.9          | –         |
| Stylet support insertion point | 20 | 44.7–60.8     | 80.1–82.4 | 52.4          | 81.1      | 4.5           | 0.6       | 56.4          | 80.7      |
| Buccal tube external width     | 20 | 7.9–13.4      | 14.5–18.5 | 10.6          | 16.4      | 1.4           | 1.0       | 11.6          | 16.6      |
| Buccal tube internal width     | 20 | 5.9–10.5      | 10.8–13.9 | 8.1           | 12.5      | 1.1           | 0.8       | 8.5           | 12.2      |
| Ventral lamina length          | 20 | 33.1–48.2     | 60.0–68.7 | 41.5          | 64.2      | 4.4           | 2.9       | 46.6          | 66.7      |
| Placoid lengths                |    |               |           |               |           |               |           |               |           |
| Macroplacoid 1                 | 20 | 15.5–24.3     | 28.4–34.4 | 20.3          | 31.3      | 2.6           | 1.8       | 22.8          | 32.6      |
| Macroplacoid 2                 | 20 | 10.1–17.0     | 18.2–22.6 | 13.1          | 20.2      | 1.9           | 1.4       | 13.4          | 19.2      |
| Microplacoid                   | 20 | 6.3–9.6       | 9.3–13.5  | 7.5           | 11.6      | 1.0           | 1.0       | 8.4           | 12.0      |
| Macroplacoid row               | 20 | 27.1–43.3     | 48.5–59.0 | 35.3          | 54.4      | 4.8           | 3.0       | 38.9          | 55.7      |
| Placoid row                    | 20 | 35.2–55.1     | 62.7–75.2 | 44.5          | 68.6      | 5.7           | 3.2       | 49.6          | 71.0      |
| Claw I heights                 |    |               |           |               |           |               |           |               |           |
| External primary branch        | 19 | 13.7–21.7     | 24.6–29.3 | 17.4          | 26.8      | 2.2           | 1.5       | 17.5          | 25.0      |
| External secondary branch      | 18 | 10.5–15.5     | 18.7–23.5 | 13.7          | 21.1      | 1.4           | 1.1       | 14.7          | 21.0      |
| Internal primary branch        | 19 | 12.4–19.8     | 22.1–26.3 | 15.9          | 24.7      | 1.8           | 1.1       | 16.9          | 24.2      |
| Internal secondary branch      | 18 | 10.5–14.5     | 17.5–21.6 | 12.5          | 19.6      | 1.2           | 1.0       | 14.1          | 20.2      |
| Claw II heights                |    |               |           |               |           |               |           |               |           |
| External primary branch        | 20 | 13.6–21.4     | 25.0–30.7 | 18.0          | 27.8      | 2.2           | 1.5       | 19.9          | 28.5      |
| External secondary branch      | 15 | 10.9–16.9     | 19.7–24.1 | 14.1          | 22.1      | 1.8           | 1.4       | 16.7          | 23.9      |
| Internal primary branch        | 20 | 11.9–20.9     | 21.8–27.8 | 16.3          | 25.2      | 2.1           | 1.5       | 17.3          | 24.7      |
| Internal secondary branch      | 19 | 9.3–17.2      | 17.1–22.8 | 13.2          | 20.3      | 1.8           | 1.6       | 14.3          | 20.5      |
| Claw III heights               |    |               |           |               |           |               |           |               |           |
| External primary branch        | 20 | 14.5–23.2     | 26.4–30.8 | 18.3          | 28.2      | 2.2           | 1.3       | 19.5          | 27.9      |
| External secondary branch      | 18 | 12.3–18.4     | 21.5–25.0 | 15.0          | 23.1      | 1.6           | 1.0       | 16.2          | 23.2      |
| Internal primary branch        | 20 | 13.2–19.9     | 23.5–27.0 | 16.5          | 25.5      | 1.8           | 1.0       | 17.7          | 25.3      |
| Internal secondary branch      | 17 | 11.2–16.5     | 18.4–22.1 | 13.4          | 20.8      | 1.5           | 1.1       | 15.2          | 21.7      |
| Claw IV heights                |    |               |           |               |           |               |           |               |           |
| Anterior primary branch        | 20 | 16.1–25.1     | 28.0–33.6 | 19.6          | 30.2      | 2.4           | 1.6       | 19.6          | 28.0      |
| Anterior secondary branch      | 15 | 12.5–17.5     | 20.9–24.9 | 15.0          | 23.0      | 1.6           | 1.1       | 15.6          | 22.3      |
| Posterior primary branch       | 20 | 17.1–27.0     | 30.7–35.9 | 20.9          | 32.3      | 2.5           | 1.5       | 21.7          | 31.0      |
| Posterior secondary branch     | 10 | 13.5–18.9     | 21.9–25.3 | 15.6          | 23.9      | 1.6           | 1.2       | 16.2          | 23.2      |



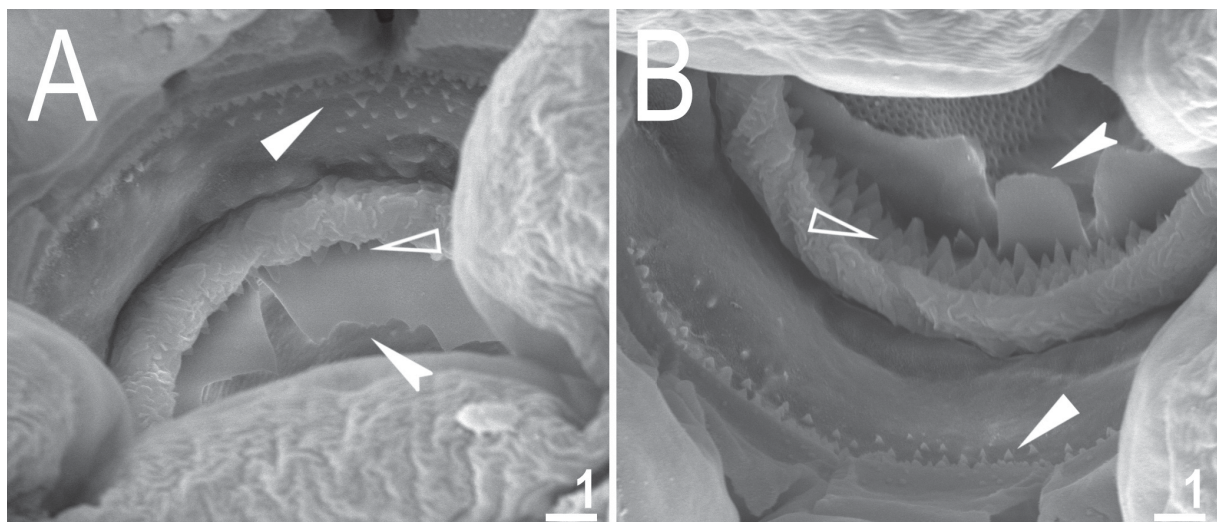
solid without any pores or light refracting dots, their teeth, are covered by small granules (visible only under SEM) that probably serve to improve the adhesive properties of the egg processes (Fig. 8C–F). The measurements and statistics of eggs are given in Table 3.

### Reproduction

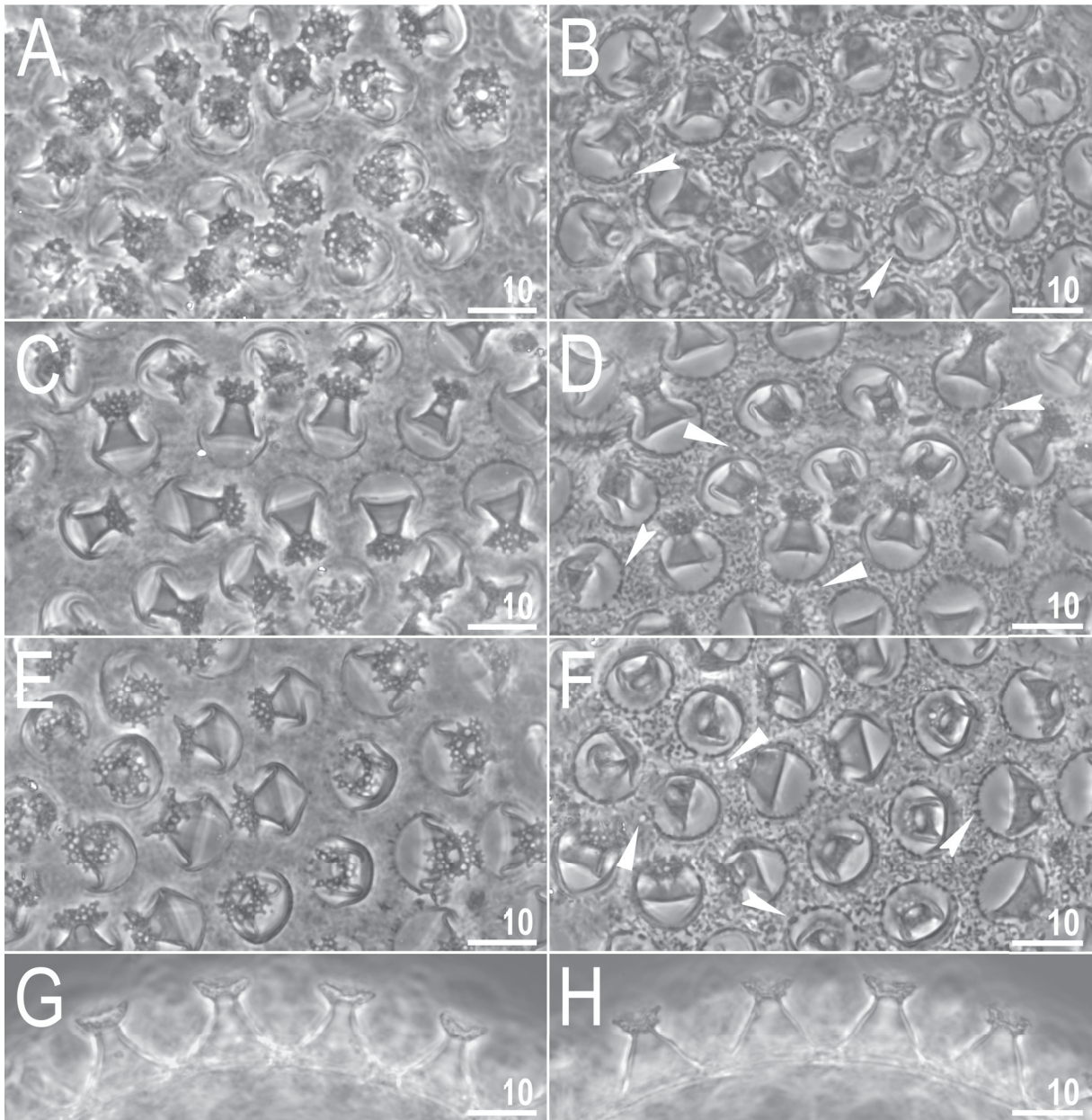
The reproduction mode of *M. ovovittatus* sp. nov. is unknown. Examination of orcein-stained specimens revealed no spermatozoa, but only developing oocytes. The same was true for the observation of freshly mounted individuals in Hoyer's medium that did not reveal any sperm either but only developing oocytes. Therefore, this population could be parthenogenetic or hermaphroditic (considering its close relationship with hermaphroditic taxa in Fig. 21). In the second case, it might have been possible that there were no specimens in the development stage when the sperm could be detectable.

### Differential diagnosis

By having (i) three bands of teeth in the oral cavity armature that are well visible under light microscope, (ii) entire body cuticle covered by granulation (sometimes visible only under SEM), (iii) eggs with inverted goblet shaped processes, the new species is the most similar to four other taxa of *Macrobiotus*, namely *Macrobiotus joannae* Pilato & Binda, 1983 reported from its type locality in Australia (Pilato & Binda 1983), and several uncertain localities in central, eastern, and southeastern Russia (Biserov 1990) and from Italy (Bertolani *et al.* 2014), *Macrobiotus hanna*e Nowak & Stec, 2018 known only from its type locality in Poland (Nowak & Stec 2018), *Macrobiotus punctillus* Pilato, Binda & Azzaro, 1990 known only from its type locality in Chile (Pilato *et al.* 1990) and *Macrobiotus rebecchii* Stec, 2022 known only from its type locality in Kyrgyzstan (Stec 2022b). However, it can be easily distinguished from all of them by having a different morphology of the egg surface (the surface is continuous/solid and clearly wrinkled with sparse, very small and irregularly spaced pores in the new species vs chorion surface covered by evident reticulum in the other species), a different appearance of the terminal discs under PCM (the terminal discs are covered by multiple light-refracting dots, and as such resemble crocheted napkins vs terminal discs without light-refracting dots in other species).

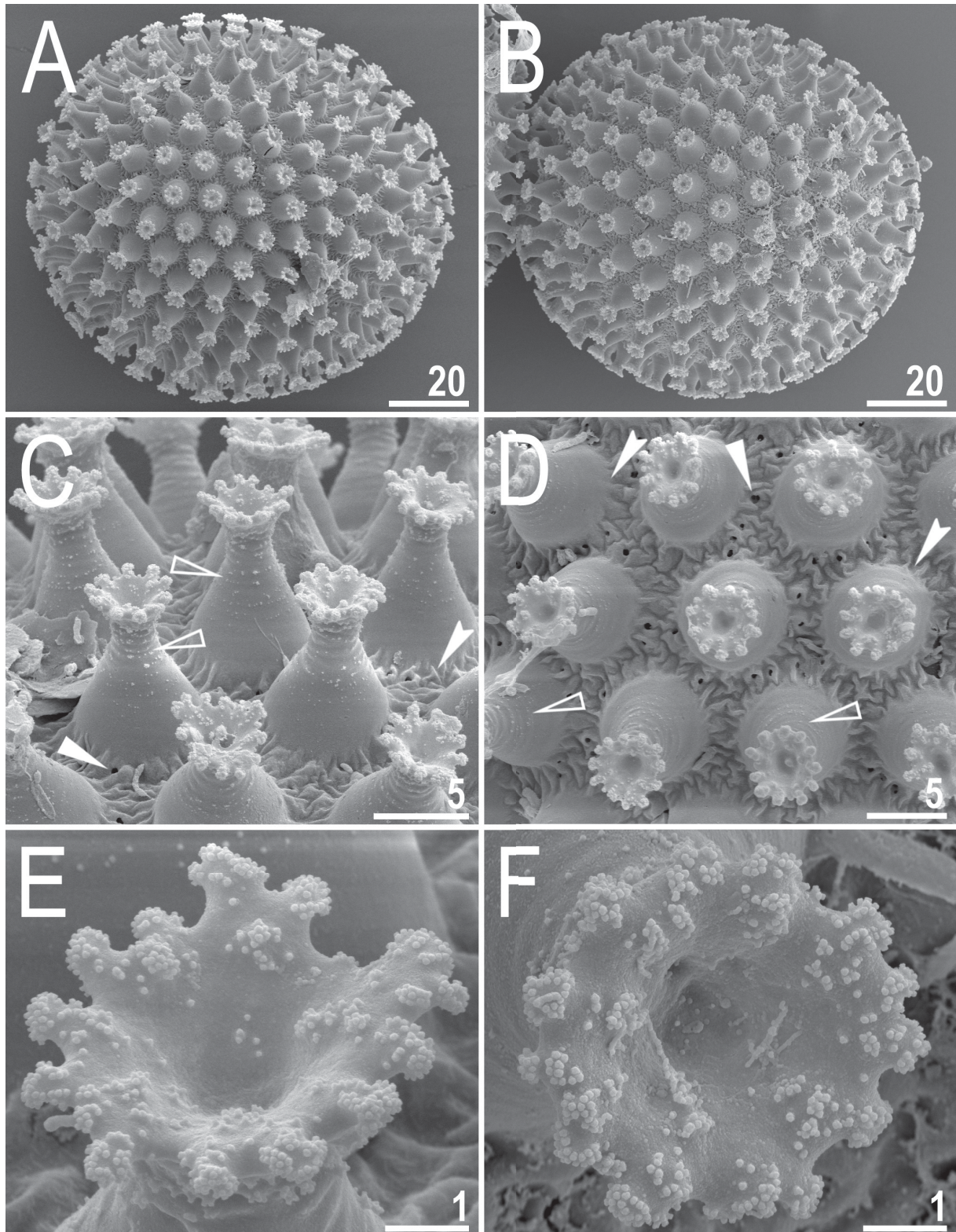


**Fig. 6.** *Macrobiotus ovovittatus* sp. nov., mouth opening and the oral cavity armature of a single paratype (ISEA PAS) seen under SEM from different angles. **A.** Dorsal view. **B.** Ventral view. The filled flat arrowheads indicate the first band of teeth, the empty flat arrowheads indicate the second band of teeth, and the filled indented arrowheads indicate the third band of teeth. Scale bars in  $\mu\text{m}$ .



**Fig. 7.** *Macrobiotus ovovittatus* sp. nov., PCM images of the eggs (ISEAPAS) under  $\times 1000$  magnification. **A, C, E.** Egg surface with focus on egg processes and terminal discs. **B, D, F.** Egg surface, focus on the surface between processes. **G–H.** Midsections of egg processes. The filled indented arrowheads indicate thickenings around the process bases and filled flat arrowheads indicate faintly visible pores. Scale bars in  $\mu\text{m}$ .





**Fig. 8.** *Macrobiotus ovovittatus* sp. nov., SEM images of eggs (ISEAPAS). **A–B.** Entire egg. **C–D.** Details of egg processes and the surface between them. **E–F.** Details of the terminal disc. The filled indented arrowheads indicate thickenings around the process bases and filled flat arrowheads indicate faintly visible pores. Scale bars in µm.



**Table 3.** Measurements [in  $\mu\text{m}$ ] of the eggs of *Macrobiotus ovovittatus* sp. nov.; eggs mounted in Hoyer's medium; process base/height ratio is expressed as percentage; N: number of eggs/structures measured; range: refers to the smallest and the largest structure among all measured specimens; SD: standard deviation.

| Character                                    | N  | Range       | Mean  | SD   |
|----------------------------------------------|----|-------------|-------|------|
| Egg bare diameter                            | 9  | 100.6–129.8 | 117.6 | 12.8 |
| Egg full diameter                            | 9  | 125.5–155.4 | 142.2 | 12.6 |
| Process height                               | 27 | 9.5–13.5    | 11.9  | 0.9  |
| Process base width                           | 27 | 9.4–13.6    | 11.1  | 0.9  |
| Process base/height ratio                    | 27 | 78%–115%    | 95%   | 11%  |
| Terminal disc width                          | 27 | 6.1–8.7     | 7.4   | 0.7  |
| Inter-process distance                       | 27 | 2.2–4.1     | 3.4   | 0.5  |
| Number of processes on the egg circumference | 9  | 28–34       | 31.2  | 2.2  |

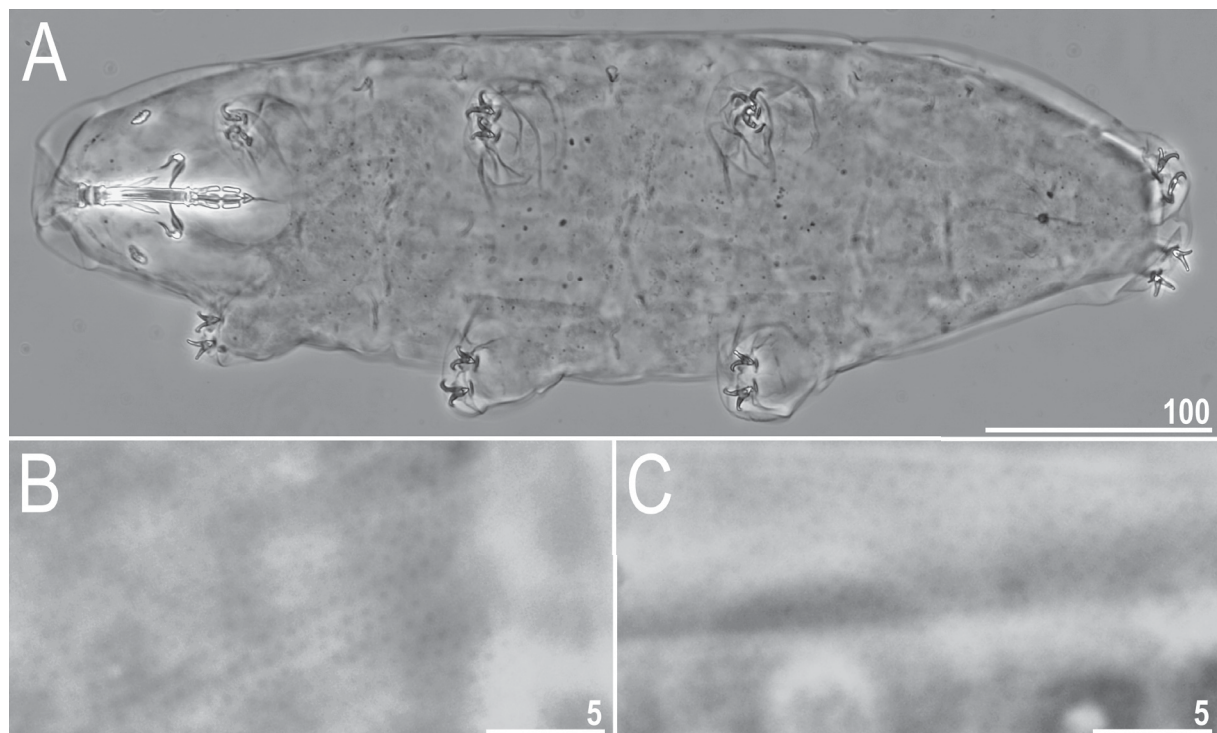
***Macrobiotus mileri* sp. nov.**

urn:lsid:zoobank.org:act:99372E2F-595A-4AB2-8398-21198A2CBD7E

Figs 9–20, Tables 4–5

**Etymology**

The species is named in honour of Krzysztof Miler, who has developed an impressive tolerance for the daily tardigrade madness that surrounds him.



**Fig. 9.** *Macrobiotus mileri* sp. nov., PCM images of habitus and body granulation. **A.** Dorso-ventral projection (holotype, IL.001.11, ISEA PAS, forma aporata). **B.** Granulation in the dorsal cuticle (paratype, ISEA PAS, forma aporata). **C.** Granulation in the dorsal body cuticle (paratype, ISEA PAS, forma porata). Scale bars in  $\mu\text{m}$ .

### Material examined

39 animals, 7 eggs mounted on microscope slides in Hoyer's medium, 7 animals and 3 eggs examined under SEM, and 4 animals processed for DNA sequencing.

### Type material

#### Holotype

ISRAEL • Tel-Aviv; 32°2'42.82" N, 34°46'14.88" E; 19 m a.s.l.; Nov. 2019; K. Miler leg.; moss growing on a stone wall in urban park; ISEA PAS, slide IL.001.11.

#### Paratypes

ISRAEL • 45 animals; same collection data as for the holotype; ISEA PAS, slides IL.001.08 to IL.001.12, SEM stub TAR.014 • 10 eggs; same collection data as for the holotype; ISEA PAS, slides IL.001.06 to IL.001.07, SEM stub TAR.014.

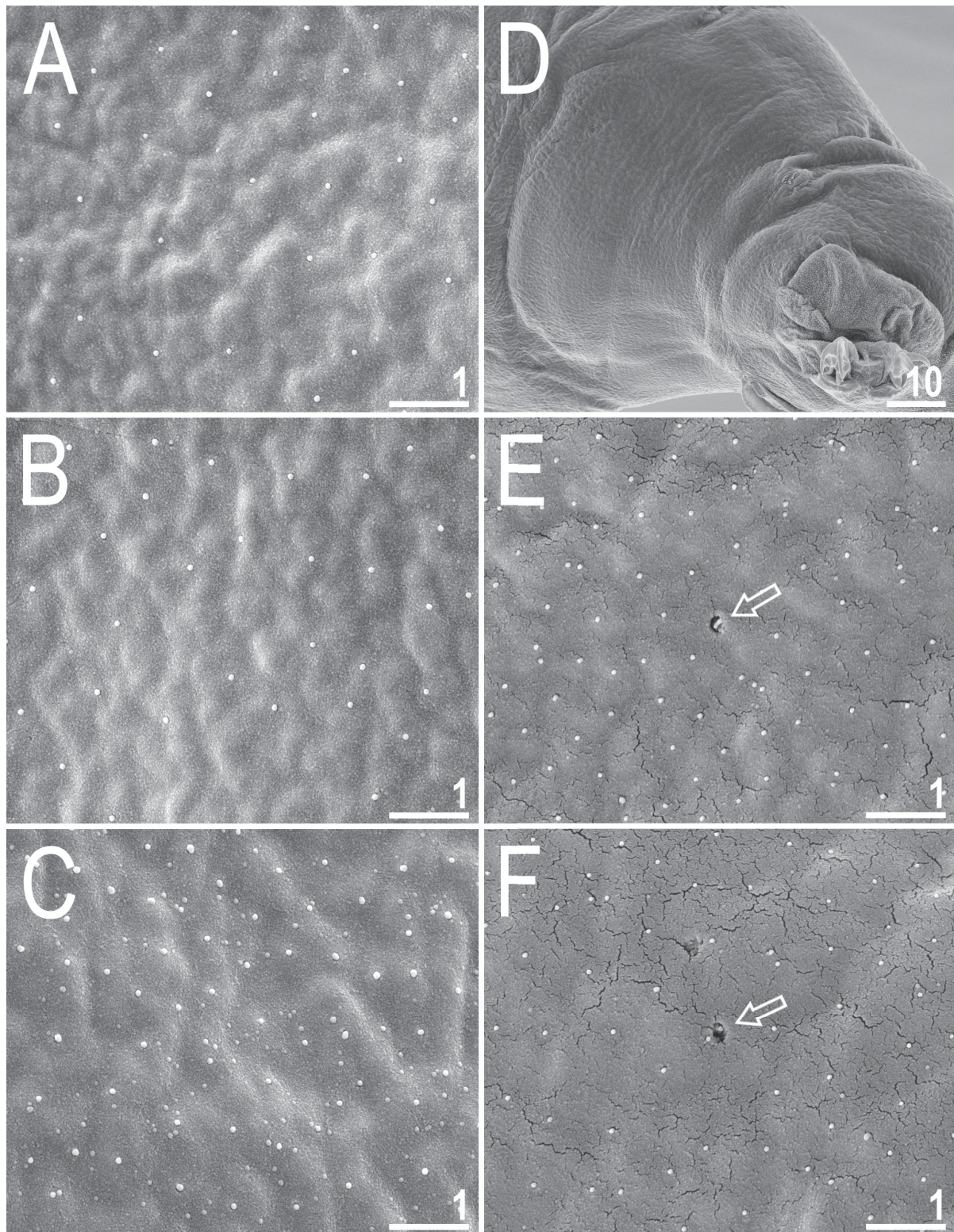
### Description

#### Animals

Body transparent in juveniles and white in adults, after fixation in Hoyer's medium transparent (Fig. 9A). Eyes present. Granulation is present on the entire body cuticle and is visible under PCM and SEM, but granulation on the ventral side of the body is less dense (Figs 9B–C, 10A–F). In terms of cuticular pores, two morphological forms of animals are present in this species. One form (*forma porata*) with large, evident pores arranged specifically in five patches (Figs 11A–B, 12A–D, 13) and second form (*forma aporata*) with only small, single pores randomly distributed on the body (almost undetectable under PCM and hardly detectable also under SEM; 0.2–0.4 µm in diameter; Figs 10E–F, 14A, 16C, 18A–B). In *forma porata*, the round and oval pores (0.4–0.7 µm in diameter) are arranged into five distinct patches: (I) a sparse patch of pores on the external surface of the distal portion of leg I (Figs 13, 15A); (II) a dense patch of pores on the external surface of the proximal portion of leg II extending also towards the lateral body cuticle (Figs 11A, 13); (III) a dense patch of pores on the lateral body surface between legs II and II (Figs 11A, 12A, C, 13); (IV) a dense large patch of pores covering the whole external surface of leg III, extending also towards the lateral body cuticle (Figs 11A–B, 12A, C, 13); and (V) the largest patch of pores that extends from the left caudo-lateral surface, through the dorsal caudal surface to the right caudo-lateral surface, extending also towards lateral and dorsal surfaces of legs IV (Figs 11B, 12A–D, 13, 15D). Only the V patch is single and continuous, while patches I–IV are doubled and present symmetrically on each side of the body. In both forms, some evident dense granulation patches are visible on the external and internal surfaces of all legs I–III, as well as on the lateral and dorsal surfaces of legs IV under PCM and SEM (Figs 14A–D, 15A–D). Small pores, visible only under SEM, can be seen in between the granulation on the hind legs (Fig. 15D). A pulvinus-shaped cuticular bulge is not visible on the internal surface of legs I–III, but there is a garter-shaped structure on the external surface of all legs I–III (Figs 13, 14A–B, 15A–B) above which there is a small cuticular bulge/fold (visible only under SEM; Fig. 15A–B).

Small and robust *hufelandi*-type claws (Fig. 16A–E). Primary branches with distinct accessory points, a moderately long common tract, and an evident stalk connecting the claw to the lunula (Fig. 16A–F). The lunulae on legs I–III are smooth (Fig. 16A, D), while there is dentation in the lunulae on legs IV (Fig. 16B, C, E). The cuticular bars are absent, but double muscle attachments are present above the claws I–III (Fig. 16A, D). Shadowed extensions extending from lunulae on legs I–III are present and faintly visible only under PCM (Fig. 16A). A horseshoe-shaped structure connects the anterior and posterior lunules on leg IV (Fig. 16C).

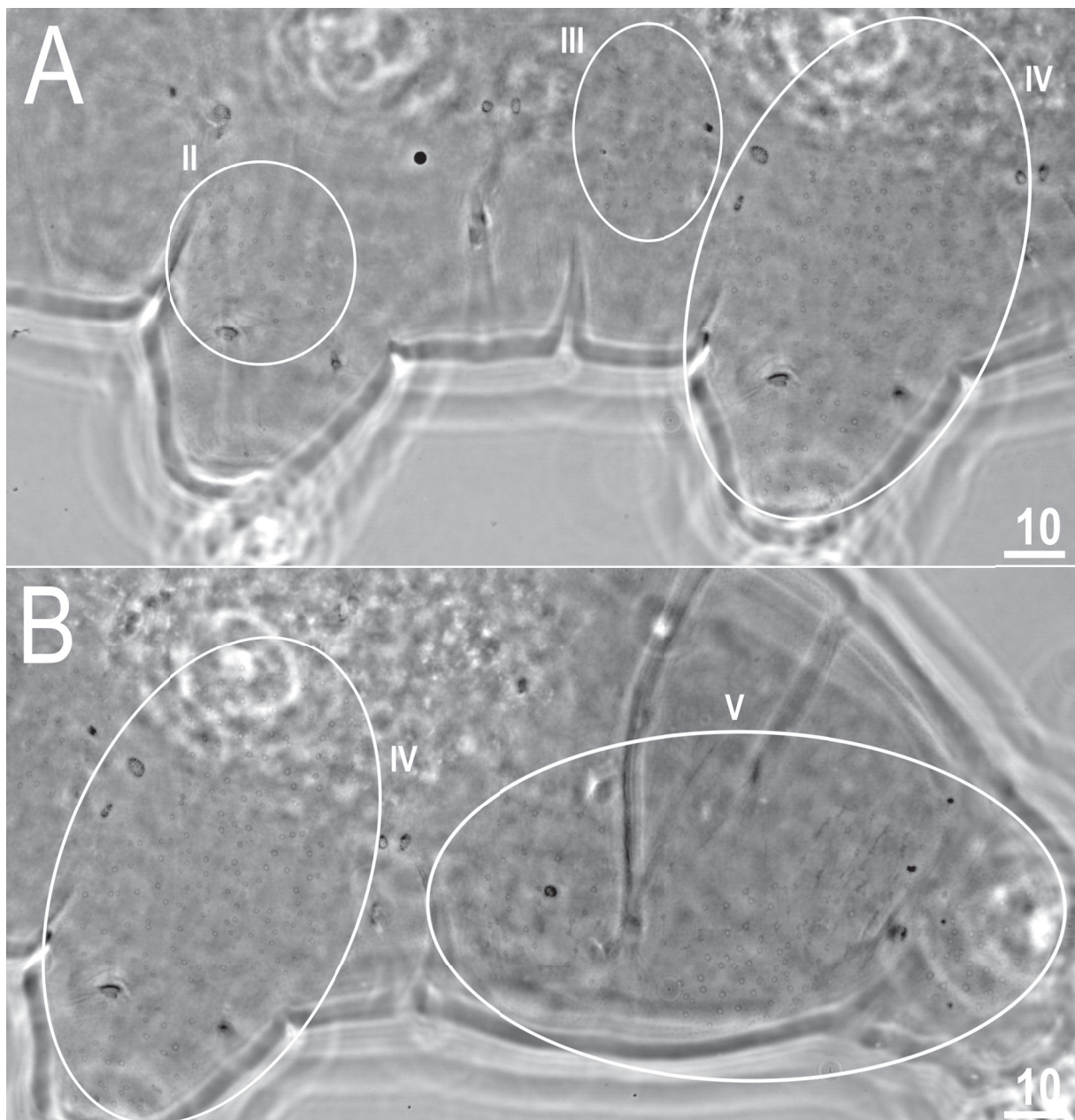




**Fig. 10.** *Macrobiotus mileri* sp. nov., SEM images of body granulation and cuticular pores (paratype, ISEA PAS, forma aporata). **A.** Body granulation in the dorsal head region. **B.** Body granulation in the dorsal central body region. **C.** Body granulation in the dorsal caudal body region. **D.** General view of body granulation in the lateral caudal body region. **E–F.** Singular pores in the body cuticle. Scale bars in  $\mu\text{m}$ .

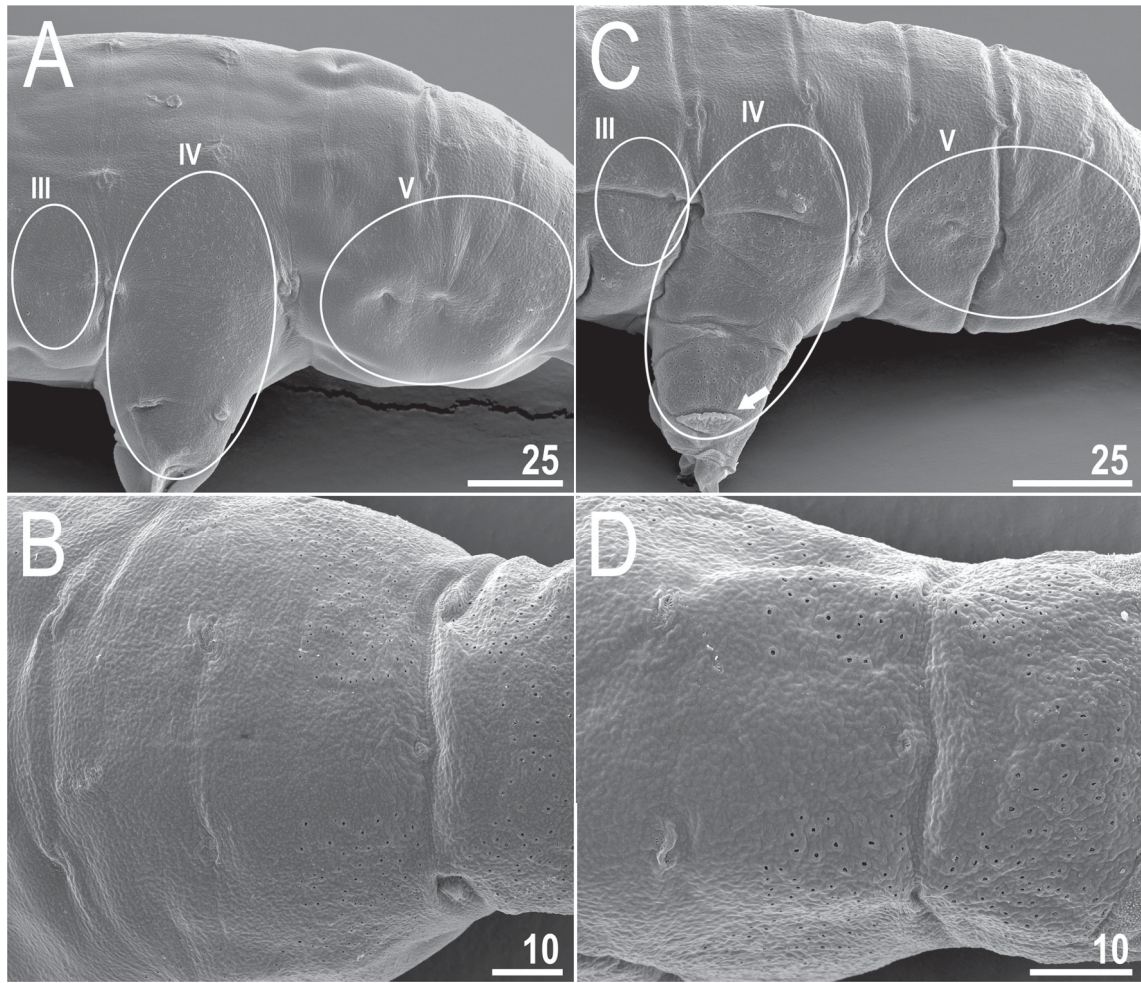


Mouth antero-ventral. Bucco-pharyngeal apparatus of *Macrobiotus* type, with ventral lamina and ten small peribuccal lamellae followed by six buccal sensory lobes (Figs 17A, 18A–D). Under PCM, the oral cavity armature is of *hufelandi* type – three bands of teeth are always visible (Fig. 17B–C). The first band of teeth is composed of numerous extremely small cones arranged in four to six rows located anteriorly in the oral cavity, just behind the bases of the peribuccal lamellae (Figs 17B–C, 18C–D). The second band of teeth is located between the ring fold and the third band of teeth and comprises about four rows of small cones, larger than those of the first band (Figs 17B–C, 18C–D). The teeth of the third band are located within the posterior portion of the oral cavity, between the second band of teeth and the opening of the buccal tube (Figs 17B–C, 18C–D). The third band of teeth is discontinuous

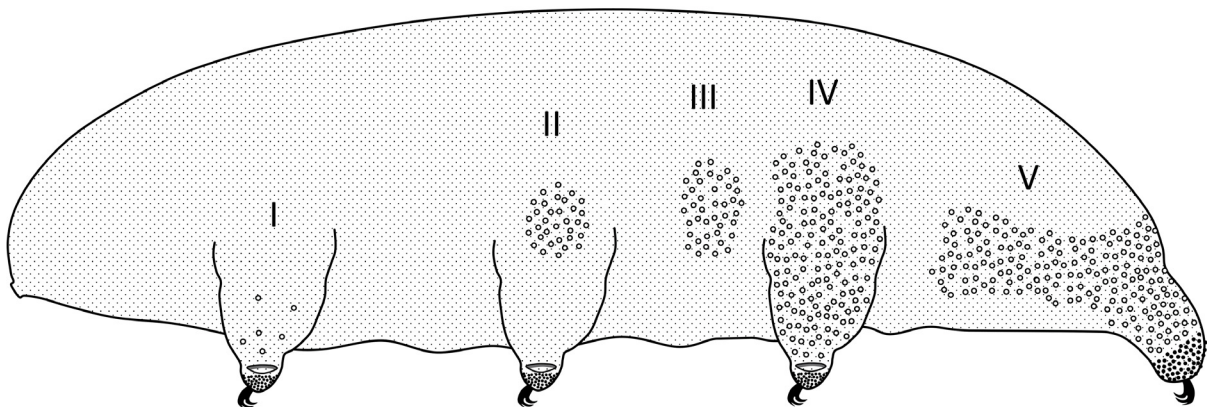


**Fig. 11.** *Macrobiotus mileri* sp. nov., PCM images of cuticular pores patches in forma porata (paratype, ISEA PAS). **A.** Patches II, III and IV. **B.** Patches IV and V. Scale bars in  $\mu\text{m}$ .





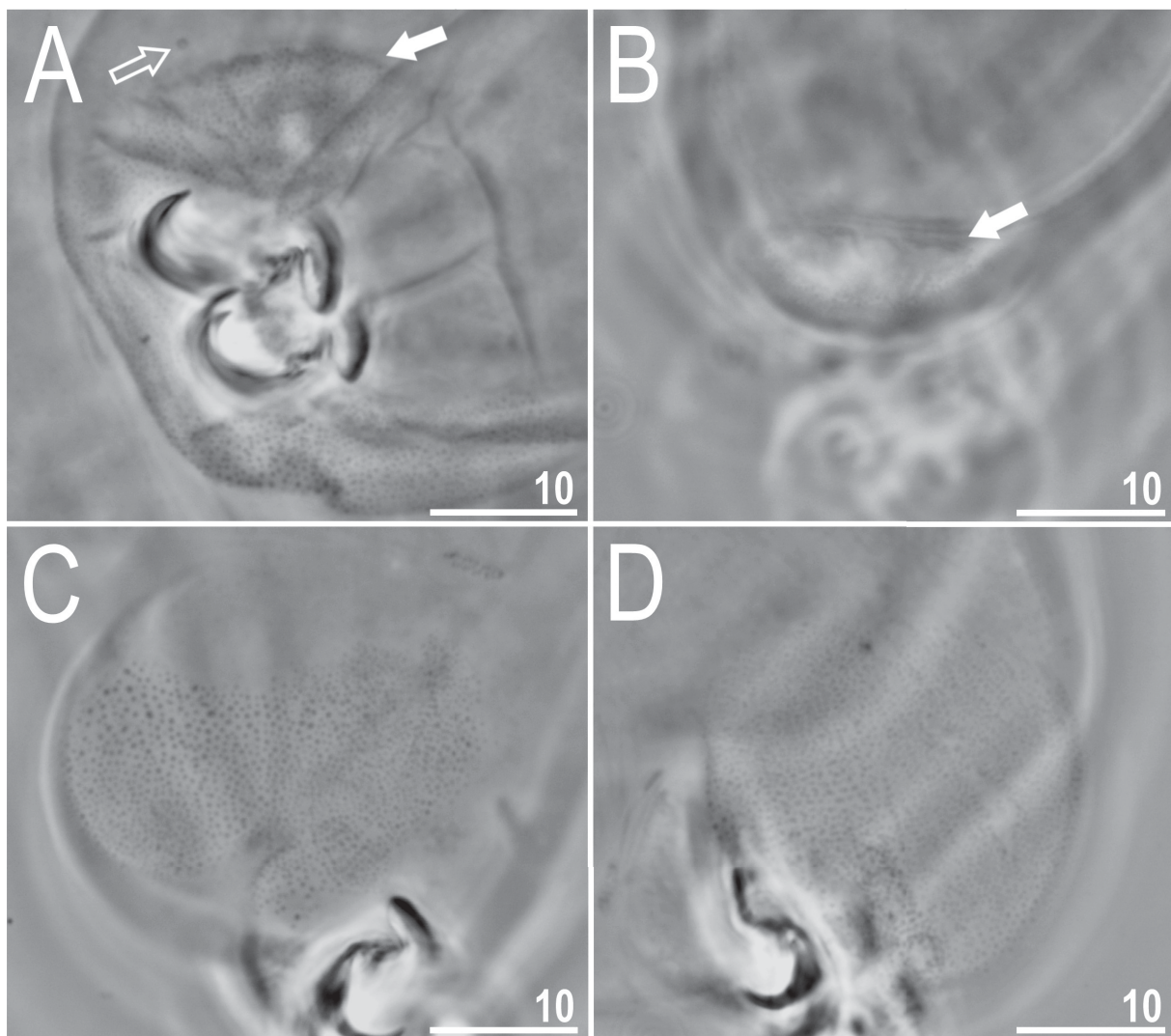
**Fig. 12.** *Macrobiotus mileri* sp. nov., SEM images of cuticular pores patches in two paratypes of forma porata (ISEA PAS). **A, C.** Patches II, III and IV. **B, D.** Dorsal view on the caudal body region with continuous patch V of pores. Scale bars in  $\mu\text{m}$ .



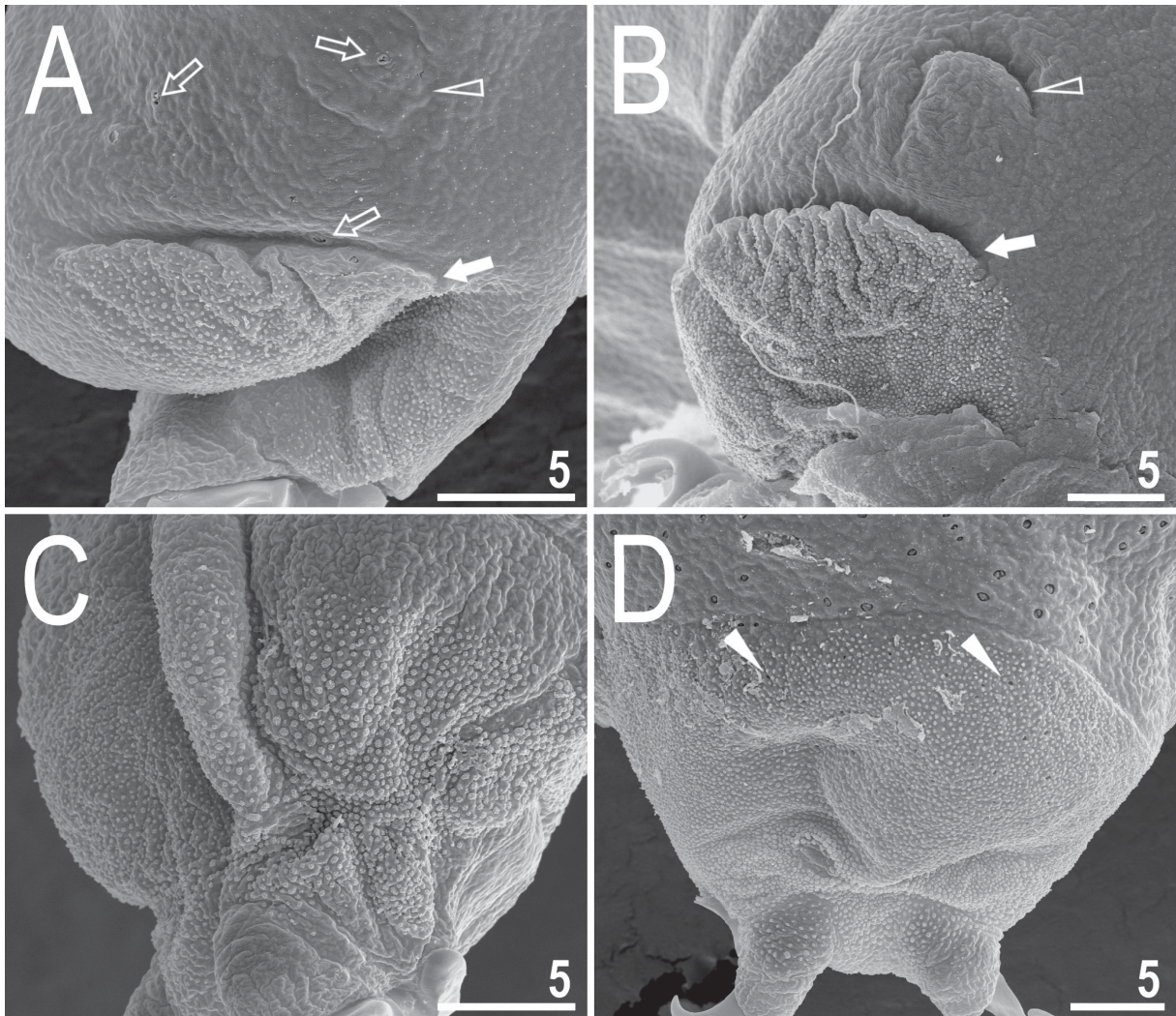
**Fig. 13.** *Macrobiotus mileri* sp. nov., a schematic drawing of a specimen belonging to forma porata, showing the distribution of patches of cuticular pores, body and leg granulation as well as the garter-shaped structures on legs I–III.



and divided into the dorsal and ventral portions. Under PCM, dorsal teeth are seen as three distinct transverse ridges, and the medio-dorsal tooth is evidently longer than the latero-dorsal teeth (Fig. 17B). The ventral teeth appear as two separate lateral transverse ridges between which a median tooth is visible and rarely divided into two teeth (Fig. 17C). Under SEM, the dorsal and ventral teeth are also clearly distinct (Fig. 18C–D). Under SEM, the margins of the dorsal teeth are serrated and the medio-dorsal tooth is clearly longer than latero-dorsal teeth (Fig. 18C) whereas the ventral teeth are smaller and their margins are less serrated (Fig. 18D). Pharyngeal bulb spherical, with triangular apophyses, two rod-shaped macropylacoids and a large triangular micropylacoid (Fig. 17A). The macropylacoid length sequence being  $2 < 1$ . The first and the second macropylacoid are constricted centrally and subterminally, respectively (Fig. 17D–E). The animals' measurements and statistics are given in Table 4.

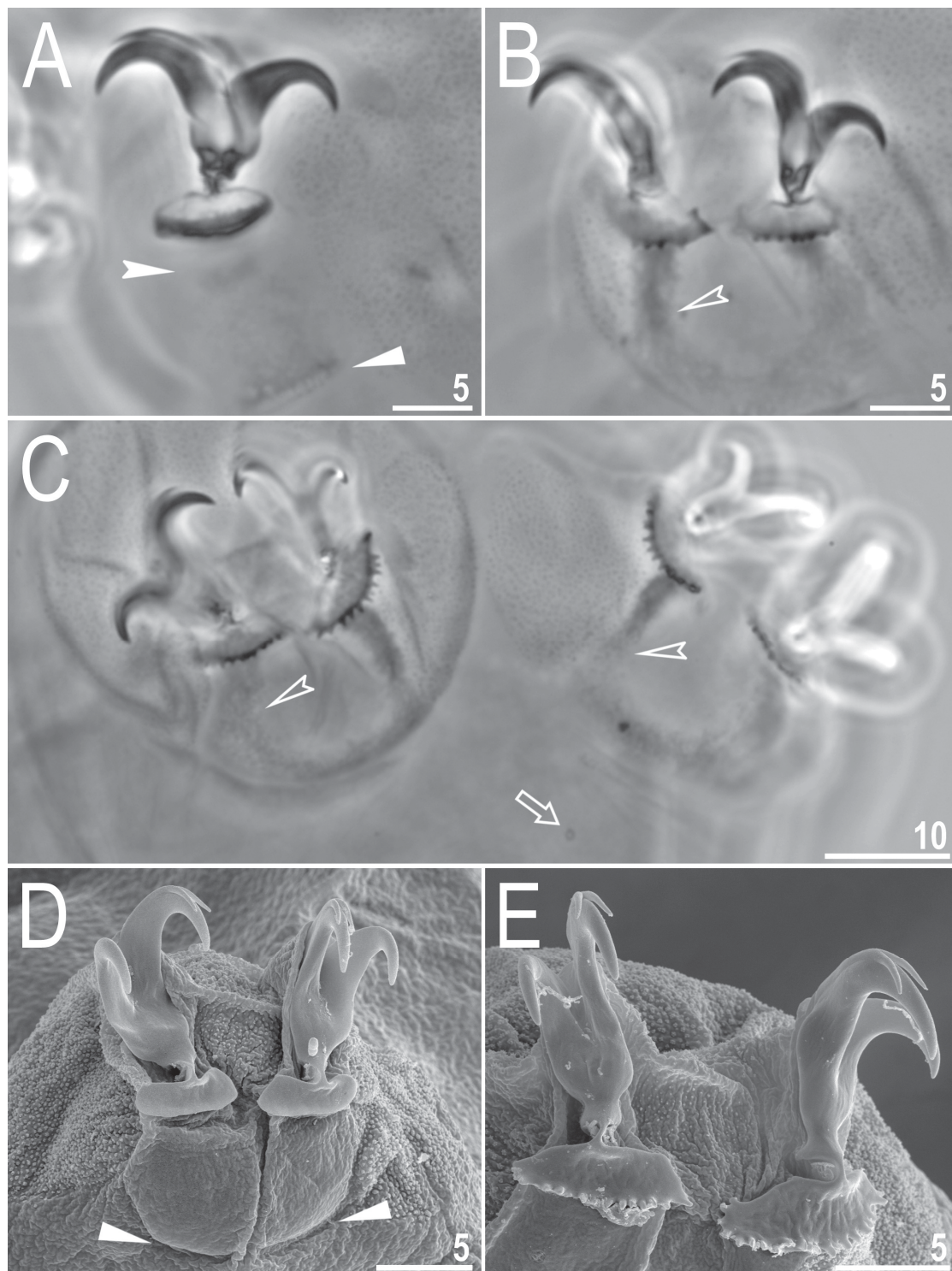


**Fig. 14.** *Macrobiotus mileri* sp. nov., PCM images of dense granulation patches and cuticular structures on legs (paratypes, ISEA PAS). **A.** Granulation on the external, proximal and internal surface of leg II. **B.** Garter-shaped structure and granulation on the external surface of leg II. **C.** Granulation on the internal surface of leg III. **D.** Granulation on the hind leg. The empty arrow indicates a cuticular pore, filled arrows indicate the garter-shaped structure. All photographs taken from specimens belonging to forma *aporata*; scale bars in  $\mu\text{m}$ .

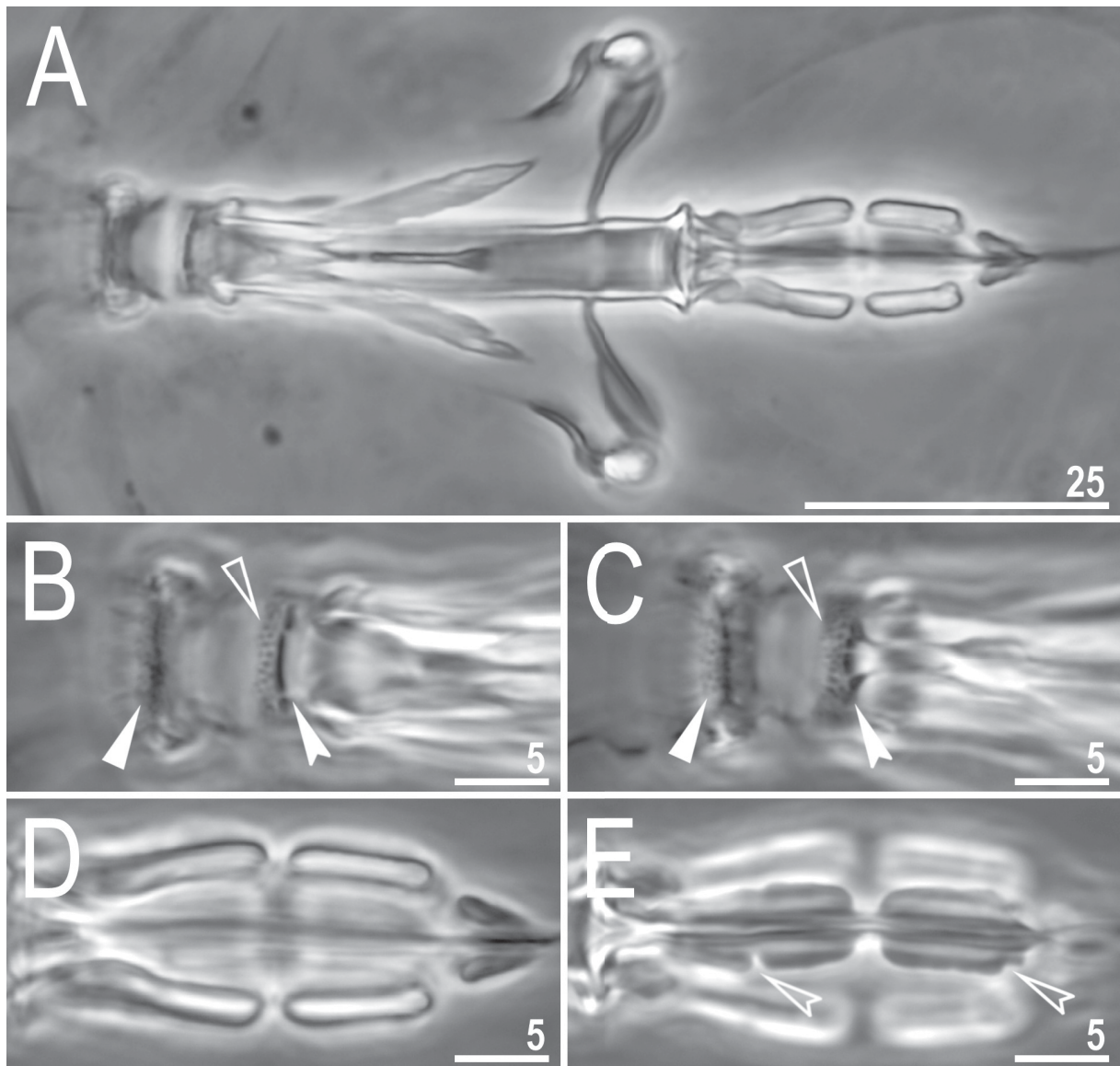


**Fig. 15.** *Macrobiotus mileri* sp. nov., SEM images of dense granulation patches and cuticular structures on legs (paratypes, ISEA PAS). **A.** Granulation and garter-shaped structure on the external surface of leg I (forma porata). **B.** Granulation and garter-shaped structure on the external surface of leg II (forma aporata). **C.** Granulation on the internal surface of leg II (forma porata). **D.** Granulation on the hind leg (forma porata). Empty arrows indicate cuticular pores, filled arrows indicate the garter-shaped structure, empty flat arrowheads indicate the small cuticular bulge/fold, filled flat arrowheads indicate small pores in between granulation that are visible only under SEM. Scale bars in  $\mu\text{m}$ .





**Fig. 16.** *Macrobiotus mileri* sp. nov., images of claws (paratypes, ISEA PAS). **A.** Claws II with smooth lunulae (PCM, forma aporata). **B.** Claws IV with dentate lunulae (PCM, forma aporata). **C.** Dentate lunulae (PCM, forma aporata). **D.** Claws II with smooth lunulae, respectively (SEM, forma porata). **E.** Claws IV with dentate lunulae (SEM, forma porata). The empty arrow indicates a singular cuticular pore, filled indented arrowheads indicate shadowed extensions extending from the lunulae (under PCM), filled flat arrowheads indicate paired muscles attachments, and empty indented arrowheads indicate the horseshoe structure connecting the anterior and the posterior claw. Scale bars in  $\mu\text{m}$ .



**Fig. 17.** *Macrobiotus mileri* sp. nov., PCM images of the buccal apparatus (all from holotype, IL.001.11, ISEA PAS, forma aporata). **A.** An entire buccal apparatus. **B–C.** The oral cavity armature, dorsal and ventral teeth respectively. **D–E.** Placoid morphology, dorsal and ventral placoids, respectively. The filled flat arrowheads indicate the first band of teeth, the empty flat arrowheads indicate the second band of teeth, the filled indented arrowheads indicate the third band of teeth, and the empty indented arrowheads indicate central and subterminal constrictions in the first and second macroplacoid, respectively. Scale bars in µm.



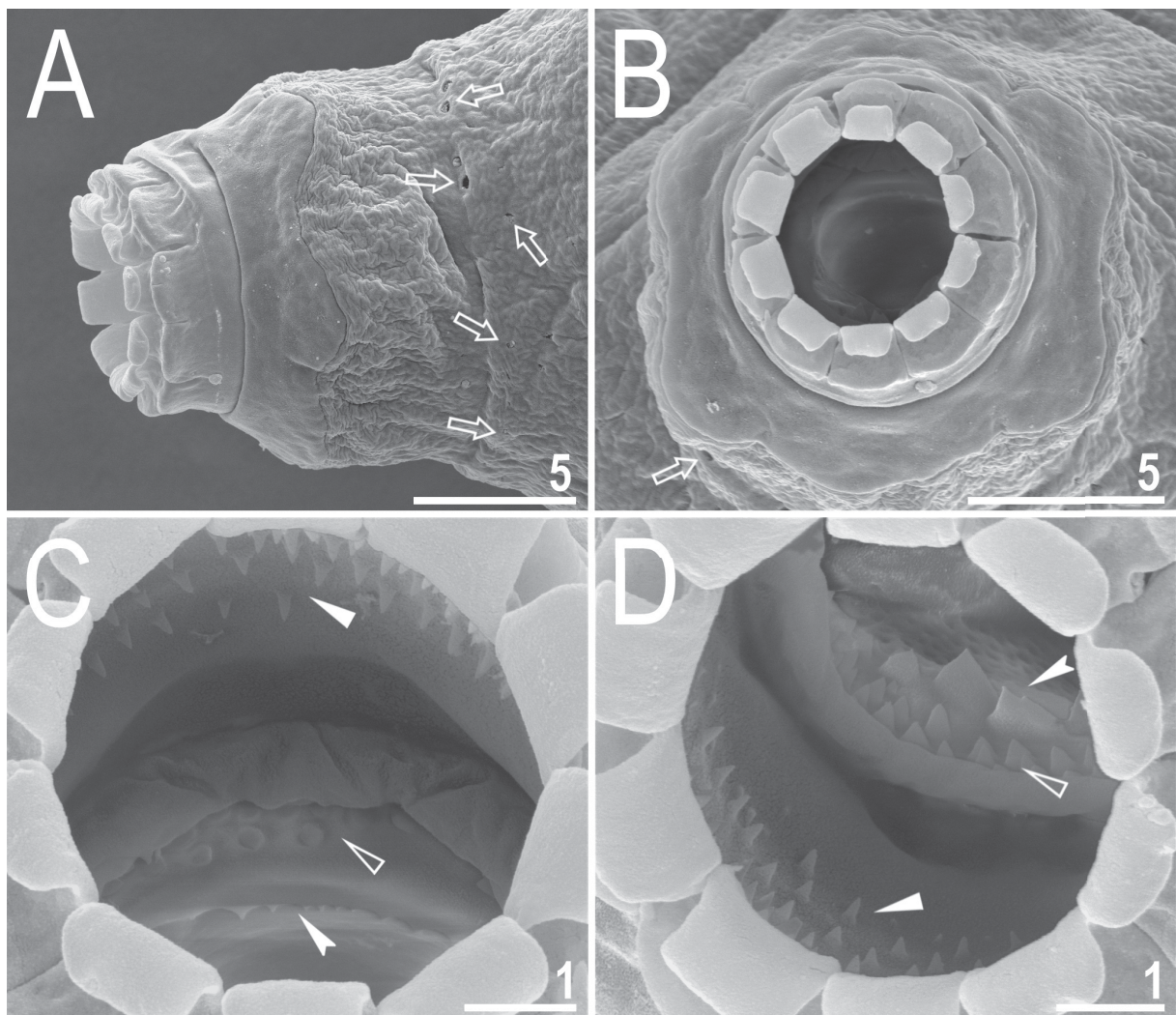
**Table 4.** Measurements [in  $\mu\text{m}$ ] and *pt* values of selected morphological structures of animals of *Macrobiotus mileri* sp. nov.; specimens mounted in Hoyer's medium; N: number of specimen/structures measured; range: refers to the smallest and the largest structure among all measured specimens; SD: standard deviation.

| Character                      | N  | Range         |           | Mean          |           | SD            |           | Holotype      |           |
|--------------------------------|----|---------------|-----------|---------------|-----------|---------------|-----------|---------------|-----------|
|                                |    | $\mu\text{m}$ | <i>pt</i> | $\mu\text{m}$ | <i>pt</i> | $\mu\text{m}$ | <i>pt</i> | $\mu\text{m}$ | <i>pt</i> |
| Body length                    | 20 | 326–523       | 1101–1392 | 449           | 1251      | 54            | 71        | 488           | 1217      |
| Buccal tube                    |    |               |           |               |           |               |           |               |           |
| Buccal tube length             | 20 | 29.5–40.8     | –         | 35.8          | –         | 3.4           | –         | 40.1          | –         |
| Stylet support insertion point | 20 | 23.3–32.8     | 78.9–81.3 | 28.5          | 79.7      | 2.7           | 0.6       | 32.0          | 79.8      |
| Buccal tube external width     | 20 | 4.3–6.7       | 14.4–17.3 | 5.8           | 16.1      | 0.7           | 0.8       | 6.4           | 16.0      |
| Buccal tube internal width     | 20 | 3.0–5.2       | 9.7–13.1  | 4.2           | 11.7      | 0.6           | 0.9       | 5.1           | 12.7      |
| Ventral lamina length          | 19 | 17.2–24.2     | 53.5–60.4 | 20.8          | 57.6      | 1.9           | 1.9       | 23.4          | 58.4      |
| Placoid lengths                |    |               |           |               |           |               |           |               |           |
| Macroplacoid 1                 | 20 | 7.0–12.2      | 23.5–30.2 | 9.7           | 26.9      | 1.6           | 2.2       | 12.1          | 30.2      |
| Macroplacoid 2                 | 20 | 4.6–7.5       | 12.3–20.8 | 6.1           | 17.1      | 0.9           | 1.8       | 7.3           | 18.2      |
| Microplacoid                   | 20 | 2.5–4.9       | 8.4–13.3  | 3.8           | 10.5      | 0.6           | 1.2       | 4.1           | 10.2      |
| Macroplacoid row               | 20 | 12.9–20.9     | 42.7–52.2 | 17.2          | 47.9      | 2.4           | 2.9       | 20.9          | 52.1      |
| Placoid row                    | 20 | 16.3–26.6     | 54.3–67.7 | 22.1          | 61.6      | 3.0           | 3.8       | 26.6          | 66.3      |
| Claw I heights                 |    |               |           |               |           |               |           |               |           |
| External primary branch        | 18 | 7.2–12.9      | 24.4–34.1 | 10.5          | 29.0      | 1.4           | 2.3       | 11.0          | 27.4      |
| External secondary branch      | 11 | 7.1–10.0      | 22.8–30.2 | 9.1           | 25.2      | 0.9           | 2.1       | 10.0          | 24.9      |
| Internal primary branch        | 18 | 7.1–11.5      | 24.1–31.1 | 9.9           | 27.3      | 1.1           | 1.6       | 10.7          | 26.7      |
| Internal secondary branch      | 12 | 7.0–10.1      | 21.4–27.1 | 9.0           | 24.3      | 1.0           | 1.6       | 9.1           | 22.7      |
| Claw II heights                |    |               |           |               |           |               |           |               |           |
| External primary branch        | 18 | 8.3–13.0      | 26.8–38.1 | 11.3          | 31.3      | 1.5           | 2.8       | 12.5          | 31.2      |
| External secondary branch      | 10 | 7.7–12.0      | 23.1–32.3 | 10.0          | 27.2      | 1.4           | 3.0       | 10.9          | 27.2      |
| Internal primary branch        | 15 | 7.4–11.0      | 24.8–29.5 | 9.7           | 26.9      | 1.1           | 1.4       | 10.1          | 25.2      |
| Internal secondary branch      | 10 | 6.2–10.2      | 20.8–27.3 | 8.9           | 23.9      | 1.2           | 2.1       | 9.8           | 24.4      |
| Claw III heights               |    |               |           |               |           |               |           |               |           |
| External primary branch        | 16 | 8.1–12.9      | 27.2–34.6 | 11.2          | 30.9      | 1.2           | 1.9       | 12.3          | 30.7      |
| External secondary branch      | 11 | 7.5–11.9      | 22.8–31.9 | 10.0          | 27.1      | 1.3           | 2.6       | 10.6          | 26.4      |
| Internal primary branch        | 13 | 7.3–11.2      | 24.5–30.0 | 9.9           | 27.2      | 1.2           | 1.5       | 10.8          | 26.9      |
| Internal secondary branch      | 10 | 6.0–10.4      | 20.1–27.9 | 9.0           | 24.0      | 1.5           | 2.6       | 10.0          | 24.9      |
| Claw IV heights                |    |               |           |               |           |               |           |               |           |
| Anterior primary branch        | 18 | 7.4–12.1      | 24.8–31.4 | 10.4          | 28.8      | 1.4           | 2.0       | 12.1          | 30.2      |
| Anterior secondary branch      | 13 | 8.2–10.7      | 20.1–27.8 | 9.1           | 24.5      | 0.7           | 2.1       | 10.7          | 26.7      |
| Posterior primary branch       | 20 | 8.2–13.1      | 23.8–38.4 | 11.1          | 31.1      | 1.5           | 3.2       | 12.2          | 30.4      |
| Posterior secondary branch     | 9  | 6.9–10.9      | 20.8–27.9 | 9.3           | 25.0      | 1.3           | 2.2       | ?             | ?         |

### Eggs

Laid freely, white, spherical and ornamented (Figs 19A–E, 20A–F). The surface between processes is of intermediate state between the *maculatus* and the *persimilis* types, that is, the surface is solid and wrinkled with very small pores, which are present mainly around the bases of the egg processes, and only some are sparse and irregularly distributed in the egg surface between processes (Figs 19A–C, 20C–F). These pores are visible under PCM, but better visible under SEM (0.1–0.3)  $\mu\text{m}$  in diameter; Figs 19A–C,

20C–F). The processes are not in the shape of inverted goblets with mostly sigmoidal (sometimes concave) conical trunks and weakly defined convex terminal discs with smooth edges (Figs 19A–C, 20C–F). Very faint annulations are visible on the process trunk, especially on the distal portion of the process (character visible only under SEM; Fig. 20D). A crown of gently marked thickenings is visible around the bases of the processes as darker dots under PCM (Fig. 19A–C) and as thicker wrinkles at the processes bases under SEM (Fig. 20D–F). In some processes under SEM the terminal discs have pores in the center (Fig. 20D–F), which under PCM are visible as large light-refracting dot in the disc center (Fig. 19A–C). However, it cannot be excluded that the actual pores in the terminal discs are preparation artefacts, while light refracting dots visible under PCM are caused by thinner chorion layers in this place. The measurements and statistics of eggs are given in Table 5.

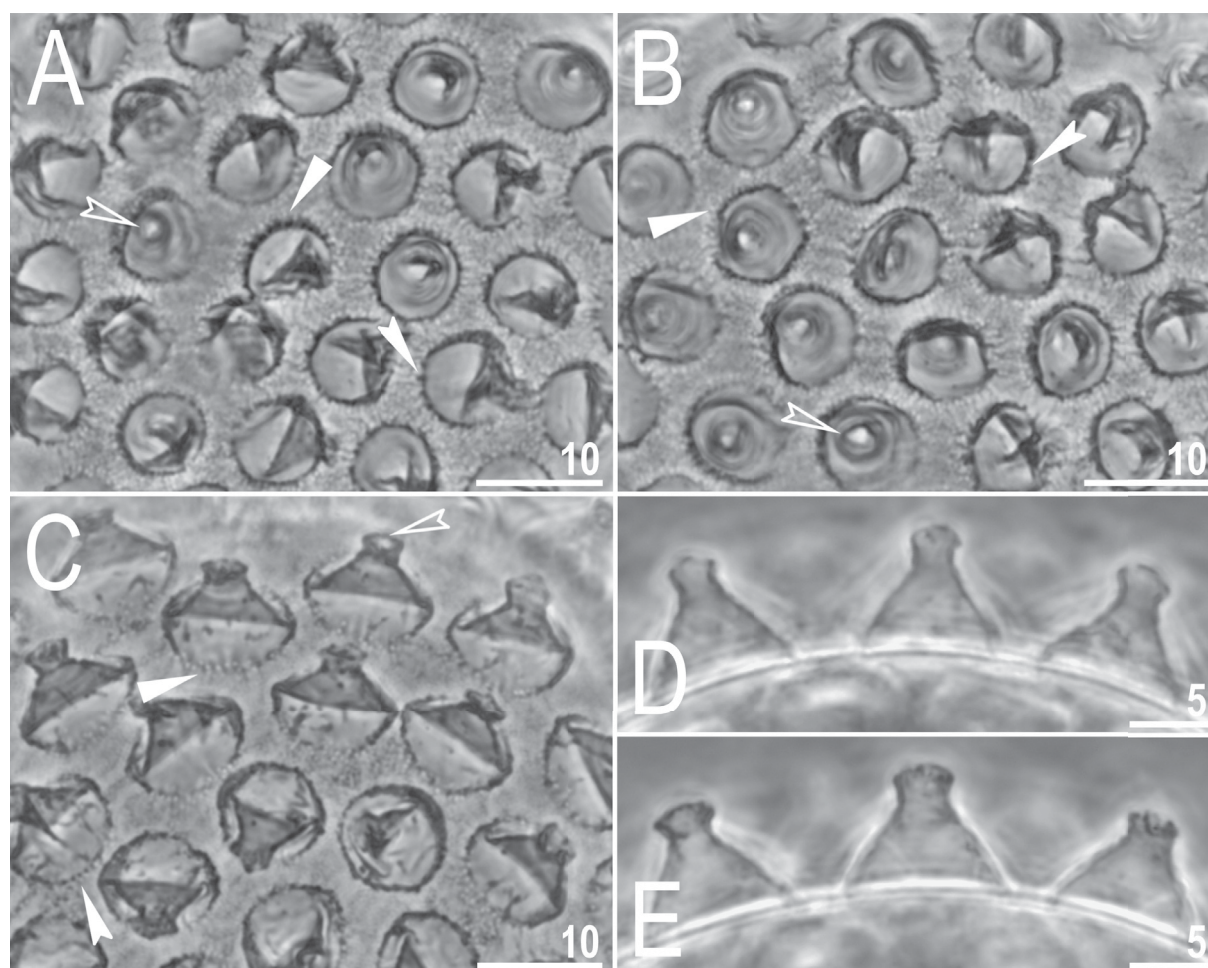


**Fig. 18.** *Macrobiotus mileri* sp. nov., mouth opening and the oral cavity armature seen under SEM (paratypes, ISEA PAS). **A–B.** The mouth opening of a single paratype visible from lateral and frontal view respectively. **C–D.** The oral cavity armature of a single paratype seen under SEM from different angles, dorsal (C) and ventral (D) view, respectively. Empty arrows indicate cuticular pores, filled flat arrowheads indicate the first band of tenth, empty flat arrowheads indicate the second band of teeth, and filled indented arrowheads indicate the third band of teeth. Scale bars in  $\mu\text{m}$ .



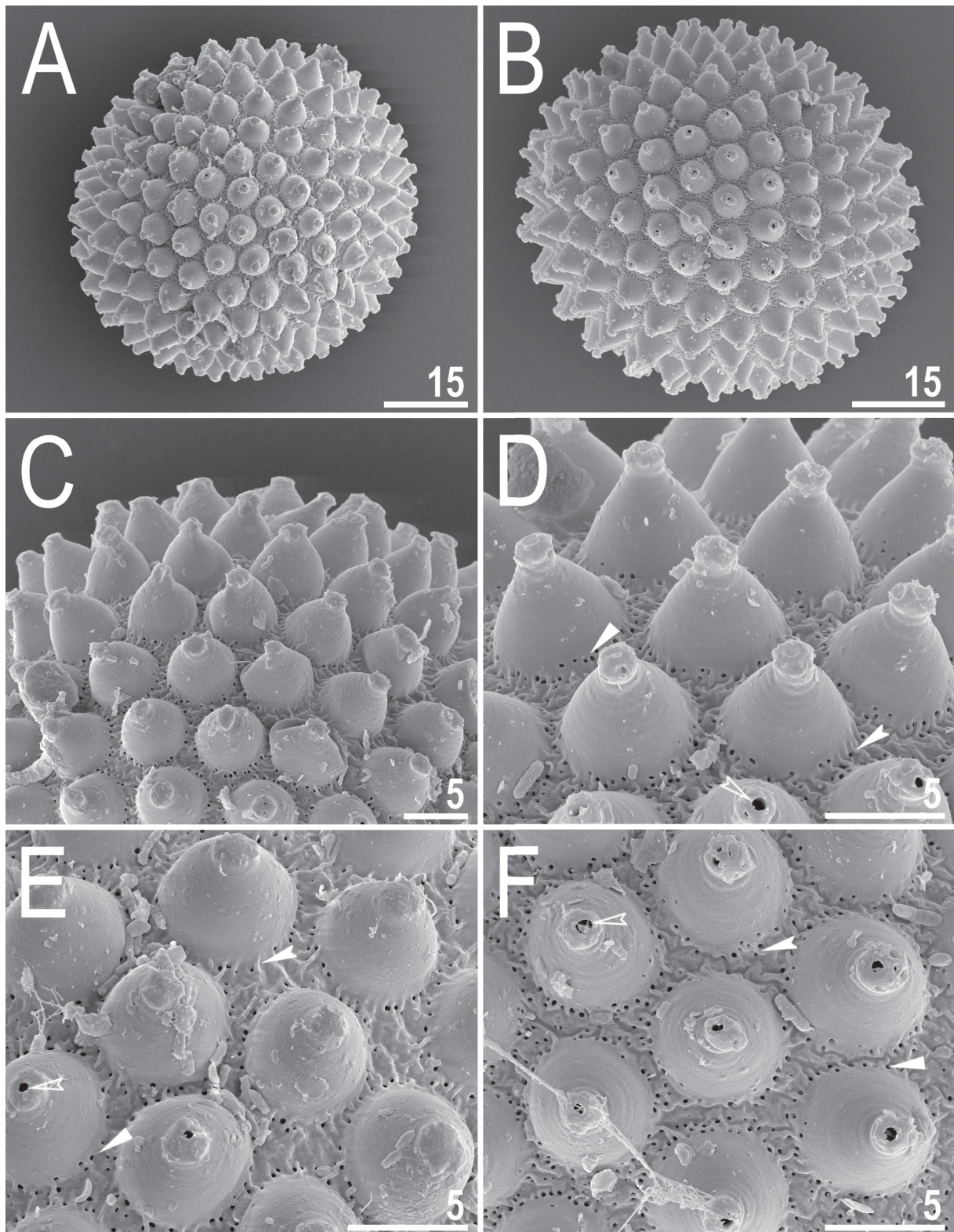
**Table 5.** Measurements [in  $\mu\text{m}$ ] of the eggs of *Macrobiotus mileri* sp. nov.; eggs mounted in Hoyer's medium; process base/height ratio is expressed as percentage; N: number of eggs/structures measured; range: refers to the smallest and the largest structure among all measured specimens; SD: standard deviation.

| Character                                    | N  | Range      | Mean | SD  |
|----------------------------------------------|----|------------|------|-----|
| Egg bare diameter                            | 7  | 76.5–92.6  | 82.5 | 6.2 |
| Egg full diameter                            | 7  | 93.0–107.6 | 98.2 | 6.3 |
| Process height                               | 21 | 6.2–8.7    | 7.6  | 0.8 |
| Process base width                           | 21 | 7.3–10.3   | 8.5  | 0.6 |
| Process base/height ratio                    | 21 | 93%–140%   | 113% | 14% |
| Terminal disc width                          | 21 | 2.2–4.2    | 3.2  | 0.4 |
| Inter-process distance                       | 21 | 1.2–3.3    | 2.4  | 0.5 |
| Number of processes on the egg circumference | 7  | 23–25      | 24.0 | 0.8 |



**Fig. 19.** *Macrobiotus mileri* sp. nov., PCM images of the eggs under  $\times 1000$  magnification (ISEA PAS). A–C. Egg surface. D–E. Midsections of egg processes. Empty indented arrowheads indicate pores/light-refracting dots in the center of the terminal discs, filled flat arrowheads indicate small pores around bases of the egg processes, filled indented arrowheads indicate dark thickenings around bases of the egg processes. Scale bars in  $\mu\text{m}$ .





**Fig. 20.** *Macrobiotus mileri* sp. nov., SEM images of eggs (ISEA PAS). **A–B.** Entire egg. **C–F.** Details of egg processes and the surface between them. Empty indented arrowheads indicate pores in center of terminal discs, filled flat arrowheads indicate small pores around bases of egg processes, filled indented arrowheads indicate thickenings around bases of egg processes. Scale bars in  $\mu\text{m}$ .



## Reproduction

The type population of *M. mileri* sp. nov. is dioecious. Both males with testes filled with sperm and females with ovaries containing oocytes were observed in specimens freshly mounted in Hoyer's medium in both specimens ascribed to each of the two morphological forms.

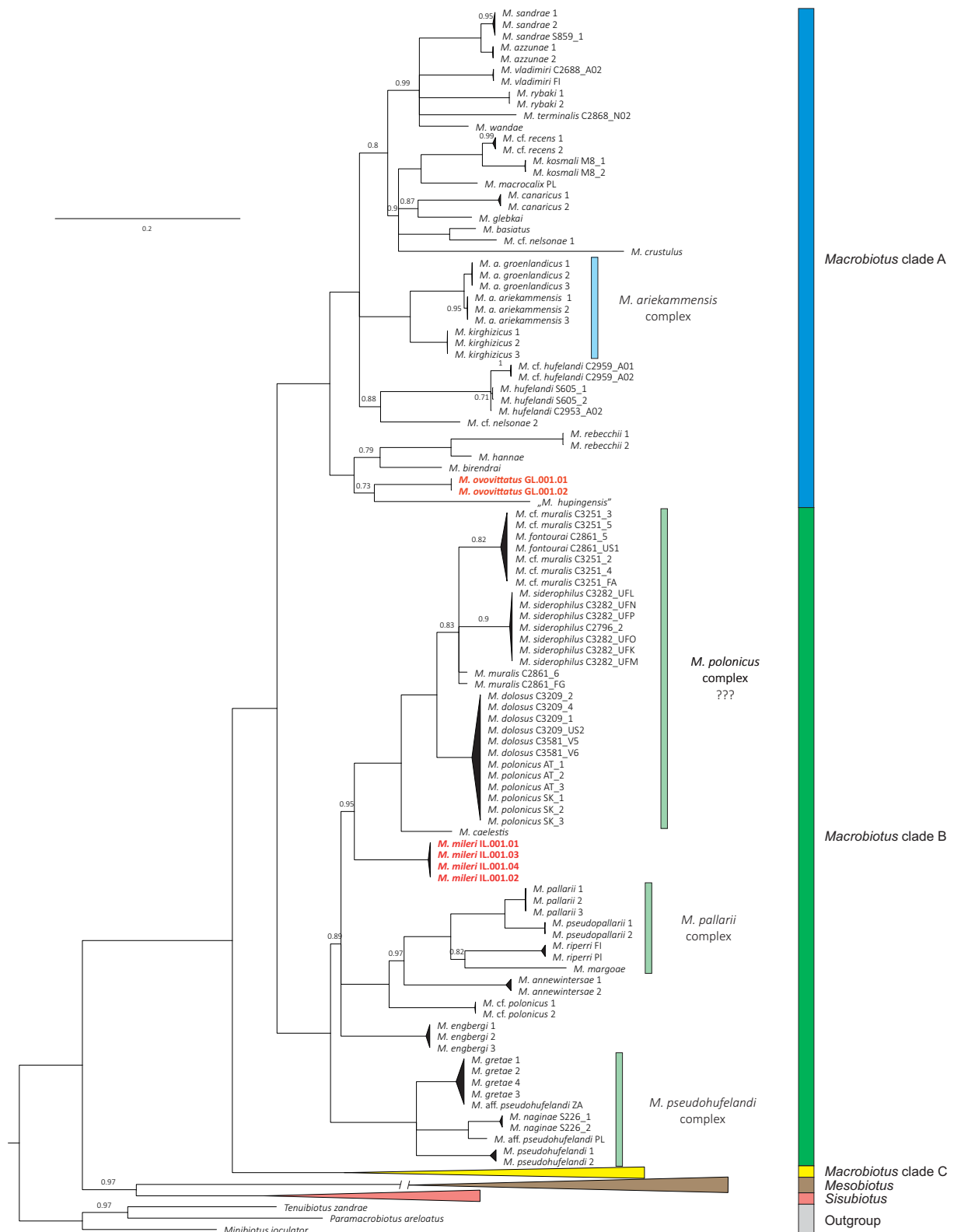
## Differential diagnosis

By having (i) three bands of teeth in the oral cavity armature that are well visible under light microscope, (ii) the entire body cuticle covered by granulation (sometimes visible only under SEM), the new species is the most similar to five other taxa of *Macrobiotus*, namely *Macrobiotus joannae* Pilato & Binda, 1983 reported from its type locality in Australia (Pilato & Binda 1983), and several uncertain localities in central, eastern, and south-eastern Russia (Biserov 1990) and from Italy (Bertolani *et al.* 2014), *Macrobiotus hanna*e Nowak & Stec, 2018 known only from its type locality in Poland (Nowak & Stec 2018), *Macrobiotus punctillus* Pilato, Binda & Azzaro, 1990 known only from its type locality in Chile (Pilato *et al.* 1990), *Macrobiotus rebecchii* Stec, 2022 known only from its type locality in Kyrgyzstan (Stec 2022b) and *M. ovovittatus* sp. nov. described above. However, it can be easily distinguished from all of them by having different pores arrangements on the body cuticles (two forms: porata with pores arranged in five distinct patches; and aporata with singular, small, almost undetectable pores vs typical, more or less evenly distributed cuticular pores in the other species) and a different morphology of the terminal discs (weakly defined convex terminal discs with smooth edges in the new species vs cog-shaped terminal discs, with a concave central area and 10–18 distinct teeth in the other species).

## Phylogenetic and delimitation results

Both phylogenetic analyses resulted in trees of similar topology, and most of the nodes well and moderately supported, in which three distinct monophyletic *Macrobiotus* lineages (*Macrobiotus* clades A, B, and C) were confidently recovered (Fig. 21, Supp. file 3). The analyses confirmed also monophyly for the *M. ariekammensis*, *M. pallarii*, and *M. pseudohufelandi* complexes (Fig. 21). At first, it seems that the *Macrobiotus polonicus-persimilis* complex as defined by Bertolani *et al.* (2023) has also been recovered to be monophyletic. However, the position of *M. cf. polonicus* 1 and 2 from Sweden (Vecchi & Stec 2021), whose morphology also fits this definition, makes this species complex paraphyletic. Also, the *M. polonicus* species complex as defined by Stec *et al.* (2021a) or the *M. persimilis* morpho-group as defined by Bertolani *et al.* (2023) is paraphyletic, since *M. cf. polonicus* from Sweden and *Macrobiotus annewintersae* Vecchi & Stec, 2021 cluster together with species of the *M. pallarii* complex. *Macrobiotus mileri* sp. nov. belongs to the *Macrobiotus* clade B staying in sister relationship with the clade containing *Macrobiotus caelestis* Coughlan, Michalczyk & Stec, 2019 and nominal taxa of the *M. polonicus-persimilis* complex (Fig. 21). The second new species, *M. ovovittatus* sp. nov., belongs to the *Macrobiotus* clade A, as the closest relative of *Macrobiotus hupingensis* Yuan, Wang, Liu, Liu & Li, 2022 and together they cluster with *Macrobiotus birendrai* Kayastha, Roszkowska, Mioduchowska, Gawlak & Kaczmarek, 2021, *M. hanna*e and *M. rebecchii* (Fig. 21). Importantly, *M. hupingensis* is a species of the *M. pallarii* complex, but the DNA sequences associated with this description belong to different unspecified *Macrobiotus* and the authors are working on correcting this mistake (Z. Yuan, Shaanxi Normal University, pers. com.). Therefore, the species name is given within quotation marks in the phylogenetic tree (Fig. 21).

The delimitation results of both ASAP analyses were congruent (Supp. file 4). The number of delimited species from the COI data set representing Macrobiotidae superclade I, and for the data set comprising only taxa of *Macrobiotus*, were 73 and 47, respectively. The number of taxa of *Macrobiotus* delimited in the larger data set was the same as for the smaller data set (47; Supp. file 4). Two new species described in this study have always been distinguished as two distinct entities (Supp. file 4). Both morphological forms within *M. mileri* sp. nov. have also been recognized as a single species (Supp. file 4). Interestingly,



**Fig. 21.** Bayesian phylogenetic reconstruction of Superclade I of the family Macrobiotidae. Values at nodes are BI posterior probability supports, nodes with support < 0.70 were collapsed whereas nodes with maximum support values (1.00) are not shown. Newly sequenced taxa are bolded and marked with red font. The scale bar represents substitutions per position.



there were several cases where taxa that were potentially thought as being distinct have been lumped together into singular putative species. These were: (i) *Macrobotus sandrae* Bertolani & Rebecchi, 1993 and *Macrobotus azzunae* Ben Marnissi, Cesari, Rebecchi & Bertolani, 2021, (see Ben Marnissi *et al.* 2021), (ii) *Macrobotus hufelandi* Schultze, 1834 and *M. cf. hufelandi* (see Bertolani *et al.* 2011), (iii) *Macrobotus fontourai* Bertolani, Cesari, Giovannini, Rebecchi, Guidetti, Kaczmarek & Pilato, 2022 and *M. cf. muralis* (see Bertolani *et al.* 2023), (iv) *Macrobotus kosmali* Kayastha, Mioduchowska, Gawlak, Sługocki, Gonçalves Silva & Kaczmarek, 2023 and *M. cf. recens* (see Kayastha *et al.* 2023).

## Discussion

### *Macrobotus ovovittatus* sp. nov.

The phylogenetic analysis recovered a clade that, together with *M. ovovittatus* sp. nov., includes *M. hannaе*, *M. rebecchii*, *M. birendrai*, and “*M. hupingensis*”. The latter species, was described as belonging to the *Macrobotus pallarii* group by Yuan *et al.* (2022); however, it is most likely that the published sequences belong to a different species. Yuan *et al.* (2022) only compared the COI sequences to other species of the *Macrobotus pallarii* group, thus making it impossible to detect the mistake. Two other species in the clade, *M. hannaе* and *M. rebecchii*, have been confirmed to be hermaphroditic (Nowak & Stec 2018; Stec 2022a). The reproductive mode of *M. birendrai* and “*M. hupingensis*” is unfortunately unknown (Kayastha *et al.* 2021; Yuan *et al.* 2022). However, it should be noted that in the original description of *M. birendrai*, more than 60 animals were analysed without finding any evidence of the presence of males. Similarly, also for *M. ovovittatus* sp. nov., males or sperm have not been observed despite a careful examination of specimens freshly mounted in Hoyer’s medium and specimens stained with orcein. Moreover, it is also important to point out that all these nominal taxa are morphologically very similar to two other species, *M. punctillus* and *M. joannae*, of which the latter is likewise confirmed to be hermaphroditic. Morphological similarity between the abovementioned species can also be observed in the egg chorion, especially when comparing the SEM data (Nowak & Stec 2018; Stec 2022b; Kayastha *et al.* 2021; this study). Taking all these fragmentary information into consideration, it might be hypothesized that in the recovered clade hermaphroditism is common (opposed to its overall rarity in tardigrades).

### *Macrobotus mileri* sp. nov.

The second newly described species, *M. mileri* sp. nov., is found in the same clade as the species of the *M. persimilis* morphogroup sensu Bertolani *et al.* (2023), species in this morphogroup exhibit a solid egg surface without reticulation or pores. However, this new species can be considered as an exception due to three main peculiarities. The first is that the animals have different alternative morphological forms (one with big, specifically arranged cuticular pores and one with singular, rare, almost impossible to notice and small pores distributed randomly on the body cuticle). Such different morphologies within one species might be ascribed to phenomena already noted in tardigrades, like cyclomorphosis (e.g., Kristensen 1982; Møbjerg *et al.* 2007) or ontogenetic changes (e.g., Surmacz *et al.* 2019, 2020). However, since cyclomorphosis is a seasonal cyclic change in morphology (thus linked to specific temporal windows), a more probable explanation for these different forms (found together at the same time) in *M. mileri* sp. nov. is an ontogenetic change. In this case, different morphologies are exhibited by different developmental stages of an animal, hence a greater possibility of finding different forms within the same population at the same time. Although such drastically disjunct animal morphologies regarding cuticular pores have not been reported so far for any member of the family Macrobotidae, one example is known within the superfamily Macrobotioidea. Specifically, in the genus *Richtersius* Pilato & Binda, 1989 only the first instar animals (hatchlings) possess cuticular pores (Guidetti *et al.* 2016; Kayastha *et al.* 2020a; Stec *et al.* 2020b; Pogwizd & Stec 2022; Kiosya & Stec 2022). The second peculiarity can be noticed in the egg chorion morphology. The fact that the new species, have reduced terminal discs but cluster together with taxa having well-developed terminal discs in egg processes once

again underlines a large evolutionary plasticity regarding morphological evolution of egg characters (Guidetti *et al.* 2013; Stec *et al.* 2016b, 2021a). Although the new species is related to taxa exhibiting so-called *persimilis* morphology, the reduced terminal discs and small pores in the egg surface between the processes prevent its inclusion in the *persimilis* morphogroup sensu Bertolani *et al.* (2023). This further stresses that the morphogroups do not reflect phylogenetic relatedness, thus no formal taxonomic rank or nomenclatural acts should be attributed to them. The third peculiarity of the new species is the presence of a garter-shaped structure (Massa *et al.* 2021) on legs I–III, which was previously suggested to be an adaptation to soil and sandy habitats characteristic of the *M. pseudohufelandi* complex, previously known as *Xerobiotus* Bertolani & Biserov, 1996 (Massa *et al.* 2021; Stec *et al.* 2021a, 2022).

### ***Macrobiotus mileri* sp. nov., in the debate on the *Xerobiotus*/*Macrobiotus pseudohufelandi* complex**

The presence of a garter-shaped structure in *Macrobiotus mileri* sp. nov., a species not related to the *Xerobiotus*/*M. pseudohufelandi* complex, invalidates this trait as diagnostic synapomorphy of the previously suppressed genus *Xerobiotus*. A similar structure was also identified in *Eremobiotus* by Gąsiorek *et al.* (2019); however, given the evolutionary distance between *Eremobiotus* and Macrobiotidae, there is the possibility that those structure are not homologous, but evolved independently as an adaptation to the same habitat. Contrarily to *Eremobiotus*, *M. mileri* sp. nov. is instead a closer relative of the *Xerobiotus*/*M. pseudohufelandi* complex, supporting the homology of this structure and dispelling its usefulness as a diagnostic synapomorphic trait for *Xerobiotus*. Bertolani *et al.* (2023) proposed to reinstate the genus *Xerobiotus* due to its clear morphological synapomorphies, keeping at the same time the genus *Macrobiotus* paraphyletic, and argued that “confirmation through synapomorphies must be sought before erecting and/or suppressing taxa”. However, the identification of one putative synapomorphy of *Xerobiotus* in another species of *Macrobiotus* erodes the set of synapomorphies supporting *Xerobiotus*. Recently, a paper proposing the resurrection of *Xerobiotus* has been published (Vincenzi *et al.* 2023). The authors found the phylogenetic position of *Xerobiotus* to be the same as in previous studies (which used the phylogenetic position as an argument for its suppression), but decided to reinstate the taxon based on its morphological distinctiveness. Surprisingly, their phylogenetic analyses seem to recover *Xerobiotus* as not monophyletic, since the newly sequenced *Pseudohexapodibius degenerans* (Biserov, 1990) is placed within it. On the other hand, genetic species delimitation performed in this new study suggested *P. degenerans* to be a synonym of one *Xerobiotus* species analysed therein. This indicates that distinguishing *Pseudohexapodibius* Bertolani & Biserov, 1996 by its extreme hind leg and claw reduction does not reflect the phylogenetic reconstruction. Such results support the suppression of *Pseudohexapodibius*, but this action has not been undertaken (Vincenzi *et al.* 2023). While the genetic data from this new publication were not available at the time, hindering reanalyses, it is unlikely that the outcome of this study would have differed significantly. The monophyly and phylogenetic position of the taxa within the *Macrobiotus* clade B (including the *Xerobiotus*/*M. pseudohufelandi* complex and *M. mileri* sp. nov.) have remained consistent across all published studies on this subject (Massa *et al.* 2021; Stec *et al.* 2021a, 2022; Vincenzi *et al.* 2023).

The approach, where a young evolutionary lineage is placed within a clade that exhibits a more conservative form, will disrupt the unity of the larger group. To ensure that the taxonomy accurately reflects the evolutionary history of tardigrades, it is crucial to avoid disrupting the unity of higher systematic ranks as already extensively explained in recent revisions (Stec *et al.* 2021a, 2022). Therefore, it should be advised that instead of hastily establishing or maintaining genera based on limited supporting evidence, it would be more appropriate to identify species complexes that emphasize the morphological and evolutionary characteristics of these lineages. Importantly, species complexes carry no nomenclatural implications and do not affect the hierarchical status of higher systematic ranks. Nonetheless, they contribute significantly to a better organization and navigation within the field of systematics. This approach aligns with the modern goal of recognizing monophyletic groups, and I



believe that subdividing the diverse yet monophyletic *Macrobiotus* into species complexes and morpho-groups provides a more flexible perspective. As such, it should be considered a preferable standpoint for future systematic and phylogenetic studies within Macrobiotidae.

## Conclusions

Comprehensive analyses that incorporate multiple biological dimensions, including morphology, behavior, molecular characteristics, and genetic variation, are now commonly employed in species delineation (Goldstein & DeSalle 2011; Vinarski 2020). These analyses generally result in coherent and stable taxonomic units that consider evolutionary histories. Thanks to comprehensive taxon sampling and combined comparative analyses of morphological and genetic data, I was able to (i) formally describe two new tardigrade species and (ii) provide an updated perspective on the *Macrobiotus* phylogeny. The results emphasize that the morphological diagnostic characters traditionally and commonly used to differentiate and identify species within this tardigrade group appear to be considerably flexible, making it challenging to recognize monophyletic entities. In addition, I discuss how one of the newly described species provides insights into the debate about the reinstatement of the genus *Xerobiotus*.

## Acknowledgments

I am particularly grateful to Krzysztof Miler (Institute of Systematics and Evolution of Animals, Polish Academy of Sciences), Piotr Łukasik, and Michał Kolasa (both from Jagiellonian University) for generously providing the samples that enabled me to conduct this study. The collections were made possible through the support of the Polish National Agency for Academic Exchange, with the Bekker scholarship awarded to KM, and the International Network for Terrestrial Research and Monitoring in the Arctic (INTERACT) Transnational Access grant in 2021 awarded to PŁ. I would also like to express my appreciation to Edoardo Massa (University of Modena and Reggio Emilia) for his valuable advice on the naming of the new species, as well as to two anonymous reviewers for their constructive comments and suggestion which improved the manuscript. This study received support from the European Commission's program 'Transnational Access to Major Research Infrastructures' through the SYNTHESYS grant (grant no. DK-TAF-TA4-005 to DS) and from the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences.

## References

- Astrin J.J. & Stüben P.E. 2008. Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics* 22: 503–522. <https://doi.org/10.1071/IS07057>
- Ben Marnissi J., Cesari M., Rebecchi L. & Bertolani R. 2021. Integrative description of a new Tunisian tardigrade species, *Macrobiotus azzunae* sp. nov. (Eutardigrada, Macrobiotidae, *hufelandi* group). *European Journal of Taxonomy* 758: 122–146. <https://doi.org/10.5852/ejt.2021.758.1429>
- Bertolani R. 1971. Rapporto-sessi e dimorfismo sessuale in *Macrobiotus* (Tardigrada). *Atti della Accademia Nazionale dei Lincei* 50: 377–382.
- Bertolani R., Rebecchi L., Giovannini I. & Cesari M. 2011. DNA barcoding and integrative taxonomy of *Macrobiotus hufelandi* C.A.S. Schultze 1834, the first tardigrade species to be described, and some related species. *Zootaxa* 2997: 19–36. <https://doi.org/10.11646/zootaxa.2997.1.2>
- Bertolani R., Guidetti R., Marchioro T., Altiero T., Rebecchi L. & Cesari M. 2014. Phylogeny of Eutardigrada: New molecular data and their morphological support lead to the identification of new evolutionary lineages. *Molecular Phylogenetics and Evolution* 76: 110–126. <https://doi.org/10.1016/j.ympev.2014.03.006>

- Bertolani R., Cesari M., Giovannini I., Rebecchi L., Guidetti R., Kaczmarek Ł. & Pilato G. 2023. The *Macrobiotus persimilis-polonicus* complex (Eutardigrada, Macrobiotidae), another example of problematic species identification, with the description of four new species. *Organisms Diversity & Evolution* 23: 329–368. <https://doi.org/10.1007/s13127-022-00599-z>
- Biserov V.I. 1990. On the revision of the genus *Macrobiotus*. The subgenus *Macrobiotus* s. st. is a new systematic status of the *hufelandi* group (Tardigrada, Macrobiotidae). Communication 2. *Zoologicheskij Zhurnal* 69 (12): 38–50.
- Casquet J.T., Thebaud C. & Gillespie, R.G. 2012. Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Molecular Ecology Resources* 12: 136–141. <https://doi.org/10.1111/j.1755-0998.2011.03073.x>
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Cesari M., Giovannini I., Altiero T., Guidetti R., Cornette R., Kikawada T. & Rebecchi L. 2022. Resistance to extreme stresses by a newly discovered Japanese tardigrade species, *Macrobiotus kyoukenus* (Eutardigrada, Macrobiotidae). *Insects* 13: 634. <https://doi.org/10.3390/insects13070634>
- Coughlan K. & Stec D. 2019. Two new species of the *Macrobiotus hufelandi* complex (Tardigrada: Eutardigrada: Macrobiotidae) from Australia and India, with notes on their phylogenetic position. *European Journal of Taxonomy* 573: 1–38. <https://doi.org/10.5852/ejt.2019.573>
- Coughlan K., Michalczyk Ł. & Stec D. 2019. *Macrobiotus caelestis* sp. nov., a new tardigrade species (Macrobiotidae: *hufelandi* group) from the Tien Shan mountains (Kyrgyzstan). *Annales Zoologici* 69 (3): 499–513. <https://doi.org/10.3161/00034541ANZ2019.69.3.002>
- Degma P. & Guidetti R. 2023. *Actual Checklist of Tardigrada Species*. Università di Modena e Reggio Emilia. [https://doi.org/10.25431/11380\\_1178608](https://doi.org/10.25431/11380_1178608)
- Gąsiorek P., Stec D., Morek W. & Michalczyk Ł. 2019. Deceptive conservatism of claws: distinct phyletic lineages concealed within Isohypsibioidea (Eutardigrada) revealed by molecular and morphological evidence. *Contributions to Zoology* 88 (1): 78–132. <https://doi.org/10.1163/18759866-20191350>
- Goldstein P.Z. & DeSalle R. 2011. Integrating DNA barcode data and taxonomic practice: Determination, discovery, and description. *Bioessays* 33: 135–147. <https://doi.org/10.1002/bies.201000036>
- Guidetti R., Peluffo J.R., Rocha A.M., Cesari M. & Moly de Peluffo M.C. 2013. The morphological and molecular analyses of a new South American urban tardigrade offer new insights on the biological meaning of the *Macrobiotus hufelandi* group of species (Tardigrada: Macrobiotidae). *Journal of Natural History* 47 (37–38): 2409–2426. <https://doi.org/10.1080/00222933.2013.800610>
- Guidetti R., Rebecchi L., Bertolani R., Jönsson K.I., Kristensen R.M. & Cesari M. 2016. Morphological and molecular analyses on *Richtersius* (Eutardigrada) diversity reveal its new systematic position and lead to the establishment of a new genus and a new family within Macrobiotidae. *Zoological Journal of the Linnean Society* 178 (4): 834–845. <https://doi.org/10.1111/zoj.12428>
- Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hoang D.T., Chernomor O., von Haeseler A., Minh B.Q. & Vinh L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522. <https://doi.org/10.1093/molbev/msx281>



Itang L.A.M., Stec D., Mapalo M.A., Mirano-Bascos D. & Michalczyk Ł. 2020. An integrative description of *Mesobiotus dilimanensis*, a new tardigrade species from the Philippines (Eutardigrada: Macrobiotidae: *furciger* group). *Raffles Bulletin of Zoology* 68: 19–31.

Kaczmarek Ł. & Michalczyk Ł. 2017. The *Macrobiotus hufelandi* (Tardigrada) group revisited. *Zootaxa* 4363 (1): 101–123. <https://doi.org/10.11646/zootaxa.4363.1.4>

Kaczmarek Ł., Cytan J., Zawierucha K., Diduszko D. & Michalczyk Ł. 2014. Tardigrades from Peru (South America), with descriptions of three new species of Parachela. *Zootaxa* 3790 (2): 357–379. <https://doi.org/10.11646/zootaxa.3790.2.5>

Kaczmarek Ł., Zawierucha K., Buda J., Stec D., Gawlak M., Michalczyk Ł. & Roszkowska M. 2018. An integrative redescription of the nominal taxon for the *Mesobiotus harmsworthi* group (Tardigrada: Macrobiotidae) leads to descriptions of two new *Mesobiotus* species from Arctic. *PLoS ONE* 13 (10): e0204756. <https://doi.org/10.1371/journal.pone.0204756>

Kaczmarek Ł., Bartylak T., Stec D., Kulpa A., Kepel M., Kepel A. & Roszkowska M. 2020. Revisiting the genus *Mesobiotus* Vecchi *et al.*, 2016 (Eutardigrada, Macrobiotidae) – remarks, updated dichotomous key and an integrative description of new species from Madagascar. *Zoologischer Anzeiger* 287: 121–146. <https://doi.org/10.1016/j.jcz.2020.05.003>

Kaczmarek Ł., Rutkowski T., Zacharyasiewicz M., Surmacki A., Osiejuk T.S. & Hayastha P. 2023. New species of Macrobiotidae (Eutardigrada) from Cameroon (Africa), characteristics of *Macrobiotus* morpho-groups and a key to the *nelsonae* group. *Annales Zoologici* 73: 1–15. <https://doi.org/10.3161/00034541ANZ2023.73.1.001>

Kalyanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A. & Jermini L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>

Katoh K. & Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298. <https://doi.org/10.1093/bib/bbn013>

Katoh K., Misawa K., Kuma K. & Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066. <https://doi.org/10.1093/nar/gkf436>

Kayastha P., Berdi D., Mioduchowska M., Gawlak M., Łukasiewicz A., Gołdyn B. & Kaczmarek Ł. 2020a. Some tardigrades from Nepal (Asia) with integrative description of *Macrobiotus wandae* sp. nov. (Macrobiotidae: *hufelandi* group). *Annales Zoologici* 70 (1): 121–142. <https://doi.org/10.3161/00034541ANZ2020.70.1.007>

Kayastha P., Berdi D., Mioduchowska M., Gawlak M., Łukasiewicz A., Gołdyn B., Jędrzejewski S. & Kaczmarek Ł. 2020b. Description and molecular characterization of *Richtersius ziemowiti* sp. nov. (Richtersiidae) from Nepal (Asia) with evidence of heterozygous point mutation events in the 28S rRNA. *Annales Zoologici* 70 (3): 381–396. <https://doi.org/10.3161/00034541ANZ2020.70.3.010>

Kayastha P., Roszkowska M., Mioduchowska M., Gawlak M. & Kaczmarek Ł. 2021. Integrative descriptions of two new tardigrade species along with the new record of *Mesobiotus skorackii* Kaczmarek *et al.*, 2018 from Canada. *Diversity* 13: 394. <https://doi.org/10.3390/d13080394>

Kayastha P., Mioduchowska M., Gawlak M., Sługocki Ł., Gonçalves Silva A.J.J. & Kaczmarek Ł. 2023. Integrative description of *Macrobiotus kosmali* sp. nov. (*hufelandi* group) from the Island of Madeira (Portugal). *The European Zoological Journal* 90 (1): 126–138. <https://doi.org/10.1080/24750263.2022.2163312>

- Kiosya Y. & Stec D. 2022. New species of the genus *Richtersius* Pilato & Binda, 1989 (Tardigrada: Eutardigrada: Richtersiusidae) from Uzbekistan. *Folia Biologica (Kraków)* 70 (4): 141–150. [https://doi.org/10.3409/fb\\_70-4.18](https://doi.org/10.3409/fb_70-4.18)
- Kiosya Y., Pogwizd J., Matsko Y., Vecchi M. & Stec D. 2021. Phylogenetic position of two *Macrobiotus* species with a revisional note on *Macrobiotus sottilei* Pilato, Kiosya, Lisi & Sabella, 2012 (Tardigrada: Eutardigrada: Macrobiotidae). *Zootaxa* 4933 (1): 113–135. <https://doi.org/10.11646/zootaxa.4933.1.5>
- Kristensen R.M. 1982. The first record of cyclomorphosis in Tardigrada based on a new genus and species from arctic meiobenthos. *Zeitschrift für Zoologische Systematik und Evolution Forschung* 20: 249–270. <https://doi.org/10.1111/j.1439-0469.1983.tb00552.x>
- Kuzdrowska K.A., Mioduchowska M., Gawlak M., Bartylak T., Kepel A., Kepel M. & Kaczmarek Ł. 2021. Integrative description of *Macrobiotus porifini* sp. nov. (Macrobiotidae) from Madagascar and its phylogenetic position within the *hufelandi* group. *The European Zoological Journal* 88 (1): 375–389. <https://doi.org/10.1080/24750263.2021.1883752>
- Lanfear R., Frandsen P.B., Wright A.M., Senfeld T. & Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773. <https://doi.org/10.1093/molbev/msw260>
- Mapalo M., Stec D., Mirano-Bascos D.M. & Michalczyk Ł. 2016. *Mesobiotus philippinicus* sp. nov., the first limnoterrestrial tardigrade from the Philippines. *Zootaxa* 4126 (3): 411–426. <https://doi.org/10.11646/zootaxa.4126.3.6>
- Mapalo M., Stec D., Mirano-Bascos D. & Michalczyk Ł. 2017. An integrative description of a limnoterrestrial tardigrade from the Philippines, *Mesobiotus insanis*, new species (Eutardigrada: Macrobiotidae: *harmsworthi* group). *Raffles Bulletin of Zoology* 65: 440–454.
- Marley N.J., McInnes S.J. & Sands C.J. 2011. Phylum Tardigrada: A re-evaluation of the Parachela. *Zootaxa* 2819: 51–64. <https://doi.org/10.11646/zootaxa.2819.1.2>
- Massa E., Guidetti R., Cesari M., Rebecchi L. & Jönsson K.I. 2021. Tardigrades of Kristianstads Vattenrike Biosphere Reserve with description of four new species from Sweden. *Scientific Reports* 11: 4861. <https://doi.org/10.1038/s41598-021-83627-w>
- Michalczyk Ł. & Kaczmarek Ł. 2013. The Tardigrada Register: a comprehensive online data repository for tardigrade taxonomy. *Journal of Limnology* 72 (S1): 175–181. <https://doi.org/10.4081/jlimnol.2013.s1.e22>
- Mironov S.V., Dabert J. & Dabert M. 2012. A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit *Aegithalos caudatus* (Passeriformes: Aegithalidae): morphological description with DNA barcode data. *Zootaxa* 3253: 54–61. <https://doi.org/10.11646/zootaxa.3253.1.2>
- Møbjerg N., Jørgensen A., Eibye-Jacobsen J., Agerlin Halberg K., Persson D. & Kristensen R.M. 2007. New records on cyclomorphosis in the marine eutardigrade *Halobiotus crispae* (Eutardigrada: Hypsibiidae). *Journal of Limnology* 66 (Suppl. 1): 132–140. <https://doi.org/10.4081/jlimnol.2007.s1.132>
- Morek W., Stec D., Gąsiorek P., Schill R.O., Kaczmarek Ł. & Michalczyk Ł. 2016. An experimental test of eutardigrade preparation methods for light microscopy. *Zoological Journal of the Linnean Society* 178 (4): 785–793. <https://doi.org/10.1111/zoj.12457>
- Nelson D.R., Bartels P.J. & Guil N. 2019. Tardigrade ecology. In: Schill R.O. (ed.) *Water Bears: The Biology of Tardigrades*: 163–210. [https://doi.org/10.1007/978-3-319-95702-9\\_7](https://doi.org/10.1007/978-3-319-95702-9_7)



- Nelson D.R., Adkins Fletcher R., Guidetti R., Roszkowska M., Grobys D. & Kaczmarek Ł. 2020. Two new species of Tardigrada from moss cushions (*Grimmia* sp.) in a xerothermic habitat in northeast Tennessee (USA, North America), with the first identification of males in the genus *Viridiscus*. *PeerJ* 8: e10251. <https://doi.org/10.7717/peerj.10251>
- Nguyen L.-T., Schmidt H.A., von Haeseler A. & Minh B.Q. 2015. IQ- TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274. <https://doi.org/10.1093/molbev/msu300>
- Nowak B. & Stec D. 2018. An integrative description of *Macrobiotus hanna* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Poland. *Turkish Journal of Zoology* 42: 269–286. <https://doi.org/10.3906/zoo-1712-31>
- Pilato G. 1981. Analisi di nuovi caratteri nello studio degli Eutardigradi. *Animalia* 8: 51–57.
- Pilato G. & Binda M.G. 1983. Descrizione di una nuova specie di Eutardigrado d’Australia *Macrobiotus joannae* n. sp. *Animalia* 10: 262–272.
- Pilato G. & Binda M.G. 2010. Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa* 2404: 1–52. <https://doi.org/10.11646/zootaxa.2404.1.1>
- Pilato G., Binda M.G. & Azzaro M. 1990. Tardigradi di Terra del Fuoco e Magallanes. III. *Macrobiotus punctillus*, nuova specie di Macrobiotidae del gruppo *hufelandi*. *Animalia* 17: 123–129.
- Pogwizd J. & Stec D. 2022. An integrative description of a new *Richtersius* species from Greece (Tardigrada: Eutardigrada: Richtersiusidae). *Acta Zoologica Academiae Scientiarum Hungaricae* 68 (1): 1–21. <https://doi.org/10.17109/AZH.68.1.1.2022>
- Rambaut A., Suchard M.A., Xie D. & Drummond A.J. 2014. Tracer v.1.6. Available from <https://beast.bio.ed.ac.uk/Tracer> [accessed 10 Sep. 2018].
- Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Roszkowska M., Ostrowska M., Stec D., Janko K. & Kaczmarek Ł. 2017. *Macrobiotus polypiformis* sp. nov., a new tardigrade (Macrobiotidae: *hufelandi* group) from the Ecuadorian Pacific coast, with remarks on the claw abnormalities in eutardigrades. *European Journal of Taxonomy* 327: 1–19. <https://doi.org/10.5852/ejt.2017.327>
- Roszkowska M., Stec D., Gawlak M. & Kaczmarek Ł. 2018. An integrative description of a new tardigrade species *Mesobiotus romani* sp. nov. (Macrobiotidae: *harmsworthi* group) from the Ecuadorian Pacific coast. *Zootaxa* 4450 (5): 550–564. <https://doi.org/10.11646/zootaxa.4450.5.2>
- Short K.A., Sands C.J., McInnes S.J., Pisani D., Stevens M.I. & Convey. P. 2022. An ancient, Antarctic-specific species complex: large divergences between multiple Antarctic lineages of the tardigrade genus *Mesobiotus*. *Molecular Phylogenetics and Evolution* 170: 107429. <https://doi.org/10.1016/j.ympev.2022.107429>
- Stec D. 2019. *Mesobiotus datanlanicus* sp. nov., a new tardigrade species (Macrobiotidae: *Mesobiotus harmsworthi* group) from Lâm Đồng Province in Vietnam. *Zootaxa* 4679 (1): 164–180. <https://doi.org/10.11646/zootaxa.4679.1.10>
- Stec D. 2021. Integrative descriptions of two new *Mesobiotus* species (Tardigrada, Eutardigrada, Macrobiotidae) from Vietnam. *Diversity* 13: 605. <https://doi.org/10.3390/d13110605>

- Stec D. 2022a. An integrative description of two new *Mesobiotus* Species (Tardigrada: Eutardigrada: Macrobiotidae) with updated genus phylogeny. *Zoological Studies* 61: 85. <https://doi.org/10.6620/zs.2022.61-85>
- Stec D. 2022b. *Macrobiotus rebecchii* sp. nov.: a new limno-terrestrial and hermaphroditic tardigrade from Kyrgyzstan. *Animals* 12 (21): 2906. <https://doi.org/10.3390/ani12212906>
- Stec D. & Kristensen R.M. 2017. An integrative description of *Mesobiotus ethiopicus* sp. nov. (Tardigrada: Eutardigrada: Parachela: Macrobiotidae: *harmsworthi* group) from the Northern Afrotropic region. *Turkish Journal of Zoology* 41: 800–811. <https://doi.org/10.3906/zoo-1701-47>
- Stec D., Smolak R., Kaczmarek Ł. & Michalczyk Ł. 2015. An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya. *Zootaxa* 4052 (5): 501–526. <https://doi.org/10.11646/zootaxa.4052.5.1>
- Stec D., Gąsiorek P., Morek W., Kosztyła P., Zawierucha K., Michno K., Kaczmarek Ł., Prokop Z.M. & Michalczyk Ł. 2016a. Estimating optimal sample size for tardigrade morphometry. *Zoological Journal of the Linnean Society* 178 (4): 776–784. <https://doi.org/10.1111/zoj.12404>
- Stec D., Morek W., Gąsiorek P., Kaczmarek Ł. & Michalczyk Ł. 2016b. Determinants and taxonomic consequences of extreme egg shell variability in *Ramazzottius subanomalus* (Biserov, 1985) (Tardigrada). *Zootaxa* 4208 (2): 176–188. <https://doi.org/10.11646/zootaxa.4208.2.5>
- Stec D., Zawierucha K. & Michalczyk Ł. 2017a. An integrative description of *Ramazzottius subanomalus* (Biserov, 1985) (Tardigrada) from Poland. *Zootaxa* 4300(3): 403–420. <https://doi.org/10.11646/zootaxa.4300.3.4>
- Stec D., Morek W., Gąsiorek P., Blagden B. & Michalczyk Ł. 2017b. Description of *Macrobiotus scoticus* sp. nov. (Tardigrada: Macrobiotidae: *hufelandi* group) from Scotland by means of integrative taxonomy. *Annales Zoologici* 67 (2): 181–197. <https://doi.org/10.3161/00034541ANZ2017.67.2.001>
- Stec D., Morek W., Gąsiorek P. & Michalczyk Ł. 2018a. Unmasking hidden species diversity within the *Ramazzottius oberhaeuseri* complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela). *Systematics and Biodiversity* 16 (4): 357–376. <https://doi.org/10.1080/14772000.2018.1424267>
- Stec D., Krzywański Ł. & Michalczyk Ł. 2018b. Integrative description of *Macrobiotus canaricus* sp. nov. with notes on *M. recens* (Eutardigrada: Macrobiotidae). *European Journal of Taxonomy* 452: 1–36. <https://doi.org/10.5852/ejt.2018.452>
- Stec D., Kristensen R.M. & Michalczyk Ł. 2018c. Integrative taxonomy identifies *Macrobiotus papei*, a new tardigrade species of the *Macrobiotus hufelandi* complex (Eutardigrada: Macrobiotidae) from the Udzungwa Mountains National Park (Tanzania). *Zootaxa* 4446 (2): 273–291. <https://doi.org/10.11646/zootaxa.4446.2.7>
- Stec D., Arakawa K. & Michalczyk Ł. 2018d. An integrative description of *Macrobiotus shonaicus* sp. nov. (Tardigrada: Macrobiotidae) from Japan with notes on its phylogenetic position within the *hufelandi* group. *PLoS ONE* 13 (2): e0192210. <https://doi.org/10.1371/journal.pone.0192210>
- Stec D., Roszkowska M., Kaczmarek Ł. & Michalczyk Ł. 2018e. An integrative description of a population of *Mesobiotus radiatus* (Pilato, Binda and Catanzaro, 1991) from Kenya. *Turkish Journal of Zoology* 42: 523–540. <https://doi.org/10.3906/zoo-1802-43>
- Stec D., Kristensen R.M. & Michalczyk Ł. 2020a. An integrative description of *Minibiotus ioculator* sp. nov. from the Republic of South Africa with notes on *Minibiotus pentannulatus* Londoño *et al.*, 2017 (Tardigrada: Macrobiotidae). *Zoologischer Anzeiger* 286: 117–134. <https://doi.org/10.1016/j.jcz.2020.03.007>



- Stec D., Krzywański Ł., Arakawa K. & Michalczyk Ł. 2020b. A new redescription of *Richtersius coronifer*, supported by transcriptome, provides resources for describing concealed species diversity within the monotypic genus *Richtersius* (Eutardigrada). *Zoological Letters* 6: 2. <https://doi.org/10.1186/s40851-020-0154-y>
- Stec D., Dudziak M. & Michalczyk Ł. 2020c. Integrative descriptions of two new Macrobiotidae species (Tardigrada: Eutardigrada: Macrobiotioidea) from French Guiana and Malaysian Borneo. *Zoological Studies* 59: 23. <https://doi.org/10.6620/ZS.2020.59-23>
- Stec D., Tumanov D.T. & Kristensen R.M. 2020d. Integrative taxonomy identifies two new tardigrade species (Eutardigrada: Macrobiotidae) from Greenland. *European Journal of Taxonomy* 614: 1–40. <https://doi.org/10.5852/ejt.2020.614>
- Stec D., Krzywański Ł., Zawierucha K. & Michalczyk Ł. 2020e. Untangling systematics of the *Paramacrobiotus areolatus* species complex by an integrative redescription of the nominal species for the group, with multilocus phylogeny and species delineation within the genus *Paramacrobiotus*. *Zoological Journal of the Linnean Society* 188 (3): 694–716. <https://doi.org/10.1093/zoolinlean/zlzl63>
- Stec D., Vecchi M., Calhim S. & Michalczyk Ł. 2021a. New multilocus phylogeny reorganises the family Macrobiotidae (Eutardigrada) and unveils complex morphological evolution of the *Macrobiotus hufelandi* group. *Molecular Phylogenetics & Evolution* 160: 106987. <https://doi.org/10.1016/j.ympev.2020.106987>
- Stec D., Vecchi M., Dudziak M., Bartels P.J., Calhim S. & Michalczyk Ł. 2021b. Integrative taxonomy resolves species identities within the *Macrobiotus pallarii* complex (Eutardigrada: Macrobiotidae). *Zoological Letters* 7: 9. <https://doi.org/10.1186/s40851-021-00176-w>
- Stec D., Vončina K., Kristensen R.M. & Michalczyk Ł. 2022. The *Macrobiotus ariekammensis* species complex provides evidence for parallel evolution of claw elongation in macrobiotid tardigrades. *Zoological Journal of the Linnean Society* 195: 1067–1099. <https://doi.org/10.1093/zoolinlean/zlab101>
- Surmacz B., Morek W. & Michalczyk Ł. 2019. What if multiple claw configurations are present in a sample: a case study with the description of *Milnesium pseudotardigradum* sp. nov. with unique developmental variability. *Zoological Studies* 58: 32. <https://doi.org/10.6620/ZS.2019.58-32>
- Surmacz B., Morek W. & Michalczyk Ł. 2020. What to do when ontogenetic tracking is unavailable: a morphometric method to classify instars in *Milnesium* (Tardigrada). *Zoological Journal of the Linnean Society* 188 (3): 797–808. <https://doi.org/10.1093/zoolinlean/zlz099>
- Tamura K., Stecher G. & Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 38 (7): 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Trifinopoulos J., Nguyen L.-T., von Haeseler A. & Minh B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: 232–235. <https://doi.org/10.1093/nar/gkw256>
- Tumanov D.V. 2020. Integrative description of *Mesobiotus anastasiae* sp. nov. (Eutardigrada, Macrobiotioidea) and first record of *Lobohalacarus* (Chelicerata, Trombidiformes) from the Republic of South Africa. *European Journal of Taxonomy* 726 (1): 102–131. <https://doi.org/10.5852/ejt.2020.726.1179>
- Vaidya G., Lohman D.J. & Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Vecchi M. & Stec D. 2021. Integrative descriptions of two new *Macrobiotus* species (Tardigrada, Eutardigrada, Macrobiotidae) from Mississippi (USA) and Crete (Greece). *Zoosystematics and Evolution* 97 (1): 281–306. <https://doi.org/10.3897/zse.97.65280>

- Vecchi M., Cesari M., Bertolani R., Jönsson K.I., Rebecchi L. & Guidetti R. 2016. Integrative systematic studies on tardigrades from Antarctica identify new genera and new species within Macrobiotidea and Echiniscoidea. *Invertebrate Systematics* 30 (4): 303–322. <https://doi.org/10.1071/IS15033>
- Vecchi M., Stec D., Tommi V., Ryndov S., Chartrain J. & Calhim S. 2022a. *Macrobiotus naginae* sp. nov., a new xerophilous tardigrade species from Rokua sand dunes (Finland). *Zoological Studies* 61: 22. <https://doi.org/10.6620/ZS.2022.61-22>
- Vecchi M., Choong H. & Calhim S. 2022b. *Sisubiotus hakaensis* sp. nov. (Tardigrada, Macrobiotidae), a new tardigrade species from Calvert Island (British Columbia, Canada). *European Journal of Taxonomy* 823: 64–81. <https://doi.org/10.5852/ejt.2022.823.1815>
- Vincenzi J., Cesari M., Kaczmarek Ł., Roszkowska M., Mioduchowska M., Rebecchi L., Kiosya Y. & Guidetti R. 2023. The xerophilic genera *Xerobiotus* and *Pseudohexapodibius* (Macrobiotidae; Tardigrada): biodiversity, biogeography and phylogeny. *Zoological Journal of the Linnean Society* 200 (1): 111–141. <https://doi.org/10.1093/zoolinnean/zlad129>
- Vinarski M.V. 2020. Roots of the taxonomic impediment: Is the “integrativeness” a remedy? *Integrative Zoology* 15: 2–15. <https://doi.org/10.1111/1749-4877.12393>
- Yuan Z., Wang Y., Liu Q., Liu L. & Li X.-C. 2022. *Macrobiotus hupingensis*, a new tardigrade species in the *Macrobiotus pallarii* complex from China. *Zoological Studies* 61: 86. <https://doi.org/10.6620/ZS.2022.61-86>

*Manuscript received: 16 September 2023*

*Manuscript accepted: 14 November 2023*

*Published on 29 March 2024*

*Topic editor: Magalie Castelin*

*Section editor: Fabio Stoch*

*Desk editor: Natacha Beau*

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the EJT consortium: Muséum national d’histoire naturelle, Paris, France; Meise Botanic Garden, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands; Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain; Leibniz Institute for the Analysis of Biodiversity Change, Bonn – Hamburg, Germany; National Museum of the Czech Republic, Prague, Czech Republic.

## Supplementary files

**Supp. file 1.** Raw morphometric data underling the description of *Macrobiotus ovovittatus* sp. nov. <https://doi.org/10.5852/ejt.2024.930.2481.11047>

**Supp. file 2.** Raw morphometric data underling the description of *Macrobiotus mileri* sp. nov. <https://doi.org/10.5852/ejt.2024.930.2481.11049>

**Supp. file 3.** Model selections of sequence evolution for BI and ML analyses and raw trees. <https://doi.org/10.5852/ejt.2024.930.2481.11051>

**Supp. file 4.** Outputs from the ASAP analyses. <https://doi.org/10.5852/ejt.2024.930.2481.11055>



**Appendix 1** (continued on next 5 pages). Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study.

| Species                                                | 18S             | 28S             | ITS-2           | COI             | Source                         |
|--------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|--------------------------------|
| <i>Macrobiotus ovovittatus</i><br>GL.001.01            | <b>OR543310</b> | <b>OR543318</b> | <b>OR543314</b> | <b>OR544395</b> | this study                     |
| <i>Macrobiotus ovovittatus</i><br>GL.001.02            | <b>OR543311</b> | <b>OR543319</b> | <b>OR543315</b> | <b>OR544396</b> | this study                     |
| <i>Macrobiotus mileri</i><br>IL.001.01 (forma porata)  | <b>OR543312</b> | <b>OR543320</b> | <b>OR543316</b> | <b>OR544397</b> | this study                     |
| <i>Macrobiotus mileri</i><br>IL.001.02 (forma aporata) | <b>OR543313</b> | <b>OR543321</b> | <b>OR543317</b> | <b>OR544398</b> | this study                     |
| <i>Macrobiotus mileri</i><br>IL.001.03 (forma porata)  |                 |                 |                 | <b>OR544399</b> | this study                     |
| <i>Macrobiotus mileri</i><br>IL.001.04 (forma aporata) |                 |                 |                 | <b>OR544400</b> | this study                     |
| <i>Macrobiotus kosmali</i> M8.1                        | OP142472        | OP143765        | OP153786        | OP141639        | Kayastha <i>et al.</i> (2023)  |
| <i>Macrobiotus kosmali</i> M8.2                        | OP142473        | OP143766        |                 | OP141640        | Kayastha <i>et al.</i> (2023)  |
| <i>Macrobiotus hupingensis</i>                         | MW183923        | MZ470349        | MZ474842        | MW186952        | Yuan <i>et al.</i> (2022)      |
| <i>Macrobiotus dolosus</i><br>C3209_2                  | OP596290        |                 |                 | OP561772        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus dolosus</i><br>C3209_US2                |                 |                 |                 | OP561773        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus dolosus</i><br>C3209_4                  |                 |                 |                 | OP561774        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus dolosus</i><br>C3581_V6                 | OP596292        |                 |                 | OP561775        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus dolosus</i><br>C3209_1                  | OP596289        |                 |                 |                 | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus dolosus</i><br>C3581_V5                 | OP596291        |                 |                 |                 | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C2796_2             | OP596293        |                 |                 | OP561776        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C3282_UFK           |                 |                 |                 | OP561777        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C3282_UFL           |                 |                 |                 | OP561778        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C3282_UFM           |                 |                 |                 | OP561779        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C3282_UFN           | OP596294        |                 |                 | OP561780        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C3282_UFO           |                 |                 |                 | OP561781        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus siderophilus</i><br>C3282_UFP           |                 |                 |                 | OP561782        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus fonturai</i><br>C2861_5                 | OP596295        |                 |                 | OP561783        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus fonturai</i><br>C2861_US1               | OP596296        |                 |                 | OP561784        | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus muralis</i><br>C2861_FG                 | OP596297        |                 |                 |                 | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus muralis</i><br>C2861_6                  | OP596298        |                 |                 |                 | Bertolani <i>et al.</i> (2023) |
| <i>Macrobiotus</i> cf. <i>muralis</i><br>C3251_2       | OP596299        |                 |                 | OP561785        | Bertolani <i>et al.</i> (2023) |

**Appendix 1.** Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study.

| Species                                               | 18S      | 28S      | ITS-2    | COI      | Source                                                                  |
|-------------------------------------------------------|----------|----------|----------|----------|-------------------------------------------------------------------------|
| <i>Macrobilotus</i> cf. <i>muralis</i><br>C3251_3     | OP596300 |          |          | OP561786 | Bertolani <i>et al.</i> (2023)                                          |
| <i>Macrobilotus</i> cf. <i>muralis</i><br>C3251_FA    | OP596301 |          |          | OP561787 | Bertolani <i>et al.</i> (2023)                                          |
| <i>Macrobilotus</i> cf. <i>muralis</i><br>C3251_4     |          |          |          | OP561788 | Bertolani <i>et al.</i> (2023)                                          |
| <i>Macrobilotus</i> cf. <i>muralis</i><br>C3251_5     |          |          |          | OP561789 | Bertolani <i>et al.</i> (2023)                                          |
| <i>Macrobilotus</i> <i>hufelandi</i><br>C2953_A02     | OP596302 |          |          | HQ876586 | Bertolani <i>et al.</i> (2023),<br>Bertolani <i>et al.</i> (2011)       |
| <i>Macrobilotus</i> cf. <i>hufelandi</i><br>C2959_A01 | OP596303 |          |          | HQ876590 | Bertolani <i>et al.</i> (2023),<br>Bertolani <i>et al.</i> (2011)       |
| <i>Macrobilotus</i> cf. <i>hufelandi</i><br>C2959_A02 | OP596304 |          |          | HQ876591 | Bertolani <i>et al.</i> (2023),<br>Bertolani <i>et al.</i> (2011)       |
| <i>Macrobilotus</i> <i>vladimiri</i><br>C2688_A02     | OP596305 |          |          | HM136932 | Bertolani <i>et al.</i> (2023),<br>Bertolani <i>et al.</i> (2011)       |
| <i>Macrobilotus</i> <i>terminalis</i><br>C2868_N02    | OP596308 |          |          | JN673959 | Bertolani <i>et al.</i> (2023),<br>Bertolani <i>et al.</i> (2011)       |
| <i>Macrobilotus</i> cf. <i>nelsonae</i> 1             | HQ604965 |          |          |          | Bertolani <i>et al.</i> (2014)                                          |
| <i>Macrobilotus</i> cf. <i>nelsonae</i> 2             | HQ604966 |          |          |          | Bertolani <i>et al.</i> (2014)                                          |
| <i>Macrobilotus</i> <i>rebecchii</i> 1                | OP479887 |          |          | OP477442 | Stec (2022b)                                                            |
| <i>Macrobilotus</i> <i>rebecchii</i> 2                | OP479888 |          |          | OP477443 | Stec (2022b)                                                            |
| <i>Macrobilotus</i> <i>kyoukenus</i> v3               | ON818312 |          | ON818300 | ON809461 | Cesari <i>et al.</i> (2022)                                             |
| <i>Macrobilotus</i> <i>kyoukenus</i> us3              | ON818314 |          | ON818301 | ON809462 | Cesari <i>et al.</i> (2022)                                             |
| <i>Macrobilotus</i> <i>kyoukenus</i> us4              | ON818315 |          | ON818302 | ON809463 | Cesari <i>et al.</i> (2022)                                             |
| <i>Macrobilotus</i> <i>kyoukenus</i> us5              | ON818316 |          | ON818303 | ON809464 | Cesari <i>et al.</i> (2022)                                             |
| <i>Macrobilotus</i> <i>scoticus</i> DK 1              | OK663218 | OK663228 | OK663207 | OK662989 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>scoticus</i> DK 2              | OK663217 | OK663229 | OK663206 | OK662988 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>naginae</i><br>S226_1          | OK663219 | OK663230 | OK663209 | OK662990 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>naginae</i><br>S226_2          | OK663220 | OK663231 | OK663208 | OK662991 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>naginae</i><br>S605_1          | OK663221 | OK663232 | OK663210 | OK662992 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>naginae</i><br>S605_2          | OK663222 | OK663233 | OK663211 | OK662993 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>sandrae</i><br>S859_1          | OK663223 | OK663234 | OK663212 | OK662994 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> cf. <i>sapiens</i>                | OK663226 | OK663237 | OK663215 | OK662997 | Vecchi <i>et al.</i> (2022a)                                            |
| <i>Macrobilotus</i> <i>sandrae</i> 1                  | MW695445 |          |          | HQ876573 | Bertolani <i>et al.</i> (2011);<br>Ben Marnissi <i>et al.</i><br>(2021) |
| <i>Macrobilotus</i> <i>sandrae</i> 2                  | MW695446 |          |          | HQ876577 | Bertolani <i>et al.</i> (2011);<br>Ben Marnissi <i>et al.</i><br>(2021) |
| <i>Macrobilotus</i> <i>azzunae</i> 1                  | MW695447 |          | MW695454 | MW698697 | Ben Marnissi <i>et al.</i><br>(2021)                                    |
| <i>Macrobilotus</i> <i>azzunae</i> 2                  | MW695448 |          | MW695455 | MW698698 | Ben Marnissi <i>et al.</i><br>(2021)                                    |



**Appendix 1.** Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study.

| Species                                           | 18S      | 28S      | ITS-2    | COI      | Source                        |
|---------------------------------------------------|----------|----------|----------|----------|-------------------------------|
| <i>Macrobiotus birendrai</i>                      | MW680641 | MW680644 | MW680418 | MW656266 | Kayastha <i>et al.</i> (2021) |
| <i>Macrobiotus a. groenlandicus</i> 1             | MZ463664 | MZ463679 | MZ463654 | MZ461006 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus a. groenlandicus</i> 2             | MZ463663 | MZ463678 | MZ463655 | MZ461007 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus a. groenlandicus</i> 3             | MZ463662 | MZ463677 | MZ463653 | MZ461005 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus kirghizicus</i> 1                  | MZ463666 | MZ463672 | MZ463659 | MZ461002 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus kirghizicus</i> 2                  | MZ463665 | MZ463671 | MZ463660 | MZ461003 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus kirghizicus</i> 3                  | MZ463667 | MZ463673 | MZ463661 | MZ461004 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus a. ariekammensis</i> 1             | MZ463668 | MZ463674 | MZ463656 | MZ460999 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus a. ariekammensis</i> 2             | MZ463669 | MZ463675 | MZ463657 | MZ461000 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus a. ariekammensis</i> 3             | MZ463670 | MZ463676 | MZ463658 | MZ461001 | Stec <i>et al.</i> (2022)     |
| <i>Macrobiotus</i> aff. <i>pseudohufelandi</i> PL | MN888373 | MN888358 | MN888345 | MN888325 | Stec <i>et al.</i> (2021a)    |
| <i>Macrobiotus</i> aff. <i>pseudohufelandi</i> ZA | MN888374 | MN888359 | MN888345 | MN888326 | Stec <i>et al.</i> (2021a)    |
| <i>Macrobiotus annewintersae</i> 1                | MW588024 | MW588030 | MW588019 | MW593927 | Vecchi & Stec (2021)          |
| <i>Macrobiotus annewintersae</i> 2                | MW588025 | MW588031 | MW588018 | MW593928 | Vecchi & Stec (2021)          |
| <i>Macrobiotus basiatius</i>                      | MT498094 | MT488397 | MT505165 | MT502116 | Nelson <i>et al.</i> (2020)   |
| <i>Macrobiotus caelestis</i>                      | MK737073 | MK737071 | MK737072 | MK737922 | Coughlan <i>et al.</i> (2019) |
| <i>Macrobiotus canaricus</i> 1                    | MH063925 | MH063934 | MH063928 | MH057765 | Stec <i>et al.</i> (2018b)    |
| <i>Macrobiotus canaricus</i> 2                    |          |          | MH063929 | MH057766 | Stec <i>et al.</i> (2018b)    |
| <i>Macrobiotus</i> cf. <i>polonicus</i> 1         | MW588026 | MW588032 | MW588021 | MW593929 | Vecchi & Stec (2021)          |
| <i>Macrobiotus</i> cf. <i>polonicus</i> 2         | MW588027 | MW588033 | MW588020 | MW593930 | Vecchi & Stec (2021)          |
| <i>Macrobiotus</i> cf. <i>recens</i> 1            | MH063927 | MH063936 | MH063932 | MH057768 | Stec <i>et al.</i> (2018b)    |
| <i>Macrobiotus</i> cf. <i>recens</i> 2            |          |          | MH063933 | MH057769 | Stec <i>et al.</i> (2018b)    |
| <i>Macrobiotus crustulus</i>                      | MT261912 | MT261903 | MT261907 | MT260371 | Stec <i>et al.</i> (2020c)    |
| <i>Macrobiotus engbergi</i> 1                     | MN443039 | MN443034 | MN443036 | MN444824 | Stec <i>et al.</i> (2020d)    |
| <i>Macrobiotus engbergi</i> 2                     |          |          | MN443037 | MN444825 | Stec <i>et al.</i> (2020d)    |
| <i>Macrobiotus engbergi</i> 3                     |          |          |          | MN444826 | Stec <i>et al.</i> (2020d)    |
| <i>Macrobiotus glebkai</i>                        | MW247177 | MW247176 | MW247180 | MW246134 | Kiosya <i>et al.</i> (2021)   |
| <i>Macrobiotus hanna</i>                          | MH063922 | MH063924 | MH063923 | MH057764 | Nowak & Stec (2018)           |
| <i>Macrobiotus kamilae</i> 1                      | MK737070 | MK737064 | MK737067 | MK737920 | Coughlan & Stec (2019)        |
| <i>Macrobiotus kamilae</i> 2                      |          |          |          | MK737921 | Coughlan & Stec (2019)        |
| <i>Macrobiotus kristenseni</i>                    | KC193577 |          |          | KC193573 | Guidetti <i>et al.</i> (2013) |
| <i>Macrobiotus macrocalix</i>                     | MH063926 | MH063935 | MH063931 | MH057767 | Stec <i>et al.</i> (2018b)    |
| <i>Macrobiotus noongaris</i> 1                    | MK737069 | MK737063 | MK737065 | MK737919 | Coughlan & Stec (2019)        |

**Appendix 1.** Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study.

| Species                              | 18S      | 28S      | ITS-2    | COI      | Source                          |
|--------------------------------------|----------|----------|----------|----------|---------------------------------|
| <i>Macrobiotus noongaris</i> 2       |          |          | MK737066 |          | Coughlan & Stec (2019)          |
| <i>Macrobiotus papei</i>             | MH063881 | MH063880 | MH063921 | MH057763 | Stec <i>et al.</i> (2018c)      |
| <i>Macrobiotus paulinae</i>          | KT935502 | KT935501 | KT935500 | KT951668 | Stec <i>et al.</i> (2015)       |
| <i>Macrobiotus polonicus</i> AT 1    | MN888369 | MN888355 | MN888337 | MN888317 | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus polonicus</i> AT 2    |          |          | MN888338 | MN888318 | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus polonicus</i> AT 3    |          |          |          | MN888319 | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus polonicus</i> SK 1    | MN888370 | MN888356 | MN888332 | MN888320 | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus polonicus</i> SK 2    |          |          | MN888333 | MN888321 | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus polonicus</i> SK 3    |          |          | MN888334 |          | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus porifini</i> 1        | MT241900 | MT241897 |          | MT246659 | Kuzdrowska <i>et al.</i> (2021) |
| <i>Macrobiotus porifini</i> 2        |          | MT241898 |          | MT246660 | Kuzdrowska <i>et al.</i> (2021) |
| <i>Macrobiotus porifini</i> 3        |          | MT241899 |          | MT246661 | Kuzdrowska <i>et al.</i> (2021) |
| <i>Macrobiotus polypiformis</i> 1    | KX810008 | KX810009 | KX810010 | KX810011 | Roszkowska <i>et al.</i> (2017) |
| <i>Macrobiotus polypiformis</i> 2    |          |          |          | KX810012 | Roszkowska <i>et al.</i> (2017) |
| <i>Macrobiotus pseudohufelandi</i> 1 | HQ604989 |          |          | AY598776 | Bertolani <i>et al.</i> (2014)  |
| <i>Macrobiotus pseudohufelandi</i> 2 | HQ604990 |          |          | AY598777 | Bertolani <i>et al.</i> (2014)  |
| <i>Macrobiotus rybaki</i> 1          | MW588029 | MW588034 | MW588022 | MW593931 | Vecchi & Stec (2021)            |
| <i>Macrobiotus rybaki</i> 2          | MW588028 | MW588035 | MW588023 | MW593932 | Vecchi & Stec (2021)            |
| <i>Macrobiotus scoticus</i>          | KY797265 | KY797266 | KY797268 | KY797267 | Stec <i>et al.</i> (2017b)      |
| <i>Macrobiotus shonaicus</i> 1       | MG757132 | MG757133 | MG757134 | MG757136 | Stec <i>et al.</i> (2018d)      |
| <i>Macrobiotus shonaicus</i> 2       |          |          | MG757135 | MG757137 | Stec <i>et al.</i> (2018d)      |
| <i>Macrobiotus sottilei</i>          | MW247178 | MW247175 | MW247179 | MW246133 | Kiosya <i>et al.</i> (2021)     |
| <i>Macrobiotus vladimiri</i> FI      | MN888375 | MN888360 | MN888347 | MN888327 | Stec <i>et al.</i> (2021a)      |
| <i>Macrobiotus wandae</i>            | MN435112 | MN435116 | MN435120 | MN482684 | Kayastha <i>et al.</i> (2020b)  |
| <i>Macrobiotus pallarii</i> 1        | MT809069 | MT809081 | MT809094 | MT807924 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus pallarii</i> 2        | MT809070 | MT809082 | MT809095 | MT807925 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus pallarii</i> 3        | MT809071 | MT809083 | MT809096 | MT807926 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus pseudopallarii</i> 1  | MT809067 | MT809079 | MT809091 | MT807921 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus pseudopallarii</i> 2  | MT809068 | MT809080 | MT809092 | MT807922 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus ripperi</i> FI        | MT809076 | MT809089 | MT809103 | MT807930 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus ripperi</i> PL        | MT809074 | MT809086 | MT809100 | MT807933 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus margoae</i> US        | MT809072 | MT809084 | MT809098 | MT807927 | Stec <i>et al.</i> (2021b)      |
| <i>Macrobiotus gretae</i> 1          | MW588434 |          | MW588431 | MW581665 | Massa <i>et al.</i> (2021)      |
| <i>Macrobiotus gretae</i> 2          | MW588437 |          | MW588433 | MW581668 | Massa <i>et al.</i> (2021)      |

**Appendix 1.** Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study.

| Species                                              | 18S      | 28S      | ITS-2    | COI      | Source                          |
|------------------------------------------------------|----------|----------|----------|----------|---------------------------------|
| <i>Macrobotus gretae</i> 3                           | MW588435 |          | MW588432 | MW581666 | Massa <i>et al.</i> (2021)      |
| <i>Macrobotus gretae</i> 4                           | MW588436 |          |          | MW581667 | Massa <i>et al.</i> (2021)      |
| <i>Sisubiotus hakaensis</i> 1                        | OM523054 | OM523059 | OM523057 | OM523181 | Vecchi <i>et al.</i> (2022b)    |
| <i>Sisubiotus hakaensis</i> 2                        | OM523055 | OM523060 | OM523058 | OM523182 | Vecchi <i>et al.</i> (2022b)    |
| <i>Sisubiotus spectabilis</i> FI 1                   | MN888371 | MN888357 | MN888331 | MN888322 | Stec <i>et al.</i> (2021a)      |
| <i>Sisubiotus spectabilis</i> FI 2                   |          |          |          | MN888323 | Stec <i>et al.</i> (2021a)      |
| <i>Sisubiotus spectabilis</i> NO                     | MN888372 | MN888364 | MN888344 | MN888324 | Stec <i>et al.</i> (2021a)      |
| <i>Mesobiotus anastasiae</i>                         | MT903468 | MT903612 | MT903470 | MT904513 | Tumanov (2020)                  |
| <i>Mesobiotus hilariae</i>                           | KT226070 |          |          | KT226108 | Vecchi <i>et al.</i> (2016)     |
| <i>Mesobiotus</i> cf. <i>barabanovi</i>              | MN310392 | MN310388 | MN310390 | MN313170 | Kaczmarek <i>et al.</i> (2020)  |
| <i>Mesobiotus datanlanicus</i>                       | MK584659 | MK584658 | MK584657 | MK578905 | Stec (2019)                     |
| <i>Mesobiotus dilimanensis</i>                       | MN257048 | MN257049 | MN257050 | MN257047 | Itang <i>et al.</i> (2020)      |
| <i>Mesobiotus ethiopicus</i>                         | MF678793 | MF678792 | MN122776 | MF678794 | Stec & Kristensen (2017)        |
| <i>Mesobiotus fiedleri</i>                           | MH681585 | MH681693 | MH681724 | MH676056 | Kaczmarek <i>et al.</i> (2020)  |
| <i>Mesobiotus harmsworthi</i>                        | MH197146 | MH197264 | MH197154 | MH195150 | Kaczmarek <i>et al.</i> (2018)  |
| <i>Mesobiotus insanis</i>                            | MF441488 | MF441489 | MF441490 | MF441491 | Mapalo <i>et al.</i> (2017)     |
| <i>Mesobiotus occultatus</i>                         | MH197147 |          | MH197155 | MH195152 | Kaczmarek <i>et al.</i> (2018)  |
| <i>Mesobiotus philippinicus</i>                      | KX129793 | KX129794 | KX129795 | KX129796 | Mapalo <i>et al.</i> (2016)     |
| <i>Mesobiotus radiatus</i> 1                         | MH197153 | MH197152 | MH197267 | MH195147 | Stec <i>et al.</i> (2018e)      |
| <i>Mesobiotus radiatus</i> 2                         |          |          | MH197268 | MH195148 | Stec <i>et al.</i> (2018e)      |
| <i>Mesobiotus romani</i>                             | MH197158 | MH197151 | MH197150 | MH195149 | Roszkowska <i>et al.</i> (2018) |
| <i>Mesobiotus imperialis</i> 1                       | OL257854 | OL257866 |          | OL311514 | Stec (2021)                     |
| <i>Mesobiotus imperialis</i> 2                       | OL257855 | OL257867 |          | OL311515 | Stec (2021)                     |
| <i>Mesobiotus marmoreus</i> 1                        | OL257856 | OL257868 | OL257861 | OL311516 | Stec (2021)                     |
| <i>Mesobiotus marmoreus</i> 2                        | OL257857 | OL257869 | OL257862 | OL311517 | Stec (2021)                     |
| <i>Mesobiotus marmoreus</i> 3                        | OL257858 | OL257870 | OL257863 | OL311518 | Stec (2021)                     |
| <i>Mesobiotus skoracki</i>                           |          | MW680636 |          | MW656257 | Kayastha <i>et al.</i> (2021)   |
| <i>Mesobiotus</i> sp.<br>Macro07_042                 | MW751942 |          |          | MW727957 | Short <i>et al.</i> (2022)      |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>Macro06_296 | MW751936 |          |          | MW727958 | Short <i>et al.</i> (2022)      |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>Macro06_310 | MW751937 |          |          | MW727961 | Short <i>et al.</i> (2022)      |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>Macro06_313 | MW751939 |          |          | MW727960 | Short <i>et al.</i> (2022)      |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>CC_MF_4     | MW751949 |          |          | MW727933 | Short <i>et al.</i> (2022)      |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>ABDC_MF_3   | MW751944 |          |          | MW727932 | Short <i>et al.</i> (2022)      |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>KPRI_MF_1   | MW751962 |          |          | MW727934 | Short <i>et al.</i> (2022)      |



**Appendix 1.** Sequences used for phylogenetic analysis. Bold font indicates sequences obtained in this study.

| Species                                              | 18S      | 28S      | ITS-2    | COI      | Source                     |
|------------------------------------------------------|----------|----------|----------|----------|----------------------------|
| <i>Mesobiotus</i> cf. <i>furciger</i><br>HMI_MF_1    | MW751957 |          |          | MW727941 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>EBNI_MF_2   | MW751952 |          |          | MW727937 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>EBNI_MF_4   | MW751954 |          |          | MW727938 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>PSAI_MF_2   | MW751967 |          |          | MW727939 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>Macro06_162 | MW751934 |          |          | MW727955 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>Macro06_171 | MW751935 |          |          | MW727956 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>JN07_MF_1   | MW751959 |          |          | MW727951 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>JN07_MF_4   | MW751960 |          |          | MW727953 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>JN07_MF_8   | MW751961 |          |          | MW727947 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus</i> cf. <i>furciger</i><br>FN01_MF_6   | MW751955 |          |          | MW727945 | Short <i>et al.</i> (2022) |
| <i>Mesobiotus diegoi</i> 1                           | OP142527 | OP142520 | OP142514 | OP143858 | Stec (2022a)               |
| <i>Mesobiotus diegoi</i> 2                           | OP142526 | OP142521 | OP142515 | OP143857 | Stec (2022a)               |
| <i>Mesobiotus maklowiczi</i> 1                       | OP142525 | OP142518 |          | OP143855 | Stec (2022a)               |
| <i>Mesobiotus maklowiczi</i> 2                       | OP142524 | OP142519 |          | OP143856 | Stec (2022a)               |
| <i>Mesobiotus peterseni</i> 1                        | OP142528 | OP142522 | OP142516 | OP143859 | Stec (2022a)               |
| <i>Mesobiotus peterseni</i> 2                        | OP142529 | OP142523 | OP142517 | OP143860 | Stec (2022a)               |
| <i>Minibiotus ioculator</i>                          | MT023998 | MT024041 | MT024000 | MT023412 | Stec <i>et al.</i> (2020a) |
| <i>Paramacrobiotus areolatus</i>                     | MH664931 | MH664948 | MH666080 | MH675998 | Stec <i>et al.</i> (2020e) |
| <i>Tenuibiotus zandrae</i>                           | MN443040 | MN443035 | MN443038 | MN444827 | Stec <i>et al.</i> (2020d) |

# ZOBODAT - [www.zobodat.at](http://www.zobodat.at)

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [European Journal of Taxonomy](#)

Jahr/Year: 2024

Band/Volume: [0930](#)

Autor(en)/Author(s): Stec Daniel

Artikel/Article: [Integrative taxonomy supports two new species of Macrobiotus \(Tardigrada: Eutardigrada: Macrobiotidae\) allowing further discussion on the genus phylogeny 79-123](#)