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Integrative systematic studies on tardigrades from Antarctica identify new genera and new species within Macrobiotoidea and Echiniscoidea

Matteo Vecchi[A], *Michele Cesari*[A,C], *Roberto Bertolani*[A], *K. Ingemar Jönsson*[B], *Lorena Rebecchi*[A] *and Roberto Guidetti*[A]

[A]Department of Life Sciences, University of Modena and Reggio Emilia, via Campi 213/D, Modena, 41125, Italy.
[B]School of Education and Environment, Kristianstad University, SE-291 88 Kristianstad, Sweden.
[C]Corresponding author. Email: michele.cesari@unimore.it

Abstract. Tardigrades represent one of the most abundant groups of Antarctic metazoans in terms of abundance and diversity, thanks to their ability to withstand desiccation and freezing; however, their biodiversity is underestimated. Antarctic tardigrades from Dronning Maud Land and Victoria Land were analysed from a morphological point of view with light microscopy and scanning electron microscopy, and from a molecular point of view using two genes (18S, 28S) analysed in Bayesian inference and maximum-likelihood frameworks. In addition, indel-coding datasets were used for the first time to infer tardigrade phylogenies. We also compared Antarctic specimens with those from Italy and Greenland. A combined morphological and molecular analysis led to the identification of two new evolutionary lineages, for which we here erect the new genera *Acanthechiniscus*, gen. nov. (Echiniscidae, Echiniscoidea) and *Mesobiotus*, gen. nov. (Macrobiotidae, Macrobiotoidea). Moreover, two species new to science were discovered: *Pseudechiniscus titianae*, sp. nov. (Echiniscidae : Echiniscoidea) and *Mesobiotus hilariae*, sp. nov. (Macrobiotidae : Macrobiotoidea). This study highlights the high tardigrade diversity in Antarctica and the importance of an integrated approach in faunal and taxonomic studies.

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Introduction

Tardigrada is one of the few animal phyla that is very abundant in Antarctica, and it represents an important element of the microfauna of the entire continent (Continental Antarctica, Antarctic Peninsula, and Maritime Antarctica) in terms of diversity, abundance, distribution, and colonised substrates. On the coastal snow-free areas and nunataks, they colonise lichens, mosses, detritus, organic material, freshwater sediments (living on algae, aquatic plants, and biofilms) and even cryoconite holes (Zawierucha *et al*. 2015). In these habitats, tardigrades can survive desiccation and freezing by temporarily suspending their active life by entering cryptobiosis (for reviews see Guidetti *et al*. 2011; Møbjerg *et al*. 2011; Wełnicz *et al*. 2011). Like many other Antarctic animals (Chown and Convey 2007; Velasco-Castrillón *et al*. 2014), Antarctic terrestrial and freshwater tardigrades are characterised by a high number (33) of endemic species (Velasco-Castrillón *et al*. 2014), probably due to the fragmentation and isolation of habitats in the glacial environment (Stevens and Hogg 2003, 2006).

In Continental Antarctica only seven heterotardigrade species (three of which are endemic for Antarctica and two are undetermined species: Utsugi and Ohyama 1989; Tsujimoto *et al*. 2014) have been reported (Dastych 1984; Utsugi and Ohyama 1991; Miller *et al*. 1996; McInnes 2010; Guidetti *et al*. 2014) dating back to the beginning of the 20th century. Even though molecular studies have shown a potentially high diversity of eutardigrades from Antarctica (Czechowski *et al*. 2012; Velasco-Castrillón *et al*. 2015), only four new species have been described in the past 15 years (Tumanov 2006; Pilato *et al*. 2012; Kaczmarek *et al*. 2014), with only one eutardigrade species being from continental Antarctica (Binda and Pilato 2000). Probably, the lower number of tardigrade species in continental Antarctica compared with the Antarctic Peninsula and subantarctic islands is due mainly to the larger number of

research stations and ease of access in the latter areas (Convey and McInnes 2005). Therefore, the tardigrade biodiversity in continental Antarctica is probably underestimated due to a low number of sampling campaigns devoted to exploring the tardigrade fauna there.

The main aim of our study was the identification of the still undescribed tardigrade biodiversity in the Antarctic continent. Additionally, we used biological samples and data obtained from this study to address important questions in tardigrade phylogeny, systematics and taxonomy, such as testing the monophyly of two of the largest tardigrade genera, *Macrobiotus* and *Pseudechiniscus*. The combined molecular and morphological analyses allowed us to reject the hypothesis of monophyly of these two genera, leading to the erection of two new genera, and to identify and describe two new species.

Material and methods

Tardigrade collection

Ten moss and freshwater sediment samples were collected in continental Antarctica during two sampling campaigns (Table 1). Four samples were collected by K. I. Jönsson in January 2002 near the Novolazarevskaya Russian Research Station (Dronning Maud Land) during the Swedish Antarctic Expedition (SWEDARP 2001–02). These samples were initially dried and stored frozen until the current analyses. Six samples were collected by R. Guidetti along the coastline of Victoria Land in 2011 during the Italian National Antarctic Program XXVI expedition (2010–11). These samples were also kept dry and frozen until analyses were performed.

In order to extract tardigrades from the samples, the collected substrates were placed and maintained in tap water at room temperature (20°C) for about half an hour. The samples were then sieved to separate tardigrades and their eggs from the substrate, and both animals and eggs were isolated using a glass pipette under a stereomicroscope.

Dry residual fragments of each sample were stored at the Department of Life Sciences, University of Modena and Reggio Emilia (UNIMORE), Italy, for possible future investigations.

As comparative material, specimens of *Pseudechiniscus victor* (Ehrenberg, 1853) collected in Greenland, and specimens of three unidentified species of the *Macrobiotus harmsworthi* group, collected in Italy, were analysed from a morphological and molecular point of view (Table 1).

Morphological analysis

The collected specimens (eggs and animals) were mounted on slides in Faure–Berlese fluid or stained with acetic–lactic orcein for light microscopy (LM) observations. Observations and measurements were carried out with LM under phase contrast (PhC) and differential interference contrast (DIC) up to the maximum magnification (100× oil objective) with a Leica DM RB microscope equipped with a Nikon DS-Fi 1 digital camera, at the Department of Life Sciences, UNIMORE. Morphospecies were identified by comparing the specimens with the original species descriptions and, if possible, with the original type material. Measurements of animals belonging to Eutardigrada were taken according to Pilato (1981). The internal buccal tube diameter was measured at the level of the stylet support insertion point. For heterotardigrades, scapular plate length was taken in the median part of the plate, and pseudosegmental plate projection length was taken from the posterior margin of the pseudosegmental plate to the point of the projection.

Additional specimens were prepared for scanning electron microscopy (SEM) analyses according to Bertolani *et al*. (2014), and observed with a Philips SEM XL 40, available at the Centro Interdipartimentale Grandi Strumenti of UNIMORE.

For comparison with the Antarctic material, mounted specimens from Ramazzotti's and Maucci's collections of the Natural History Museum of Verona (Italy) (slides: CT6081 *Macrobiotus liviae* Ramazzotti, 1962 syntype; CT6947 *M. mauccii* Pilato, 1974; CT7298 *M. arguei* Pilato & Sperlinga, 1975 paratype; CT9277 *M. harmsworthi* Murray, 1907*a*; CT11185 *M. lusitanicus* Maucci & Durante Pasa, 1984 paratype; CT12946 *M. furciger* Murray, 1907*b*) and Bertolani's collection of the Department of Life Sciences, UNIMORE

Table 1. List of sampled localities, geographic coordinates of sampling sites, altitude, and substrate of origin
n.a., not available

Sample code	Location	Latitude	Longitude	Altitude a.s.l. (m)	Substrate
C2257	Lyngmark Glacier, Greenland	n.a.	n.a.	250	Moss on rock
C2749	Andalo, Trento, Italy	46°9.742′N	10°59.499′E	1100	Leaf litter
C2780	Lake Baccio, Modena, Italy	44°7.871′N	10°35.377′E	1750	Leaf litter
C2827	Portella Mandrazzi, Messina, Italy	37°58.535′N	15°8.366′E	1145	Moss on trunk
C3321	Crater Cirque, Victoria Land, Antarctica	72°36.199′S	169°20.937′E	132	Moss on soil
C3324	Crater Cirque, Victoria Land, Antarctica	72°36.207′S	169°20.945′E	133	Algae/aq. plant on gravel
C3326	Crater Cirque, Victoria Land, Antarctica	72°36.174′S	169°20.047′E	139	Lichen on rocks
C3326	Crater Cirque, Victoria Land, Antarctica	72°36.174′S	169°20.047′E	139	Lichen on rocks
C3431	Vegetation Island, Victoria Land, Antarctica	74°47.033′S	163°38.745′E	221	Moss on soil
C3434	Inespressible Land, Victoria Land, Antarctica	74°53.022′S	163°43.057′E	41	Sediment and algae/ bacteria in water
C3610	Novolazarevskaya, Dronning Maud Land, Antarctica	70°46.557′S	11°48.837′E	116	Moss on ground
C3620	Novolazarevskaya, Dronning Maud Land, Antarctica	70°46.657′S	11°49.063′E	137	Moss on rock
C3623	Novolazarevskaya, Dronning Maud Land, Antarctica	70°45.566′S	11°46.899′E	41	Moss on ground
C3624	Novolazarevskaya, Dronning Maud Land, Antarctica	70°45.566′S	11°46.899′E	41	Moss on ground

(slides: C460-S97 *M. sandrae* Bertolani & Rebecchi, 1993 holotype; 5N05 *M. nelsonae* Guidetti, 1998 holotype) were analysed and photographed under LM.

Molecular analysis

Genomic DNA was extracted from single adult animals following the protocol described by Cesari *et al*. (2009). Molecular investigations were carried out using fragments of the nuclear 18S and 28S rDNA genes. In addition, a fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene was sequenced to provide barcode data for species identification. For 18S, primers and cycles used for amplification in eutardigrades were those described in Bertolani *et al*. (2014), whereas those described in Vicente *et al*. (2013) were used for heterotardigrades. For 28S, primers and cycles for both heterotardigrades and eutardigrades were those described in Guidetti *et al*. (2014). Sequences of *cox1* were analysed only for the two new species identified in this study, and were amplified and sequenced according to Bertolani *et al*. (2011*b*). The amplified products were gel purified using the Wizard Gel and PCR cleaning (Promega) kit. Both strands were subjected to Sanger sequencing using the Big Dye Terminator 1.1 kit (Applied Biosystems) and an ABI Prism 3100 sequencer (Applied Biosystems) available at Centro Interdipartimentale Ricerche Genomiche of UNIMORE. Nucleotide sequences of the newly analysed specimens were submitted to GenBank (accession numbers in Table 2).

For phylogenetic analyses, the 18S and 28S nucleotide sequences were aligned with the MUSCLE algorithm (Edgar 2004), using default parameters implemented in MEGA5 (Tamura *et al*. 2011). The GBlocks program (Castresana 2000) was used with relaxed settings and parameters (values are as specified in Bertolani *et al*. 2014) to find and discard ambiguous regions of the alignment. Two datasets were used: Eutardigrada dataset (EUD) and Echiniscoidea dataset (ECD). The EUD dataset was the same as in Bertolani *et al*. (2014) with the addition of the 12 newly sequenced eutardigrades and additional 28S sequences from four animals used in Bertolani *et al*. (2014); sequences pertaining to the genus *Milnesium* (Eutardigrada, Apochela) were used as outgroups as they have been shown to be in sister-group relationships to all other eutardigrades in the dataset (Bertolani *et al*. 2014). The ECD dataset comprised 10 newly sequenced echiniscid specimens and other Echiniscoidea sequences retrieved from GenBank; a sequence of *Florarctus* sp. (Heterotardigrada, Arthrotardigrada) was used as the outgroup (see Table S1) as it has been shown to have a sister-group relationship with echiniscid tardigrades (Jørgensen *et al*. 2011).

For both the ECD and EUD datasets, combined analyses using both 18S and 28S sequences were performed, applying Bayesian inference (BI) and maximum likelihood (ML). Best-fitting model evaluations were performed taking into account the Akaike information criterion and Bayes information criterion (jModelTest 0.0.1: Posada 2008), which identified the GTR+G model as the most suitable. The BI analyses were performed with the program MrBayes 3.2 (Ronquist *et al*. 2012). For each analysis, two independent runs, each of four Metropolis-coupled Markov Chain Monte Carlo (MCMC), were launched and the

Table 2. GenBank accession numbers of obtained sequences
n.a., not available

Specimen	Sample code	18S	28S	*cox1*
Echiniscus cf. *jenningsi* 1	C3326	KT226088	KT226098	n.a.
Echiniscus cf. *jenningsi* 2	C3326	KT226089	KT226099	n.a.
Echiniscus cf. *jenningsi* 3	C3326	KT226090	KT226100	n.a.
Mopsechiniscus franciscae 1	C3321	KT226091	KT226101	n.a.
Mopsechiniscus franciscae 2	C3321	KT226092	KT226102	n.a.
Mopsechiniscus franciscae 3	C3321	KT226093	KT226103	n.a.
Pseudechiniscus titianae, sp. nov.	C3623	KT226094	KT226104	n.a.
Pseudechiniscus victor 1	C2257	KT226095	KT226105	n.a.
Pseudechiniscus victor 2	C2257	KT226096	KT226106	n.a.
Pseudechiniscus victor 3	C2257	KT226097	KT226107	n.a.
Mesobiotus hilariae, sp. nov. A 1	C3610	KT226068	n.a.	n.a.
Mesobiotus hilariae, sp. nov. A 2	C3610	KT226069	n.a.	n.a.
Mesobiotus hilariae, sp. nov. B 1	C3620	KT226070	KT226076	n.a.
Mesobiotus hilariae, sp. nov. C 1	C3623	KT226071	n.a.	KT226108
Macrobiotus cf. *mottai* Crater Cirque 1	C3324	KT226072	n.a.	n.a.
Macrobiotus cf. *mottai* Crater Cirque 2	C3324	n.a.	KT226080	n.a.
Macrobiotus gr. *harmsworthi* Trento 1	C2749	n.a.	KT226081	n.a.
Macrobiotus gr. *harmsworthi* Trento 2	C2749	n.a.	KT226032	n.a.
Macrobiotus gr. *harmsworthi* Messina 1	C2827	KT226073	KT226083	n.a.
Macrobiotus gr. *harmsworthi* Messina 2	C2827	KT226074	KT226084	n.a.
Macrobiotus gr. *harmsworthi* Modena 1	C2780	n.a.	KT226085	n.a.
Macrobiotus gr. *harmsworthi* Modena 2	C2780	n.a.	KT226086	n.a.
Macrobiotus polaris Vegetation Island1	C3431	KT226075	n.a.	n.a.
Macrobiotus polaris Vegetation Island 2	C3431	KT226076	n.a.	n.a.
Macrobiotus polaris Inespressible Island 1	C3434	KT226077	KT226087	n.a.
Macrobiotus polaris Inespressible Island 2	C3434	KT226078	n.a.	n.a.

program was set to stop at convergence ($P < 0.005$) with trees sampled every 1000 generations. The obtained parameter value files of each run were analysed with Tracer 1.5 (Rambaut and Drummond 2007) to verify the convergence of the MCMC runs and their effective sample sizes, then 25% of the sampled generations were discarded as burn-in. The ML analysis was performed with the program RAxML 7.2.4 (Stamatakis 2006). Bootstrap resampling with 1000 replicates was undertaken via the rapid bootstrap procedure of Stamatakis *et al*. (2008) to assign support to branches in the ML tree.

To improve the phylogenetic tree resolution obtained from previous analyses of the Echiniscoidea (Kristensen 1987; Jørgensen *et al*. 2011; Vicente *et al*. 2013; Guidetti *et al*. 2014), the ECD was also used to create two matrices based on Simple Indel Coding (SIC) (Simmons and Ochoterena 2000) and Modified Complex Indel Coding (MCIC) (Müller 2006) using the software SeqState 1.4.1 (Müller 2005). Indel-coding datasets were integrated in two concatenated datasets (one for each indel-coding method) with the nucleotide sequences. A partitioned analysis (18S+28S+Indel) was performed in MrBayes and the indel data-partition coded as standard characters, using the same parameters as described above for MCMC runs for nucleotidic data, and NST = 1 + G for the indel data.

Results

Morphological results

The morphological analyses of the tardigrades extracted from the samples collected in Antarctica led to the identification of six morphospecies. Four species were found in Victoria Land (*Macrobiotus* cf. *mottai*, *M. polaris* Murray, 1910, *Echiniscus* cf. *jenningsi*, *Mopsechiniscus franciscae* Guidetti *et al*., 2014), whereas two species new to science, belonging to the genera *Pseudechiniscus* and *Macrobiotus*, were found in Dronning Maud Land.

The newly discovered species of *Pseudechiniscus* is characterised by long cirri A, no other lateral appendages in the trunk, first and second median plates transversally divided, and a longitudinally divided pseudosegmental plate with two triangular projections in the caudal margin. Spurs on the internal claws are present. For a detailed description of this new species see Taxonomic account below.

The newly discovered species of *Macrobiotus* is characterised by three roundish macroplacoids and a relatively large microplacoid close to the last macroplacoid in the pharynx, and ventral transversal crests of the buccal armature reduced to two small teeth. The eggs are laid freely and their shell has conical processes. The base of the processes presents expansions, connected to other processes' expansions, forming an areolation around the processes themselves; the internal walls of the egg processes are reticulated. For a detailed description of this new species see Taxonomic account below.

The analyses of *Pseudechiniscus victor* (Fig. 1) from Disko Island (Greenland) led to the identification of morphological characters not reported in previous descriptions. On the first three pairs of legs, three cushion-like structures of different sizes are located on each side of the distal part of the leg (Fig. 1*A*, *E*): the two structures on the external side are smaller than the single structure located on the internal side. A similar pattern was described in *Mopsechiniscus* (Guidetti *et al*. 2014). A small cushion-like structure is present also in the external side of the fourth leg (Fig. 1*D*). Moreover, the ornamentation of the dorsal cuticle is characterised by small hemispherical projections (which appear as dots with LM) densely and uniformly distributed, and joined by striae; these ornamentations form a polygonal net design (Fig. 1*C*).

Molecular results

Sequences of 18S (654–688 bp for ECD; 850–889 bp for EUD) and 28S (970–1002 bp for ECD; 891–913 bp for EUD) genes were obtained for 26 tardigrade specimens belonging to the six species found in Antarctic samples (*Macrobiotus* cf. *mottai*, *M. polaris*, *Macrobiotus* sp. nov., *Echiniscus* cf. *jenningsi*, *Mopsechiniscus franciscae*, and *Pseudechiniscus* sp. nov.), the Greenland species *Pseudechiniscus victor*, and the three Italian undetermined species of *Macrobiotus harmsworthi* group (Table 2). The amplification and sequencing of *cox1* gene (681 bp) was successful for only one specimen of *Macrobiotus* sp. nov.

The phylogenetic reconstructions carried out using ML and BI show similar topologies in both ECD (Fig. 2) and EUD (Fig. 3), albeit with several differences in the support values of some nodes. Moreover, the ECD BI trees (Fig. 2), computed with different indel-codification systems, are consistent with BI and ML topologies, with only very small differences in support values.

Echiniscoidea. In Echiniscoidea (Fig. 2, Supplementary Fig. S2), the phylogenetic relationships of the basal nodes are unresolved. Nevertheless, all genera except *Pseudechiniscus* are well supported. Lineage 1 (Fig. 2) contains the genera *Hypechiniscus*, *Testechiniscus*, *Diploechiniscus* and *Echiniscus*. This monophyletic group has been recovered previously, and here the relationships within this group are the same as those found by Vicente *et al*. (2013). Lineage 2 (Fig. 2) comprises the genera *Proechiniscus* and *Cornechiniscus*, and two species of the genus *Pseudechiniscus* belonging to the *P. victor* group: *P. victor* and *P. islandicus* (Richters, 1904). Lineage 3 (Fig. 2) comprises two species of the genus *Pseudechiniscus* belonging to the *P. suillus*/*P. conifer* group (*P. suillus* (Ehrenberg, 1853) and *P. facettalis* Petersen, 1951), and the newly discovered *Pseudechiniscus* species from Dronning Maud Land, Antarctica. The *Pseudechiniscus* sp. 1 sequence is attributed to *Echiniscus* in GenBank, but according to the authors that analysed it for the first time (Sands *et al*. 2008), and also according to our data, it should be attributed to a *Pseudechiniscus* species belonging to the P. *suillus*/*P. conifer* group. The phylogenetic relationships of *Oreella*, *Parechiniscus*, *Mopsechiniscus*, and the clade *Bryochoerus* + *Bryodelphax* are not resolved.

Macrobiotoidea. The phylogenetic relationships within Eutardigrada reflect those found in previous studies (Bertolani *et al*. 2014). Here we focus on the results obtained for Macrobiotoidea, as the eutardigrade species we detected in Antarctica belong to this superfamily. The relationships in

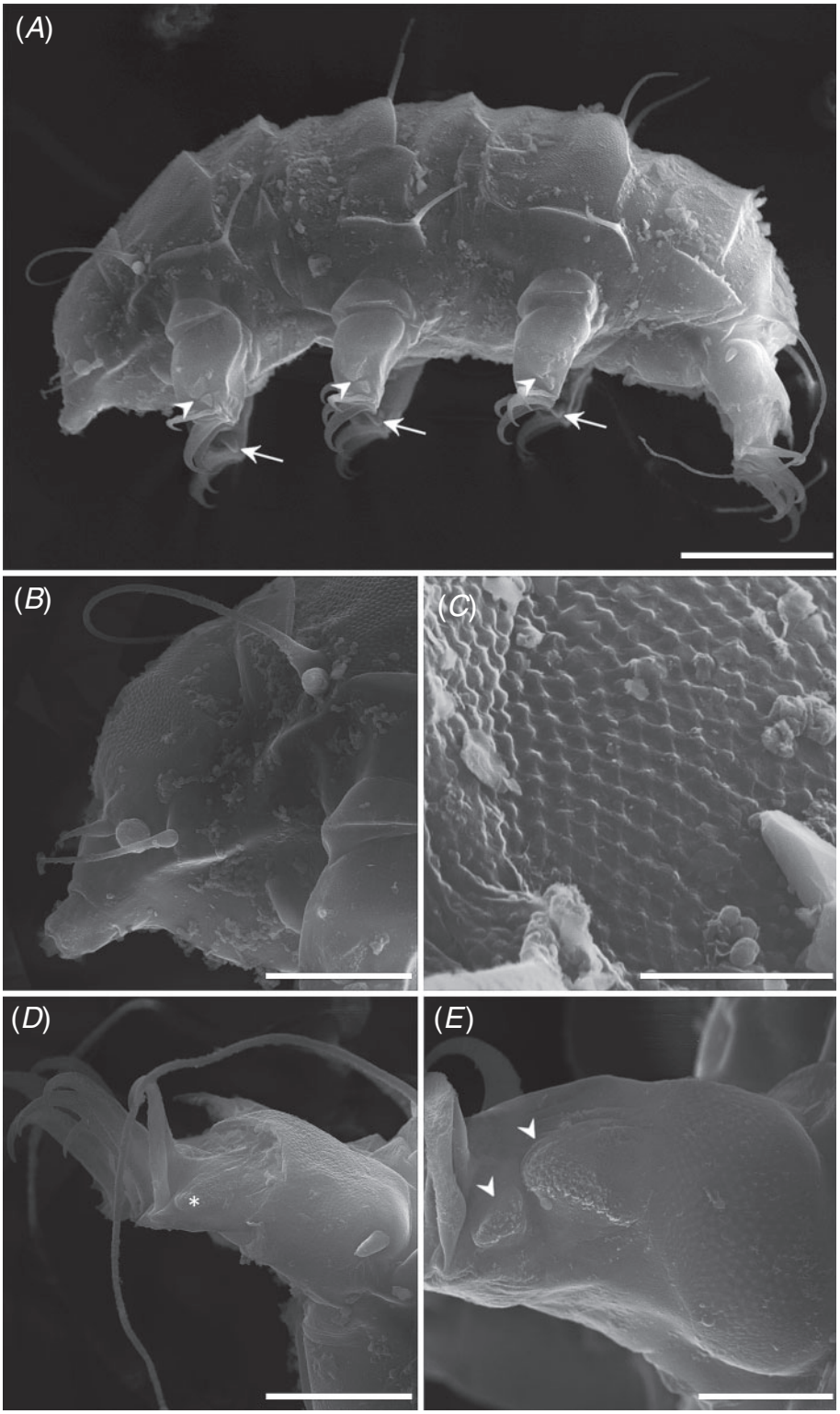

Fig. 1. (*A–E*) *Pseudechiniscus victor* (SEM). (*A*) *In toto* specimen, lateral view. (*B*) Head. (*C*) Cuticle ornamentation. (*D*) Hind leg. (*E*) Second leg. Scale bars: A, 50 µm; B, 20 µm; C, 5 µm; D, 20 µm; E, 10 µm. Arrows, cushion-like structures in the internal side of the legs; arrowheads, cushion-like structures in the external side of the legs; asterisk, cushion-like structure in the hind leg.

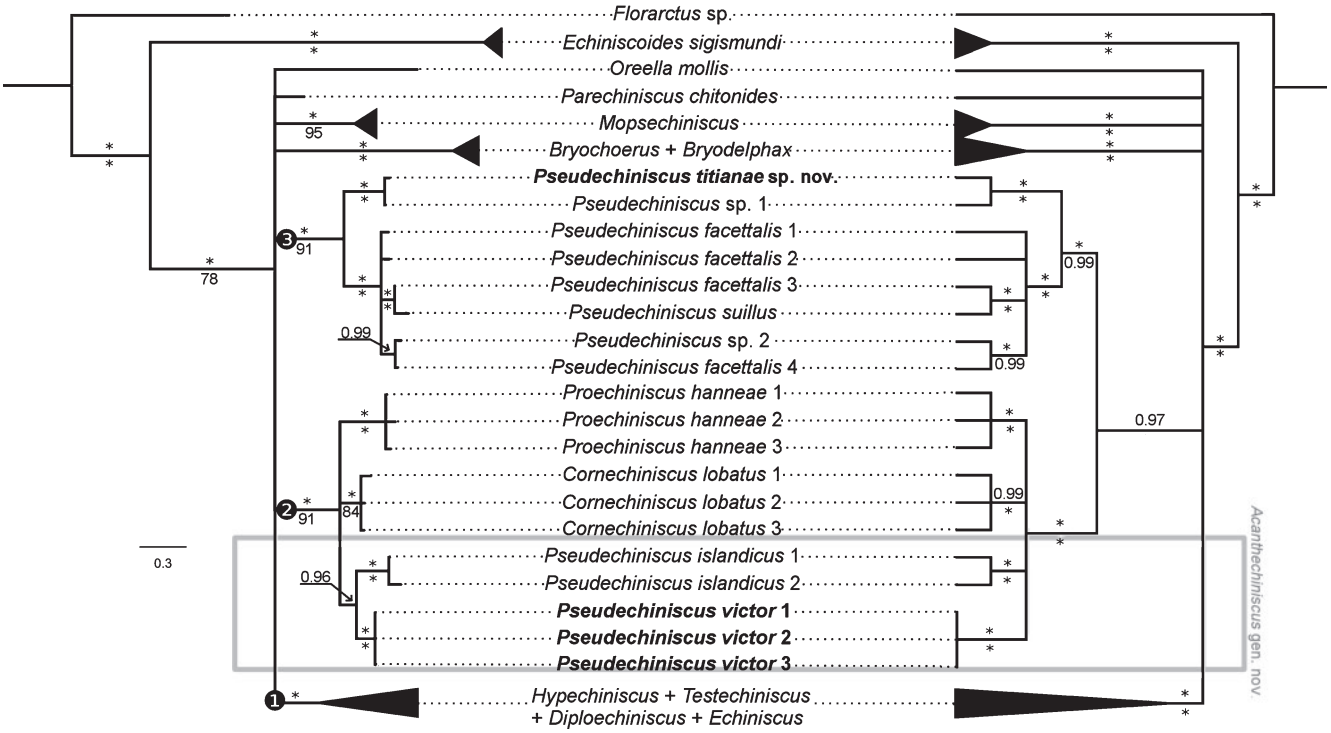

Fig. 2. Phylogeny of Echiniscidae. Left: Bayesian inference phylogenetic trees (BI); values above branches: BI posterior probability values (PP); values below branches: maximum likelihood bootstrap values (BS). Right: BI consensus phylogeny with modified complex indel-coding; PP values above branches from analysis using modified complex indel-coding; PP values below branches from analysis using simple indel-coding. Branches with <0.95 PP and <75 BS have been collapsed. Asterisks denote PP = 1.0 or BS = 100. Newly generated sequences are bolded. Numbers in black circles identify the three echiniscid phylogenetic lineages described in the text. Scale bar shows number of substitutions per site.

Macrobiotoidea (Fig. 3, Supplementary Fig. S3) are the same as those recovered by Bertolani *et al*. (2014). It should be noted that species of *Macrobiotus* do not all group together, such that they are clearly divided into four phylogenetic lineages. Lineage 1 (Fig. 3) is characterised by *Macrobiotus islandicus* Richters, 1904, which clusters together with *Richtersius coronifer* (Richters, 1903) and genera in the family Murrayidae. Lineage 2 (Fig. 3) comprises *M. polonicus* Pilato *et al.*, 2003 and the genus *Xerobiotus*. Lineage 3 (Fig. 3) comprises species belonging to the *Macrobiotus hufelandi* group (*sensu* Guidetti *et al*. 2013): *M. hufelandi* Schultze, 1834, *M. kristenseni* Guidetti *et al.*, 2013, *M. macrocalix* Bertolani & Rebecchi, 1993, *M. joannae* Pilato & Binda, 1983, *M. sapiens* Binda & Pilato, 1984, *M. vladimiri* Bertolani *et al.*, 2011*a*, *M.* gr. *hufelandi*, and *M. nelsonae.* Lineage 4 (Fig. 3) contains species belonging to the *Macrobiotus harmsworthi* group (*sensu* Kaczmarek *et al*. 2011): *M.* cf. *mottai*, *M. polaris*, the Italian specimens of the *M. harmsworthi* group, the newly discovered Antarctic *Macrobiotus* and one species belonging to the *Macrobiotus furciger* group (see Binda *et al.* 2005): *M. furciger* Murray, 1907*b*. This latter phylogenetic lineage is formed by two sister groups, one comprising the Italian specimens and the other the Antarctic specimens. Within the Antarctic group of species, two evolutionary lineages can be identified: one grouping *M. furciger* and *M.* cf. *mottai*, and the other comprising *M. polaris* and the newly recognised species of *Macrobiotus*.

Discussion

The description of *Pseudechiniscus titianae*, sp. nov. (see Taxonomic account) increases the number of identified heterotardigrade species in continental Antarctica to six, all belonging to Echiniscoidea: three *Echiniscus* (Dastych 1984; McInnes 2010), one *Mopsechiniscus* (Guidetti *et al*. 2014), and two *Pseudechiniscus* (Dastych 1984; Miller *et al*. 1996; present study). Two additional undetermined species of *Pseudechiniscus* have been found by Utsugi and Ohyama (1989) and Tsujimoto *et al*. (2014). All *Pseudechiniscus* species previously found in continental Antarctica belong to the *P. suillus* group, and were found in Enderby Land (*P. suillus*: Dastych 1984), Wilkes Land (*P. suillus*: Miller *et al*. 1996) and Dronning Maud Land (two undetermined species: Utsugi and Ohyama 1989; Tsujimoto *et al*. 2014). The undetermined species of *Pseudechiniscus* found by Utsugi and Ohyama (1989) in moss collected at Strandnibba (close to the Japanese Syowa Station in Dronning Maud Land) corresponds well with *P. titianae*, sp. nov., and therefore this species might also be distributed along the coast of Dronning Maud Land.

The use of molecular data, supported by the identification of morphological synapomorphies, leads to the identification of two evolutionary lineages within *Pseudechiniscus* that are not each other's sister, i.e. *Pseudechiniscus* as currently recognised

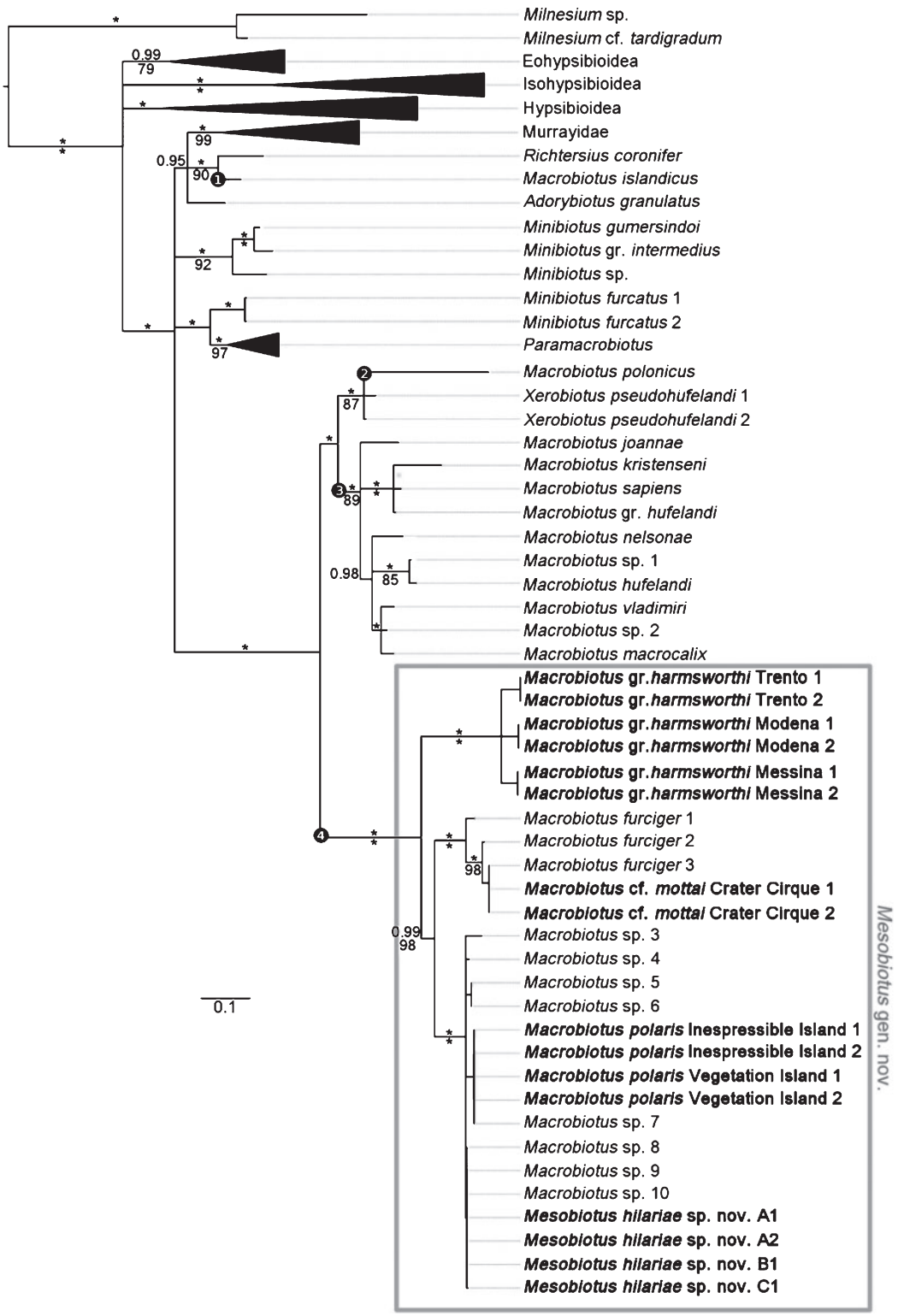

Fig. 3. Phylogeny of Macrobiotoidea obtained by Bayesian inference (BI) and maximum likelihood (ML). Value above branches: BI posterior probability values (PP); values below branches: ML boostrap values (BS). Branches with <0.95 PP and <75 BS have been collapsed. Asterisks denote PP = 1.0 or BS = 100. Newly generated sequences are bolded. Numbers in black circles identify the three *Macrobiotus* phylogenetic lineages described in the text. Scale bar shows number of substitutions per site.

is not monophyletic. According to Kristensen (1987), two main groups of species can be distinguished within *Pseudechinicus* Thulin, 1911: the '*victor* group', comprising species with large filaments or spines other than cirrus A, and the '*suillus*/*conifer* group', with species characterised by the absence of filaments or spines other than cirrus A. The species of the *P. suillus*/*P. conifer* group (*P. facettalis*, *P. suillus*, *P. titianae*, sp. nov.) are separated from the species of the *P. victor* group (*P. victor*, *P. islandicus*). The more obvious morphological characters that characterise the *P. victor* group and distinguish it from the *P. suillus*/*P. conifer* group, are the presence of a notched collar (or spine fringe) on the hind legs, and filaments in all lateral positions along the body trunk. With *P. suillus* as the type species of the genus *Pseudechiniscus*, the erection of the new genus *Acanthechiniscus* for the species of the *P. victor* group and the emendation of the *Pseudechiniscus* genus are proposed (see Taxonomic account).

All species of *Macrobiotus* found in continental Antarctica belong to the so-called *M. harmsworthi* group. In particular, all these species, except *M. mottai* Binda & Pilato, 1994, have been found in Dronning Maud Land: *M. blocki* Dastych, 1984 (Sohlenius *et al*. 1995; Sohlenius and Boström 2005), *M. furciger* (Sohlenius *et al*. 1995), *M. krynauwi* Dastych & Harris, 1995 (Dastych and Harris 1995; Sohlenius and Boström 2005), and *M. polaris* (Sanyal 2004). *Macrobiotus harmsworthi coronatus* de Barros, 1942 and *M. montanus* Murray, 1910, reported by Utsugi and Ohyama (1991) at the border between Dronning Maud Land and Wilkes Land, very likely correspond to *M. blocki* and to *M. mottai*/*M. polaris*, respectively.

On the basis of molecular data, *Macrobiotus* also seems to be non-monophyletic, being divided into at least four well supported evolutionary lineages. According to our LM and SEM observations, the phylogenetic lineage of the *M. harmsworthi* group is characterised by three roundish macroplacoids arranged along a curved line, a microplacoid clearly close (less than its length) to the third macroplacoid (Fig. 4), and the common tract of the claw with an internal septum defining a distal part (Fig. 5). On the other hand, species of the *M. hufelandi* group (comprising *M. polonicus*) and *Xerobiotus* are characterised by two bands of small teeth in

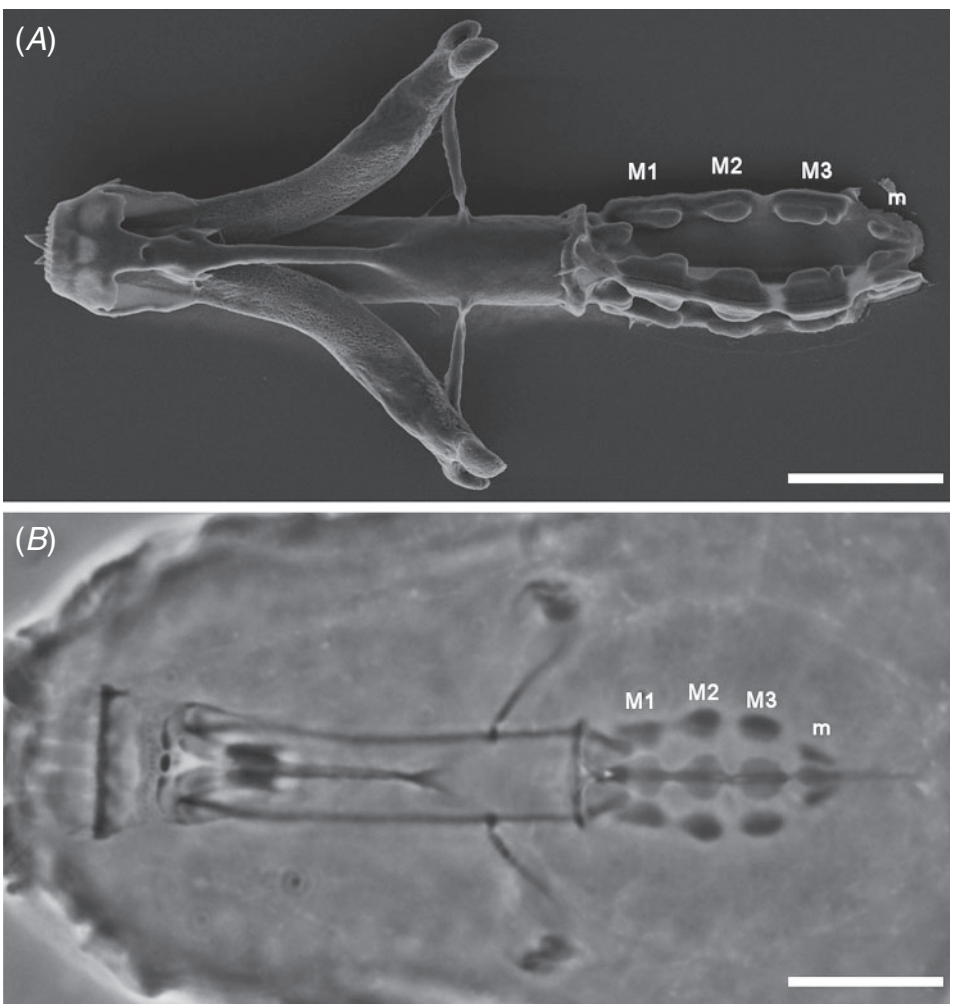

Fig. 4. (*A*, *B*) *Mesobiotus*, gen. nov. buccal pharyngeal apparatus. (*A*) *Mesobiotus* sp. (SEM). (*B*) *Mesobiotus mauccii*, comb. nov. (PhC). Scale bars: 10 µm. M1, first macroplacoid; M2, second macroplacoid; M3, third macroplacoid; m, microplacoid.

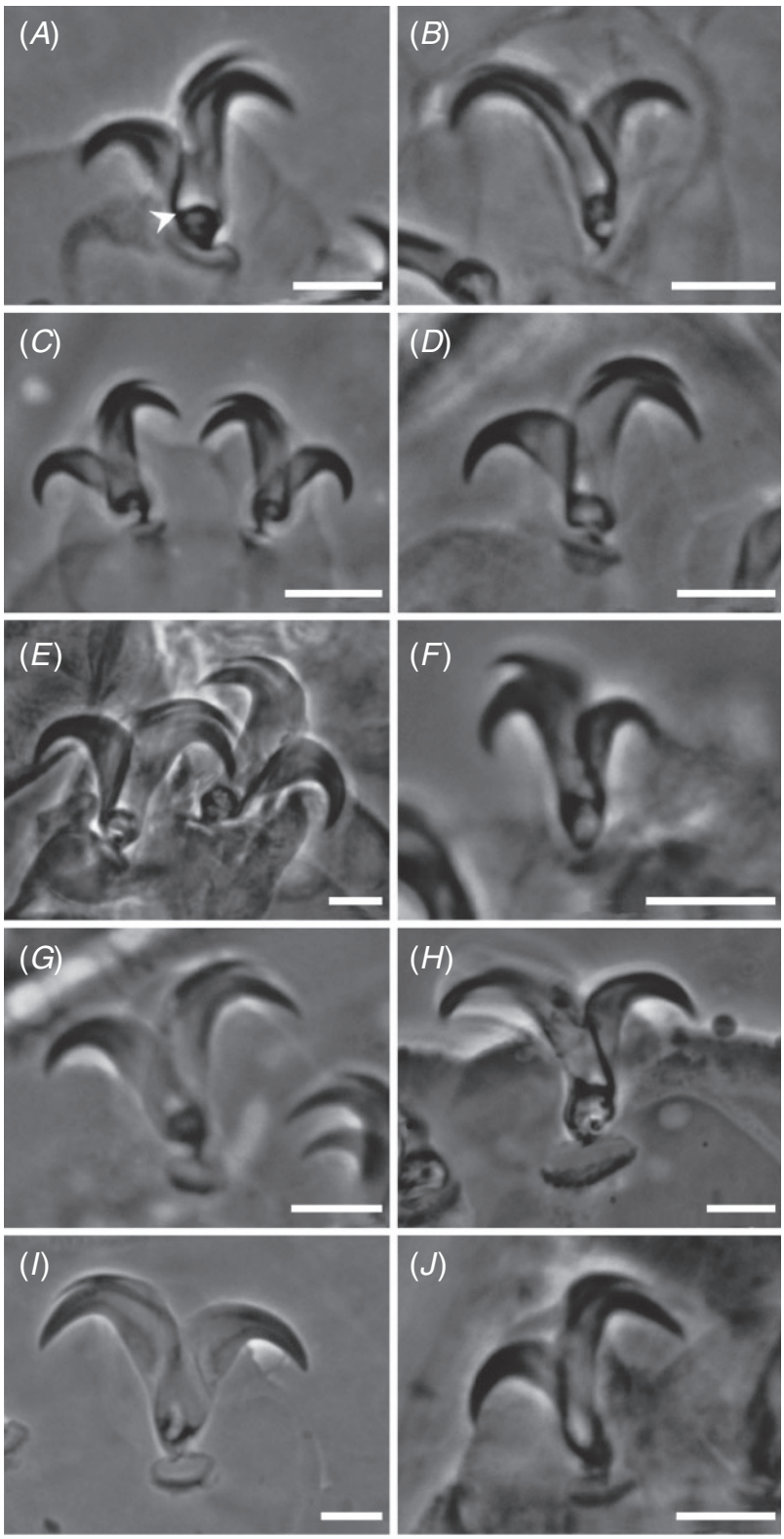

Fig. 5. (*A–J*) Claws of the third leg pairs of *Mesobiotus*, gen. nov. (*A–H*) and *Macrobiotus* (*I–J*) (PhC). (*A*) *Mesobiotus mottai*, comb. nov. (*B*) *M. polaris*, comb. nov. (*C*) *M.* sp. Lake Baccio. (*D*) *M. harmsworthi*, comb. nov. (*E*) *M. liviae*, comb. nov. (*F*) *M. lusitanicus*, comb. nov. (*G*) *M. furciger*, comb. nov. (*H*) *M. arguei*, comb. nov. (*I*) *Macrobiotus nelsonae*. (*J*) *M. sandrae*. Scale bars: 5 μm. Arrowhead, claw septum.

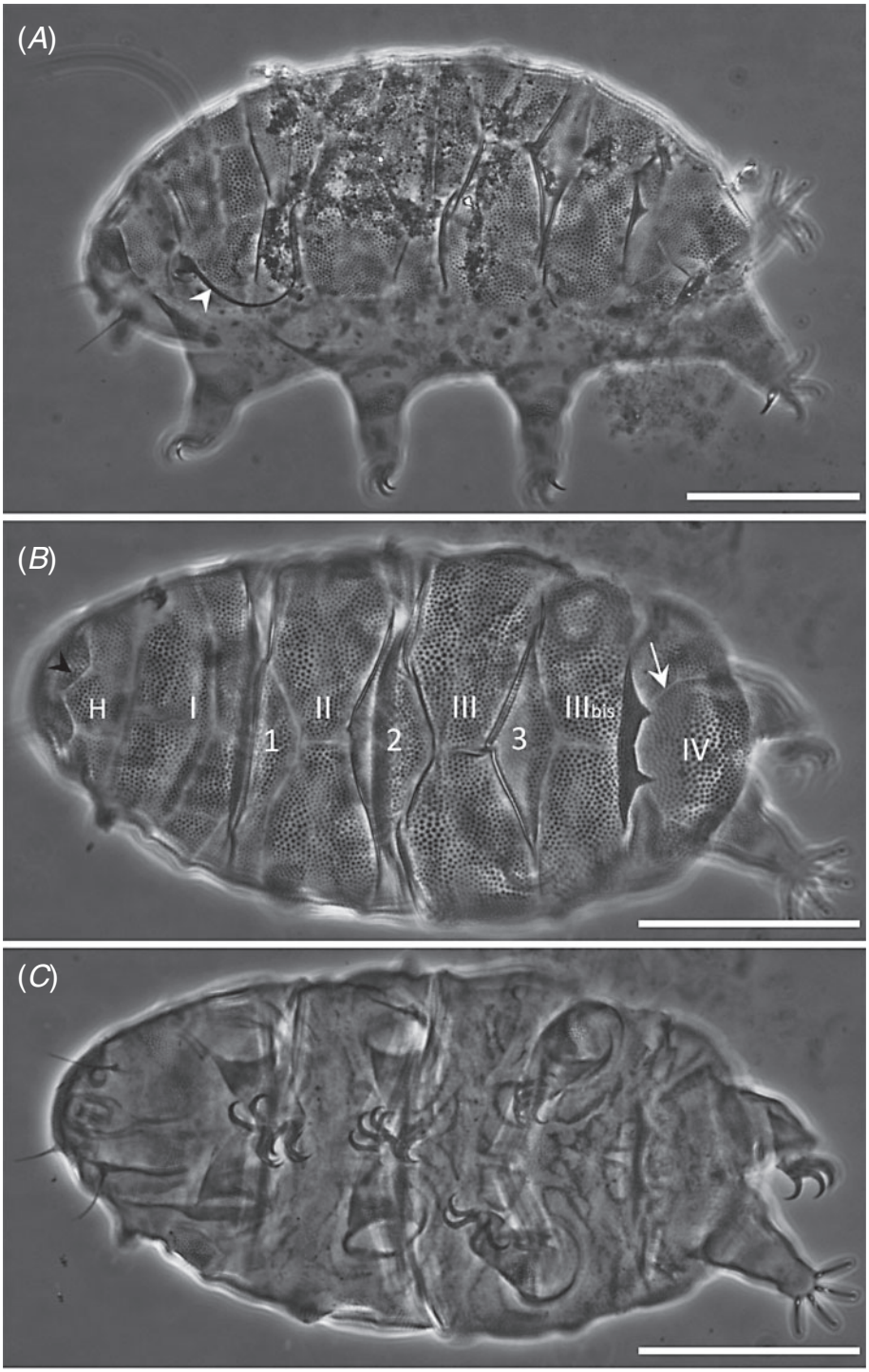

Fig. 6. (*A–C*) *Pseudechiniscus titianae*, sp. nov. (PhC). (*A*) Holotype, dorso-lateral view. (*B*) Paratype, dorsal view. (*C*) Paratype, ventral view. Scale bars: 50 µm. Abbreviations: H, head plate; I, scapular plate; 1–3, median plates; II–III, paired plates; IIIbis, pseudosegmental plate; IV, caudal plate. Dorsal plates are numbered according to Ramazzotti and Maucci (1983). Arrowhead, cirrus A; black arrow, W-shaped fold in the head plate; white arrow, indentation of caudal plate.

the buccal armature, two macroplacoids and a microplacoid positioned close to the second macroplacoid, pores (pearls) on the cuticular surface, and egg processes generally in the form of inverted goblets (Guidetti *et al*. 2013). *Macrobiotus islandicus* is characterised by the lack of the small teeth bands in the buccal armature and microplacoids, and by the presence of two macroplacoids, pores on the cuticular surface, and egg processes in the form of cones.

With *M. hufelandi* as the type species of *Macrobiotus*, the erection of the new genus *Mesobiotus* is proposed for the species belonging to the *M. harmsworthi* and *M. furciger* groups (see Taxonomic account).

As previously reported by Guidetti *et al*. (2009) for the erection of the genus *Paramacrobiotus*, the new genus *Mesobiotus* cannot correspond to the *Macrobiotus* subgenus *Orthomacrobiotus* proposed by Biserov (1990*a*, 1990*b*). Biserov correctly identified a phylogenetic lineage for the *M. hufelandi* group, but he defined the new *Macrobiotus* subgenus *Orthomacrobiotus* only by absence of characters, i.e. simply defining them as different from those he attributed to *Macrobiotus* in his definition of the nominal subgenus. On the basis of our results, the genus *Macrobiotus* remains polyphyletic. The eventual separation of the *M. islandicus* and *M. polonicus* lineages needs further molecular analyses, especially with respect to increased taxon and gene sampling.

Taxonomic accounts

Genus ***Acanthechiniscus***, gen. nov. Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti

http://zoobank.org/urn:lsid:zoobank.org:act:2E30053F-31FE-4857-BCD8-DBA24C004E2A

Type species: *Echiniscus victor* Ehrenberg, 1853: 530. (Synonym: *Pseudechiniscus tridentifer* Bartoš, 1935: 45–47.)

Description

Echiniscid with pseudosegmental cuticular plate. Black eyes. Buccal cirri present, filamentous cirri A present. Rigid buccal tube. Filaments and/or spines (may be small) present, generally in all lateral positions (B, C, D, E). Dorsal spines present. Notched collar (or spine fringe) on hind legs present.

Species of Acanthechiniscus*, gen. nov.*

Acanthechiniscus victor (Ehrenberg, 1853), comb. nov., *Acanthechiniscus distinctus* (Mihelčič, 1951), comb. nov., *Acanthechiniscus goedeni* (Grigarick *et al.*, 1964) (pending further studies; see Remarks), *Acanthechiniscus islandicus* (Richters, 1904), comb. nov., *Acanthechiniscus sinensis* (Rahm, 1937) (although not well described).

Remarks

Acanthechiniscus goedeni, comb. nov. shows red eyes and multidivided dorsal plates, so it is provisionally placed in this new genus pending further morphological and molecular analyses. According to several morphological characters, it could belong to the genus *Multipseudechiniscus* Schulte & Miller, 2011.

Etymology

The name derives from the presence of spines and filaments (*acantho* – thorny, spiny (Greek)) on the body of the animals of the species of this genus.

Genus ***Pseudechiniscus*** Thulin (Emended)

http://zoobank.org/urn:lsid:zoobank.org:act:2044028D-C545-42FA-A0AC-4D8C887BFAA4

Type species: *Echiniscus suillus* Ehrenberg, 1853: 530. (Synonym: *Echiniscus mutabilis* Murray, 1906: 683–684 and plate I, figs 2a to 2d.)

Emended description

Echiniscid with pseudosegmental plate. Black eyes. Buccal cirri present, filamentous cirri A present. Rigid buccal tube, stylet supports may be present, but very tiny and located close to the margin of pharyngeal bulb. Lateral and dorsal trunk filaments or spines generally absent or reduced in number and/or size. Notched collar (or spine fringe) on hind legs absent.

Remarks

Pseudechiniscus alberti Dastych, 1987 shows a spine fringe with 1–2 teeth in the hind legs, lateral plates with short spines, and head cirri with bifurcated tips as in *P. victor*, so it is kept in this genus pending further morphological and molecular analyses.

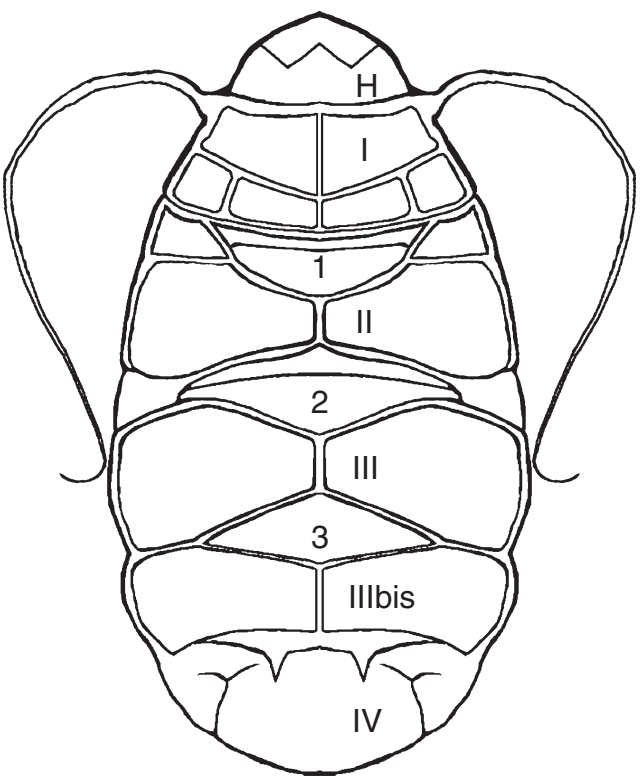

Fig. 7. *Pseudechiniscus titianae*, sp. nov. Schematic drawing of the dorsal cuticular plates, and lateral and dorsal appendages. Scale bar: 50 µm. Legend for plates as in Fig. 6.

Genus ***Mesobiotus***, gen. nov. Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti

http://zoobank.org/urn:lsid:zoobank.org:act:44AB6EAB-753B-4EE6-93F0-C3237FDF23B0

Type species: *Macrobiotus harmsworthi* Murray, 1907*a*: 677–678 and plate I, figs 7a to 7d.

Description

Macrobiotids with Y-type double-claws with a common tract characterised by an internal septum defining a distal part. Cuticle without pores. Mouth ring with 10 peribuccal lamellae; rigid buccal tube; three roundish macroplacoids arranged along a curved line; microplacoid clearly close (less than its length) to the third macroplacoid. Eggs laid freely and characterised by conical or hemispherical processes, generally with pointed tips.

Species of Mesobiotus*, gen. nov.*

Mesobiotus harmsworthi (Murray, 1907*a*), comb. nov., *Mesobiotus altitudinalis* (Biserov, 1997/98), comb. nov., *Mesobiotus aradasi* (Binda *et al.*, 2005), comb. nov., *Mesobiotus arguei* (Pilato & Sperlinga, 1975), comb. nov., *Mesobiotus armatus* (Pilato & Binda, 1996), comb. nov., *Mesobiotus australis* (Pilato & D'Urso, 1976), comb. nov., *Mesobiotus baltatus* (McInnes, 1991), comb. nov., *Mesobiotus barabanovi* (Tumanov, 2005), comb. nov., *Mesobiotus barbarae* (Kaczmarek *et al.*, 2007), comb. nov., *Mesobiotus binieki* (Kaczmarek *et al.*, 2011), comb. nov., *Mesobiotus blocki* (Dastych, 1984), comb. nov., *Mesobiotus contii* (Pilato & Lisi, 2006), comb. nov., *Mesobiotus coronatus* (de Barros, 1942), comb. nov., *Mesobiotus creber* (Pilato & Lisi, 2009), comb. nov., *Mesobiotus diffusus* (Binda & Pilato, 1987), comb. nov., *Mesobiotus diguensis* (Pilato & Lisi, 2009), comb. nov.,

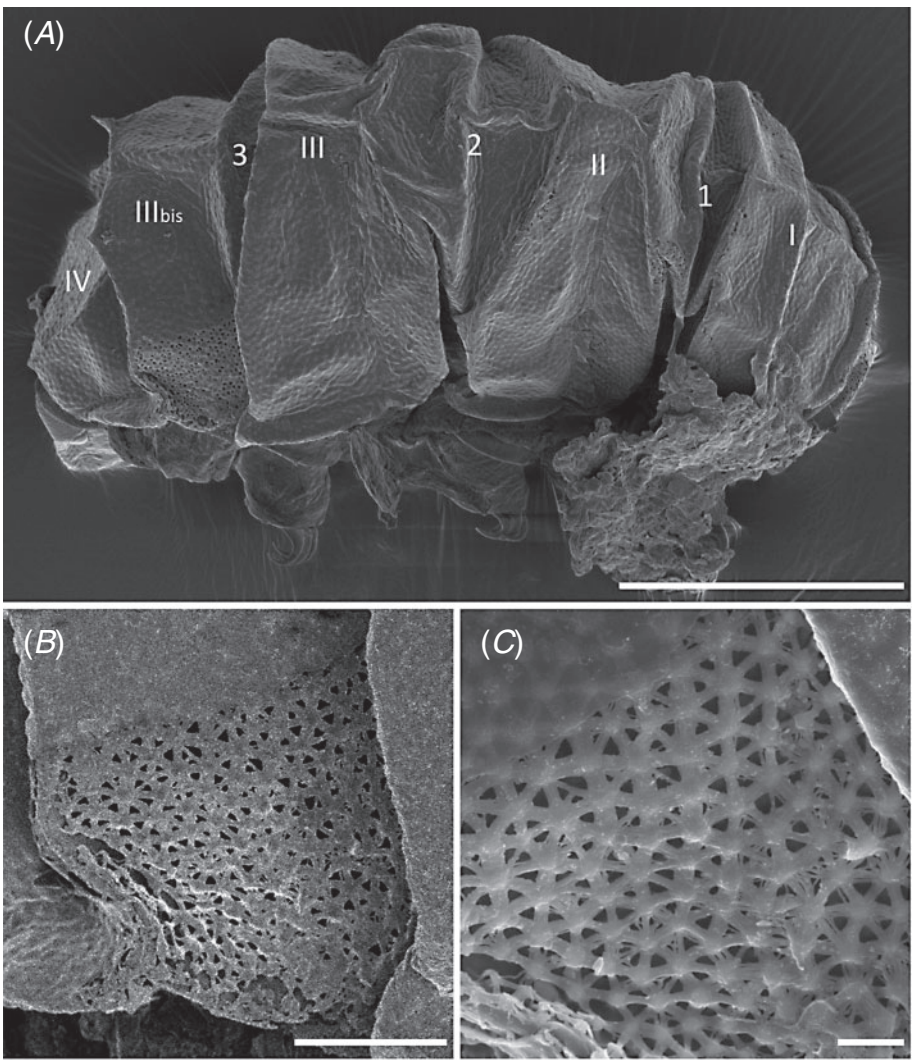

Fig. 8. (*A–C*) *Pseudechiniscus titianae*, sp. nov. (SEM). (*A*) Paratype, dorso-lateral view of *in toto* animal. (*B*) Broken cuticle of the pseudosegmental plate. (*C*) Magnification of the broken cuticle of pseudosegmental plate. Scale bars: A, 30 µm; B, 5 µm; C, 2 µm. Legend for plates as in Fig. 6.

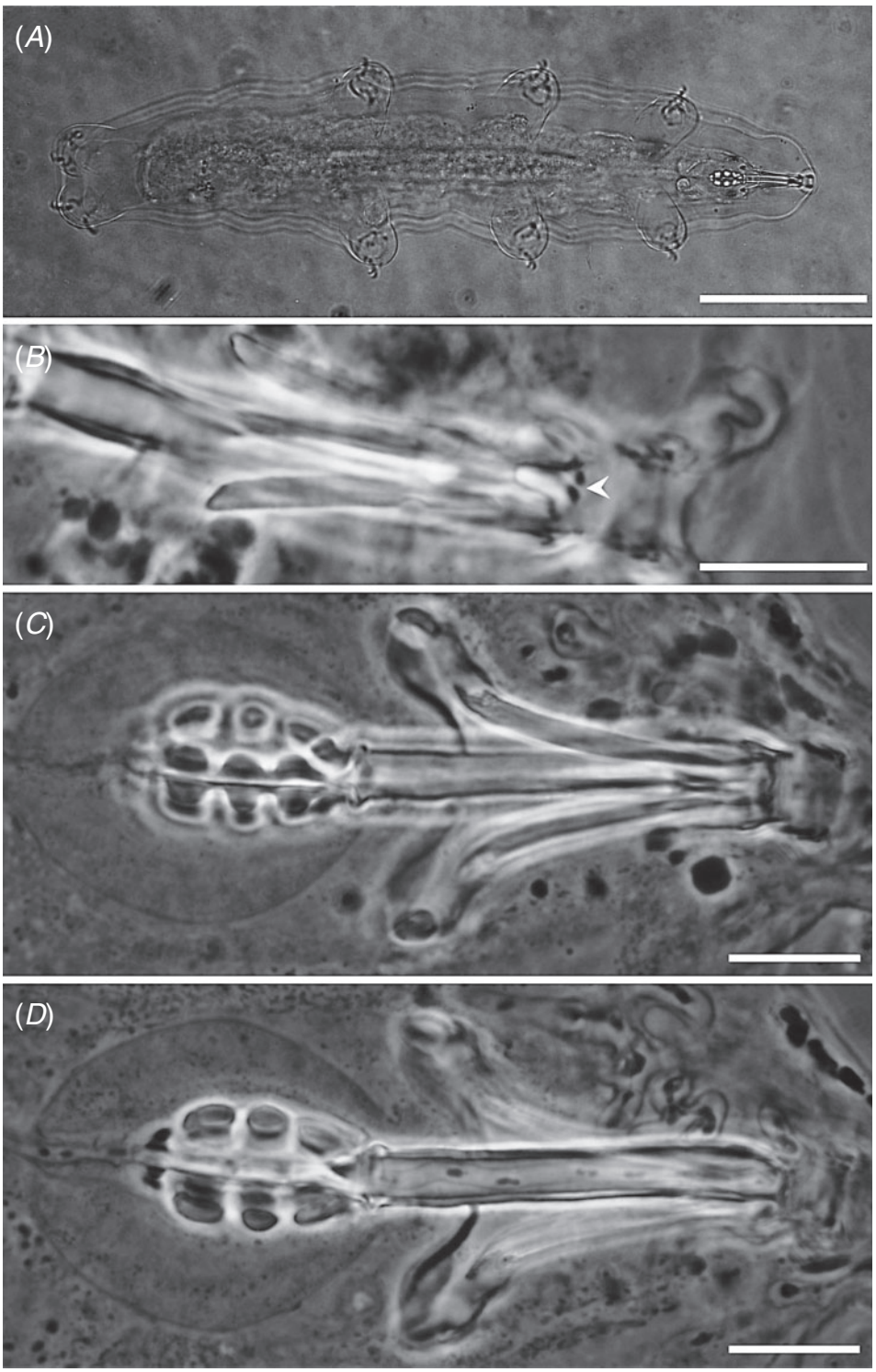

Fig. 9. (*A–D*) *Mesobiotus hilariae*, sp. nov. (PhC). (*A*) Holotype, ventral view. (*B*) Buccal armature, ventral view. (*C*) Buccal pharyngeal apparatus, ventral view. (*D*) Buccal pharyngeal apparatus, dorsal view. Scale bars: A, 100 μm; B–D, 10 μm. Arrowhead, transversal crests of the buccal armature.

Mesobiotus dimentmani (Pilato *et al.*, 2010), comb. nov., *Mesobiotus divergens* (Binda *et al.*, 2005), comb. nov., *Mesobiotus erminiae* (Binda & Pilato, 1999), comb. nov., *Mesobiotus furciger* (Murray, 1907*a*), comb. nov., *Mesobiotus hieronimi* (Pilato & Claxton, 1988), comb. nov., *Mesobiotus hilariae*, sp. nov., *Mesobiotus insuetus* (Pilato *et al.*, 2014), comb. nov., *Mesobiotus kovalevi* (Tumanov, 2004), comb. nov., *Mesobiotus krynauwi* (Dastych & Harris, 1995), comb. nov., *Mesobiotus liviae* (Ramazzotti, 1962), comb. nov., *Mesobiotus lusitanicus* (Maucci & Durante Pasa, 1984), comb. nov., *Mesobiotus mauccii* (Pilato, 1974), comb. nov., *Mesobiotus meridionalis* (Richters, 1909), comb. nov., *Mesobiotus montanus* (Murray, 1910), comb. nov., *Mesobiotus mottai* (Binda & Pilato, 1994), comb. nov., *Mesobiotus neuquensis* (Rossi *et al.*, 2009), comb. nov., *Mesobiotus nuragicus* (Pilato & Sperlinga, 1975), comb. nov., *Mesobiotus orcadensis* (Murray, 1907*a*), comb. nov., *Mesobiotus ovostriatus* (Pilato & Patanè, 1998), comb. nov., *Mesobiotus pallarii* (Maucci, 1954), comb. nov., *Mesobiotus patiens* (Pilato *et al.*, 2000), comb. nov., *Mesobiotus perfidus* (Pilato & Lisi, 2009), comb. nov., *Mesobiotus peterseni* (Maucci, 1991), comb. nov., *Mesobiotus pilatoi* (Binda & Rebecchi, 1992), comb. nov., *Mesobiotus polaris* (Murray, 1910), comb. nov., *Mesobiotus pseudocoronatus* (Pilato *et al.*, 2006), comb. nov., *Mesobiotus pseudoliviae* (Pilato & Binda, 1996), comb. nov., *Mesobiotus pseudonuragicus* (Pilato *et al.*, 2004), comb. nov., *Mesobiotus radiatus* (Pilato *et al.*, 1991), comb. nov., *Mesobiotus reinhardti* (Michalczyk & Kaczmarek, 2003), comb. nov., *Mesobiotus rigidus* (Pilato & Lisi, 2006), comb. nov., *Mesobiotus siamensis* (Tumanov, 2006), comb. nov., *Mesobiotus sicheli* (Binda *et al.*, 2005), comb. nov., *Mesobiotus simulans* (Pilato *et al.*, 2000), comb. nov., *Mesobiotus snaresensis* (Horning *et al.*, 1978), comb. nov., *Mesobiotus stellaris* (du Bois-Reymond Marcus, 1944), comb. nov., *Mesobiotus szeptyckii* (Kaczmarek & Michalczyk, 2009), comb. nov., *Mesobiotus tehuelchensis* (Rossi *et al.*, 2009), comb. nov., *Mesobiotus wuzhishanensis* (Yin*et al.*, 2011), comb. nov., *Mesobiotus zhejiangensis* (Yin *et al.*, 2011), comb. nov.

Remarks

According to the original description, *Mesobiotus krynauwi* (Dastych & Harris, 1995), comb. nov. differs from the description of the genus *Mesobiotus*, gen. nov. only by the presence of pores in the cuticle. Nevertheless, it is placed in the new genus due to its high similarity to the other *Mesobiotus*

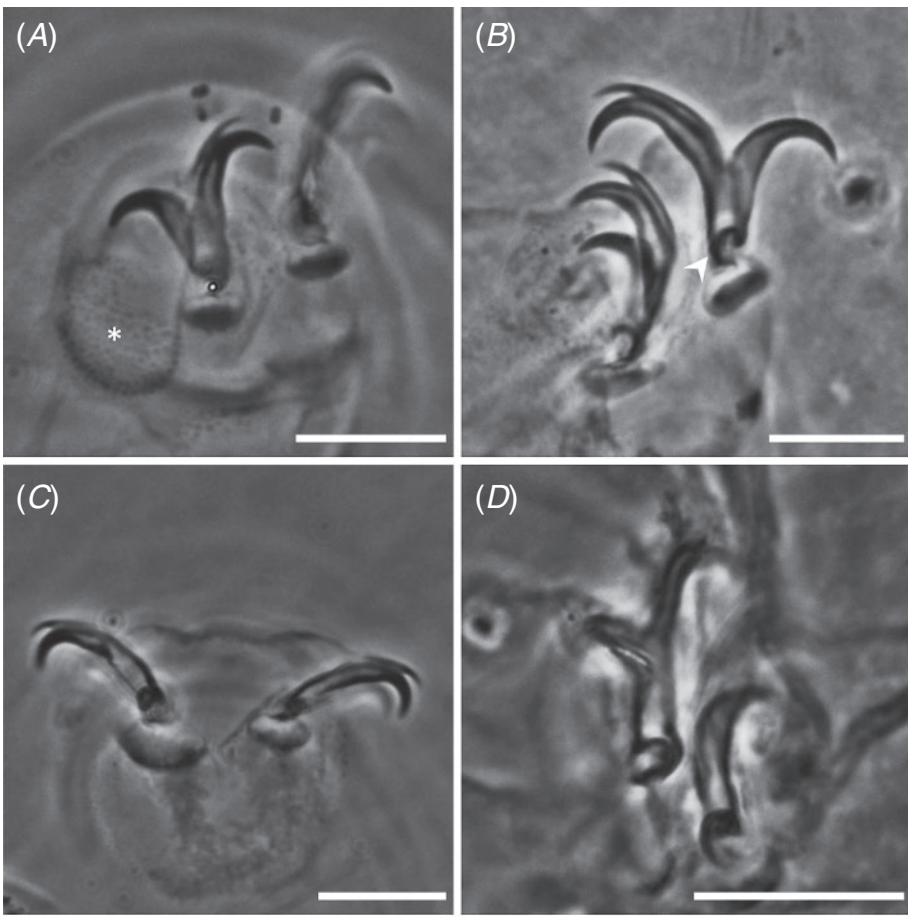

Fig. 10. (*A–D*) Claws of *Mesobiotus hilariae*, sp. nov. (PhC). (*A*) Holotype, second leg pair claws. (*B*) Paratype, third leg pair claws. (*C*) Holotype, hind leg claws. (*D*) Paratype, hind leg claws. Scale bars: 10 μm. Asterisk, swelling on the internal side of the leg; arrowhead, claw septum.

species, pending further molecular and morphological investigations.

Etymology

The name refers to the phylogenetic position of this genus among (*meso* – in between, among (Latin)) the other macrobiotid genera.

Mesobiotus hilariae, sp. nov. Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti

http://zoobank.org/urn:lsid:zoobank.org:act:95D05839-3E29-47DB-841A-48BFB860E012

Material examined

Holotype. Sex undetermined, Antarctica, Dronning Maud Land, Novolazarevskaya Station (70°46.557′S, 11°48.837′E, 116 m a.s.l.), moss sample, Jan. 2002. Mounted on slide (C3610-S2-A) and deposited in the Bertolani collection (Department of Life Sciences, University of Modena and Reggio Emilia, Italy).

Paratypes. 21 paratypes (9 animals, 12 eggs). Paratypes are mounted on slides. Sixteen (C3610-S2-B; C3610-S3-A,B; C3610-S5-A; C3620-S2-A; C3623-S4-A,B; C3623-S5-A,B; C3623-S6-A; C3623-S7-A,B; C3623-S10-A; C3624-S4-A; C3624-S5-A,D) are deposited in the Bertolani collection (Department of Life Sciences, University of Modena and Reggio Emilia, Italy), five (C3610-S4-A; C3620-S1-A; C3620-S4-A,B,C) in the tardigrade slide collections of the Swedish Museum of Natural History (Stockholm, Sweden).

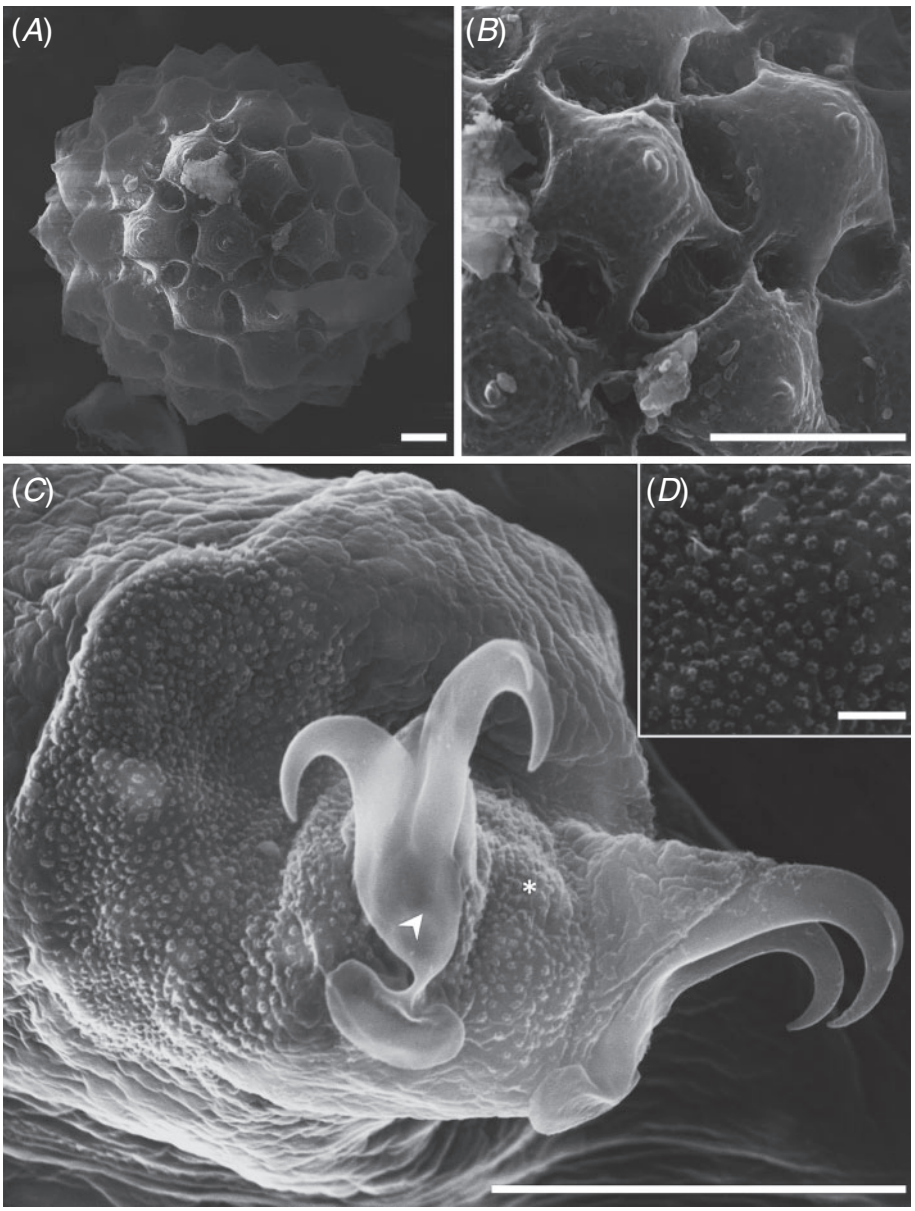

Fig. 11. (*A–D*) *Mesobiotus hilariae*, sp. nov. (SEM). (*A*) *In toto* egg. (*B*) Egg shell detail. (*C*) Second leg pair claws. (*D*) Leg granulation detail. Scale bars: A–C, 10 μm; D, 1 μm. Arrowhead, claw septum; asterisk, leg granulation.

18S DNA sequences: GenBank KT226068, KT226069, KT226070, KT226071
28S DNA sequence: GenBank KT226079
cox1 *DNA sequence*: GenBank KT226108

Diagnosis

Macrobiotidae with Y-shaped claws. Colour whitish. Three roundish macroplacoids and a relatively large microplacoid close (less than its length) to the last macroplacoid in the pharynx. Ventral transversal crests of the buccal armature reduced to two small teeth. Ornamented eggs, laid freely. Egg shell with conical processes connected at their base by expansions that form an areolation around the processes. Internal walls of the egg processes reticulated.

Description

Animals

Size range: 189.1–527.4 µm long. Colour whitish. Anterior black eye spots present. Cuticle smooth and without pores. Terminal mouth opening surrounded by a wreath of buccal lamellae. Buccal armature not well distinguishable under LM, a fine posterior band of teeth visible only in larger specimens; transversal crests present: dorsally, median transversal crest larger than the laterals; ventrally, only two small triangular crests present (Fig. 9*B*). Buccal tube relatively narrow with anterior curvature, ending in the pharynx with a thick margin. Long ventral lamina (~65% of the buccal tube length). Insertion of the stylet support placed posteriorly at ~3/4 (72%) of the buccal tube length. Pharynx round or oval in shape. In the pharynx, large pharyngeal apophyses, three rounded macroplacoids, and a large microplacoid close to the third macroplacoid present (Fig. 9*C*, *D*). The first macroplacoid usually the biggest and with a triangular shape, the second rounded/quadrangular, and the third with a posterior constriction; the second and third macroplacoids similar in size.

Main branch of the double-claws relatively long, with well developed accessory points not reaching the distal tip of the branch; secondary branch with a wide curvature. In the first three leg pairs (Figs 10*A*, *B*, 11*C*), claw common tract wide, and shorter than half of the claw length, with a small rounded basal portion delimited by a transversal septum, and an evident stalk connecting the claw to the lunules. The claw of the hind legs (Fig. 10*C*, *D*) different: the common tract longer, but still with a transversal septum, and the secondary branch shorter with respect to the first three pairs of claws. Lunules small and

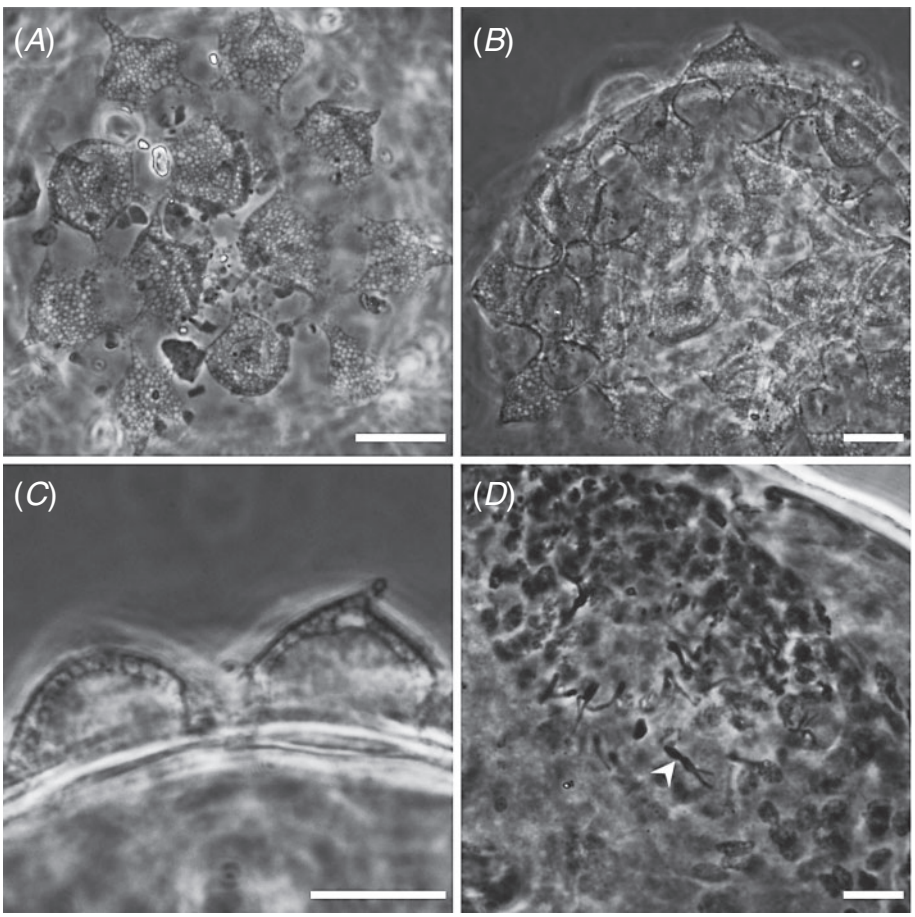

Fig. 12. (*A–D*) *Mesobiotus hilariae*, sp. nov. (PhC). (*A–C*) Egg shell details. (*D*) Male gonad with spermatozoa. Scale bars: 10 µm. Arrowhead, spermatozoon.

Table 3. Mean, minimum and maximum values of the measurements of *Macrobiotus hilariae*, sp. nov.

	Mean (μm)	s.d. (μm)	Minimum (μm)	Maximum (μm)	Mean (*pt*)	s.d. (*pt*)	Min (*pt*)	Max (*pt*)	*N*
Body length	408.1	93.5	238.8	527.4					9
Buccal tube length	34.7	4.6	23.8	39.6					9
Buccal tube diameter	2.6	0.4	2.0	3.0	7.6	0.6	7.1	8.6	9
Stylets support insertion	24.9	3.4	20.8	29.7	72.4	8.5	60	87.5	9
Macroplacoids length row	15.4	1.7	12.9	18.8	44.8	4.5	40	54.2	9
Macroplacoids length 1st	4.8	0.6	4.0	5.9	14.1	2.8	11.4	20.8	9
Macroplacoids length 2nd	3.6	0.5	3.0	4.0	10.6	1.4	8.6	12.5	9
Macroplacoids length 3rd	4.2	0.9	2.5	5.9	12.0	1.6	10.4	15.0	9
Microplacoid length	2.3	0.4	2.0	3.0	6.7	1.1	5.0	8.3	9
Claws length I	10.1	1.5	7.4	12.4	29.3	2.2	25.7	32.4	9
Claws length II–III	11.4	1.8	7.9	13.9	33.0	2.6	28.6	37.1	9
Claws length IV	13.1	2.5	8.4	16.8	37.5	3.7	34.3	45.9	8
Eggs diameter	78.6	5.4	71.3	89.1					11
Eggs processes diameter	14.4	2.8	8.9	19.8					57
Eggs processes height	10.0	2.0	5.9	14.8					53
Eggs processes on diameter	13.4	1.1	12.0	15.0					8

smooth in the first three pairs of legs (Figs 10*A*, *B*, 11*C*); larger with small teeth barely visible with LM in the hind legs (the internal lunules evidently larger than the external).

The first three pairs of legs with a fine granulation in the external and internal sides, close to the claws and between the claws (Figs 10*A*, 11*C*). Dorsal surface of the hind legs covered by granulation. Each dot of the granulation (~0.2 μm in diameter) formed by small clustered granules (about five granules for each dot) (Fig. 11*D*). A small swelling present in the internal side of the first three pairs of legs and in the external side of the hind legs, also this swelling with a fine granulation (Fig. 10*A*).

Males and females present. The type of gametes within the gonad allowed identification of one female and one male (Fig. 12*D*).

Mean, maximum and minimum values of the measures of the cuticular structures of animals are reported in Table 3.

Eggs

Ornamented eggs laid freely (Figs 11*A*, *B*, 12*A*–*C*). Egg ornamentation formed by broad conical processes with a pointed tip, sometimes bifurcated. The process wall formed by two sides (an internal and an external), between them a net of trabecular structures is present, forming irregular meshes (Fig. 12*C*). Processes connected to one another at their base by lateral expansions. These connections form areolation (usually six) around each process (Figs 11*A*, *B*, 12*B*). The shell surface within the areolation wrinkled with some small 'holes' in some areas. An animal exiting from the egg was found. The measurements of the eggs are reported in Table 3.

Measurements for every single animal and egg are provided in Table S3 (newborn C3623-S5-A was not considered in summary measures). A *cox1* sequence of an individual from *locus typicus* has been obtained and it is available in GenBank (KT226108).

Differential diagnosis

Within *Mesobiotus*, gen. nov. two species groups can be recognised: the *harmsworthi* group (see Kaczmarek *et al.* 2011) and the *furciger* group (see Binda *et al.* 2005). According to Kaczmarek *et al*. (2011), 41 species belong to the *harmsworthi* group and are characterised by three macroplacoids with short and rounded rods, a microplacoid situated very close to them, and conical or hemispherical egg processes. *Mesobiotus hilariae*, sp. nov. can be attributed to this group and differs from all the other species of the group by the presence, in correspondence with the ventral transversal ridges of the buccal armature, of two small triangular teeth. The only species of the *harmsworthi* group with similar teeth is *Mesobiotus polaris*. *Mesobiotus hilariae*, sp. nov. differs from *M. polaris* by having smaller ventral triangular teeth and by the shape of the egg processes.

Table 4. Mean, minimum and maximum values of the measurements of *Pseudechiniscus titianae*, sp. nov.

IIIbis pr., pseudosegmental plate projection; s.d., standard deviation

		Mean (μm)	s.d. (μm)	Minimum (μm)	Maximum (μm)	*N*
Body length		169.6	10.6	147.8	180.7	8
Scapular plate length		28.6	1.8	26.0	30.2	4
Buccal cirrus length	Int.	10.6	0.1	10.5	10.6	2
	Ext.	15.0	1.7	13.86	16.2	2
Papilla length		3.8				1
Clava length		5.6				1
Cirrus A length		69.2	6.9	64.3	74.1	2
IIIbis pr. length		5.3	1.9	4.0	7.5	3
Claws I length	Int.	7.2				1
	Ext.	7.0				1
Claws II–II length	Int.	7.9	0.7	7.4	8.4	2
	Ext.	7.6	0.8	7.0	8.2	2
Claws IV length	Int.	10.3	0.9	9.6	10.9	2
	Ext.	9.2	0.4	8.8	9.8	4

Etymology

This species is dedicated to the tardigradologist and friend Dr Ilaria Giovannini.

Table 5. Differential diagnostic characters among *Pseudechiniscus titianae*, sp. nov. and similar species
IIIbis = pseudosegmental plate. Cirri A: short, <1/3 of the body length; long, >1/3 of the body length

	Median plate 1	Median plate 2	IIIbis	No. of projections on IIIbis	Spurs on internal claws	Cirri A
P. titianae, sp. nov.	Divided	Divided	Divided	2	Present	Long
P. bartkei	Undivided	Undivided	Divided	1,2	Present	Short
P. quadrilobatus	Undivided	Undivided	Undivided	1	Absent	Short
P. spinerectus	Undivided	Divided	Divided	2	Present	Short
P. gullii	Divided	Undivided	Divided	1	Absent	Short
P. pilatoi	Undivided	Undivided	Divided	1	Absent	Short
P. yunnanensis	Undivided	Undivided	Undivided	2	Absent	Short
P. santomensis	Divided	Divided	Undivided	2	Present	Short

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http://zoobank.org/urn:lsid:zoobank.org:act:8BE41AE4-D353-4A11-A43A-9C6EF0CF0EBF

Material examined

Holotype. ♀, Antarctica, Dronning Maud Land, near the Novolazarevskaya Station (70°45.566′S, 11°46.899′E, 41 m a.s.l.), moss sample, Jan. 2002. Mounted on slide (C3623-S2-A) and deposited in the Bertolani collection (Department of Life Sciences, University of Modena and Reggio Emilia, Italy),

Paratypes. 8 paratypes (8 animals, ♀) mounted on slides. Five (C3623-S1-A,B,C; C3623-S2-B; C3624-S3-A) are deposited in the Bertolani collection (Department of Life Sciences, University of Modena and Reggio Emilia, Italy), two (C3623-S11-A,B) in the tardigrade slide collections of the Swedish Museum of Natural History (Stockholm, Sweden).

18S DNA sequence: GenBank KT226094

28S DNA sequence: GenBank KT226104

Diagnosis

Echiniscidae orange-red in colour, with pseudosegmental plate. Eyes present. Buccal cirri and clavae present. Cirri A long. No other lateral appendages in the trunk. First and second median plates transversally divided. Longitudinally divided pseudosegmental plate with two triangular projection in the caudal margin. Spurs on the internal claws present.

Description

Size range: 147.9–180.8 µm long. Orange-red in colour. Anterior eye spots present. Dorsal cuticle with evident cuticular plates (Fig. 6*A*, *B*). Head plate (H) with a W-shaped fold. Scapular plate (I) divided into an anterior and a posterior portion by a transverse fold; anterior portion divided in two by a longitudinal fold; posterior portion divided into four parts by three longitudinal folds. Median plates 1 and 2 divided in two portions by transverse fold (the posteriors the largest); median plate 3 undivided. Pseudosegmental plate (IIIbis) divided by a longitudinal fold, two triangular pointed projections (short spines) present in its posterior margin (Figs 6, 7*A*). Terminal plate (IV) not faceted but with two Y-shaped notches reaching the base of the pseudosegmental plate (Fig. 7). Ornamentation of the dorsal plates formed by small hemispherical projections (dots by LM) ~0.5 µm in diameter, densely and uniformly distributed, and joined by striae, forming a hexagonal net design. Observations with SEM show that these striae are formed by bundles of thin filaments positioned in the inner layer of the cuticle (Fig. 8*B*, *C*). Ventral ornamentation similar to the dorsal one but more delicate and made by small dots (Fig. 6*C*). Ornamented plate on the external side of the legs with no clear borders. Cephalic appendages and filaments A (cirri) present (Fig. 6*A*). Filaments A with a large basal part, cephalic papillae and clavae very well developed. Four short claws in each leg. Internal claws with a large and straight spur oriented towards the claw base. Small spine on first legs absent. A papilla present on hind legs. Dentate collar on hind legs absent.

Only females were found, the female has a gonopore with the typical six-petal rosette shape. Two-clawed larvae have not been found. Eggs unknown.

Mean, maximum and minimum values of the measurements of the cuticular structures of animals are reported in Table 4; measurements for every single specimen are provided in Table S2.

Differential diagnosis

Within the genus *Pseudechiniscus*, seven species have the following characters in common with *P. titianae*, sp. nov.: absence of lateral papillae, presence of projections at the posterior margin of the pseudosegmental plate, scapular plate with a transverse fold, terminal plate not faceted, and cuticular ornamentation comprised of dots joined by striae. These taxa are: *P. bartkei* Węglarska, 1962; *P. quadrilobatus* Iharos, 1969; *P. spinerectus* Pilato *et al.*, 2001; *P. gullii* Pilato & Lisi, 2006; *P. pilatoi* Li, 2007; *P. yunnanensis* Wang, 2009, and *P. santomensis* Fontoura *et al.*, 2010. The new species is clearly distinguishable from these species by a combination of characters listed in Table 5.

Etymology

This species is dedicated to the tardigradologist and friend Dr Tiziana Altiero.

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