European Journal of Taxonomy 958: 77–113
https://doi.org/10.5852/ejt.2024.958.2663
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2024 · Rocha A. *et al.*

Research article

urn:lsid:zoobank.org:pub:94D3C714-B808-436F-B84B-1398196B1E09

Integrative description of a new species of *Minibiotus* (Tardigrada: Macrobiotidae) from Salta City (Argentina)

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[6] urn:lsid:zoobank.org:author:92C61F57-2A95-40D2-8917-60660575F2E5
[7] urn:lsid:zoobank.org:author:416958C5-064F-4E10-BA23-DA81B63D7946
[8] urn:lsid:zoobank.org:author:1EE34EFB-1742-49A8-BC11-109B67E2BEA3

Abstract. The limno-terrestrial tardigrade fauna of Argentina has been investigated methodically and with modern criteria just in the last two decades, but current knowledge is still incomplete. So far, about 119 limno-terrestrial species are known for the country, of which only 6 belong to the genus *Minibiotus* R.O. Schuster, 1980. Until 1988, this genus was monotypic, with only *Minibiotus intermedius* (Plate, 1888), but today the number of species of the genus has risen to 55. In the present contribution, we describe with an integrated approach (PCM, SEM, morphometry and DNA analysis with COI, ITS2, 18S and 28S genes) a new species of *Minibiotus* from Salta City (Argentina). *Minibiotus dispositus* sp. nov. has ten transverse bands of variously shaped cuticular pores, arranged in transverse rows, with differences between smaller and larger specimens. Three macroplacoids and a microplacoid are present in the pharynx. The eggs have small conical processes and granulated chorion. The new species is morphologically and morphometrically well differentiated from all other species of the genus, and genetically from the up to date sequenced species. The new species description gave the occasion to broaden knowledge on taxonomy, morphology and faunistics of the genus *Minibiotus*, and on the tardigrade fauna of Argentina and the Neotropical region.

Keywords. Tardigrades, South America, taxonomy, morphology, DNA analysis.

Rocha A., Doma I., Camarda D., Ostertag B., Meier F., Frigieri F., Cesari M. & Lisi O. 2024. Integrative description of a new species of *Minibiotus* (Tardigrada: Macrobiotidae) from Salta City (Argentina). *European Journal of Taxonomy* 958: 77–113. https://doi.org/10.5852/ejt.2024.958.2663

Introduction

Tardigrada Doyère, 1840 is a phylum of microscopic animals (usually 250–600 µm in length) belonging to the superclade Ecdysozoa Aguinaldo *et al.* 1997, inhabiting terrestrial, freshwater and marine environments throughout the world (Nelson *et al.* 2015; Schill *et al.* 2018). To date, 1488 species have been formally described (Degma & Guidetti 2009–2024) and this number is systematically growing.

The tardigrade fauna of the Republic of Argentina has begun to be investigated continuously just over the last two decades; however, the current state of knowledge is very far from complete. Up to date, 119 limno-terrestrial species have been reported for the country (Rocha *et al.* 2023) of which only 6 belong to the genus *Minibiotus* R.O. Schuster, 1980, with *M. claxtonae* Rossi, Claps & Ardohain, 2009 and *M. pseudostellarus* Roszkowska, Stec, Ciobanu & Kaczmarek, 2016 currently considered endemic. This genus is characterized by the presence of an antero-ventral mouth with 10 peribuccal 'papulae' but without lamellae; a short, rigid, and narrow buccal tube usually with 2 bends; a relatively cephalic stylet support insertion point, and an extra thickening on the buccal tube wall immediately caudal to the stylet support insertion point (Claxton 1998; Michalczyk & Kaczmarek 2004). For one hundred years, most species of *Minibiotus* were hidden under the diagnosis of *Macrobiotus intermedius* Plate, 1888, or of *Minibiotus intermedius* after the genus institution (R.O. Schuster, 1980 in Schuster *et al.* 1980), and the genus remained monotypic until 1988, when *Minibiotus maculartus* Pilato & Claxton, 1988 was described. Additional species were later described, and Claxton (1998) published a revision of the genus *Minibiotus* redescribing the type species and describing many new species. Since then, very numerous new species have been described and several have also been transferred to *Minibiotus* from the genus *Macrobiotus* C.A.S. Schultze, 1834 based on the characters defined by Claxton (1998) and later supplemented by Guidetti *et al.* (2007) and Dueñas-Cedillo *et al.* (2021). Very recently, Kaczmarek *et al.* (2022) redescribed with the most modern criteria *M. intermedius* solving several past problems about this species and, consequently, the genus. Currently, the total number of species of *Minibiotus* has risen to 55 (Guidetti & Bertolani 2005; Degma & Guidetti 2007, 2009–2024).

The genus was recorded for the first time in Argentina by Iharos (1963) from Río Negro Province, with *M. intermedius*. Later, Mihelčič (1972) reported *M. acontistus* (de Barros, 1942) with no geographic indication but Claps & Rossi (1988) reported this species for Misiones Province; besides, Claps & Rossi (1981) reported *M. subintermedius* (Ramazzotti, 1962) for Neuquén and Chubut Provinces; Rossi *et al.* (2009) described *M. claxtonae* Rossi, Claps & Ardohain, 2009 for Neuquén Province; Claps *et al.* (2008) reported *M. furcatus* (Ehrenberg, 1859) for Buenos Aires and Tierra del Fuego Provinces and, more recently, *M. pseudostellarus* Roszkowska, Stec, Ciobanu & Kaczmarek, 2016 was described for Río Negro Province.

In the present paper, we broaden the knowledge on Argentinian tardigrades describing a new species of *Minibiotus* from Salta Province, from which the genus was up to date unreported.

Material and methods

The present contribution is part of a tardigradological collection revision work of the Rocha and Doma collection of the Department of Natural Sciences at the National University of La Pampa, Argentina (deposited within 2014 and 2017). Having found the new species (at first identified only morphologically through PCM and morphometry), we carried out resampling in order to obtain new fresh specimens for SEM and molecular analyses.

The former sampling had taken place in May of 2014 in Salta Province, Salta City, 24°47′18″ S, 65°24′38″ W, and one sample was collected, producing 49 specimens (of various body sizes) and 6 eggs all mounted on microscopy slides (slide Nos. in "Material Examined") and deposited in the collection as mentioned above. Resampling took place in June 2022, collecting one sample perfectly corresponding to that of 2014 (same geographic coordinates, tree and position on it, substrate type); this produced only 13 big, dead specimens (no young and no eggs), of which 2 were mounted on a microscopy slide, 10 prepared for SEM analysis, and 1 for DNA analysis.

In both cases, the material was extracted from an undetermined lichen and moss sample growing on a sidewalk tree (*Handroanthus* Mattos); this was about 5 m tall, collecting the sample at chest height on the trunk (1.3 m), on the trunk surface facing the street. The samples were stored in paper bags at room temperature. For processing, they were hydrated for 24 hours each in a plastic sieve (1.1 mm mesh) placed in Petri dishes filled with mineral water. Tardigrades and eggs were sorted using a stereoscopic microscope and extracted with a micropipette.

The material intended for light microscopy studies was mounted on microscopic slides with polyvinyl-lactophenol medium.

Tardigrades were mainly identified using a Leica DM500 phase contrast microscope, equipped with a ICC 50 HD digital camera; observations were made also with a Zeiss Axio Scope A1 Differential Interference Contrast (DIC) microscope equipped with a Zeiss AxioCam ICc5CCD digital camera. Specimens prepared for Scanning Electron Microscope (SEM) were processed according to Camarda *et al.* (2023). The specimens were examined using a Zeiss Crossbeam 340, column Gemini 1 Scanning Electron Microscope in the Institute of Nanoscience and Nanotechnology Bariloche node (CNEA/CONICET), Bariloche, Argentina.

Morphometric data were obtained using AxioVision SE64 software, given in micrometers (μm). Structures were measured only if their orientation was appropriate/suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. Buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). Buccal tube width was measured as the external diameter at the level of the stylet support insertion point. Lengths of the claw branches were measured from the base of the claw to the top of the branch including accessory points. The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981). Macroplacoid length sequence is given according to Kaczmarek *et al.* (2014). Morphometric data were handled using the "Parachela" ver. 1.7 template available from the Tardigrada Register (Michalczyk & Kaczmarek 2013). Tardigrade taxonomy follows Bertolani *et al.* (2014) and Stec *et al.* (2020b). Student *t*-tests (one-side tests) for statistical significance of differences between species morphometry (only when ranges of the given characters of the two compared species overlapped) were performed through Microsoft Office Excel software and the results are reported in

Table 9 (relative to the various differential diagnoses); when ranges did not overlap, they are reported directly in the text of the differential diagnoses.

For species identification and differentiation, dichotomous keys reported in Claxton (1998) and papers regarding descriptions and redescriptions of species (Ehrenberg 1859; Ramazzotti 1962; Horning *et al.* 1978; Dastych 1988, 1990; Binda & Pilato 1992; Claxton 1998; Michalczyk & Kaczmarek 2004; Michalczyk *et al.* 2005; Miller *et al.* 2005; Fontoura *et al.* 2009a, 2009b; Meyer & Hinton 2009; Meyer *et al.* 2011; Dueñas-Cedillo *et al.* 2020, 2021; Kaczmarek *et al.* 2022) were used.

For comparison with our material, the following type specimens from the Pilato and Binda Collection (Museum of the Section of Animal Biology, Department of Biological, Geological and Environmental Sciences, University of Catania) were examined: paratypes of *Minibiotus eichhorni* Michalczyk & Kaczmarek, 2004 (slide No. 5403), holotype and paratypes of *Minibiotus sidereus* Pilato, Binda & Lisi, 2003 (slides Nos. 4925, 6018, 6021, 6022), one egg of the type series of *Minibiotus ethelae* (slide No. 4211). Slides from the Zoological Museum of the Jagiellonian University with paratypes of *Minibiotus constellatus* Michalczyk & Kaczmarek, 2003 (slides Nos. Peru–17, Peru–18, Peru–19) were also examined.

Culturing was not possible, preventing exact determination of life stages, since we worked with already mounted slides (first sampling), or with dead specimens (resampling). In the latter case, only big specimens were found, also preventing having SEM material of young specimens and eggs, or a genetic analysis of young specimens. We were only able, depending on body size, cuticular pore pattern and metric characters, to distinguish between smaller specimens (called ‘young’) and larger (called ‘senior’ specimens).

Total genomic DNA of the analysed senior specimen was extracted with the QuickExtract™ DNA Extraction Solution (Lucigen, Middleton, WI, USA) following the manufacturer’s protocol. The animal was previously observed in vivo up with LM up to 100 × oil immersion magnification to avoid mistakes in determining the morphology and then photographed, using the method described by Cesari *et al.* (2011) in order to obtain the voucher specimen.

Molecular investigations were carried out using fragments of the mitochondrial cytochrome oxidase 1 (COI) gene, the nuclear internal transcribed spacer ITS2, the small ribosome subunit (18S rRNA) and the large ribosome subunit (28S rRNA) using the primers and protocols described by Cesari *et al.* (2009), Stec *et al.* (2018), Bertolani *et al.* (2014), and Guidetti *et al.* (2014), respectively. The amplified products were gel purified using the Wizard Gel and PCR Cleaning (Promega) kit, while sequencing reactions were performed using the ABIPRISM® BigDye™ Terminator ver. 1.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on purified amplicons. Each sequencing reaction contained 0.2 μM of a single PCR primer to initiate the sequencing reaction, 2 μL of BigDye™, 70 ng of purified products, 4 μL of 5 × BigDye™ Terminator ver. 1.1 Sequencing Buffer (Applied Biosystems, Foster City, CA, USA) and bi-distilled H2O for a final volume of 20 μL. Cycling conditions for sequencing reactions consisted of 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min.

Both strands were sequenced using an ABI Prism 3100 (Applied Biosystems, Foster City, CA, USA) at UNIMORE. Chromatograms obtained and nucleotide sequences were checked by visual inspection and the sequences were aligned with the MUSCLE algorithm. In order to perform proper molecular comparisons, we included sequences from GenBank of other specimens of *Minibiotus* in our analysis (Table 1). Pairwise nucleotide sequence divergences between scored haplotypes were calculated by using MEGA 11 (Tamura *et al.*, 2021). The distance-based ASAP species delimitation analysis was performed on COI gene (Table 7) and ITS2 (Table 8) on the ASAP website (https://bioinfo.mnhn.fr/abi/public/asap/, accessed on 29 Feb. 2024, Puillandre *et al.*, 2021). Nucleotide sequences of the newly analyzed specimen

Table 1 (continued on next page). Sequences from GenBank of specimens of *Minibiotus* R.O. Schuster, 1980 used for comparison in the molecular analysis for all analyzed genes.

		GenBank accession no.				
Taxon	**Locality**	**COI**	**ITS2**	**18S**	**28S**	**References**
Minibiotus gumersindoi	Madrid, Spain	FJ435803		FJ435748	FJ435761	Guil & Giribet 2012
Minibiotus furcatus	Madrid, Spain	FJ435802		FJ435746		Guil & Giribet 2012
Minibiotus furcatus	Portugal	JX683828				Vicente *et al.* 2013
Minibiotus furcatus	Portugal	JX683829				Vicente *et al.* 2013
Minibiotus furcatus	Madrid, Spain			FJ435747		Guil & Giribet 2012
Minibiotus furcatus	Madrid, Spain			FJ435745		Guil & Giribet 2012
Minibiotus furcatus	Gaianello, Italy			HQ604977		Bertolani *et al.* 2014
Minibiotus furcatus	Gaianello, Italy			HQ604978		Bertolani *et al.* 2014
Minibiotus ioculator	South Africa	MT023412	MT024000	MT023998		Stec *et al.* 2020a
Minibiotus pentannulatus	Tanzania	MT023413	MT024001	MT023999		Stec *et al.* 2020a
Minibiotus pentannulatus	Tanzania	MT023414				Stec *et al.* 2020a
Minibiotus intermedius	Marburg, Germany	ON005160				Kaczmarek *et al.* 2022
Minibiotus intermedius	Alaska, USA			JX888504		Adams *et al.* (unpublished)
Minibiotus intermedius	Alaska, USA			JX888505		Adams *et al.* (unpublished)
Minibiotus cf. *intermedius*	Lembolovo Russia	OP013287	OP035707	OP035718		Tumanov *et al.* 2022
Minibiotus cf. *intermedius*	Lembolovo Russia	OP013286	OP035708	OP035719		Tumanov *et al.* 2022
Minibiotus cf. *intermedius*	Lembolovo Russia	OP013288				Tumanov *et al.* 2022
Minibiotus gr. *intermedius*	Andalo, Italy			HQ604979		Bertolani *et al.* 2014
Minibiotus gr. *intermedius*	Andalo, Italy			HQ604980		Bertolani *et al.* 2014
Minibiotus citlalium	Iztaccihuatl, Mexico	OP684765	OP696660			Amezcua-Martinez *et al.* (unpublished)
Minibiotus citlalium	Iztaccihuatl, Mexico	OP684766	OP696661			Amezcua-Martinez *et al.* (unpublished)
Minibiotus citlalium	Iztaccihuatl, Mexico	OP684767	OP696662			Amezcua-Martinez *et al.* (unpublished)
Minibiotus sidereus	Iztaccihuatl, Mexico	OP684768	OP696663			Amezcua-Martinez *et al.* (unpublished)
Minibiotus sidereus	Iztaccihuatl, Mexico	OP684769	OP696664			Amezcua-Martinez *et al.* (unpublished)

Table 1 (continued on next page). Sequences from GenBank of specimens of *Minibiotus* R.O. Schuster, 1980 used for comparison in the molecular analysis for all analyzed genes.

		GenBank accession no.				
Taxon	**Locality**	**COI**	**ITS2**	**18S**	**28S**	**References**
Minibiotus sidereus	Iztaccihuatl, Mexico	OP684770	OP696665			Amezcua-Martinez *et al.* (unpublished)
Minibiotus sp.	Iztaccihuatl, Mexico	OP684773	OP696668			Amezcua-Martinez *et al.* (unpublished)
Minibiotus sp	Iztaccihuatl, Mexico	OP684772	OP696667			Amezcua-Martinez *et al.* (unpublished)
Minibiotus sp	Iztaccihuatl, Mexico	OP684771	OP696666			Amezcua-Martinez *et al.* (unpublished)
Minibiotus sp.	Chile				MH079492	Guil *et al.* 2019
Minibiotus sp.	Dronning Maud Land, Antarctica	JX865309				Czechowski *et al.* 2012
Minibiotus sp.	Dronning Maud Land, Antarctica	JX865313		EU266932		Czechowski *et al.* 2012; Sands *et al.* 2008
Minibiotus sp.				EU266933		Sands *et al.* 2008
Minibiotus sp.				EU266934		Sands *et al.* 2008
Minibiotus sp.	Tuscan-Emilian Apennine National Park, Italy	MW306857				Vecchi *et al.* 2022a
Minibiotus sp.	Tuscan-Emilian Apennine National Park, Italy	MW306858				Vecchi *et al.* 2022a
Minibiotus sp.	Tuscan-Emilian Apennine National Park, Italy	MW306859		OK663227		Vecchi *et al.* 2022a, 2022b
Minibiotus sp.	Corniglio, Italy		OK663216			Vecchi *et al.* 2022b

were submitted to GenBank (accession numbers: PP937153 for COI gene; PP938064 for the ITS2 gene; PP938063 for the 18S; PP938062 for the 28S).

Institutional acronyms

Specimens from the following institutions and collections were examined (curator in parentheses).

MCNS = Museum of Natural Sciences, National University of Salta, Argentina (Ivanna Cruz)
UNICT = Università degli Studi di Catania, Italy, Museum of the Department of Animal Biology 'Marcello La Greca', Italy, Binda and Pilato collection (Giovanni Pilato and Oscar Lisi)
UNLPam = National University of La Pampa, Faculty of Exact and Natural Sciences, Argentina, (Rocha Alejandra Mariana)

Results

Taxonomic account

Phylum Tardigrada Doyère, 1840
Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Superfamily Macrobiotoidea Thulin, 1928 in Marley *et al.* 2011
Family Macrobiotidae Thulin, 1928
Genus *Minibiotus* R.O. Schuster, 1980 in Schuster *et al.* 1980

Minibiotus dispositus sp. nov.
urn:lsid:zoobank.org:act:780E50E1-48E8-474C-8935-070FCD6B09AE
Figs 1–8; Tables 2–6; Supp. files 1–3

Diagnosis

Minibiotus with smooth cuticle but with cuticular pores variously sized (0.9–3.1 µm) and shaped; under SEM, most pores are polygonal or multilobate (3–5 angles/lobes/arms); under PCM, pentagonal pores often appear round, and 5-lobate are rarely observable, only caudal or on legs. Dorsal pores arranged in a group of very cephalic and a group of very caudal pores, with in between a series of transverse bands; young specimens with 8 bands of 1–2 rows; senior specimens with 7 bands of about 2–5 less regular rows (band 8 joined to the very caudal pores). Ventral pores arranged in 7 transverse bands, starting posterior to legs I, of a single row each, but partially duplicated medially in senior specimens. Bucco-pharyngeal apparatus typical for the genus; oral cavity armature with three bands of teeth, better visible under SEM, with band I reduced; three macroplacoids (length sequence $3 \leq 2 < 1$) and an evident microplacoid in the pharynx. Robust double claws with short, robust accessory points and small, smooth lunules. Faint leg 'cuticular bars', divided on legs I–III, undivided on legs IV; no leg granulation, pulvini present on legs I–III. Moderate allometry regarding buccal tube width, macroplacoid and claw length.

Etymology

From the Latin word '*dispositus*' = 'ordered', in the meaning of 'with a pattern', referred to the cuticular pores forming a pattern.

Material examined

In total, 51 animals (undetermined sex; 31 senior and 20 young specimens) and 6 eggs mounted on microscope slides in Polivinil lactophenol medium; 10 additional specimens mounted on SEM stubs; one specimen (voucher) used for DNA analysis.

Holotype

ARGENTINA • senior spec.; Salta Province, Salta City; 24°47′18″ S, 65°24′38″ W; 1150 m a.s.l.; 2 May 2014; Rocha and Doma leg.; moss and lichen growing on *Handroanthus* Mattos; UNLPam 1088(3).

Paratypes

ARGENTINA • 2 senior specs; same data as for holotype; 2 May 2014; MCNS Tar. 000026(1), Tar. 000026(4) • 1 egg; same data as for holotype; 2 May 2014; MCNS Tar. 000027(1) • 2 senior specs; same data as for holotype; 5 Jun. 2022; UNICT 6010, 6011 • 1 young spec.; same data as for holotype; 2 May 2014; UNICT 6012 • 1 egg; same data as for holotype; 2 May 2014; UNICT 6013 • 26 senior specs; same data as for holotype; 2 May 2014; UNLPam 643(3), 654(1), 655(1), 655(2), 656(1), 659(1), 659(2), 1037(4), 1038(2), 1042(2), 1049(1) to 1049(3), 1050(2), 1050(3), 1056(2) to 1056(4), 1085(1), 1085(3), 1085(4), 1087(3), 1088(4), 1089(3), 1090(1), 1090(2) • 19 young specs; same data as for holotype; 2 May 2014; UNLPam 1033(1), 1034(4), 1035(1), 1035(2), 1036(2), 1038(4), 1040(3),

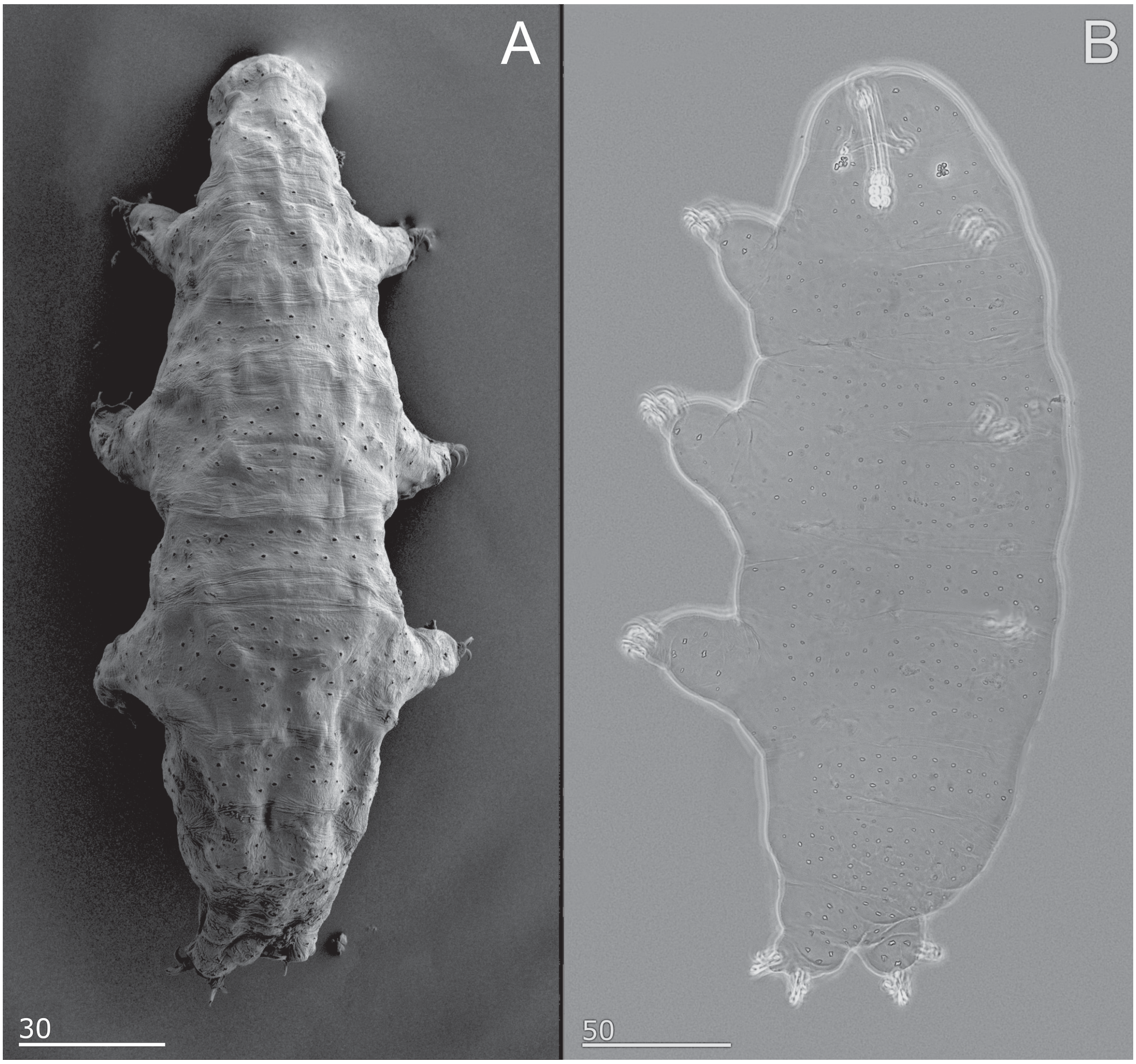

Fig. 1. *Minibiotus dispositus* sp. nov., habitus. **A**. Paratype (UNLPam), SEM. **B**. Paratype (slide No. UNICT 6011), PCM. Scale bars in µm.

1041(1), 1041(3), 1044(3), 1046(1) to 1046(4), 1047(1) to 1047(3), 1062(2), 1063(4) • 4 eggs; same data as for holotype; 2 May 2014; UNLPam 348(1), 348(2), 644(1), 1222(3).

General morphological description of the animals

Body length 97–342 µm (Fig. 1; Tables 3, 5), yellowish before mounting, transparent after mounting. Eyespots, small and very caudal, present (Fig. 1B).

Smooth cuticle with pores of different shapes. Under PCM, apparently, the smaller pores (around 1 µm) are usually roundish (though often with irregular margins), or few elliptical, while the larger typically triangular, quadrangular, trilobate or quadrilobate (Fig. 2); the pore size is 0.9–3.1 µm on the dorsum (with the biggest on the head, on the mid-dorsal line along the body, and on the caudal extremity), 1.3–3.4 µm on the legs, 1.0–1.8 µm on the ventral cuticle. Coherently, dorsal pores, on average bigger, appear more often non-roundish, while the ventral ones on average smaller, appear more often roundish. Exceptionally, some caudal or leg pore may appear pentagonal- or, extremely rarely, star-shaped (with five lobes/arms; Fig. 2) under PCM, but this occurs only in few specimens and in only 1–2 pores in each of these few specimens.

SEM reveals that pores are actually never perfectly rounded; instead, few small pores are truly elliptical, a minority (of any size) is irregular, while the rest are all (both dorsal and ventral, smaller and larger) polygonal or multilobate, from three to five angles/lobes/arms (Fig. 2); pores in an unsuitable position, and/or too small, may give the false impression to be irregularly roundish under SEM also, and, obviously, the lower magnification of PCM gives the impression of seeing a more common shape, especially where pores are smaller.

There is no clear distinction between polygonal and multilobate pores, since there are many intermediate shapes (e.g., between triangular and three-lobated/armed, or between quadrangular and four-lobated/armed), and, very probably, each pore may partially appear more polygonal or multilobate depending on the cuticle distention or contraction. Pentagonal pores are less common but more easily detectable under SEM with respect to PCM (several of them can be seen on each specimen), while properly star-shaped (with five arms/lobes) are quite rare: some of the specimens mounted for SEM apparently lack them, while the others may show one or few of them (Fig. 2).

Pores are arranged in transverse bands made of one or more transverse rows (more regular in young specimens). The cuticle along the body, as visible in most eutardigrades, forms transverse folds marking the division into the five body segments (head plus four segments of the trunk), and, additionally, each segment is subdivided into 2 ‘subsegments’, one more anterior, and one more posterior, by an additional transverse cuticular fold. In this way, there are ten cuticular subsegments of the body clearly followed by the dorsal (Tables 2, 4), and partially ventral, pore arrangement.

Smaller (97–156 µm, called young) and larger (180–342 µm, called senior) specimens show differences in the pore number and arrangement on each subsegment, but we concluded they must belong to the same species (additionally to having been found in the same sample, first sampling) for the following reasons: 1) only one egg type was found; 2) most morphological characters were the same; 3) regarding the more detailed characters for which they differed, the two groups had clear body size distinction (97–156 µm vs 180–342 µm) with no exceptions; 4) the main difference regarded the pore number and pattern, but this was consistent with ontogenetic changes documented in other species (e.g., *Minibiotus pentannulatus* Londoño, Daza, Lisi & Quiroga, 2017), and the pattern of the bigger specimens was still perfectly comparable with that of the smaller, just more complicated due to the appearance of more pores; 5) the other differences were metric, but they all appeared consistent with allometric growing (buccal tube becoming wider, and placoids and claws becoming longer) already known in eutardigrades,

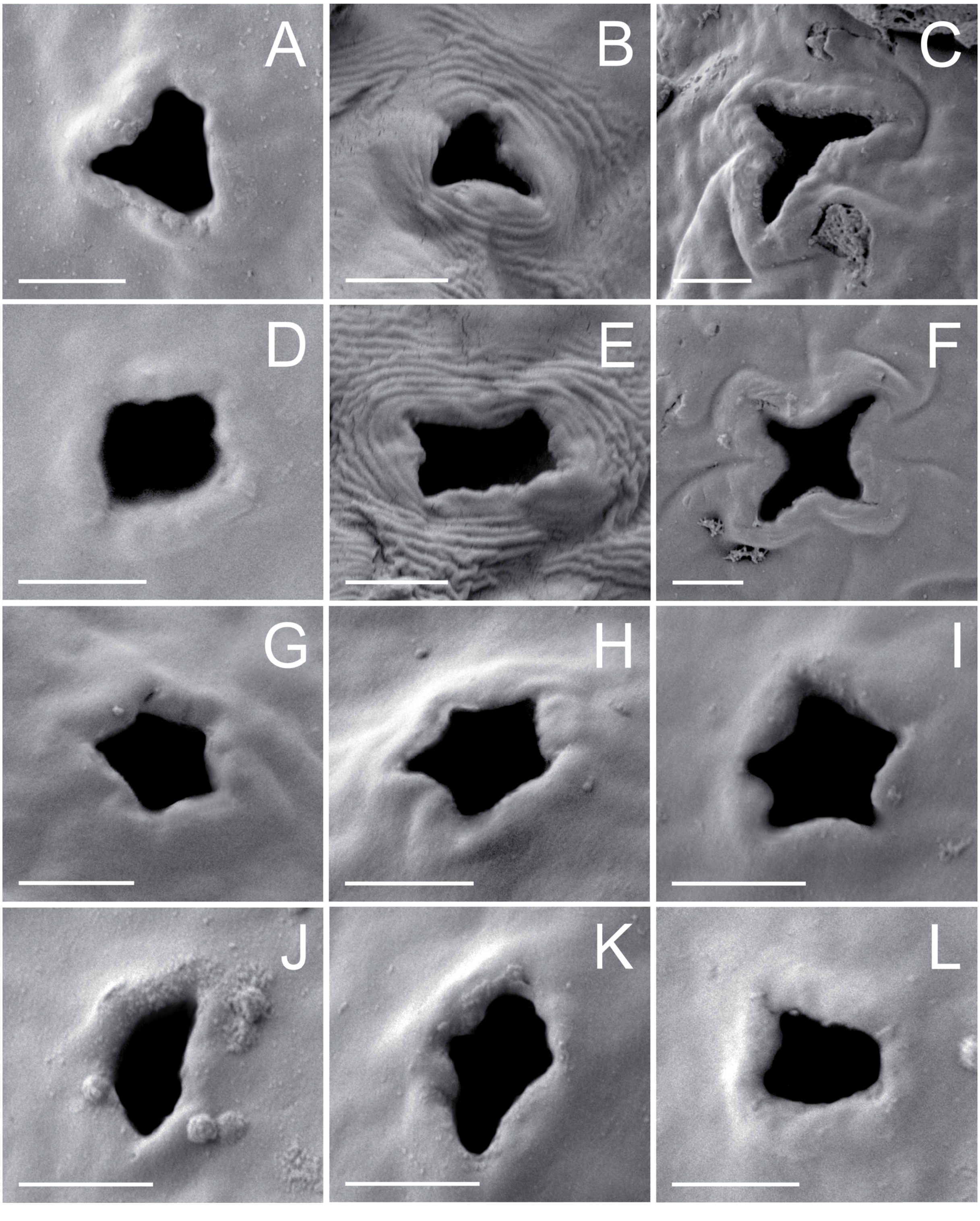

Fig. 2. *Minibiotus dispositus* sp. nov. Paratypes (UNLPam) under SEM. Type and morphological variation of pores on cuticle. **A–C**. Cuticular pores of triangular/trilobate shape. **D–F**. Quadrangular/quadrilobate. **G–I**. Pentagonal/star–shaped (with 5 lobes/arms). **J–L**. Irregular. Scale bars = 1 µm.

Table 2. Schematization of the dorsal pore pattern of young specimens of *Minibiotus dispositus* sp. nov. with reference to the precise body districts.

Body regions	Body segments	Subsegments and legs	Number of rows	Number of pores	Pore shape
Head	Head	1	4	2 4 2 4	triangular quadrilobated triangular triangular
		2	2 plus one very small pore lateral to each eye	4 6	various
Trunk	Second segment (segment I of the trunk), with legs I	3 (with legs I)	2 plus lateral circle	4/6 lateral circle of 5 4/6	various
		Legs I		1 big	3 – armed
		4	1	10 – 12 with 2 medio – lateral pores aligned longitudinally on each side	various
	Third segment (segment II of the trunk), with legs II	5 (with legs II)	2 plus lateral circle	4/6 lateral circle of 5 4/6	various
		Legs II		1 big	3 – armed
		6	1	12 with 2 medio – lateral pores aligned longitudinally on each side	
	Fourth segment (segment III of the trunk), with legs III	7 (with legs III)	2 plus lateral circle	4/6 lateral circle of 5 4/6	various
		Legs III		1 big anterior some caudal smaller	4–armed various
		8	1	10 – 12 with 2 medio – lateral pores aligned longitudinally on each side	various
	Fifth segment (segment IV of the trunk), with legs IV	9	1	10 – 12 with 2 medio – lateral pores aligned longitudinally on each side	various
		10 (with legs IV)		5 geometrically arranged: 3 medial, big, forming a triangle, 2 lateral small	roundish elongated
		Legs IV		3 forming a triangle	various

especially macrobiotids. Besides, specimens from resampling (only large individuals) matched perfectly the morphology and the morphometry of the large animals obtained from the first sampling.

As already mentioned, noticeable differences in the pore number and arrangement on each subsegment can be seen between young and senior specimens; for this reason, their pattern is indicated separately in the subsequent paragraphs with the pertinent tables and illustrations. It must be stressed that the determination of bands, rows and pore number and shape, was obtained through the observation of all specimens of each group (i.e., young and senior specimens); the bands of pores and the average pattern were always recognisable, but the precise number, disposition and shape of the pores composing the rows, or other patterns, had some degree of variability (especially in senior specimens); for this reason, descriptions and illustrations (Figs 6–7) are partially schematic trying to take into account the average situation of most specimens and have to be intended more as a tendency than an exact, constant reality.

Mouth antero-ventral; peribuccal papulae present (Fig. 3C–E, SEM), very probably corresponding to reduced lamellae (see Stec *et al.* 2020a). Oral cavity armature (Fig. 3) with three bands of teeth. The first band, very reduced and visible only under SEM, is located at the basal zone of peribuccal papulae

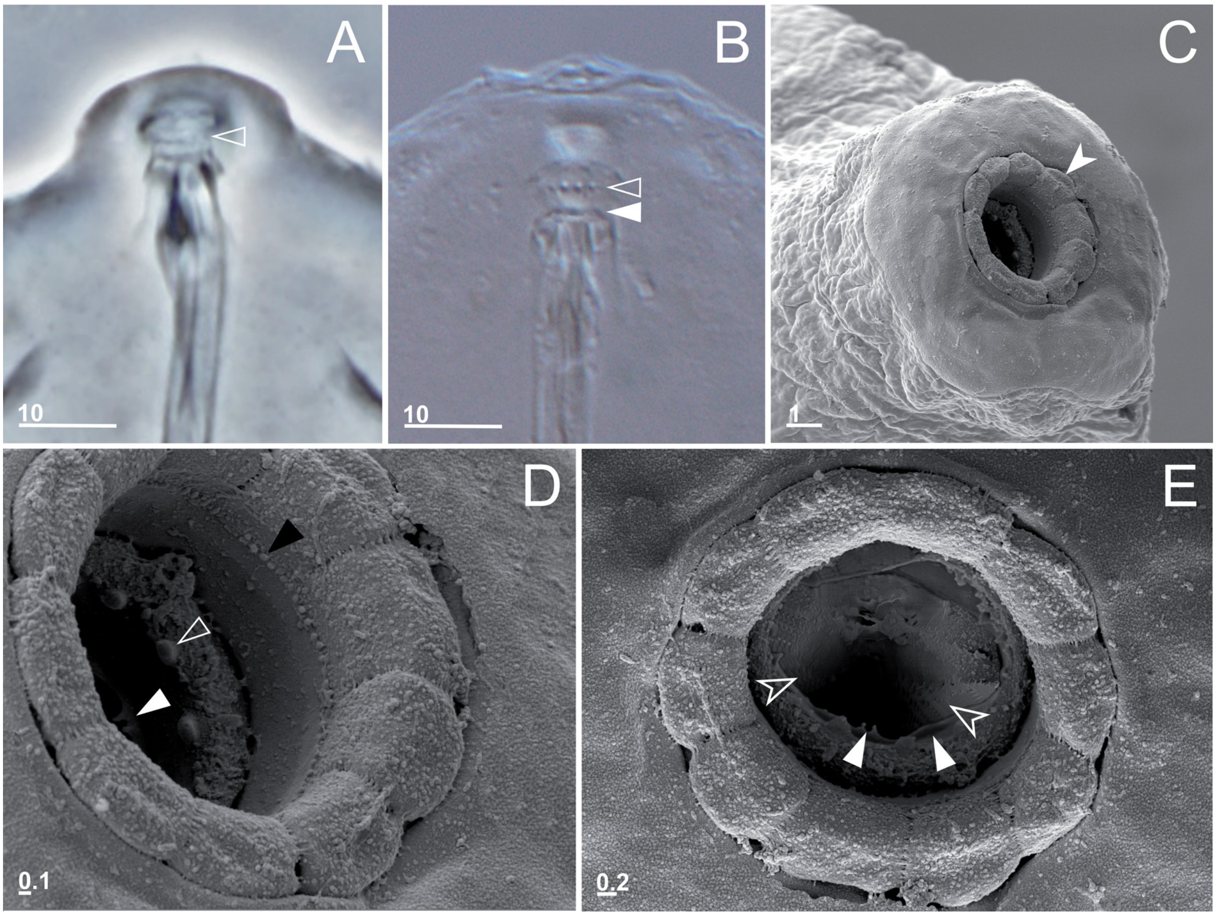

Fig. 3. *Minibiotus dispositus* sp. nov. Oral cavity armature. **A**. Paratype (UNLPam 1089 (3)), ventral lateral view seen in PCM. **B**. Paratype (MCNS Tar. 000026(1)), ventral view seen in DIC. **C–E**. Paratype (UNLPam), SEM from different angles. Black filled flat arrowhead indicates teeth of the first band, white empty flat arrowheads indicate teeth of the second band whereas white filled flat arrowheads indicate the third band of teeth. White indented filled arrowhead indicates peribuccal papulae. White indented empty arrowheads indicate cribrous areas. Scale bars in μm.

and composed of a single row of small cone-shaped teeth fused to form a continuous ring ridge around the oral cavity (Fig. 3D). The second band of teeth comprises one row of rather large, globular-shaped, separate teeth (Fig. 3D), partially visible under light microscopy (Fig. 3A–B PCM and DIC), depending on the animal size and quality of the preparation: it may be also invisible or appearing as an irregular line since teeth are not distinguishable from one another; this second band of teeth is instead very obvious under SEM (Fig. 3D–E). Third band of teeth (buccal crests) difficult to see under light microscopy; under SEM, in our specimens it was possible to see only the dorsal portion, made of three little-protruding ridges, with two lateral, and one medial bearing two sharpened teeth (Fig. 3D–E); ventral portion not visible under SEM in our specimens, but supposedly more developed than the dorsal one, since slightly better visible under DIC, showing two lateral crests and a median tooth (Fig. 3B).

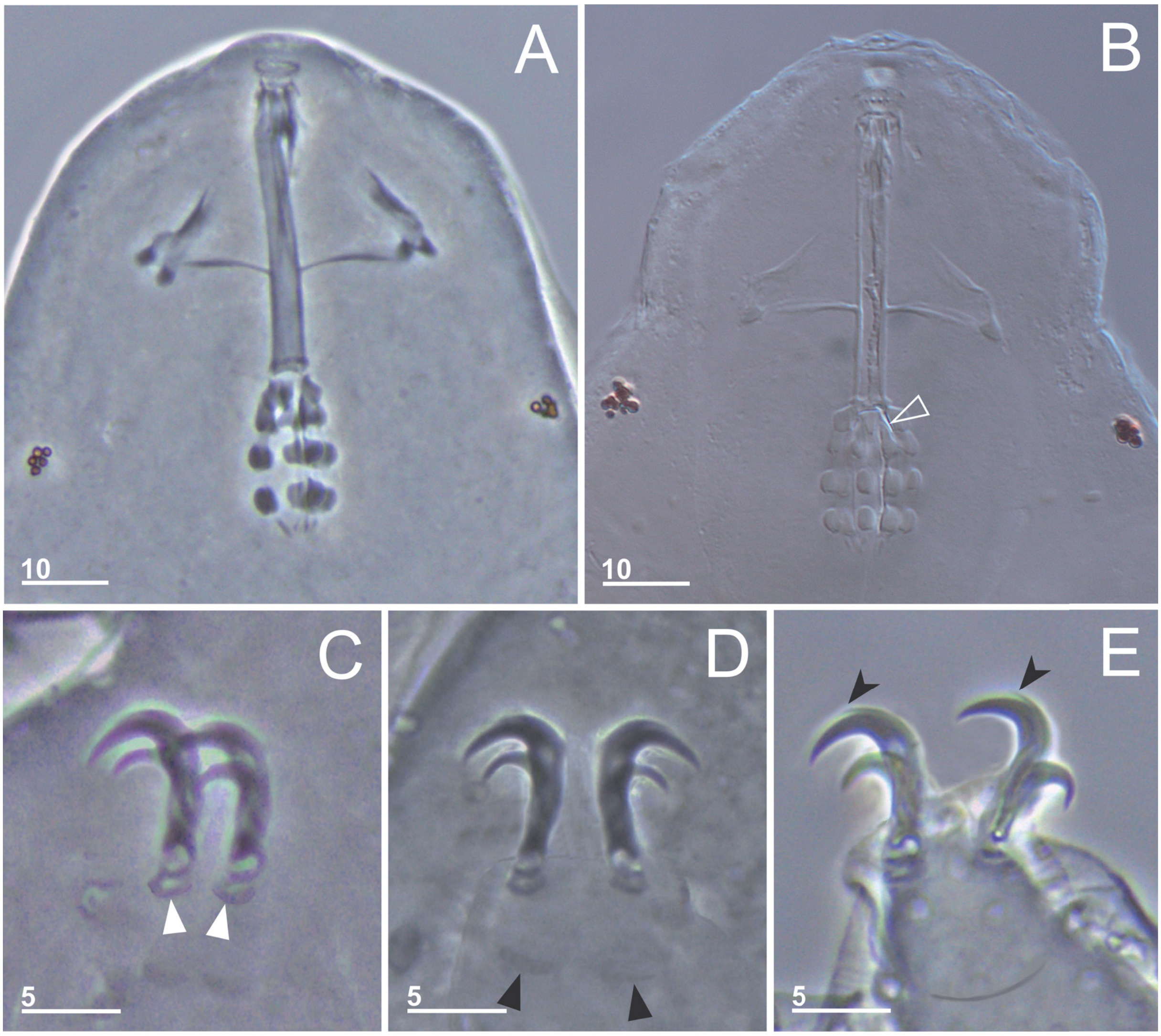

Fig. 4. *Minibiotus dispositus* sp. nov. Bucco-pharyngeal apparatus and claws. **A, C–E**. Holotype (UNLPam 1088(3)). **B**. Paratype (MCNS Tar. 000026(1)). **A**. Ventral view of the entire apparatus seen in PCM. **B**. Ventral view seen in DIC. Claws seen in PCM. **C**. Claws of legs I. **D**. Claws of legs II. **E**. Claws of legs IV. White empty flat arrowhead indicates first macroplacoid, white filled flat arrowheads indicate lunulae, black filled flat arrowheads indicate muscle attachment, black filled indented arrowheads indicate accessory points. Scale bars in µm.

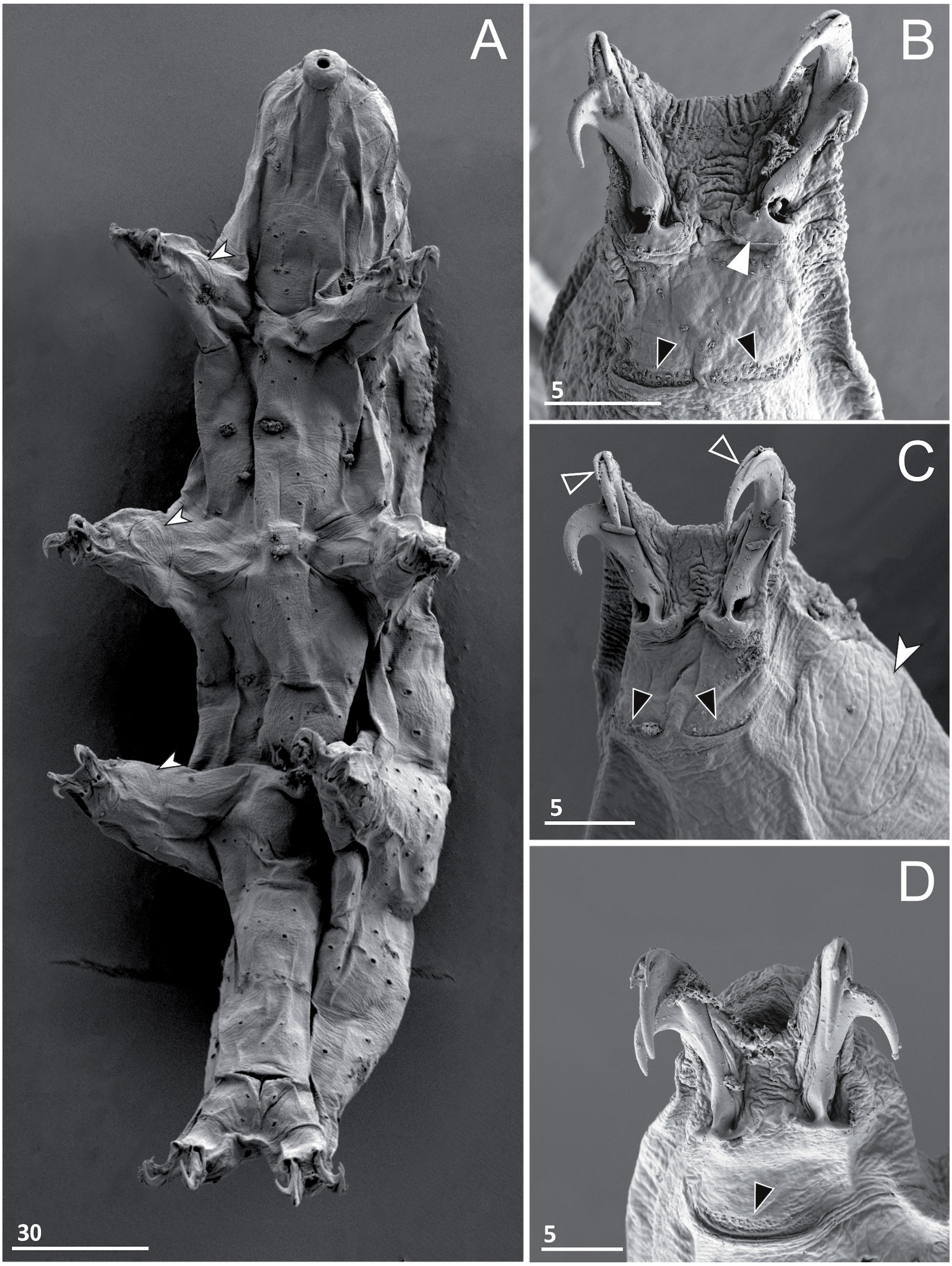

Fig. 5. *Minibiotus dispositus* sp. nov. Paratype (UNLPam). Ventral view and claws seen in SEM. **A**. Ventral cuticle. **B**. Claws of legs I. **C**. Claws of legs III. **D**. Claws of legs IV. White filled indented arrowheads indicate pulvini, white filled flat arrowhead indicates a lunula, black filled flat arrowheads indicate double muscle attachment under the claws I–III and single continuous muscle attachment under claw IV, white empty arrowheads indicate accessory points. Scale bars in µm.

Bucco-pharyngeal apparatus of the *Minibiotus* type (Fig. 4A–B). Buccal tube rigid with anterior and posterior dorsal bends; well-developed ventral lamina. Buccal tube wall with cribrous areas, at least anteriorly (Fig. 3E). Pharyngeal bulb oval, with triangular apophyses, three granular macroplacoids and a small microplacoid. The macroplacoid length sequence is $3 \leq 2 < 1$. The first macroplacoid tapering anteriorly (Fig. 4B). All macroplacoids without constrictions. Robust double claws with short and robust accessory points and small, smooth lunules (Figs 4C–E, 5). Faint leg cuticular bars (i.e., muscular attaches), divided on legs I–III, undivided on legs IV, but not visible on all specimens under PCM (Fig. 4C–E); clearly visible under SEM (Fig. 5). No granulation on the legs, pulvini present on legs I–III (Fig. 5A, C).

Young specimens (Fig. 6; Tables 2–3; Supp. file 1)
Arrangement of dorsal and dorso-lateral pores (Fig. 6; Table 2): the smaller specimens have a group of cephalic pores (in subsegment 1) forming a pattern but not exactly 'rows'; from subsegment 2 to 9 pores

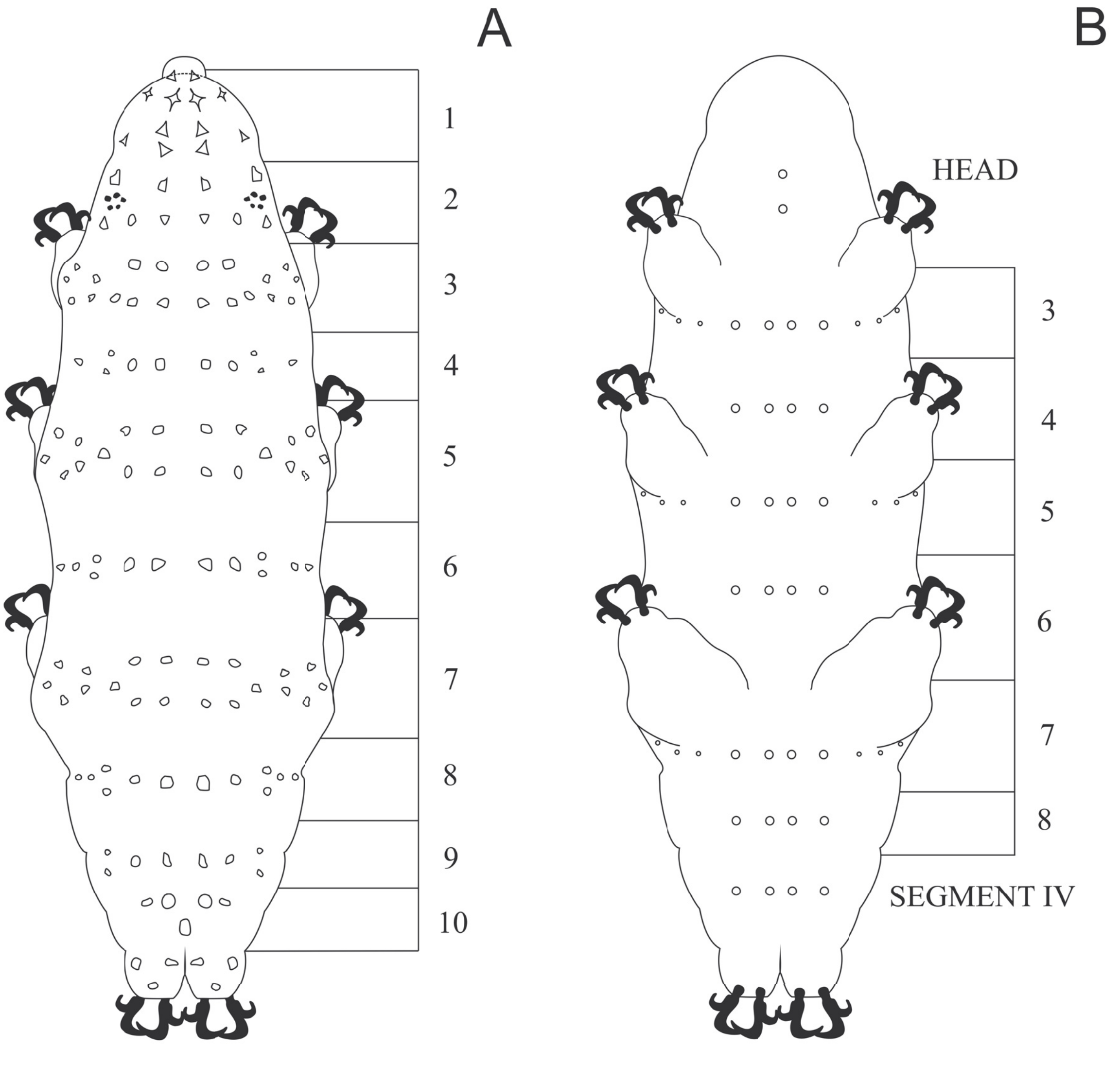

Fig. 6. *Minibiotus dispositus* sp. nov. Schematic drawing of the arrangement of the pores of the young specimens. **A**. Dorsal cuticle. **B**. Ventral cuticle.

Table 3. Measurements (in µm) and *pt* values of selected morphological structures of the young paratypes of *Minibiotus dispositus* sp. nov. 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**	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	19	97 – 156	*433 – 705*	126	*550*	17	*71*
Buccopharyngeal tube							
Buccal tube length	19	21.5 – 25.0	–	22.9	–	0.8	–
Stylet support insertion point	17	13.7 – 16.8	*62.9 – 72.9*	15.1	*66.3*	0.9	*2.1*
Buccal tube external width	19	1.4 – 2.1	*6.4 – 8.7*	1.8	*7.9*	0.2	*0.6*
Buccal tube internal width	19	0.9 – 1.2	*3.8 – 5.2*	1.0	*4.5*	0.1	*0.4*
Ventral lamina length	10	9.9 – 11.4	*45.4 – 49.5*	10.7	*47.0*	0.5	*1.6*
Placoid lengths							
Macroplacoid 1	18	1.9 – 2.3	*8.0 – 9.7*	2.0	*8.8*	0.1	*0.5*
Macroplacoid 2	18	1.6 – 2.0	*7.2 – 8.5*	1.8	*7.8*	0.1	*0.4*
Macroplacoid 3	18	1.6 – 1.9	*6.8 – 8.0*	1.7	*7.5*	0.1	*0.3*
Microplacoid	16	0.8 – 1.0	*3.2 – 4.2*	0.8	*3.7*	0.1	*0.3*
Macroplacoid row	18	5.9 – 7.3	*26.0 – 30.9*	6.3	*27.4*	0.4	*1.1*
Placoid row	16	7.0 – 8.6	*30.1 – 36.3*	7.3	*32.2*	0.4	*1.3*
Claw 1 heights							
External primary branch	13	6.5 – 7.3	*28.1 – 31.5*	6.8	*30.0*	0.2	*1.0*
External secondary branch	5	4.3 – 5.3	*18.7 – 22.4*	4.7	*20.2*	0.4	*1.5*
Internal primary branch	10	6.3 – 7.1	*27.6 – 31.1*	6.7	*29.2*	0.3	*1.2*
Internal secondary branch	6	4.0 – 5.0	*17.1 – 21.3*	4.2	*18.4*	0.4	*1.5*
Claw 2 heights							
External primary branch	11	6.7 – 7.5	*27.8 – 31.8*	6.9	*30.3*	0.2	*1.3*
External secondary branch	9	4.0 – 5.3	*17.3 – 22.3*	4.3	*18.7*	0.5	*1.6*
Internal primary branch	7	6.4 – 7.3	*29.1 – 30.6*	6.7	*29.8*	0.3	*0.6*
Internal secondary branch	4	4.0 – 4.9	*18.0 – 20.5*	4.3	*18.8*	0.4	*1.2*
Claw 3 heights							
External primary branch	10	6.6 – 7.0	*28.1 – 32.2*	6.8	*29.6*	0.1	*1.1*
External secondary branch	7	4.0 – 5.7	*17.5 – 23.8*	4.5	*19.2*	0.6	*2.2*
Internal primary branch	10	6.4 – 7.5	*27.9 – 32.0*	6.8	*29.5*	0.3	*1.4*
Internal secondary branch	5	4.0 – 5.4	*17.4 – 22.6*	4.3	*18.9*	0.6	*2.2*
Claw 4 heights							
Anterior primary branch	6	6.9 – 8.4	*28.9 – 35.4*	7.4	*31.5*	0.6	*2.4*
Anterior secondary branch	5	4.2 – 4.5	*18.2 – 19.2*	4.4	*18.8*	0.1	*0.5*
Posterior primary branch	8	6.7 – 7.9	*29.6 – 34.9*	7.2	*32.0*	0.4	*1.9*
Posterior secondary branch	6	3.8 – 4.8	*16.5 – 20.1*	4.1	*18.2*	0.3	*1.3*

are arranged in 8 bands (one per subsegment) made of transverse rows, and, lastly, subsegment 10 has pores not arranged in proper rows and continuing onto the dorsal part of legs IV.

Describing textually in detail the complete dorsal/dorso-lateral pore arrangement would be long and complicated, with continuous necessity to compare the text with Figures and Tables; for this reason, we refer to these latter (Fig. 6; Table 2) for the description of the dorsal/dorso-lateral pore arrangement.

Leg pores (Fig. 6, Table 2): on the external side of each leg (I–IV) there is a big, usually lobate (three or four lobes) pore, but sometimes just triangular/quadrangular (usually triangular or three-armed on legs I–II, while usually quadrangular or four-armed on legs III–IV); legs III show few additional, smaller and more caudal pores, while legs IV show 3–4 additional, dorsal pores.

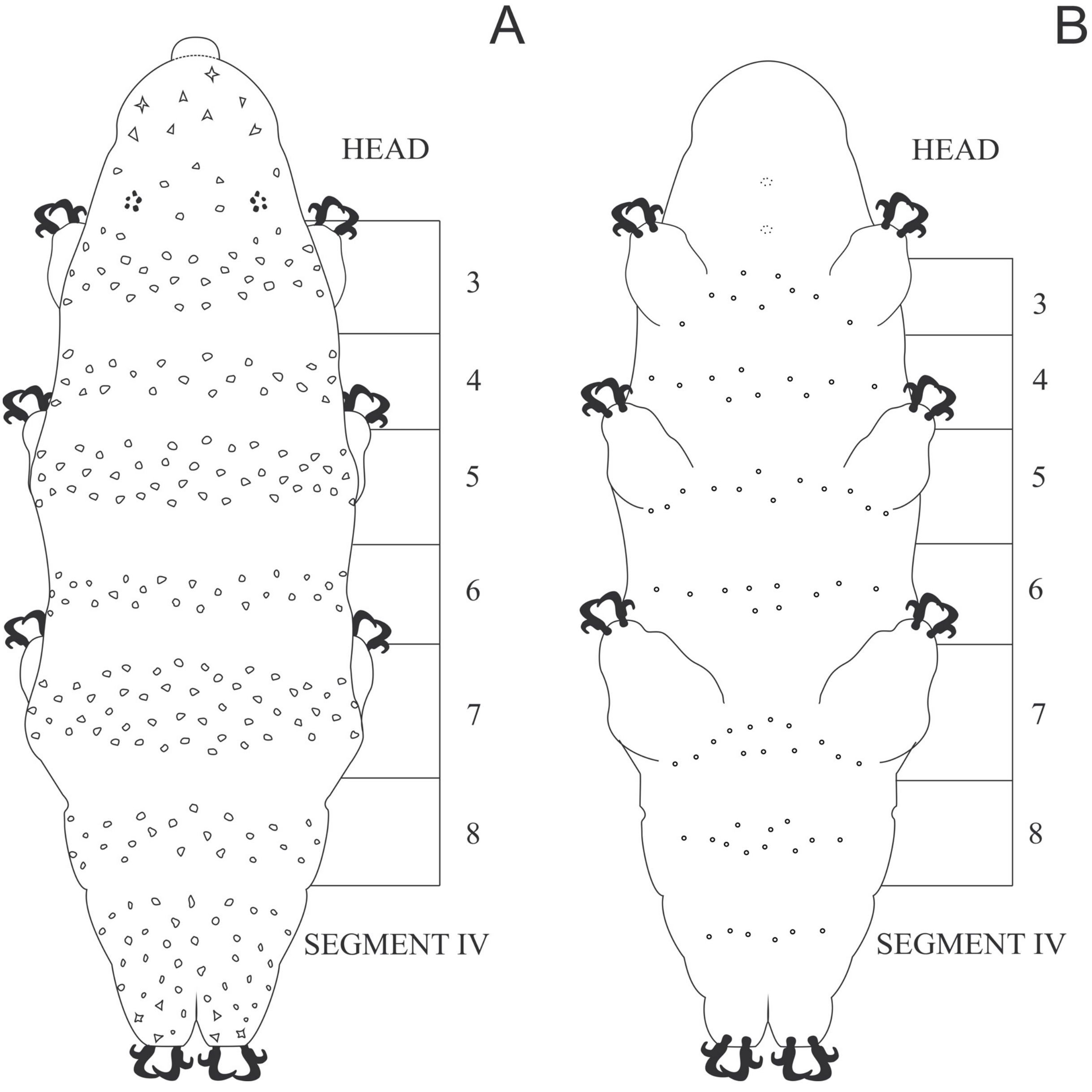

Fig. 7. *Minibiotus dispositus* sp. nov. Schematic drawing of the arrangement of the pores of the senior specimens. **A**. Dorsal cuticle. **B**. Ventral cuticle.

Arrangement of ventral pores (Fig. 6): ventral cuticle may show 1–2 medial pores, aligned longitudinally, on the caudal portion of the head (subsegment 2), and, normally, seven rows (each representing also a band) each on subsegments 3–9 (subsegment 10 without ventral pores), organized as follows: two rows are present on each of the first three segments of the trunk (subsegments 3–8), while only one on the hind segment (subsegment 9); four medial pores are present in all seven rows, but the three rows just behind each of legs I–III (subsegments 3, 5 and 7) show some (usually three) additional, smaller, pores lying more laterally just at the base of the legs; instead, the four rows consisting of only the four medial, bigger pores, are the interlegs 4, 6, 8 and 9.

Morphometry is reported in Table 3.

Senior specimens (Figs 1, 7; Tables 4–5; Supp. file 2)
In the senior specimens, the dorsal and dorso-lateral pore pattern (Figs 1, 7), and, partially, the ventral one, is basically a complication of that of the young, with an increase of the pore number (and their rows) and also introducing more variability and less 'order' especially in those that should be the pore transverse rows.

Arrangement of dorsal and dorso-lateral pores: the bigger pores are kept rather similar in shape and size from the earlier life stage(s), with rather good correspondence especially on the head and on the legs, while additional pores appear, usually smaller, resulting in the above-mentioned increase in row number and decrease in row clearness especially on the rest of the dorsum.

As stated for the young, we refer to Figures and Tables (Figs 1, 7; Table 4) for the detailed description of the dorsal/dorso-lateral pore arrangement of the senior specimens, with all the more reason since the pattern is indeed more complicated. In general, there is a group of cephalic pores (subsegment 1), 7 bands of pores (subsegments 2–8) more or less organized in transverse rows, while the eighth band that was recognisable in the smaller specimens (subsegment 9), here is joined to the group of caudal pores (subsegment 10 plus dorsum of legs IV), so that the dorsal and dorso-lateral cuticle of the whole hind segment (plus legs IV) show a unique, large, caudal group made of many pores with no clear rows recognisable.

Arrangement of leg pores: on the external side of legs I–III there are some large, usually lobate (with 3–4 lobes) pores, but sometimes just triangular/quadrangular; legs IV show several triangular, quadrangular and lobate/star-shaped (with 3–5 lobes/arms) pores.

Arrangement of ventral pores: ventrally, there is instead a good correspondence with the pattern of the young specimens; thus, to avoid repetition, we stress here only the difference, consisting of a tendency to complicate only the medial part of the transverse rows, which are partially multiplied with 4–12 pores forming in the centre a patch of pores sometimes similar to some geometric figure such as a rhombus, a circle, a square or a pentagon.

Morphometry is reported in Table 5. These senior specimens, in comparison to the young, have a slightly wider buccal tube (e.g., *pt* of external width [*8.3–11.5*] vs [*6.4–8.7*] in young) and longer macroplacoids (e.g., *pt* of macroplacoid row [*32.7–39.4*] vs [*26.0–30.9*] in young) and claws (e.g., *pt* of claw I external primary branches [*28.7–35.9*] and of claw IV posterior primary branches [*38.0–46.0*] vs [*28.1–31.5*] and [*29.6–34.9*] respectively in young).

Eggs (Fig. 8; Table 6; Supp. file 3)
Eggs are light orange in colour before mounting, spherical and laid freely. Processes in the shape of elongated cones, rarely bifurcate distally, usually ending at the tip in a filament (Fig. 8A–E). In some processes, single bubble-like structures can be seen inside the distal half portion of the processes

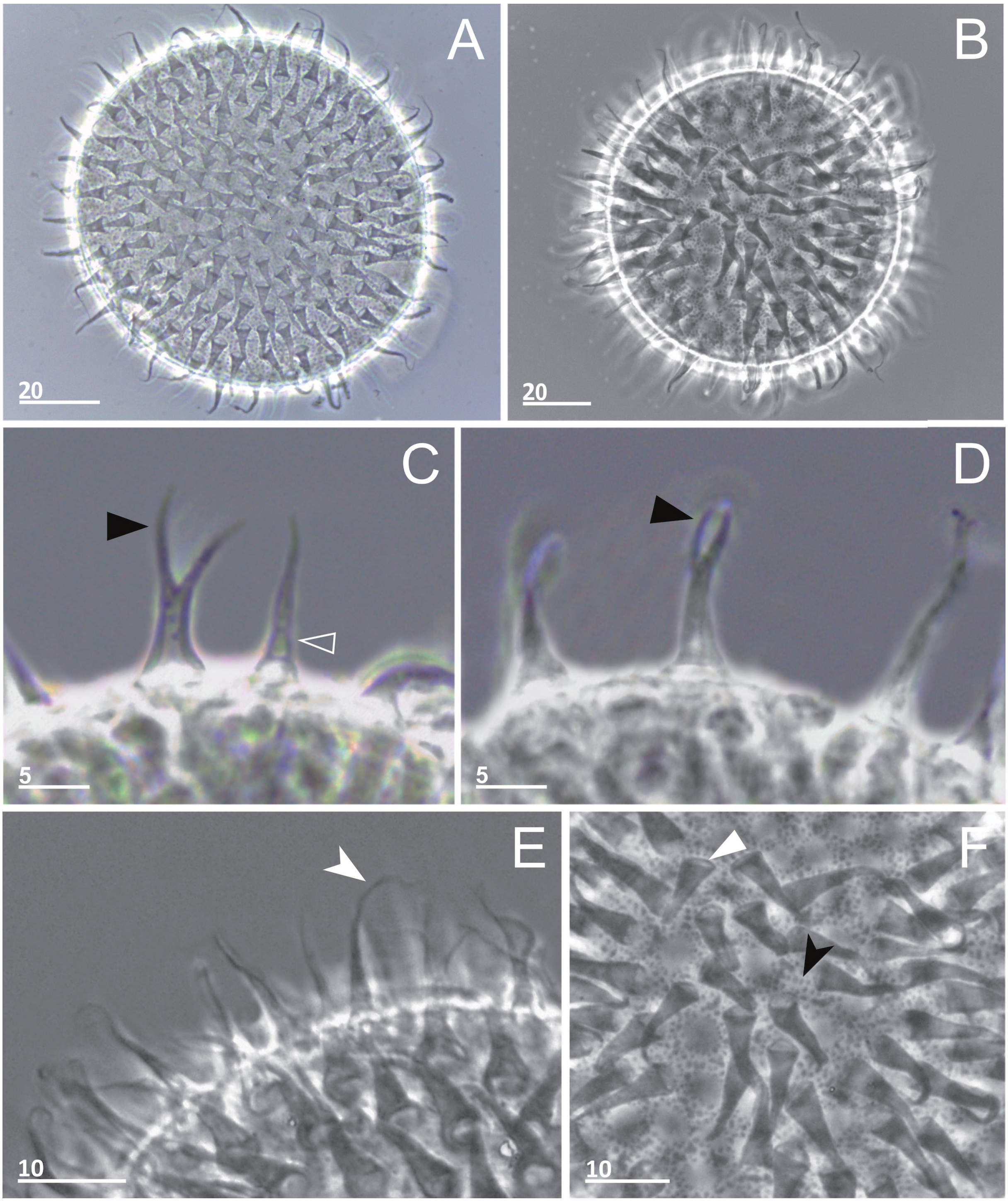

Fig. 8. *Minibiotus dispositus* sp. nov. Eggs seen in PCM. **A**. Paratype (UNLPam 1222(3)), midsection. **B, F**. Paratype (UNICT 6013). **C–E**. Paratype (UNLPam 348(1)). **B**. Surface. **C–F**. Details of egg. Black filled flat arrowheads indicate bifurcated process, white empty flat arrowhead indicates singular bubble-like structure, white filled indented arrowhead indicates filament tip, white filled flat arrowhead indicates process base whereas black filled indented arrowhead indicates granulation of the chorion. Scale bars in µm.

Table 4 (continued on next page). Schematization of the dorsal pore pattern of senior specimens of *Minibiotus dispositus* sp. nov. with reference to the precise body districts.

Body regions	Body segments	Subsegments and legs	Number of rows	Number of pores	Pore shape
Head	Head	1	4	2 4 2 6	triangular quadrilobated triangular various
		2	2	8 10	various
Trunk	Second segment (segment I of the trunk), with legs I	3 (with legs I)	4 plus lateral patch	6–10 6–10 6–10 6–10 lateral patch of about 7–12	various
		Legs I		1 big	Three – or four –armed
		4	2	8–10 8–10	various
	Third segment (segment II of the trunk), with legs II	5 (with legs II)	4 plus lateral patch	6–10 6–10 6–10 6–10 lateral patch of about 7–12	various
		Legs II		1 big	Three – or four –armed pore
		6	2	8–10 8–10	various
	Fourth segment (segment III of the trunk), with legs III	7 (with legs III)	5 plus lateral patch	4–10 4–10 4–10 4–10 4–10 lateral patch of about 7–12	various

Table 4 (continued). Schematization of the dorsal pore pattern of senior specimens of *Minibiotus dispositus* sp. nov. with reference to the precise body districts.

Body regions	**Body segments**	**Subsegments and legs**	**Number of rows**	**Number of pores**	**Pore shape**
Trunk	Fourth segment (segment III of the trunk), with legs III	7 (with legs III)	5 plus lateral patch	4–10 4–10 4–10 4–10 4–10 lateral patch of about 7–12	various
		Legs III		1 big more anterior some caudal smaller	Four – armed various
		8	3	4–10 4–10 4–10	
	Fifth segment (segment VI of the trunk), with legs VI	9		continuous, big, caudal group made of many pores with no clear rows recognisable (also on the dorsum of legs IV)	various
		10 (with legs IV)			
		Legs IV			One big, four – or five – armed, others of various shape

Table 5. Measurements (in µm) and *pt* values of selected morphological structures of senior types (including the holotype) of *Minibiotus dispositus* sp. nov. (first sampling specimens). 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	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	29	180 – 342	*656 – 1047*	258	*883*	47	*112*	300	*989*
Buccopharyngeal tube									
Buccal tube length	29	24.6 – 33.6		29.0	–	2.2	–	30.3	–
Stylet support insertion point	28	17.2 – 23.2	*65.0 – 71.4*	19.9	*68.2*	1.5	*1.7*	20.1	*66.2*
Buccal tube external width	28	2.2 – 3.6	*8.3 – 11.5*	2.8	*9.7*	0.4	*0.8*	3.5	*11.5*
Buccal tube internal width	28	1.1 – 2.3	*4.2 – 7.4*	1.6	*5.5*	0.4	*1.0*	2.2	*7.4*
Ventral lamina length	16	13.0 – 16.0	*45.1 – 53.7*	14.6	*50.6*	1.0	*2.3*	16.0	*52.8*
Placoid lengths									
Macroplacoid 1	29	2.6 – 4.0	*9.8 – 13.4*	3.3	*11.3*	0.4	*0.9*	3.6	*11.7*
Macroplacoid 2	29	2.1 – 3.5	*8.1 – 11.6*	2.7	*9.2*	0.3	*0.7*	2.9	*9.6*
Macroplacoid 3	29	1.9 – 3.3	*7.5 – 11.1*	2.6	*8.9*	0.3	*0.7*	2.9	*9.6*
Microplacoid	29	0.9 – 1.8	*3.5 – 5.9*	1.4	*4.9*	0.3	*0.8*	1.7	*5.7*
Macroplacoid row	28	8.5 – 12.5	*32.7 – 39.4*	10.5	*36.1*	1.2	*2.1*	11.9	*39.2*
Placoid row	28	9.7 – 14.9	*39.4 – 47.8*	12.5	*43.0*	1.4	*2.8*	14.5	*47.8*
Claw 1 heights									
External primary branch	27	7.8 – 10.6	*28.7 – 35.9*	9.4	*32.2*	0.8	*1.7*	9.5	*31.3*
External secondary branch	26	5.4 – 8.6	*20.6 – 26.6*	6.9	*23.5*	0.8	*1.5*	7.5	*24.7*
Internal primary branch	28	7.2 – 10.1	*27.1 – 34.9*	9.0	*30.8*	0.9	*2.1*	9.1	*29.9*
Internal secondary branch	27	5.1 – 7.8	*19.9 – 25.6*	6.5	*22.3*	0.8	*1.7*	7.0	*23.1*
Claw 2 heights									
External primary branch	28	8.0 – 11.2	*30.5 – 38.5*	9.9	*34.3*	1.0	*1.8*	10.4	*34.4*
External secondary branch	25	5.7 – 8.7	*22.1 – 28.8*	7.4	*25.4*	0.9	*1.8*	7.7	*25.2*
Internal primary branch	28	7.8 – 10.9	*29.7 – 37.7*	9.6	*33.0*	1.0	*2.1*	10.0	*32.9*
Internal secondary branch	27	5.0 – 8.7	*19.6 – 26.6*	6.9	*23.8*	0.9	*1.9*	7.1	*23.2*
Claw 3 heights									
External primary branch	29	8.4 – 11.9	*32.4 – 39.7*	10.3	*35.6*	0.9	*1.7*	10.7	*35.3*
External secondary branch	29	5.9 – 9.1	*23.1 – 29.4*	7.6	*26.2*	0.9	*1.8*	8.3	*27.3*
Internal primary branch	27	7.9 – 11.1	*30.5 – 37.9*	9.7	*33.5*	1.0	*2.0*	9.8	*32.4*
Internal secondary branch	26	5.5 – 8.2	*21.5 – 28.1*	7.2	*24.7*	0.8	*1.8*	7.3	*24.1*
Claw 4 heights									
Anterior primary branch	27	8.7 – 13.7	*35.3 – 43.6*	11.5	*39.6*	1.4	*2.6*	12.6	*41.4*
Anterior secondary branch	26	6.3 – 10.8	*25.1 – 32.9*	8.5	*29.0*	1.2	*2.3*	8.6	*28.2*
Posterior primary branch	27	9.7 – 14.1	*38.0 – 46.0*	12.2	*41.7*	1.2	*2.6*	12.7	*41.8*
Posterior secondary branch	27	6.8 – 10.7	*26.0 – 34.2*	8.7	*29.6*	1.1	*2.5*	8.9	*29.5*

Table 6. Measurements of selected morphological structures, and other metric traits, of eggs of *Minibiotus dispositus* sp. nov. mounted in polivinil lactofenol medium. N = number of eggs/structures measured; Range = refers to smallest and largest structure or value among all measured eggs/structures; SD = standard deviation.

Character	**N**	**Range**	**Mean**	**SD**
Diameter of egg without processes (in µm)	6	55.6–82.1	66.9	9.6
Diameter of egg with processes (in µm)	6	77.2–110	92.2	11.6
Process height (in µm)	6	13.0–17.0	14.8	1.4
Process base width (in µm)	6	2.0–4.5	3.2	0.9
Distance between processes	6	1.3–3.4	2.3	0.8
Number of processes on the egg circumference	6	29–34	31.3	0.9
Number of processes on the egg hemisphere	6	135–177	149.4	4.9

(Fig. 8C). On the egg circumference 29–34 processes are present and about 135–177 in the hemisphere depending also on the egg size. Process bases without projections on the chorion (Fig. 8F), but this latter, between the process, has evident granulation (Fig. 8A–B, F). Quantitative data are reported in Table 6.

DNA sequences

The sequenced senior specimen (V4) of *Minibiotus dispositus* sp. nov. is differentiated from all the other sequenced species belonging to the genus *Minibiotus*, as indicated by the ranges of genetic p-distances:

COI (523 bp dataset): 21.4 to 26.5% (Supp. file 4), with the most similar being unpublished sequences of *M. citlalium* (OP684766, OP684767) from Mexico;

ITS2 (531 bp dataset): 12.3 to 27.8% (Supp. file 5), with the most similar being *M. ioculator* (MT024000) from South Africa;

18S (778 bp dataset): 0.2 to 13.4% (Supp. file 6), with the most similar being *M. furcatus* (FJ435745) from Spain;

28S (817 bp dataset): 1.8 to 3.1% (Supp. file 7), with the most similar being *Minibiotus* sp. (MH079492) from Chile.

The ASAP analysis for both COI and ITS2 genes (Tables 7–8) further confirms the status of *Minibiotus dispositus* sp. nov. specimen V4 as a clearly distinct species from the other sequenced taxa of the genus.

The integration of the present molecular data with the morphological ones, therefore points to the validity of the erection of *Minibiotus dispositus* sp. nov.

Morphological differential diagnosis

Cuticular pores arranged in transverse bands are reported in many species of the genus, but the following are excluded from comparison for the reasons indicated in brackets: *Minibiotus formosus* Zawierucha, Dziamięcki, Jakubowska, Michalczyk & Kaczmarek, 2014, *M. granatai* (Pardi, 1941) and *M. gumersindoi* Guil & Guidetti, 2005 (they have only round/elliptical pores, lacking lobated/star-shaped ones); *M. jonesorum* Meyer, Lyons, Nelson & Hinton, 2011 (it lacks microplacoid and has polygonal pores, that also are very large and very densely distributed); *M. pseudofurcatus* (Pilato, 1972) (pores have at maximum 3 lobes/arms).

Table 7. Results of species delimitation analysis of the genus *Minibiotus* R.O. Schuster, 1980 from GenBank by automatic partitioning (ASAP) on COI gene (lower ASAP-score = 1.50; threshold p-distance = 7.86%).

Specimen	Species partition
FJ435802 *Minibiotus furcatus* Tar527 Madrid Spain	1
FJ435803 *Minibiotus gumersindoi* Tar710 Madrid Spain	2
JX683828 *Minibiotus furcatus* 2003 C3042 V10 Portugal	2
JX683829 *Minibiotus furcatus* 2007 C3039 Mini 1 Portugal	2
JX865309 *Minibiotus* sp. Mini_06_138	3
JX865313 *Minibiotus* sp. Mini_07_120	4
MT023412 *Minibiotus ioculator* ZA.274 South Africa	5
MT023413 *Minibiotus pentannulatus* TZ.027 haplotype 1 Tanzania	6
MT023414 *Minibiotus pentannulatus* TZ.027 haplotype 2 Tanzania	6
MW306857 *Minibiotus* sp. A S.297 Min Italy	7
MW306858 *Minibiotus* sp. A S.297 Min 2 Italy	7
MW306859 *Minibiotus* sp. A S.69 Min Italy	7
ON005160 *Minibiotus intermedius* Min4 GR Marburg Germany	8
OP013286 *Minibiotus* cf. *intermedius* DT274 Lembolovo Russia	9
OP013287 *Minibiotus* cf. *intermedius* DT270 Lembolovo Russia	9
OP013288 *Minibiotus* cf. *intermedius* DT279 Lembolovo Russia	9
OP684765 *Minibiotus citlalium* AI04 Iztaccihuatl Mexico	10
OP684766 *Minibiotus citlalium* AI08 Iztaccihuatl Mexico	10
OP684767 *Minibiotus citlalium* AI19 Iztaccihuatl Mexico	10
OP684770 Minibiotus sidereus AI12 Iztaccihuatl Mexico	10
OP684768 *Minibiotus sidereus* AI09 Iztaccihuatl Mexico	10
OP684769 *Minibiotus sidereus* AI11 Iztaccihuatl Mexico	10
OP684773 *Minibiotus* sp. AI33 Iztaccihuatl Mexico	10
OP684772 *Minibiotus* sp. AI31 Iztaccihuatl Mexico	10
OP684771 *Minibiotus* sp. AI13 Iztaccihuatl Mexico	10
V4 *Minibiotus* sp. nov.	**11**

For differential diagnosis, we here compare *M. dispositus* sp. nov. with the species sharing the presence of smooth cuticle, multilobated pores (3–5 arms/lobes) and three macroplacoids plus evident microplacoid. However, considering the paucity of 5-armed pores recognisable under PCM (only 0–2 per specimen, and only caudally or on legs) in the new species, we excluded from comparison the species with very numerous, evident star-shaped pores (5 arms or more) clearly observable under PCM on all the body (thus excluding: *M. citlalium* Dueñas-Cedillo & García-Román, 2020, *M. claxtonae* Rossi, Claps & Ardohain, 2009, *M. constellatus* Michalczyk & Kaczmarek, 2003, *M. pentannulatus* Londoño, Daza, Lisi & Quiroga, 2017, *M. pseudostellarus* Roszkowska, Stec, Ciobanu & Kaczmarek, 2016, *M. sidereus* Pilato, Binda & Lisi, 2003).

Table 8. Results of species delimitation analysis of the genus *Minibiotus* R.O. Schuster, 1980 from GenBank by automatic partitioning (ASAP) on ITS2 gene (lower ASAP-score = 2.00; threshold p-distance = 6.07%).

Specimen	**Species partition**
MT024000 *Minibiotus ioculator* ZA.274 South Africa	1
MT024001 *Minibiotus pentannulatus* TZ.027 Tanzania	2
OP696660 *Minibiotus citlalium* AI04 Iztaccihuatl Mexico	3
OP696661 *Minibiotus citlalium* AI08 Iztaccihuatl Mexico	3
OP696662 *Minibiotus citlalium* AI19 Iztaccihuatl Mexico	3
OP696663 *Minibiotus sidereus* AI09 Iztaccihuatl Mexico	3
OP696664 *Minibiotus sidereus* AI11 Iztaccihuatl Mexico	3
OP696666 *Minibiotus* sp. AI13 Iztaccihuatl Mexico	3
OP696667 *Minibiotus* sp. AI31 Iztaccihuatl Mexico	3
OP696665 *Minibiotus sidereus* AI12 Iztaccihuatl Mexico	3
OP696668 *Minibiotus* sp. AI33 Iztaccihuatl Mexico	3
OK663216 *Minibiotus* sp. S69 01 S69 Min 1 Italy	4
OP035707 *Minibiotus* cf. *intermedius* DT270 Lembolovo Russia	5
OP035708 *Minibiotus* cf. *intermedius* DT274 Lembolovo Russia	5
V4 *Minibiotus* sp. nov.	**6**

Lastly, we decided not to limit the comparison with species clearly having a pore pattern (described or at least well visible in the description illustrations), because such a character may not have been noticed or reported in some past descriptions; in this way, *M. aculeatus* Murray, 1910 and *M. vinciguerrae* Binda & Pilato, 1992 are included, also considering that the old illustrations available may not be perfectly accurate.

All that considered, *Minibiotus dispositus* sp. nov. is to be compared with: *M. aculeatus* (Murray, 1910); *M. bernhardi* Schuster, 2021; *M. bisoctus* (Horning, Schuster & Gregarick, 1978); *M. eichhorni* Michalczyk & Kaczmarek, 2004; *M. ethelae* Claxton, 1998; *M. furcatus* (Ehrenberg, 1859); *M. harrylewisi* Meyer & Hinton, 2009; *M. lazzaroi* (Maucci, 1986); *M. orthofasciatus* Fontoura, Pilato, Lisi & Morais, 2009; *M. pustulatus* (Ramazzotti, 1959); *M. vinciguerrae* Binda & Pilato, 1992; *M. weglarskae* Michalczyk, Kaczmarek & Claxton, 2005; *M. xavieri* Fontoura, Pilato, Morais & Lisi, 2009.

For correct morphometric comparisons, considering the allometry of some characters of the new species, we compared the morphometric characters in the present differential diagnosis taking into account the body size (available from the literature) of each compared species. Our young specimens had body sizes of up to about 156 µm while our senior specimens had body lengths starting from about 180 µm; most of the compared species had a body length starting from at least 200 µm; thus, we compared the morphometry of these species with that of our senior specimens; only two species, *M. orthofasciatus* and *M. weglarskae*, had body lengths starting from less than 180 µm (from 138 µm and 166 µm, respectively) but exceeding 200 µm in the maximum value: in this case we joined together our morphometric ranges of young and senior specimens for comparison (no compared species had a body length range compatible only with our young specimens).

Minibiotus dispositus sp. nov. specifically differs from the various compared species as follows.

Minibiotus aculeatus (according to the description, drawings and measurements by Claxton 1998), reported from the type locality in Australia, and from New Zealand: this species (also) has star-shaped pores (even 6-armed) and should have no pore bands; judging from the drawings, however, it is not clear how many such star-shaped pores are common and spread, or whether the bands are surely absent (the drawings may be schematic or not sufficiently accurate). *Minibiotus dispositus* sp. nov. and *M. aculeatus* differ in smallest pore size (smallest 0.9 µm in the new species vs 0.5 µm in *M. aculeatus*); the absence of three pairs of soft conical spines on the dorsal cuticle in connection with the segment bearing legs II–III–IV (such spines present in *M. aculeatus*); claws in *M. dispositus* less robust than in *M. aculeatus*. Different characteristics of the egg: granulated chorion in the new species (smooth in *M. aculeatus*); diameter without/with processes larger 55.6–82.1 / 77.2–110 µm (54 / 65 µm in *M. aculeatus*); 29–34 (24–30 in *M. aculeatus*) processes around the circumference; process height 13.0–17.0 µm, and with 1.3–3.4 µm process base distance (process height 9–11 µm, 3–4 µm base distance in *M. aculeatus*); base of each process smooth in the new species, indented in *M. aculeatus*.

Minibiotus bernhardi, reported only from a few localities in Germany: multilobate pores very common in *M. dispositus* sp. nov. vs scarce in *M. bernhardi*. Nine dorsal bands of pores in the new species vs ten (total band number of both species includes cephalic and caudal bands but excluding legs IV); in particular, *M. dispositus* has on all the dorsum of the hind segment, including legs IV, a continuous, large, caudal group of pores with no bands or rows distinguishable, while in *M. bernhardi* three separate bands are distinguishable. Different number of ventral pore bands: seven in the new species vs eight in *M. bernhardi*. Totally different egg since *M. bernhardi* produces eggs of the *intermedius*-type.

Minibiotus bisoctus (according to the description, drawings and measurements by Claxton 1998) reported only from the type locality in New Zealand: presence of pores on the ventral cuticle (absent in *M. bisoctus*); eyes present in the new species (absent in *M. bisoctus*); *pt* of the ventral lamina [*45.1–53.6*] (*42.0* in *M. bisoctus*); stylet supports inserted more posteriorly, *pt* [*65.0–71.4*] (more anteriorly *60.3* in *M. bisoctus*); *pt* of macroplacoid row [*32.7–39.4*] (*31.0* in *M. bisoctus*); legs IV posterior primary branches longer in *M. dispositus* sp. nov. (*pt* [*38.0–46.0*] vs *31.0* in *M. bisoctus*); leg granulation around the claws absent (present in *M. bisoctus*).

Minibiotus eichhorni reported only from the type locality in Perú: clear pattern of cuticular pores arranged in rows within the bands, while no rows are visible in *M. eichhorni*; the authors of *M. eichhorni* did not mention any pore arrangement in rows within the bands; but if any, the rows seems to be no more than 3 per band (see Michalczyk & Kaczmarek 2004: figs 1–2), while *M. dispositus* sp. nov. has 2–5 well-disposed rows per band; a clear pattern of ventral pores disposed in 8 rows while randomly distributed pores in *M. eichhorni* ("Round and oval pores present over whole cuticle, however in lower density on ventral cuticle" according to Michalczyk & Kaczmarek 2004); granulations around the claws on all legs absent (present on all legs in *M. eichhorni*). Morphometric differences regard partially overlapping morphometric ranges that were statistically tested (Table 9; Supp. file 8).

Minibiotus ethelae reported only from the type locality in Australia and from South Africa: dorsal pattern of cuticular pores distinct from ventral one in the new species, including different number between dorsal and ventral rows, while "9 bands around the body" in *M. ethelae* according to Claxton (1998); cuticle not thickened around the pores of the caudal region (while thickened in *M. ethelae*); evident microplacoid in *M. dispositus* sp. nov., while "small, indistinct" in *M. ethelae* (Claxton 1998); absence of the refractive zone at base of the claws (present in *M. ethelae*). The eggs of the two species are very similar, both morphologically and morphometrically; however, two possible differences can be singled out: the distance between the egg processes is 1.3–3.4 µm in the new species, while Claxton (1998)

Table 9 (continued on next page). Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of selected metric characters, between *Minibiotus dispositus* sp. nov. and the similar species. Values indicate the *pt* ranges and the mean in brackets; they refer, without any indication, to senior specimens for correct comparison with *M. xavieri* Fontoura, Pilato, Morais & Lisi, 2009, *M. eichhorni* Michalczyk & Kaczmarek, 2004 and *M. harrylewisi* Meyer & Hinton, 2009; otherwise "Y+S" is specified to indicate that the values of young and senior specimens are together in a single range for correct comparison with *M. weglarskae* Michalczyk, Kaczmarek & Claxton, 2005.

Species	***M. dispositus*** sp. nov.	***M. xavieri***	***M. eichhorni***	***M. harrylewisi***	***M. weglarskae***
Character					**All ranges Y+S**
Stylet support insertion point	65.0–71.4 (68.2)			61.4–67.6 (64.1) $t_{36}= 6.9$, $p<0.001$	
Buccal tube external width	8.3–11.5 (9.7)		6.9–9.7 (8.4) $t_{27}= 4.8$, $p<0.001$		
Macroplacoid 1	9.8–13.4 (11.3) Y+S 8.0–13.4 (10.3)			5.2–12.4 (8.0) $t_{28}= 4.6$, $p<0.001$	6.4–9.1 (7.8) $t_{45}= 9.8$, $p<0.001$
Macroplacoid 2	8.1–11.6 (9.2)		6.9–9.2 (8.3) $t_{28}= 5.0$, $p<0.001$	6.0–8.6 (7.2) $t_{28}= 8.3$, $p<0.001$	
Macroplacoid 3	7.5–11.1 (8.9) Y+S 6.8–11.1 (8.3)	10.9–11.9 (11.3) $t_{28}= -16.9$, $p<0.001$		4.7–10.0 (7.7) $t_{28}= 3.3$, $p<0.001$	4.4–8.7 (6.6) $t_{46}= 6.0$, $p<0.001$
Microplacoid	3.5–5.9 (4.9)	5.0–6.2 (5.5) $t_{28}= -4.9$, $p<0.001$			
Macroplacoid row	32.7–39.4 (36.1) Y+S 26.0–39.4 (32.6)			26.3–36.6 (32.3) $t_{27}= 5.8$, $p<0.001$	20.8–26.1 (23.3) $t_{45}= 12.4$, $p<0.001$
Placoid row	39.4–47.8 (43.0) Y+S 29.9–47.8 (39.0)		36.2–44.1 (40.6) $t_{27}= 4.0$, $p<0.001$		24.4–30.4 (27.3) $t_{43}= 12.4$, $p<0.001$
Claw I					
External/internal primary branch	27.1–35.9 (31.5)		25.9–31.0 (29.0) $t_{54}= 8.2$, $p<0.001$	23.1–32.6 (27.6) $t_{54}= 7.0$, $p<0.001$	

Table 9 (continued). Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of selected metric characters, between *Minibiotus dispositus* sp. nov. and the similar species. Values indicate the *pt* ranges and the mean in brackets; they refer, without any indication, to senior specimens for correct comparison with *M. xavieri* Fontoura, Pilato, Morais & Lisi, 2009, *M. eichhorni* Michalczyk & Kaczmarek, 2004 and *M. harrylewisi* Meyer & Hinton, 2009; otherwise "Y+S" is specified to indicate that the values of young and senior specimens are together in a single range for correct comparison with *M. weglarskae* Michalczyk, Kaczmarek & Claxton, 2005.

Species	***M. dispositus***	***M. xavieri***	***M. eichhorni***	***M. harrylewisi***	***M. weglarskae***
Character	sp. nov.				**All ranges Y+S**
External/internal secondary branch	19.9–26.6 (22.9) Y+S 17.1–26.6 (22.3)		18.5–23.5 (21.0) t_{52}= 5.5, $p<0.001$	16.4–23.1 (19.9) t_{52}= 7.3, $p<0.001$	13.0–17.4 (15.9) t_{63}= 17.7, $p<0.001$
Claw 2					
External/internal primary branch	29.7–38.5 (33.6) Y+S 27.6–38.5 (32.7)		26.9– 33.3 (30.7) t_{55}= 7.5, $p<0.001$	27.4–35.2 (29.7) t_{55}= 5.4, $p<0.001$	21.7–29.2 (25.2) t_{73}= 16.2, $p<0.001$
External/internal secondary branch	19.6–28.8 (24.6) Y+S 16.9–28.8 (23.4)		19.2–22.4 (21.2) t_{51}= 9.8, $p<0.001$	16.8–27.1 (20.5) t_{51}= 4.4, $p<0.001$	13.0–25.0 (17.2) t_{64}= 7.2, $p<0.001$
Claw 3					
External/internal primary branch	30.5–39.7 (34.5) Y+S 27.8–39.7 (33.2)		29.4–33.3 (31.8) t_{55}= 9.0, $p<0.001$	27.9–34.3 (31.1) t_{55}= 7.9, $p<0.001$	21.7–29.2 (25.3) t_{75}= 15.4, $p<0.001$
External/internal secondary branch	21.5–29.4 (25.4) Y+S 17.1–30.4 (24.3)		20.6–25.0 (22.8) t_{54}= 7.2, $p<0.001$	16.0–26.8 (22.4) t_{54}= 5.5, $p<0.001$	13.0–21.7 (17.2) t_{66}= 12.2, $p<0.001$
Claw 4					
Anterior/posterior primary branch	35.3–46.0 (40.8)		34.6–41.7 (36.8) t_{52}= 5.7, $p<0.001$		
Anterior/posterior secondary branch	25.1–34.2 (29.3)		23.1–27.8 (24.8) t_{52}= 10.0, $p<0.001$		

reported a distance of "about 5 µm"; this may be confirmed by the slightly higher average number of processes on the egg hemisphere in *M. dispositus* (135–177) with respect to *M. ethelae* (120–160).

Minibiotus furcatus (according to the redescription by Binda & Pilato 1992) reported from the type locality in Europe (Monte Rosa, Italian Alps), the Americas, South Africa and India: shorter ventral lamina (*pt* [*45.1–53.7*] in *M. dispositus* sp. nov. vs *62.0* in *M. furcatus*); smooth lunules of all legs, while slightly indented on legs IV of *M. furcatus*. Different egg characteristics: orange color (colorless or yellowish in *M. furcatus*), process height 13.0–17.0 µm (5–6 µm in *M. furcatus*), granulated chorion (smooth chorion in *M. furcatus*).

Minibiotus harrylewisi reported only from the type locality in South Africa: morphometric differences regarding partially overlapping morphometric ranges but statistically tested (Table 9; Supp. file 8). Different egg characteristics: long cones, 13.0–17.0 µm high, uniformly tapering from the base, with granulated chorion in the new species, while *M. harrylewisi* has shorter cones (7.6–12.8 µm high) with bulbous base, with smooth chorion.

Minibiotus lazzaroi, reported only for few Italian localities: the species was compared with senior specimens of *M. dispositus* sp. nov. as the reported body length of *M. lazzaroi* was "up to 420 µm" and no differences were reported between young and senior specimens by Maucci (1986). Roundish (PCM), elliptical and multilobate (up to 5 lobes) pores in the new species vs triangular, trapezoidal or rhomboidal pores in *M. lazzaroi*; pores having a wide dimensional range (from 0.9 to 3.1 µm) in *M. dispositus* vs pores of almost the same size on all the body (from 2 to 2.2 µm) in *M. lazzaroi*; 9 bands of pores vs 8 bands of pores (according to the drawing in the original description) in *M. lazzaroi* (band number of both species including cephalic and caudal bands). Totally different egg characteristics, since the new species has eggs with very slender, smooth, conical processes (process height 13.0–17.0 µm, process base width 2.0–4.5 µm) tapering apically, and a granulated chorion without ridges, while *M. lazzaroi* has a peculiar egg with very wide, reticulated, trunco-conical processes (process height 8–12 µm, process base width 28–30 µm) with jagged apical portion and irregular ridges on the chorion surface.

Minibiotus orthofasciatus reported only from the type locality in Portugal: cuticular pores arranged in 10 dorsal bands in the new species, while 11 in *M. orthofasciatus*; shorter ventral lamina (*pt* [*45.1–53.7*] in *M. dispositus* sp. nov. vs [*55.3–58.4*] in *M. orthofasciatus*). Totally different egg characteristics since the new species has eggs with conical processes and a granulated chorion not covered by a membrane including the processes, while *M. orthofasciatus* has eggs of the *intermedius* group, i.e., with screw-like processes joined by a membrane covering a non-granulated egg chorion.

Minibiotus pustulatus reported from the type locality in Italia, and from Chile: eye-spots present in the new species (absent in *M. pustulatus*); dorsal and leg pores occur to be multilobate (usually 3–4 lobes/arms) in the new species, while subcircular to triangular or polygonal, but with no lobes/arms, in *M. pustulatus*; pores are also smaller in the new species (about 1–2 µm diameter), while very large (4–7 µm) in *M. pustulatus*). *Minibiotus pustulatus* was not described as having a pore distribution pattern, but but if it had the number of possible pore rows (according to the original drawing) is clearly far lower than in the new species.

Minibiotus vinciguerrae, only recorded from Antarctica, by the pore shape and size: in *M. vinciguerrae* many pores are elliptical, reaching the size of 2.1 µm (largest pore size for the species), while few pores are triangular/trilobate or (even more rarely) quadrangular/quadrilobate, in any case of smaller or equal size to the elliptical pores; in the new species, instead, elliptical pores are rare and small (around 1 µm), while the triangular/trilobate and quadrangular/quadrilobate are common and bigger (up to 3.1 µm). The new species has shorter ventral lamina (*pt* [*45.1–53.7*] vs [*58–60*] in *M. vinciguerrae*); macroplacoid length sequence $3 \leq 2 < 1$ in the new species vs $2 < 3 < 1$ in *M. vinciguerrae*; robust claws (very slender in *M. vinciguerrae*); different details of the egg morphology: the egg processes have only sometimes bifurcated end (very commonly in *M. vinciguerrae*), have smooth base margin (jagged/irregular in *M. vinciguerrae*), and are higher (13.0–17.0 µm vs 8.17 µm in *M. vinciguerrae*), closer to one-another (distance between processes 1.3–3.4 µm vs 5 µm in *M. vinciguerrae*) and more numerous on the egg circumference (29–34 vs 26 in *M. vinciguerrae*).

Minibiotus weglarskae reported from the type locality in Mongolia: stylet supports inserted in more posterior position in the new species (*pt* [*62.9–72.9*] vs [*54.5–59.6*] in *M. weglarskae*); longer claws in the new species (higher *pt* indices), see Table 9 for statistical significance of differences in overlapping *pt* ranges of claw heights and other metric characters. Leg granulation absent in the new species (present in *M. weglarskae*). Totally different egg characteristics since the new species has eggs with conical processes and a granulated chorion not covered by a membrane including the processes, while *M. weglarskae* has eggs of the *intermedius* group, i.e., with screw-like processes joined by a membrane covering a non-granulated egg chorion.

Minibiotus xavieri reported from the type locality in Portugal: multilobate dorsal/leg pores with 3–4 lobes/arms, sometimes 5, in the new species, while only trilobate in *M. xavieri*; *pt* of ventral lamina length [*45.1–53.7*] in the new species (vs [*55.2–57.4*] in *M. xavieri*), additional morphometric differences regard partially overlapping morphometric ranges statistically tested (Table 9; Supp. file 8). Egg processes in the circumference far more numerous in the new species (29–34), while only 20–23 in *M. xavieri.*

Discussion

The present contribution adds a unit to the list of species of the genus *Minibiotus*, and to that of Argentinian species; it is also the first report for the genus in Salta Province and the first species of *Minibiotus* with a DNA analysis from Argentina.

It is worth mentioning that the present work started from a collection revision process, and resampling was carried out only for SEM and DNA analyses, otherwise it would have been impossible with the sole permanent slides deposited. Zoological collections are an important resource and should not be overlooked, especially when, due to lack of time, the material cannot be immediately identified with high taxonomic resolution; in such cases, but also more in general, collections may hide new taxa and other new data that deserve to be known, as is the case of the present work.

In addition to the new species integrative description and the related novelties mentioned at the beginning of this chapter, it was also possible to report some morphological characters or methodological aspects that deserve consideration. In detail, we think that from now on it is important to study with great attention the species of *Minibiotus* (both old and newly described) with cuticular pores, to ensure whether there is, or not (really), some dorsal and ventral pore pattern, at least at some life stages; these latter, in turn, must be also carefully studied in order to reveal possible differences, both qualitative and quantitative, which otherwise may even lead to the false impression to have two different species; this is already well known and demonstrated for other taxa, such as *Milnesium* Doyère, 1840 (e.g., Surmacz *et al.* 2019; Rocha *et al.* 2022), but less advanced for *Minibiotus* (e.g., Londoño *et al.* 2017). This was the reason why, after having provided the general description of the species, we reported separate detailed descriptions and morphometric analyses for the young and the senior specimens of *Minibiotus dispositus* sp. nov., and we strongly suggest doing so every time this is applicable, or, better, describing in detail every single life stage if their distinction is possible.

Besides, we consider important to standardize the way how to report, in species descriptions, patterns of cuticular pores or sculpturings, gibbosities, pseudoplates, etc.; though this was done for some genera (e.g., Michalczyk & Kaczmarek 2010; Moreno-Talamantes *et al.* 2019), there is not always a clear reference to the body segments and subsegments (an attempt was that of Rocha *et al.* 2022), and a more universal method is still lacking. We are not proposing to abandon the methods already in use, but to integrate them with reference to the body segments and subsegments, and a more detailed indication of the patterns, which can be done graphically like our Figures 6 and 7, and textually through using the scheme of our Tables 2 or 4 (with all the necessary adjustments for precise taxa/structures/patterns), whose template we are also furnishing as supplementary material (Supp. file 9).

Acknowledgments

We are grateful to the section editor and the anonymous reviewers who improved the quality of the article; to Prof. Giovanni Pilato for making his collection available for our study; to Andrea González-Reyes for valuable collaboration in the process of collection of the samples for this work; to Łukasz Michalczyk and Łukasz Kaczmarek for the donation, in the past, of paratypes of *Minibiotus eichhorni*. We are also grateful to Łukasz Michalczyk and Piotr Gąsiorek for the loan of paratypes of *Minibiotus constellatus* and for having made accessible (during the 15th International Symposium on Tardigrada) the Zeiss Axio Scope A1 DIC microscope equipped with a Zeiss AxioCam ICc5CCD digital camera, thanks to which we produced our Figs 3B and 4B. We would also like to thank Carlos Ignacio Bertoli of the Instituto de Nanociencia y Nanotecnología nodo Bariloche (CNEA/CONICET) for the preparation and photographs of the specimens under SEM. Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 – Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project Code [CN_00000033], Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP E93C22001090001, Project title “National Biodiversity Future Center – NBFC”.

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Manuscript received: 17 December 2023
Manuscript accepted: 6 May 2024
Published on: 20 September 2024
Topic editor: Magalie Castelin
Section editor: Daniel Stec
Desk editor: Pepe Fernández

Supplementary files

For the Excel files (1–2), the Excel template "Parachela" (ver. 1.7) from the tardigrade Register (Michalczyk & Kaczmarek 2013) was used.

Supp. file 1. Complete Excel version of measurements [in μm] and *pt* values of selected morphological structures of the young paratypes of *Minibiotus dispositus* sp. nov.
https://doi.org/10.5852/ejt.2024.958.2663.12321

Supp. file 2. Complete Excel version of measurements [in μm] and *pt* values of selected morphological structures of the senior types of *Minibiotus dispositus* sp. nov.
https://doi.org/10.5852/ejt.2024.958.2663.12323

Supp. file 3. Complete Excel version of the quantitative characters of the eggs of *Minibiotus dispositus* sp. nov. https://doi.org/10.5852/ejt.2024.958.2663.12325

Supp. file 4. Genetic distance values (p-distance) for the COI gene among all available *Minibiotus* species. The analysis was carried out on a dataset of 523 bp. Newly analyzed specimen is given in bold.
https://doi.org/10.5852/ejt.2024.958.2663.12327

Supp. file 5. Genetic distance values (p-distance) for the ITS2 gene among all available *Minibiotus* species. The analysis was carried out on a dataset of 531 bp. Newly analyzed specimen is given in bold. https://doi.org/10.5852/ejt.2024.958.2663.12329

Supp. file 6. Genetic distance values (p-distance) for the 18S gene among all available *Minibiotus* species. The analysis was carried out on a dataset of 778 bp. Newly analyzed specimen is given in bold. https://doi.org/10.5852/ejt.2024.958.2663.12331

Supp. file 7. Genetic distance values (p-distance) for the 28S gene among all available *Minibiotus* species. The analysis was carried out on a dataset of 817 bp. Newly analyzed specimen is given in bold. https://doi.org/10.5852/ejt.2024.958.2663.12333

Supp. file 8. Complete Excel version of statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of selected metric characters between specimens of *Minibiotus dispositus* sp. nov. and similar species. https://doi.org/10.5852/ejt.2024.958.2663.12335

Supp. file 9. Template from Tables 2–4; schematization for the indication of the disposition of cuticular structures (pores, pseudoplates, gibbosities etc.) with reference to the precise body districts. https://doi.org/10.5852/ejt.2024.958.2663.12337