

Taxonomy and systematics

New records of tardigrades from Mexico with the description of *Paramacrobiotus puma* sp. nov. (Eutardigrada: Macrobiotidae)

Nuevos registros de tardígrados de México con la descripción de Paramacrobiotus puma sp. nov. (Eutardigrada: Macrobiotidae)

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Abstract

Five samples of mosses and 2 samples of lichens were collected within the Cantera Oriente, in southern Mexico City. In total, 110 tardigrades and 27 eggs, belonging to 2 orders (Apochela and Parachela), 4 families (Milnesiidae, Hypsibiidae, Macrobiotidae, and Ramazzottiidae), 6 genera (*Milnesium*, *Hypsibius*, *Notahypsibius*, *Minibiotus*, *Paramacrobiotus*, and *Ramazzottius*) and 9 species were recorded. *Milnesium longiungue* Tumanov, 2006, *Notahypsibius pallidoides* Pilato, Kiosya, Lisi, Inshina and Biserov, 2011 and *Paramacrobiotus gadabouti* Kayastha, Stec, Mioduchowska and Kaczmarek, 2023, represent new records for Mexico. The species *Paramacrobiotus puma* sp. nov. is new to science and its description is provided and supported by phase contrast light microscopy (PCM), scanning electron microscopy (SEM) and analysis of nucleotide sequences of 3 nuclear and 1 mitochondrial markers. The new records increase the diversity of the phylum to 87 species in the country.

Keywords: Tardigrada; Diversity; REPSA; Mexico City

Resumen

Se recolectaron 5 muestras de musgo y 2 muestras de líquenes dentro de la Cantera Oriente en el sur de la Ciudad de México. En total, se registraron 110 tardígrados y 27 huevos pertenecientes a 2 órdenes (Apochela y Parachela), 4 familias (Milnesiidae, Hypsibiidae, Macrobiotidae y Ramazzottiidae), 6 géneros (*Milnesium*, *Hypsibius*, *Notahypsibius*, *Minibiotus*, *Paramacrobotus* y *Ramazzottius*) y 9 especies. *Milnesium longiungue* Tumanov, 2006, *Notahypsibius pallidoides* Pilato, Kiosya, Lisi, Inshina y Biserov, 2011 y *Paramacrobotus gadabouti* Kayastha, Stec, Mioduchowska y Kaczmarek, 2023, representan nuevos registros para México. La especie *Paramacrobotus puma* sp. nov. es nueva para la ciencia y se proporciona su descripción, respaldada por microscopía óptica de contraste de fases (MCF), microscopía electrónica de barrido (MEB) y análisis de secuencias de nucleótidos de 3 marcadores nucleares y 1 mitocondrial. Estos nuevos registros incrementan la diversidad del grupo a 87 especies en el país.

Palabras clave: Tardigrada; Diversidad; REPSA; Ciudad de México

Introduction

Tardigrades (*tardus* = slow, *gradus* = step or “slow-stepper”) have commonly been called “water bears” due to their bear-like appearance, legs with claws, and slow lumbering gait (Nelson et al., 2015, 2018). These micrometazoans are found in a wide variety of marine, freshwater and terrestrial habitats, such as tropical forests and polar and arid deserts, from mountains to the depths of the oceans, and inhabit mainly mosses and lichens (Nelson & Marley, 2000; Nelson et al., 2015; Ramazzotti & Maucci, 1983). Being hydrophilic organisms, they need an aqueous medium to move and carry out their life cycle. Particularly terrestrial tardigrades depend on water films that adhere to the substrates where they live (Glime, 2017; Nelson et al., 2015).

To date, more than 1,400 species of tardigrades have been described in 3 classes (Eutardigrada, Heterotardigrada, and Mesotardigrada) (Bertolani et al., 2014; Degma & Guidetti, 2024; Guidetti & Bertolani, 2005). As for Mexico, 83 species have been recorded in 16 of the 32 states: Baja California, Chiapas, Chihuahua, Coahuila, Mexico City, Michoacán, Morelos, Nuevo León, Oaxaca, Quintana Roo, San Luis Potosí, Sinaloa, Sonora, Estado de México, Tamaulipas and Yucatán (Anguas-Escalante et al., 2020; Beasley, 1972; Beasley et al., 2008; Dueñas-Cedillo et al., 2020, 2024; García-Román et al., 2022; Heinis, 1911; Kaczmarek et al., 2011; León-Espinosa et al., 2017, 2019; Moreno-Talamantes & León-Espinosa, 2019; Moreno-Talamantes et al., 2015, 2019, 2020; May, 1948; Núñez et al., 2021; Pérez-Pech et al., 2017, 2018, 2020; Pilato, 2006; Pilato & Lisi, 2006; Ramazzotti & Maucci, 1983; Schuster, 1971). The presence of tardigrades in the rest of the Mexican territory is unknown. In this article, we present new records of tardigrades found in samples of mosses and lichens collected in the Cantera Oriente, buffer zone that belongs to the Pedregal de San Angel

Ecological Reserve (REPSA), southern Mexico City. We describe a new species, *Paramacrobotus puma* sp. nov., by using an integrative approach, including morphological analysis with phase-contrast microscopy (PCM), scanning electron microscopy (SEM) as well as DNA sequencing.

Materials and methods

Five samples of mosses and 2 samples of lichens were collected within the Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl) on October 2, 2015, and August 30, 2016, respectively. The area is composed mainly of high elevation xerophilic scrub and is located in southern Mexico City and corresponds to the Buffer Zone (A3) of the Pedregal de San Ángel Ecological Reserve (REPSA) (Fig. 1), produced by the Xitle Volcano's eruption approximately 1,670 years ago. This ecological reserve is a conservation area created in 1983 within the campus of the Universidad Nacional Autónoma de México (UNAM). The objective of the reserve is to maintain an area of biological and cultural diversity containing the last remnants of natural ecosystems in the southern Mexico Basin (Palacio & Guilbaud, 2015). The samples covered an area of approximately 5 cm² and were deposited in brown paper bags and taken to the laboratory for examination. The specimens were collected under the Scientific Collector Permits FAUT-0027 and FAUT-209 granted by Semarnat and with project 361 issued by the Executive Secretariat of REPSA. Tardigrade specimens were deposited in the Colección de Tardígrados associated with the Colección Nacional de Ácaros (CNAC), Instituto de Biología, UNAM, Mexico City.

Tardigrades and eggs were extracted from the samples using the technique described by Dastych (1985). Specimens for light microscopy were mounted on slides with Hoyer's liquid and were observed under the microscope Nikon Optiphot-2 using phase contrast



Figure 1. View of collection site within the buffer zone (A3), and map indicating location of the Cantera Oriente and Pedregal de San Ángel Ecological Reserve (REPSA), CDMX, Mexico. Map by D. López-Sandoval.

microscopy (PCM). Some specimens were separated for Scanning Electron Microscopy (SEM) and were prepared according to the technique described by Stec et al. (2015). Images were obtained in a SEM Hitachi, model SU 1510 at the Laboratorio Nacional de la Biodiversidad (LANABIO), Instituto de Biología, Universidad Nacional Autónoma de México.

The sample size for morphometrics was chosen following recommendations by Stec et al. (2016). All measurements are in micrometers (μm). For the measurements, terminology of the structures and claws of the buccopharyngeal apparatus follow Pilato and Binda (2010) and Michalczyk and Kaczmarek (2003). The claw measurements were according to Beasley et al. (2008) and

Kaczmarek and Michalczyk (2017). The macroplacoid length sequence was determined according to Kaczmarek, Cytan et al. (2014) and Kaczmarek and Michalczyk (2017). The terminology and measurements for Apochela follow Tumanov (2006), Michalczyk et al. (2012a, b), and Pilato et al. (2016). The *pt* ratio is the relation between the length of a given structure and the length of the buccal tube, expressed as a percentage (Pilato, 1981). Morphometric data was managed using templates “Parachela” and “Apochela”. Versions 1.2 for both templates are available in the Tardigrada Register (Michalczyk & Kaczmarek, 2013). Tardigrade taxonomy follows Bertolani et al. (2014) and Stec et al. (2021). The morphometric data is given in supplementary material (SM1).

The specimens examined were identified with taxonomic keys and compared with original species descriptions and other useful literature: Tumanov (2006, 2020), Pilato and Lisi (2006), Claxton (1998), Guidetti et al. (2009, 2022), Pilato and Binda (2010), Michalczyk et al. (2012a, b), Pilato et al. (2016), Kaczmarek et al. (2017), Gąsiorek, Stec, Morek et al. (2018), Stec, Morek et al. (2018), Moreno-Talamantes et al. (2019, 2020), Dueñas-Cedillo et al. (2020), Morek and Michalczyk (2020), Stec, Morek et al. (2018), Stec, Roszkowska et al. (2018), Stec, Kristensen et al. (2020), Stec, Krzywański et al. (2020), Rocha et al. (2022), Kayastha, Mioduchowska et al. (2023), Kayastha, Stec et al. (2023). For comparison with the new species, we also examined the following type material deposited at the Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa, Kraków, Poland: *Paramacrobiotus areolatus* (Murray, 1907) (slides NO.385.63 – NO.385.71, NO.385.75 - NO.385.78 and NO.385.81) and *P. lachowskiae* Stec, Roszkowska, Kaczmarek and Michalczyk, 2018 (slides CO.018.04 - CO.018.21).

Before DNA extraction, specimens were mounted in water on a glass slide and examined under a Nikon Optiphot-2 optical microscope to confirm identification. The DNA was extracted from individual animals following a modified protocol by Casquet et al. (2012), using the Chelex® 100 resin (Bio-Rad) extraction method. Each specimen was placed individually in a 1.5 ml Eppendorf microcentrifuge tube, in 50 µl of a 5% suspension of 75-150 µm wet bead size Chelex® 100 resin (Bio-Rad) in ddH₂O with the addition of 3.5 µl Proteinase K (A&A Biotechnology) and incubated at 56 °C for 1 h. Then, tubes were incubated at 95 °C for 30 min and centrifuged at 4,500 rpm for 15 min. After that, the supernatant was transferred to new 1.5 ml tubes and stored at -20 °C. After the extraction, the hologenophores (Pleijel et al., 2008), were mounted in Hoyer's medium. Four DNA fragments were sequenced: the small ribosome subunit (18S rRNA,

nDNA), the large ribosome subunit (28S rRNA, nDNA), the internal transcribed spacer 2 (ITS-2, nDNA), and the cytochrome oxidase subunit I (COI, mtDNA). Primers and original references for specific PCR programs for amplification of the fragments are listed in Table 1.

For every PCR reaction, the solution contained 9.5 µl ddH₂O, 3 µl 5x MyTaq Reaction Buffer (Bioline™), 0.2 µl 10 mM forward primer, 0.2 ml 10 mM reverse primer, 0.1 µl MyTaq™ DNA Polymerase, (Bioline™) (5U/µl), and 2 µl of genomic DNA extract. The PCR products were controlled by 1.5% agarose gel electrophoresis stained with GelRed Nucleic Acid Gel Stain, 10,000X (Biotium™) and purified with the ExoSap-IT enzyme (Applied Biosystems), following the manufacturer's instructions. The sequence reaction was prepared with 4 µl of water, 2 µl of Buffer 5X, 2 µl of big dye Terminator v3.1 (Applied Biosystems), 1 µl of the primer, and 2.5 µl of the purified product. The reaction was placed in a PCR 2720 with the program suggested by the manufacturer. When finished, they were purified with Sephadex CentriSep™ plates (Princeton) and read in a 3730xl sequencer (Applied Biosystems), at the LANABIO.

All sequences were assembled, manually inspected, and processed in SeqTrace (Stucky, 2012) and submitted to GenBank.

The identity of the obtained sequences was verified using the Basic Local Alignment Search Tool (Altschul et al., 1990). As a support for the morphological comparisons between the new species and other *Paramacrobiotus* species, several sequences deposited in GenBank of the 4 sequenced markers were used to calculate the uncorrected genetic distances (p-distance), applying the program MEGA X (Kumar et al., 2018). The distance matrices are provided in the supplementary material (SM2).

In order to establish the phyletic position of the new species, a phylogenetic tree was constructed using the concatenated 18S rRNA + 28S rRNA + ITS-2 + COI sequences of the genus *Paramacrobiotus* with the sequences of 2 *Minibiotus* species as an outgroup (Table 2). Sequences of the newly barcoded species and sequences of species obtained from GenBank were aligned with the MAFFT algorithm version 7 (Katoh et al., 2002) implemented in the MAFFT online service (Katoh et al., 2019). Sequences were checked by visual inspection and translated to amino acids by using the invertebrate mitochondrial code implemented in MEGA X (Kumar et al., 2018) to check for the presence of pseudogenes. The sequences were concatenated using SequenceMatrix (Vaidya et al., 2011) and before partitioning, the concatenated alignment was divided into 6 data blocks constituting 3 separate blocks of ribosomal markers and 3 separate blocks of 3 codon positions in the COI data

set. We selected the best scheme of partitioning with the program ModelFinder (Kalyaanamoorthy et al., 2017) and the best substitution model for the posterior phylogenetic analysis using ModelTest-NG (Darriba et al., 2020). The best-fit substitution model for each partition under the Bayesian Information Criterion (BIC) was: GTR+G4 for the first and the second codon positions and GTR+I+G4 for the third codon position in COI data set. As for the ribosomal markers the best-fit model was: HKY+G4 for ITS2 marker data set, HKY+I+G4 for 28S marker data set and HKY+I for 18S marker data set. Bayesian inference (BI) marginal posterior probabilities were calculated using MrBayes version 3.2 (Ronquist & Huelsenbeck, 2003). The analysis was run for 10 million generations using random starting trees and sampling the Markov chain every 1,000 generations. An average standard deviation of split frequencies of < 0.01 was used as a guide to ensure the 2 independent analyses had converged. To ensure Markov chains had reached stationarity and to determine the correct “burn-in” for the analysis (which was the first 10% of generations), the program Tracer version 1.7 (Rambaut et al., 2018) was used. The ESS values were > 200 and the consensus tree was obtained after summarizing the resulting topologies and discarding the “burn-in”. Additionally, a maximum likelihood (ML) analysis was run using Iqtree2 (Minh et al., 2020) and the branch support values of the ML tree were measured using 1,000 ultrafast bootstrap replicates (UFBoot) (Hoang et al., 2018). The final consensus trees were viewed and

edited in FigTree version 1.4.4 available from <http://tree.bio.ed.ac.uk/software/figtree>

Results

We obtained 110 tardigrades and 27 eggs from the class Eutardigrada. The specimens examined belong to 2 orders (Apochela and Parachela), 4 families (Milnesiidae, Hypsibiidae, Macrobiotidae, and Ramazzottiidae), 6 genera (*Milnesium*, *Hypsibius*, *Notahypsibius*, *Minibiotus*, *Paramacrobiotus*, and *Ramazzottius*) and 9 species are recorded. We found 3 species which correspond to new records for Mexico and for Mexico City, and 1 species is new to science.

Class Eutardigrada Richters, 1926

Order Apochela Schuster, Nelson, Grigarick and Christenberry, 1980

Family Milnesiidae Ramazzotti, 1962

Genus *Milnesium* Doyère, 1840

Milnesium longiungue Tumanov, 2006

(Fig. 2A-C)

Taxonomic summary

Type locality: Hymalaia, India.

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl), 8 specimens (CNAC-Tar000243 – CNAC-Tar000250). Coll. D. López and G. Montiel.

Table 1

PCR primers for amplification of the 4 DNA fragments sequenced in the present study.

DNA marker	Primer name	Primer direction	Primer sequence (5'-3')	Source	PCR programme source
18S rRNA	SSU01_F	forward	AACCTGGTTGATCCTGCCAGT	Sands et al. (2008)	Zeller (2010)
	SSU82_R	reverse	TGATCCTTCTGCAGGTTACCTAC		
28S rRNA	28S_Eutar_F	forward	ACCCGCTGAACTTAAGCATAT	Gąsiorek, Stec, Zawierucha et al. (2018)	Stec, Kristensen et al. (2020)
	28SR0990	reverse	CCTTGGTCCGTGTTTCAAGAC	Mironov et al. (2012)	
ITS-2	ITS2_Eutar_Ff	forward	CGTAACGTGAATTGCAGGAC	Stec, Morek et al. (2018)	Stec, Kristensen et al. (2020)
	ITS2_Eutar_Rr	reverse	TCCTCCGCTTATTGATATGC		
COI	COI_Para_F	forward	GGTCAACAAATCATAAAGATATTGG	Gąsiorek et al. (2017)	Michalczyk et al. (2012a)
	COI_Mac_Rr	reverse	TAAACTTCAGGGTGACCAAAAAATCA	Stec, Krzywański et al. (2020)	

Table 2

Accession numbers sequences used for phylogenetic analysis downloaded from GenBank.

Taxon	18S rRNA	28S rRNA	ITS-2	COI	Source
<i>Paramacrobiotus</i> aff. <i>richtersi</i> BR.009 1	MH664934	MH664952	MH666082	MH676000	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> BR.009 2	-	-	-	MH676001	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> BR.009 3	-	-	-	MH676002	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> HU.012 1	MH664936	MH664954	MH666084	MH676005	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> HU.012 2	-	-	-	MH676006	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> MG.002 1	MH664938	MH664956	MH666086	MH676008	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> MG.002 2	-	-	MH666087	-	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> NO.386	MH664939	MH664957	MH666088	MH676009	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> NZ.001	MH664940	MH664958	MH666089	MH676010	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus</i> aff. <i>richtersi</i> TZ.018	MH664933	MH664951	MH666095	MH676017	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus arduus</i> Guidetti et al., 2019	MK041032	-	-	MK041020	Guidetti et al. (2019)
<i>Paramacrobiotus areolatus</i> (Murray, 1907)	MH664931	MH664948	MH666080	MH675998	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus bengalenseis</i> Basu et al., 2023	ON923868	-	-	OP531839	Basu et al. (2023)
<i>Paramacrobiotus bifrons</i> (Pontremoli) 1 Brandoli et al., 2024	-	-	PP240910	PP236542	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Pontremoli) 2 Brandoli et al., 2024	-	-	PP240911	PP236543	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Pontremoli) 3 Brandoli et al., 2024	-	-	PP240912	PP236544	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Pontremoli) 4 Brandoli et al., 2024	-	-	PP240913	PP236545	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Gombola) 1 Brandoli et al., 2024	-	-	-	PP236546	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Gombola) 2 Brandoli et al., 2024	-	-	PP240914	PP236547	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Gombola) 3 Brandoli et al., 2024	-	-	PP240915	PP236548	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Gombola) 4 Brandoli et al., 2024	-	-	PP240916	PP236549	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Gombola) 5 Brandoli et al., 2024	-	-	PP240917	PP236550	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Gombola) 6 Brandoli et al., 2024	-	-	PP240918	PP236551	Brandoli et al. (2024)

Table 2. Continued

Taxon	18S rRNA	28S rRNA	ITS-2	COI	Source
<i>Paramacrobiotus bifrons</i> (Sassi di Varana) Brandoli et al., 2024	-	-	-	PP236552	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Monte Sant'Angelo) 1 Brandoli et al., 2024	-	-	PP240919	PP236553	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Monte Sant'Angelo) 2 Brandoli et al., 2024	-	-	PP240920	PP236554	Brandoli et al. (2024)
<i>Paramacrobiotus bifrons</i> (Monte Sant'Angelo) 3 Brandoli et al., 2024	-	-	PP240921	PP236555	Brandoli et al. (2024)
<i>Paramacrobiotus celsus</i> Guidetti et al., 2019	MK041031	-	-	MK041019	Guidetti et al. (2019)
<i>Paramacrobiotus</i> cf. <i>klymenki</i> IT.048	MH664937	MH664955	MH666085	MH676007	Stec, Dudziak et al. (2020)
<i>Paramacrobiotus</i> cf. <i>klymenki</i> PT.006	MH664943	MH664960	MH666092	MH676013	Stec, Dudziak et al. (2020)
<i>Paramacrobiotus depressus</i> Guidetti et al., 2019	MK041030	-	-	MK041015	Guidetti et al. (2019)
<i>Paramacrobiotus experimentalis</i> Kaczmarek et al., 2020	MN073468	MN073465	MN073464	MN097837	Kaczmarek et al. (2020)
<i>Paramacrobiotus fairbanksi</i>	MH664941	MH664950	MH666090	MH676011	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus filipi</i> 1 Dudziak et al., 2020	MT261913	MT261904	-	MT260372	Stec, Dudziak et al. (2020)
<i>Paramacrobiotus filipi</i> 2 Dudziak et al., 2020	-	-	-	MT260373	Stec, Dudziak et al. (2020)
<i>Paramacrobiotus gadabouti</i> MD50.1 Kayastha et al., 2023	OP394210	-	-	OP394113	Kayastha, Stec et al. (2023)
<i>Paramacrobiotus gadabouti</i> MD50.4 Kayastha et al., 2023	OP394212	-	-	OP394114	Kayastha, Stec et al. (2023)
<i>Paramacrobiotus gadabouti</i> AU.044 Kayastha et al., 2023	MH664932	MH664949	MH666081	MH675999	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus gadabouti</i> FR.077 1 Kayastha et al., 2023	MH664935	MH664953	MH666083	MH676003	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus gadabouti</i> FR.077 2 Kayastha et al., 2023	-	-	-	MH676004	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus gadabouti</i> PT.048 1 Kayastha et al., 2023	MH664944	MH664961	MH666093	MH676014	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus gadabouti</i> PT.048 2 Kayastha et al., 2023	-	-	-	MH676015	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus gadabouti</i> TN.014 Kayastha et al., 2023	MH664945	MH664962	MH666094	MH676016	Stec, Krzywański et al. (2020)
<i>Paramacrobiotus lachowskiae</i> Stec et al., 2018	MF568532	MF568533	MF568535	MF568534	Stec, Roszkowska et al. (2018)
<i>Paramacrobiotus metropolitanus</i> Sugiura et al., 2022	LC637243	LC649795	LC649794	LC637242	Sugiura et al. (2022)
<i>Paramacrobiotus puma</i> sp. nov.	PP416751	PP416752	PP416753	PP414782	Present study
<i>Paramacrobiotus richtersi</i> (Murray, 1911)	MK041023	-	-	MK040994	Guidetti et al. (2019)
<i>Paramacrobiotus richtersi</i> S38 (Murray, 1911)	OK663224	OK663235	OK663213	OK662995	Vecchi et al. (2022)

Table 2. Continued

Taxon	18S rRNA	28S rRNA	ITS-2	COI	Source
<i>Paramacrobiotus spatialis</i> Guidetti et al., 2019	MK041024	-	-	MK040996	Guidetti et al. (2019)
<i>Paramacrobiotus spatialis</i> S107 Guidetti et al., 2019	OK663225	OK663236	OK663214	OK662996	Vecchi et al. (2022)
<i>Paramacrobiotus tonolli</i> US (Ramazzotti, 1956)	MH664946	MH664963	MH666096	MH676018	Stec, Krzywański et al. (2020)
<i>Minibiotus ioculator</i> Stec et al. 2020	MT023998	MT024041	MT024000	MT023412	Stec, Kristensen et al. (2020)
<i>Minibiotus pentannulatus</i> Londoño et al., 2017	MT023999	MT024042	MT024001	MT023413	Stec, Kristensen et al. (2020)

Habitat: high elevation xerophilic scrub. Elevation: 2,260 m asl.

Microhabitat: mosses of the species *Thuidium delicatulum* (Hedw.) Schimp. collected on a rock.

Remarks. The specimens examined correspond well to the original description by Tumanov (2006). The cuticle is smooth and white colored, the eyes are present, 6 peribuccal lamellae are present, accessory points on primary branches are absent, secondary branches of external claws I-III and of posterior claws IV with 3 points (claw configuration [3-3] - [3-3]), *pt* of the primary branch IV length is between 82-91% and cuticular bars under the I-III are present. This species has been recorded previously in China (Beasley & Miller, 2007). This is the second record of the species outside the type locality in Hymalaia, India. New record for Mexico.

Milnesium cf. *reductum*

Taxonomic summary

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl), 5 specimens (CNAC-Tar000238 - CNAC-Tar000242). Coll. D. López and G. Montiel.

Habitat: high elevation xerophilic scrub. Elevation: 2,260 m asl.

Microhabitat: mosses of the species *Thuidium delicatulum* collected on a rock.

Remarks. The 5 specimens examined present a smooth and reddish colored cuticle, the eyes are present, 6 peribuccal lamellae are present, secondary branches of external claws I-III and of posterior claws IV with 2 points (claw configuration [2-3] - [3-2]), *pt* of the primary branch IV length is between 65-69% and the cuticular bars under legs I-III are present. Those characters correspond to the original description of *Milnesium reductum* Tumanov,

2006 (Tumanov, 2006); however, we were unable to confirm the presence or absence of accessory points on primary branches in all specimens. Therefore, due to the lack of a greater number of specimens for examination, for morphometric measurements and for sequencing of genetic material, this species cannot be identified with certainty.

Order Parachela Schuster, Nelson, Grigarick and Christenberry, 1980
 Superfamily Hypsibioidea Pilato, 1969
 Family Hypsibiidae Pilato, 1969
 Subfamily Hypsibiinae Pilato, 1969
 Genus *Hypsibius* Ehrenberg, 1848
Hypsibius cf. *dujardini*

Taxonomic summary

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W, 2,260 m asl), 3 specimens (CNAC-Tar000224 - CNAC-Tar000226). Coll. D. López and G. Montiel.

Habitat: high elevation xerophilic scrub. Elevation: 2,260 m asl.

Microhabitat: mosses of the species *Amblystegium varium* (Hedw.) Lindb. collected on a rock.

Remarks. The specimen traits correspond to the redescription of *Hypsibius dujardini* (Doyère, 1840) made by Gąsiorek, Stec, Morek et al. (2018). However, we cannot confirm the presence or absence of cuticular bars on legs I-III in all specimens. Therefore, due to the lack of a greater number of specimens for morphometric measurements and for sequencing of genetic material, this species cannot be identified with certainty.

Subfamily Pilatobiinae Bertolani, Guidetti, Marchioro, Altiero, Rebecchi and Cesari, 2014

Genus *Notahypsibius* Tumanov, 2020

Notahypsibius pallidoides Pilato, Kiosya, Lisi, Inshina and Biserov, 2011
(Fig. 2D-F)

Taxonomic summary

Type locality: Ukraine.

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl), 10 specimens (CNAC-Tar000214 - CNAC-Tar000223). Coll. D. López and G. Montiel.

Habitat: high elevation xerophilic scrub. Altitude: 2,260 m asl.

Microhabitat: mosses of the species *Orthostichella rigida* (Müll. Hal.) B.H. Allen & Magill collected on a rock.

Remarks. The specimens examined present *Ramazzottius*-like claws (Fig. 2E), a smooth cuticle, the *pt* of the stylet support insertion point between 56.9-57.8%, the eyes are present, a pharyngeal bulb with 2 elongated macroplacoids and a minute dot-like septulum is present (Fig. 2E). The accessory points on primary branches and the lunules under the claws are present (Fig. 2F). The cuticular bars on all legs are absent. Specimen traits correspond to the original description of *Notahypsibius pallidoides* by Pilato et al. (2011). Also, they were compared with the redescription by Tumanov (2020). The species represents a new record for Mexico.

Family Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008
Genus *Ramazzottius* Binda & Pilato, 1986
Ramazzottius cf. *oberhaeuseri*

Taxonomic summary

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl), 11 specimens (CNAC-Tar000227 - CNAC-Tar000237) and 3 eggs (CNAC-Tar000282 - CNAC-Tar000284). Coll. D. López, G. Montiel, L. Piña and M. Hernández.

Habitat: high elevation xerophilic scrub. Elevation: 2,260 m asl.

Microhabitat: lichens of the species *Heterodermia* sp. collected on a tree.

Remarks. The specimens and eggs traits correspond to the redescription of *Ramazzottius oberhaeuseri* (Doyère, 1840) made by Stec, Morek et al. (2018). However, due to the lack of a greater number of specimens for morphometric measurements and for sequencing of genetic material, this species cannot be identified with certainty.

Superfamily Macrobiotoidea Thulin, 1928

Family Macrobiotidae Thulin, 1928

Genus *Minibiotus* R.O. Schuster, 1980

Minibiotus cf. *continuus*

Taxonomic summary

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl), 7 specimens (CNAC-Tar000282 - CNAC-Tar000288) and 3 eggs (CNAC-Tar000289 - CNAC-Tar000291). Coll. D. López and G. Montiel.

Habitat: high elevation xerophilic scrub. Elevation: 2,260 m asl.

Microhabitat: mosses of the species *Syntrichia amphidiacea* (Müll. Hal.) R.H. Zander collected on a tree.

Remarks. The traits of the specimens and eggs correspond to the original description of *Minibiotus continuus* Pilato and Lisi, 2006 (Pilato & Lisi, 2006). However, the eggs examined differ in size from the egg in the original description, which has a diameter of 46.2 µm excluding the processes, and 52.2 µm including them. In contrast, the eggs examined in the present study the diameter is between 42.5 - 44.3 µm excluding the processes and between 49.8 - 51.9 µm including them. Therefore, due to the lack of a greater number of specimens for morphometric measurements, this species cannot be identified with certainty.

Genus *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar and Wolf, 2009

Paramacrobiotus gadabouti Kayastha, Stec, Mioduchowska and Kaczmarek, 2023
(Fig. 2G-I)

Taxonomic summary

Type locality: Portugal.

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl), 7 specimens (CNAC-Tar000204 - CNAC-Tar000210), 3 eggs (CNAC-Tar000211 - CNAC-Tar000213) and 2 eggs were prepared for SEM. Coll. D. López and G. Montiel.

Habitat: high elevation xerophilic scrub. Elevation: 2,260 m asl.

Microhabitat: mosses of the species *Syntrichia amphidiacea* collected on a tree.

Remarks. The specimens examined lack eyes, and present a smooth cuticle, a *pt* of the stylet support insertion point between 77.2-80.5%, granulation on the external surface of legs I-III, smooth lunules under all claws and accessory points on primary branches. The

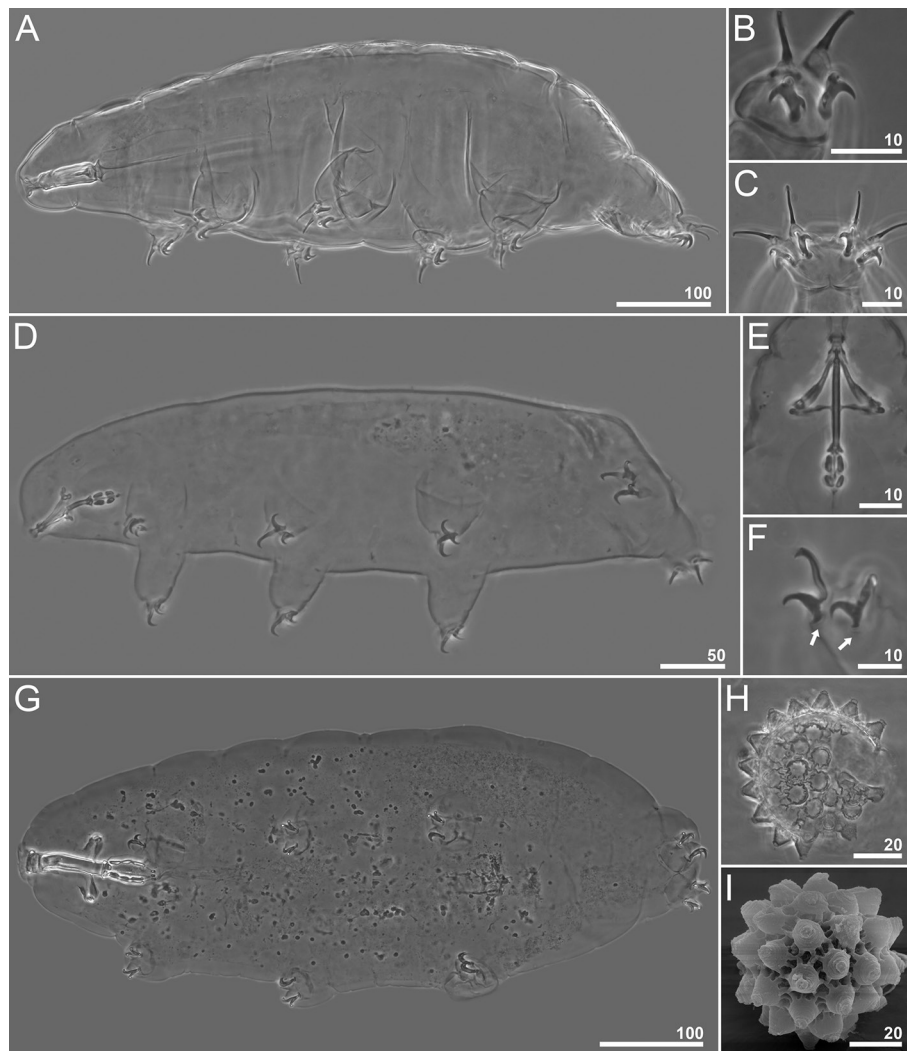


Figure 2. Tardigrade species that represent new records for Mexico. A-C, *Milnesium longiungue*: A, habitus; B, claws II; C, claws IV. D-F, *Notahypsibius pallidoides*: D, habitus; E, buccopharyngeal apparatus; F, claws III; white arrows indicate the thickened region on the lunule margin. G-I, *Paramacrobiotus gadabouti*: G, habitus; H, egg seen on PCM; I, egg seen on SEM. Scale bars = μm .

eggs are areolated, of *richtersi* type with a single ring of 10-12 areolae around each process. The top endings of the processes present cap like structures. Specimens and eggs traits correspond to the original description of *Paramacrobiotus gadabouti* (Kayastha, Stec et al., 2023), also they were examined following the diagnostic key by Kayastha, Mioduchowska et al. (2023). In addition to the type locality in Madeira Island, Portugal, this species has also been recorded in Australia, France and Tunisia (Kayastha, Stec et al., 2023). A new record for Mexico.

Paramacrobiotus puma López-Sandoval, Montiel-Parra and Pérez sp. nov.

(Tables 3, 4; Figs. 3-9)

<http://zoobank.org/urn:lsid:zoobank.org:act:4C178E55-DA78-4EFD-88A5-186B7B716A72>

Taxonomic summary

Type locality: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl). Mexico City, Mexico.

Material examined: Mexico: Mexico City: Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl). Coll. D. López, G. Montiel, L. Piña and M. Hernández.

Microhabitat: lichens of the species *Heterodermia* cf. *tremulans*, collected from a tree trunk.

Type material. Holotype and 55 paratypes (32 specimens and 23 eggs). Additionally, 2 specimens and 2 eggs were prepared for SEM photographs, and 2 specimens were processed for DNA sequencing.

Holotype (CNAC-TTar000011). Mexico City, Mexico: Reserva Ecológica del Pedregal de San Ángel, Cantera Oriente (19°19'00.5" N, 99°10'21.5" W; 2,260 m asl).

Paratypes. (CNAC- TTar000001 - CNAC-TTar000056). The same data as for the holotype.

Type specimen's depositories. Holotype (CNAC-TTar000011) and 51 paratypes (30 specimens and 21 eggs) (CNAC-TTar000001 - CNAC-TTar000056) are deposited at the Tardigrada Collection associated with the National Collection of Mites (CNAC) of the Instituto de Biología, UNAM, Mexico City (Mexico). Additionally, 4 paratypes (2 specimens and 2 eggs) (slides CNAC-TTar000030, CNAC-TTar000032, CNAC-TTar000050 and CNAC-TTar000053) are deposited in the Department of Animal Taxonomy and Ecology, Institute of Environmental Biology, Adam Mickiewicz University, Poznań, Poland, and 4 paratypes (2 specimens and 2 eggs) (slides CNAC-TTar000029, CNAC-TTar000031, CNAC-TTar000040 and CNAC-TTar000043) are deposited in the Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland.

Etymology. The new species is named after the Puma, the official mascot which is the emblem of the National Autonomous University of Mexico (UNAM).

Description (measurements and statistics in Table 3).

White body in live specimens, transparent in specimens mounted in Hoyer's medium (Fig. 3A-C). Eyes are present in live adult animals (present in 18 of the 25 specimens examined mounted in Hoyer's medium) and absent in a hatching specimen examined (Fig. 3C). Cuticle smooth, without gibbosities, papillae, pores, spines, or ornaments. Small areas of granulation in the first 3 pairs of legs are present, specifically on the external surfaces near the claw bases, and as for the hind legs, the granulation is extended from the claws onto the entire dorsal surface of the legs (Fig. 4). Claws robust, of the *hufelandi* type (Fig. 4A, B). Smooth lunules are present under all claws (Fig. 4C, D). Accessory points on primary branches are present. Cuticular bars under the claws are absent.

Mouth antero-ventral with 10 peribuccal lamellae. Buccopharyngeal apparatus of the *Macrobiotus* type with ventral lamina present (Fig. 5A). The oral cavity armature is composed of 3 bands of teeth (Fig. 6). The first band of teeth consists of small cones (granules in PCM) positioned at the base of the buccal lamellae. The second band of teeth is composed of larger cones arranged in one row around the oral cavity and positioned in the rear of the

oral cavity between the ring fold and the third band of teeth. The third band of teeth is positioned just before the buccal tube opening and is composed of dorsal and ventral transversal ridges, organized into 2 large lateral ridges and a smaller median ridge (Fig. 6C, D).

The pharyngeal bulb is spherical, with triangular apophyses and 3 rod-shaped macroplacoids (Fig. 5B). Macroplacoid length sequence is $2 < 1 < 3$. The first macroplacoid is without constrictions but narrower anteriorly. The second macroplacoid is of uniform width and without constrictions. The third macroplacoid presents a sub-terminal constriction. Microplacoid is absent, however, a sclerified line and a rudimentary microplacoid-like thickening are present after the third macroplacoid in adult specimens (Fig. 5A, B). In the hatching specimen examined, the microplacoid-like thickening is also present (Fig. 3C).

Eggs (measurements and statistics in Table 4). Eggs of the *richtersi* type. Laid free, white, spherical, and with 12-15 cone-shaped processes on the circumference with a variable termination (Figs. 3C; 7A-D; 9A-F). The apices can be very short or thin and long (Fig. 8A-D). The labyrinth layer between the walls of the processes is visible under PCM as a reticular pattern (Fig. 8B). Between 12 and 14 areolae around each process. The internal surface of the areolae is sculpted with a reticular pattern with pores (Figs. 7D; 9E, F).

DNA sequences. We obtained DNA sequences for all 4 molecular markers from 1 hologenophore (voucher number: CNAC-TTar000033). The sequence length and GenBank accession number of each marker are as follows: 18s rRNA (GenBank: PP416751), 1632-bp long; 28s rRNA (GenBank: PP416752), 737-bp long; ITS-2 (GenBank: PP416753), 419-bp long; COI (GenBank: PP414782), 631-bp long.

Phenotypic differential diagnosis. The genus *Paramacrobiotus* is divided into 2 morphologically distinct species groups: the *richtersi* group (species with a microplacoid in the pharynx) and the *areolatus* group (species without a microplacoid or with rudimentary structures in the place of microplacoid in the pharynx). Also, there are 7 types of eggs, being the *areolatus* and *richtersi* the most common types (Kaczmarek et al., 2017; Kayastha, Mioduchowska et al., 2023). Since the microplacoid is absent in *Paramacrobiotus puma* sp. nov., it belongs to the *areolatus* group along with 12 other species: *P. areolatus* (Murray, 1907), *P. bifrons* Brandoli, Cesari, Massa, Vecchi, Rebecchi and Guidetti, 2024, *P. centesimus* (Pilato, 2000), *P. csotiensis* (Iharos, 1966), *P. derkai* (Degma, Michalczyk & Kaczmarek, 2008), *P. huziori* (Michalczyk & Kaczmarek, 2006), *P. intii* Kaczmarek, Cytan, Zawierucha, Diduszko and

Table 3

Measurements (in μm), and values of the *pt* index, of some structures of the holotype and paratypes of *Paramacrobiotus puma* sp. nov.

Character	N	Range		Mean		Sd		Holotype	
		μm	<i>pt</i>	μm	<i>pt</i>	μm	<i>pt</i>	μm	<i>pt</i>
Body length	25	364-678	814-1106	470	944	76	75	576	958
Buccal tube									
Length	25	36-61.3	-	49.7		7.2	-	60.1	-
Stylet support insertion point	25	27.2-46.5	74.4-81.2	38.6	77.8	5.3	1.4	45.9	76.3
External width	25	5.6-11.8	15.6-25.2	9.6	19.3	1.6	1.2	10.5	17.5
Internal width	25	4.3-9.7	11.6-20	7.4	14.9	1.3	1.2	8.1	13.5
Ventral lamina length	15	21.7-40.3	54.8-75.5	30	62	5.6	3	34	56.6
Placoid lengths									
Macroplacoid 1	25	5.5-12.7	13.8-20.7	8.6	17.2	1.9	1.5	11.7	19.5
Macroplacoid 2	25	4.9-9.6	10.5-18.4	6.6	13.3	1.4	1	8.6	14.3
Macroplacoid 3	25	5.7-13	15.8-21.5	9.5	19	2.2	1.8	12.8	21.3
Macroplacoid row	25	18-35.9	49.4-67.3	27.8	55.8	5	2.5	33.4	55.6
Claw 1 lengths									
External primary branch	25	8.8-17.6	16.8-31.4	12.8	25.9	1.8	1.8	14.4	24
External secondary branch	22	7-12.8	13.4-26.2	9.6	19.2	1.4	2	11.2	18.6
Internal primary branch	23	6.4-14.8	12.2-28.9	11.6	23.5	1.7	1.7	14.1	23.5
Internal secondary branch	21	3.2-11.7	6.1-24.7	8.2	16.6	1.3	2	10.4	17.3
Claw 2 lengths									
External primary branch	24	10.3-17.4	22-31.4	13.1	26.4	2.1	2.6	15.1	25.1
External secondary branch	25	6.4-12.9	12.2-25.3	9.8	19.7	1.8	2.1	12.8	21.3
Internal primary branch	25	7.2-16	13.8-28.9	12	24.1	2.1	1.8	14.8	24.6
Internal secondary branch	25	5.6-12.8	10.7-23.6	9.3	18.7	1.9	2.2	11.6	19.3
Claw 3 lengths									
External primary branch	24	11.2-18.4	24.3-31.9	14.1	28.4	2	1.4	16	26.6
External secondary branch	23	8-13.2	15.7-24.5	10.3	20.7	1.7	1.5	13	21.6
Internal primary branch	23	9.6-17.6	23.6-31.5	13.3	26.9	2.1	2.1	15.2	25.3
Internal secondary branch	22	7.7-12.6	17-22.6	9.6	19.5	1.5	1.5	11.2	18.6
Claw 4 lengths									
Anterior primary branch	24	10.2-20.8	19.5-33.9	14.7	29.6	2.3	1.8	17.6	29.3
Anterior secondary branch	23	5.6-15.2	10.7-24.8	10.6	21.2	2	1.7	13.6	22.6
Posterior primary branch	25	11.4-21.6	21.7-35.2	15.6	31.4	2.5	1.4	19.2	31.9
Posterior secondary branch	24	7.2-16	13.8-27.4	11.5	23.2	1.7	1.7	13.1	21.8

N = Number of specimens/structures measured; Range = the smallest and the largest structure among all measured specimens; Sd = standard deviation.

Table 4

Measurements (μm) of morphological structures of eggs of *Paramacrobiotus puma* sp. nov.

Character	N	Range	Mean	Sd
Diameter of egg without processes	22	72-107.1	89.9	8.1
Diameter of egg with processes	22	94.4-131.9	117.1	10.4
Process height	66	11.2-27	16.5	3.2
Process base width	66	9.9-21.1	15.7	2.3
Process base/height ratio	66	49%-144%	97%	18%
Distance between processes	66	5.6-10.4	7.8	1.1
Number of processes on the egg circumference	19	12-15	13.1	0.8

N = Number of eggs/structures measured; Range = the smallest and the largest structure among all measured specimens; Sd = standard deviation.

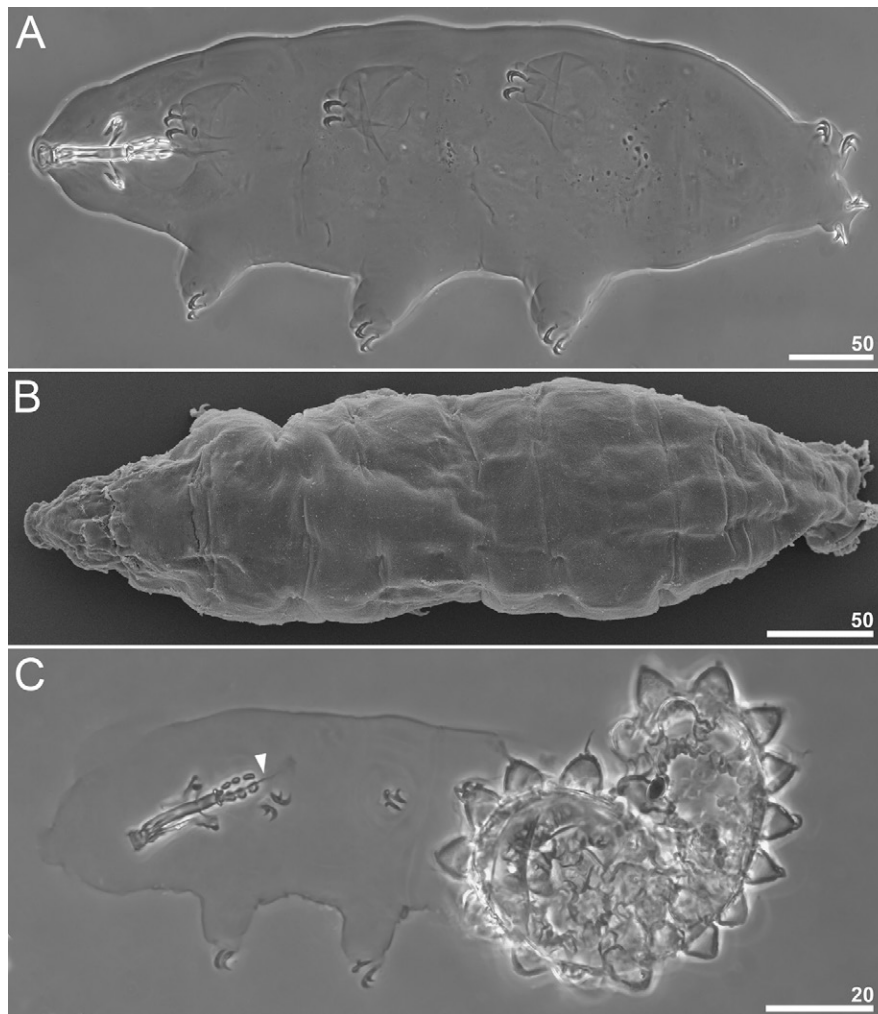


Figure 3. *Paramacrobiotus puma* sp. nov. Habitus: A, dorso-ventral projection of the entire animal (holotype, PCM); B, dorsal view of the entire animal (paratype, SEM); C, juvenile hatching from the egg (paratype); arrowhead indicates a sclerified line after the third macroplacoid. Scale bars = μm .

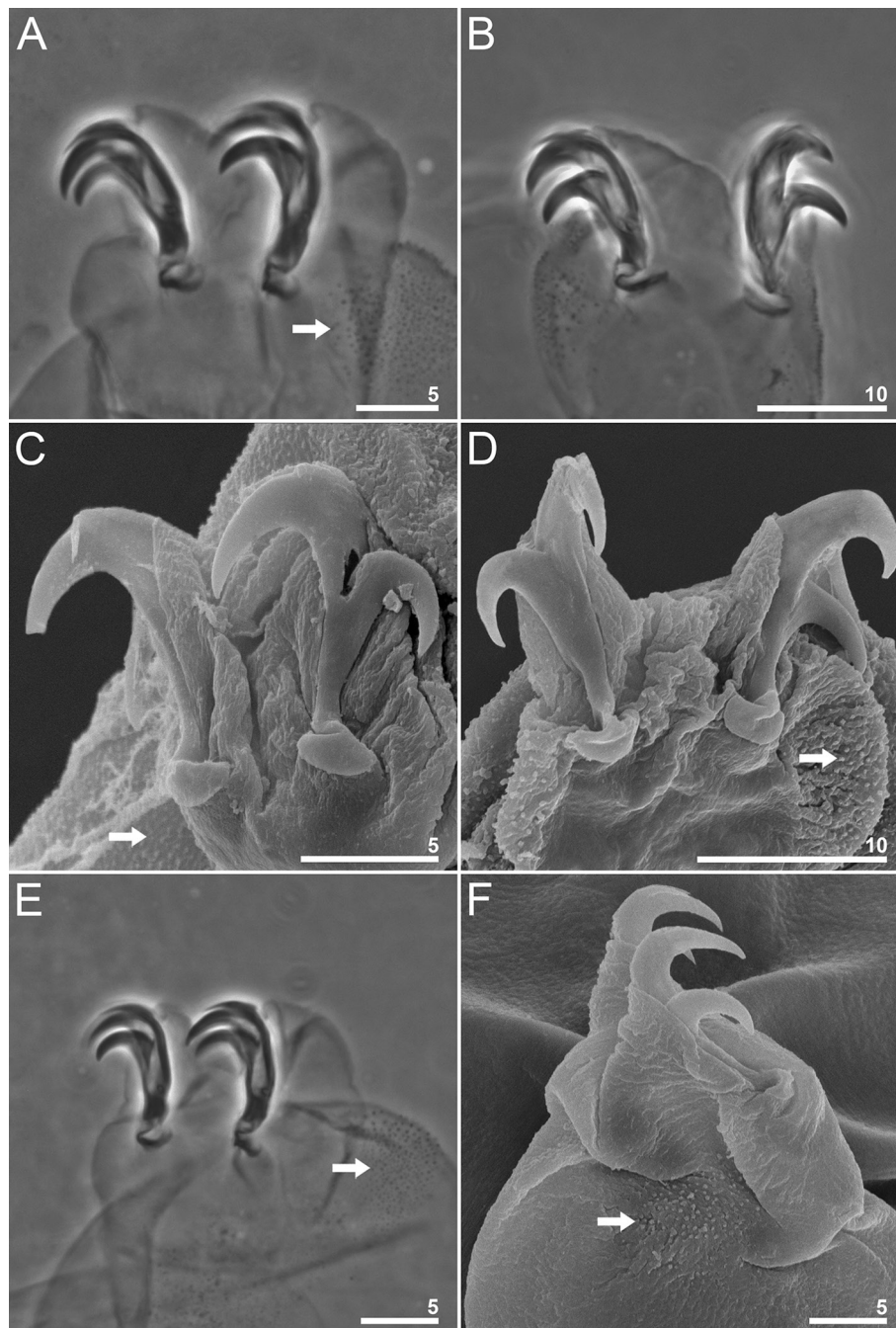


Figure 4. *Paramacrobiotus puma* sp. nov. Claws and leg granulation: A-B, claws II and IV, respectively (holotype PCM); C-D, claws II and IV, respectively, with smooth lunules seen in SEM (paratype); E-F, claws III seen in PCM (holotype) and SEM (paratype) respectively; arrows indicate the granulation on the external surface of the legs. Scale bars = μm .

Michalczyk, 2014, *P. klymenki* Pilato, Kiosya, Lisi and Sabella, 2012, *P. lachowskiae* Stec, Roszkowska, Kaczmarek and Michalczyk, 2018, *P. spinosus* Kaczmarek, Gawlak, Bartels, Nelson and Roszkowska, 2017, *P.*

tonollii (Ramazzotti, 1956) and *P. walteri* (Biserov, 1998) (Biserov, 1998; Brandoli et al., 2024; Degma et al., 2008; Iharos, 1966; Kaczmarek, Michalczyk et al., 2014, 2017; Michalczyk & Kaczmarek, 2006; Murray, 1907;

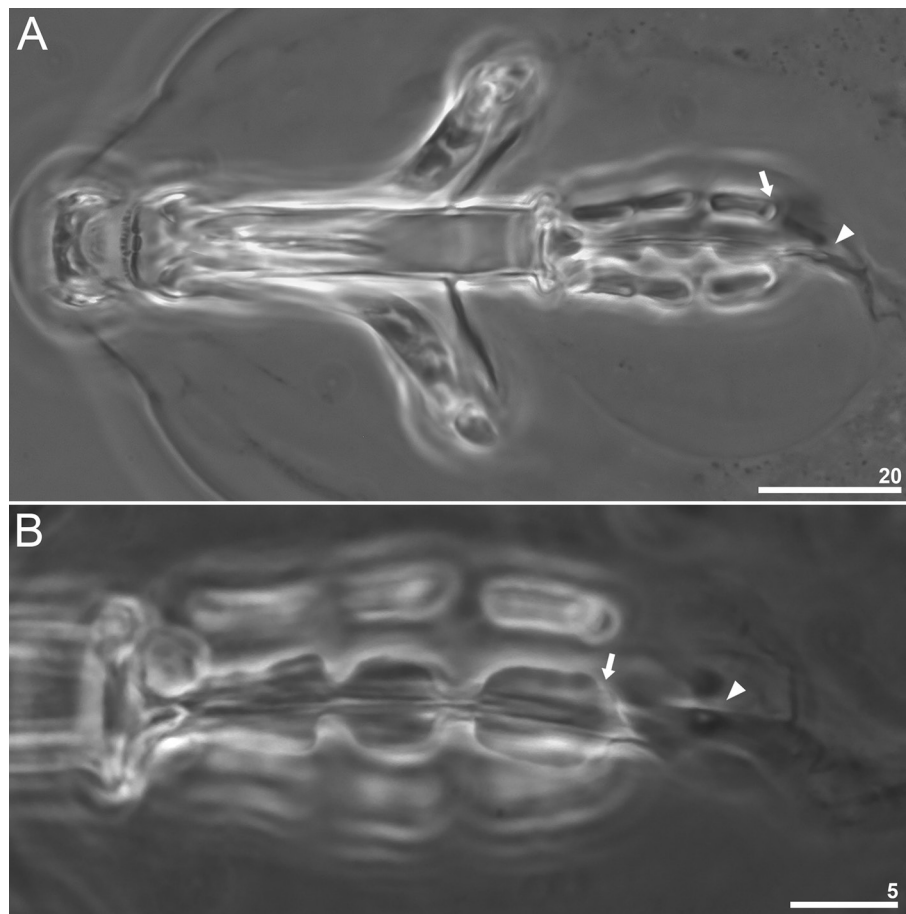


Figure 5. *Paramacrobiotus puma* sp. nov. Buccopharyngeal apparatus (dorso-ventral projection in PCM): A, general view (paratype); B, dorsal placoids (paratype); arrows indicate the subterminal constriction in the third macroplacoid; arrowhead indicates a sclerified line and a rudimentary microplacoid-like thickening after the third macroplacoid. Scale bars = μm .

Pilato, 2000; Pilato et al., 2012; Ramazzotti, 1956; Stec, Roszkowska et al., 2018). Moreover, *Paramacrobiotus puma* sp. nov., presents the *richtersi* type of egg, and is most similar to 7 species within the *areolatus* group: *P. areolatus*, *P. centesimus*, *P. intii*, *P. klymenki*, *P. lachowskiae*, *P. spinosus* and *P. walteri*. Nevertheless, the new species can be differentiated specifically from all species mentioned above by the following traits.

Paramacrobiotus puma sp. nov. differs from *P. areolatus*, restricted only to Svalbard and Greenland (McInnes, 1994; Stec, Krzywański et al., 2020), by the presence of smooth lunules under the IV claws (crenate in *P. areolatus*) and a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *areolatus* type in *P. areolatus*). From *P. bifrons*, recorded only in Italy (Brandoli et al., 2024), by the presence of smooth lunules under the IV claws (clearly indented larger lunules on legs IV in *P.*

bifrons) and by a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. 2 egg types in *P. bifrons*: *areolatus* type and *csotiensis* type). From *P. centesimus*, recorded only in Brazil and Ecuador (Kaczmarek et al., 2015; Pilato, 2000), by a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *areolatus* type in *P. centesimus*), longer egg processes ($11.2\text{--}27.0\text{ }\mu\text{m}$ in *P. puma* sp. nov. vs. $7.0\text{--}11.0\text{ }\mu\text{m}$ in *P. centesimus*), and by larger full egg diameter ($94.4\text{--}131.9\text{ }\mu\text{m}$ in *P. puma* sp. nov. vs. $76.0\text{--}91.0\text{ }\mu\text{m}$ in *P. centesimus*). From *P. csotiensis*, recorded only in Hungary (Iharos, 1966), by a different type of egg (egg processes shape blunt with a transparent cover [*csotiensis* type] in *P. csotiensis* vs egg processes shape conical without a transparent cover [*richtersi* type] in *P. puma* sp. nov.), and by different egg diameters (diameters with processes of $75\text{--}80\text{ }\mu\text{m}$ and without processes of $60\text{--}65\text{ }\mu\text{m}$ in *P. csotiensis* vs $94.4\text{--}131.9\text{ }\mu\text{m}$ with processes

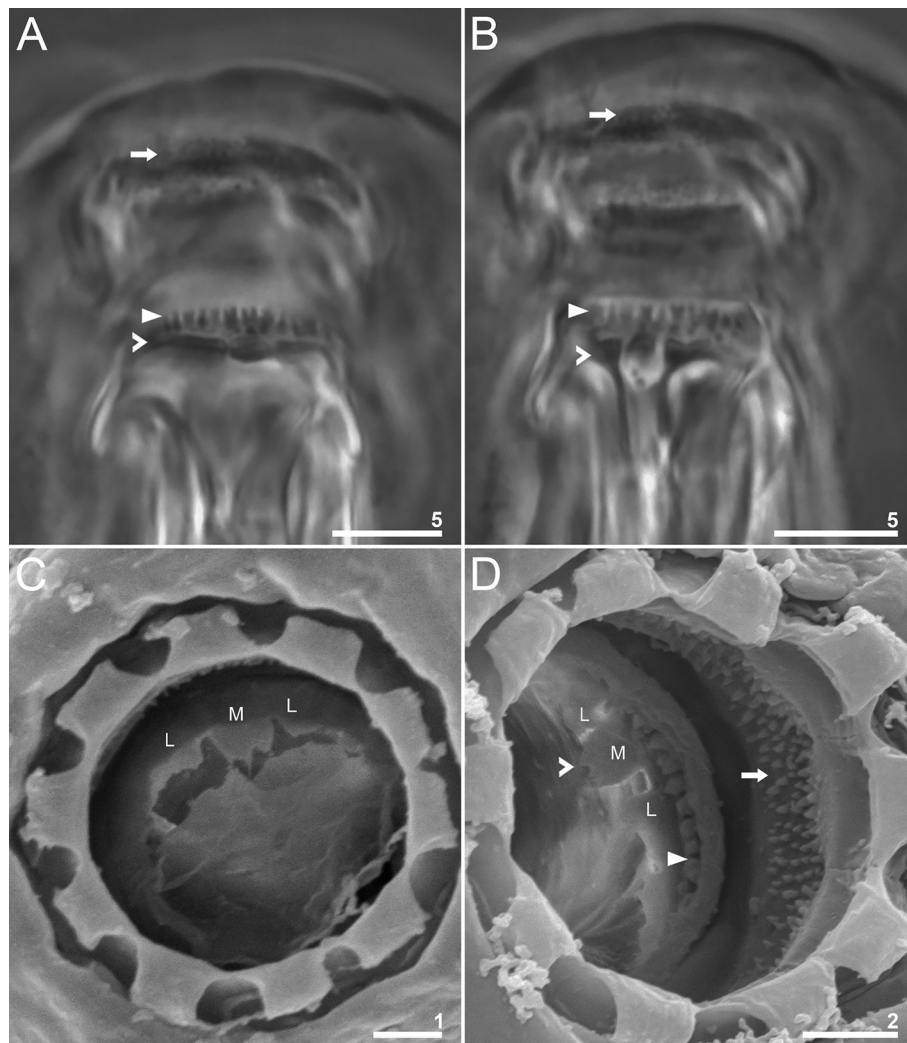


Figure 6. *Paramacrobiotus puma* sp. nov. Oral cavity armature: A-B, dorsal and ventral views, respectively (paratype, PCM); C-D, dorsal and ventral views, respectively (paratypes, SEM); the arrow indicates teeth of the first band; flat arrowheads indicate teeth of the second band; indented arrowheads indicate teeth of the third band; letters indicate lateral (L) and median (M) crests. Scale bars = μm .

and of 72.0-107.1 μm without processes in *P. puma* sp. nov.). From *P. derkai*, recorded only in Colombia and Peru (Degma et al., 2008; Kaczmarek et al., 2016), by less protruding accessory points on primary branches, for a different type of egg (*richtersi* type in *P. puma* sp. nov. vs *huziori* type in *P. derkai*), spaces between neighbor areolae (narrower than areolae widths in *P. puma* sp. nov. vs. usually broader than the areolae widths in *P. derkai*), and by longer egg processes (11.2-27.0 μm *P. puma* sp. nov. vs. 8.0-17.1 μm in *P. derkai*). From *P. huziori*, recorded only in Costa Rica (Michalczyk & Kaczmarek, 2006; Kaczmarek, Michalczyk et al., 2014), by leg granulation

in aggregations of small granules or cones absent (present in *P. huziori*), a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *huziori* type in *P. huziori*), shorter egg processes (11.2-27 μm in *P. puma* sp. nov. vs. 20.0-33.0 in *P. huziori*), and by the number of egg processes (12-15 in *P. puma* sp. nov. vs. 9-11 in *P. huziori*). From *P. intii*, recorded only in Peru (Kaczmarek, Cytan et al., 2014), by the oral cavity armature, showing bands I to III in PCM (only I and II in *P. intii*), a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *areolatus* type in *P. intii*), shorter egg processes (11.2-27 μm in *P. puma* sp. nov. vs. 15.4-24.4 in *P. intii*), and by the number of egg processes

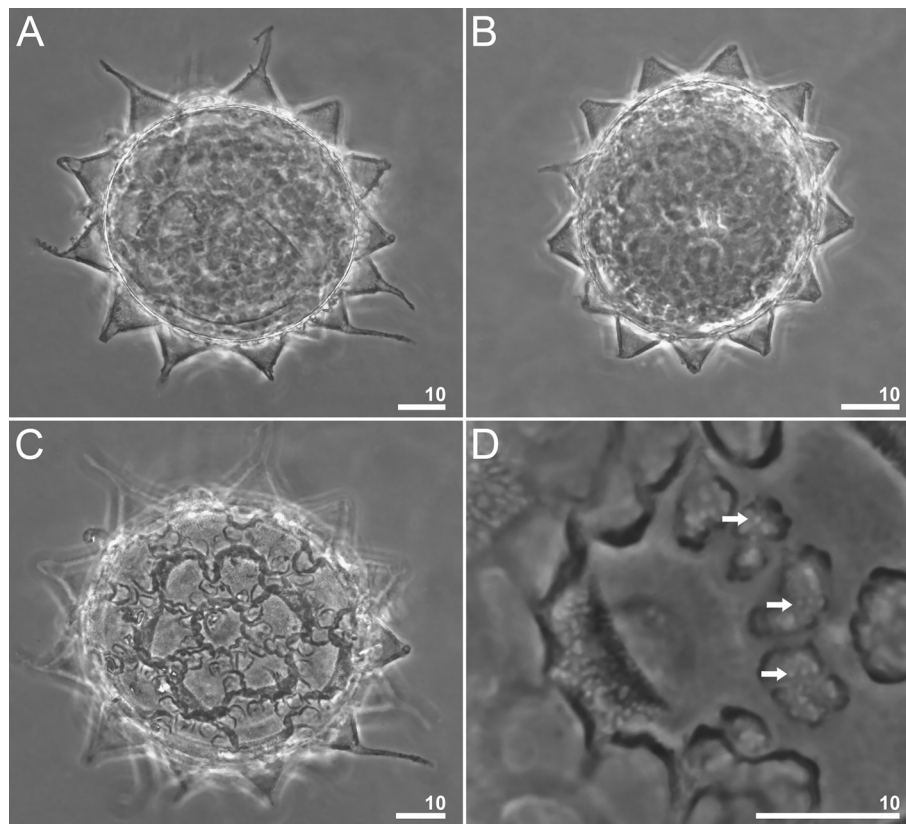


Figure 7. *Paramacrobiotus puma* sp. nov. Eggs seen in PCM: A-B, midsection; C, the surface of the egg seen in PCM; D, a closer look at the areolation around a process; the arrows indicate the smaller areoles. Scale bars = μm .

(12-15 in *P. puma* sp. nov. vs. 9-10 in *P. intii*). From *P. klymenki*, recorded only in Belarus (Pilato et al., 2012), by presenting eyes (absent in *P. klymenki*), lunules IV smooth (crenate in *P. klymenki*), a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *areolatus* type in *P. klymenki*), and by the number of egg processes (12-15 in *P. puma* sp. nov. vs. 10-11 in *P. klymenki*). From *P. lachowskiae*, recorded only in Colombia (Stec, Roszkowska et al., 2018), by macroplacoid length sequence ($2 < 1 < 3$ in *P. puma* sp. nov. vs. $2 < 3 \leq 1$ in *P. lachowskiae*), a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *areolatus* type in *P. lachowskiae*), egg processes shape (conical processes with filaments not covered with hairs in *P. puma* sp. nov. vs. dome-like, wrinkled, and with long flexible spines/filaments covered by fine short hairs in *P. lachowskiae*). From *P. spinosus*, recorded only in Ecuador (Kaczmarek et al., 2017), by the presence of eyes (absent in *P. spinosus*), egg processes shape (conical processes with transverse annulations smooth in *P. puma* sp. nov. vs. transverse annulations associated with short spines in the upper parts of egg processes in *P. spinosus*), by the

number of egg processes (12-15 in *P. puma* sp. nov. vs. 10-11 in *P. spinosus*), and by the number of areolae on egg surface (between 12 and 14 areolae around each process in *P. puma* sp. nov. vs. 10 areolae in *P. spinosus*). From *P. tonollii*, recorded in Lapland (Finland), USA, and Canada (McInnes, 1994), by a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *tonollii* type in *P. tonollii*) and by the number of egg processes (12-15 in *P. puma* sp. nov. vs. 8-10 in *P. tonollii*). And from *P. walteri*, known only from Russia (Biserov, 1998), by the presence of smooth lunules under claws IV (dentate in *P. walteri*), and by a different type of egg (*richtersi* type in *P. puma* sp. nov. vs. *areolatus* type in *P. walteri*).

Genotypic differential diagnosis. The ranges of uncorrected genetic p-distances from the most to the least conservative between the new species and other species of the genus *Paramacrobiotus* for which sequences are available from GenBank, are as follows: 18S rRNA: 0.06-5.02% (1.66% on average), with the most similar being *Paramacrobiotus lachowskiae* from Magdalena Province, Colombia (MF568532), and the least similar

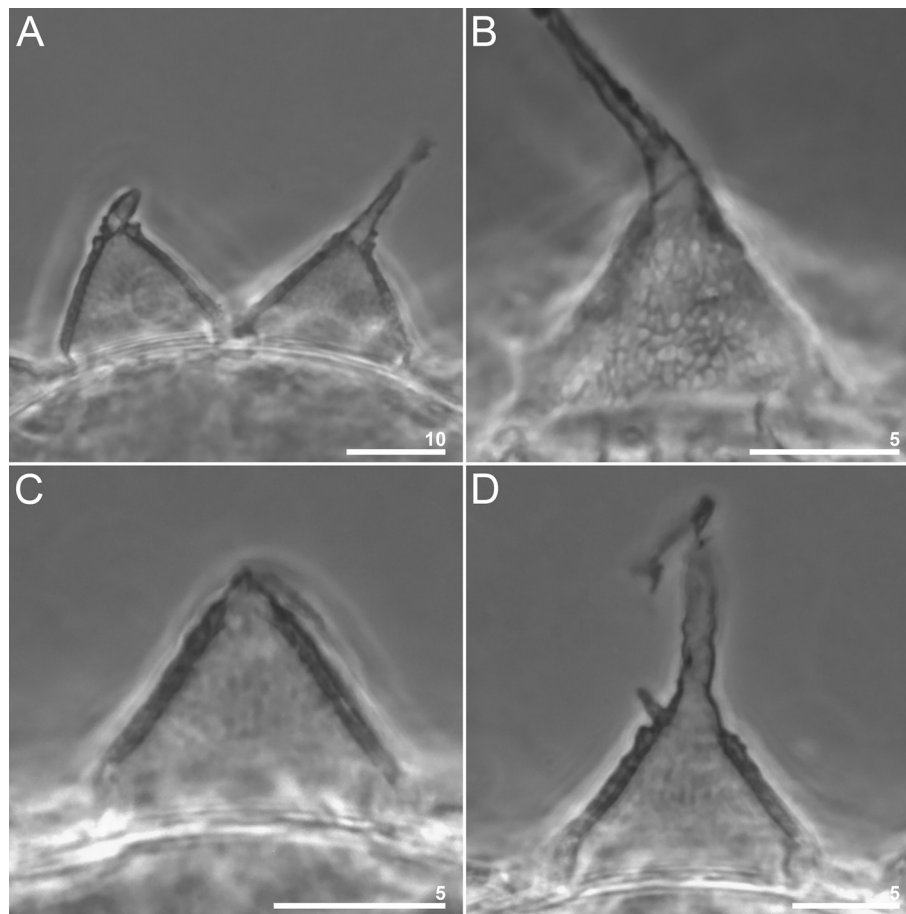


Figure 8. *Paramacrobotus puma* sp. nov. Egg processes morphology seen in PCM: A, midsection of 2 processes; B, reticulum within the process walls; C-D, midsection of 2 processes with different apices. Scale bars = μm .

being a haplotype attributed to *P. danielae* (Pilato, Binda, Napolitano & Moncada, 2001) from undetermined location (MZ081363). In the 28S rRNA: 0.00–8.57% (5.98% on average), being identical to *Paramacrobotus lachowskiae* from Magdalena Province, Colombia (MF568533), and the least similar being *P. tonollii* (Ramazzotti, 1956) from the East Tennessee State University campus, USA (MH664963). In the ITS-2: 10.36–35.69% (25.29% on average), with the most similar being *Paramacrobotus lachowskiae* from Magdalena Province, Colombia (MF568535), and the least similar *P. tonollii* (Ramazzotti, 1956) from Oregon, USA (GQ403679). In the COI: 11.33–26.94% (21.78% on average), with the most similar being *Paramacrobotus lachowskiae* Stec, Roszkowska, Kaczmarek and Michalczyk, 2018 from Magdalena Province, Colombia (MF568534), and the least similar being a haplotype attributed to *P. richtersi* (Murray, 1911) from China (unpublished) (GU339056).

Phylogenetic analysis. The phylogenetic reconstruction performed using BI and ML methods resulted in trees with similar topology and mostly well supported nodes, although the lowest support values corresponded to the ML tree (Fig. 10). The results show that the *Paramacrobotus richtersi* morphogroup was recovered as paraphyletic, and the *areolatus* morphogroup as polyphyletic, as the sequences of the new species obtained in this study clustered together with *P. lachowskiae* from Colombia within the clade of the *richtersi* morphogroup. These results are largely consistent with the phylogenies previously presented by Stec, Krzywański et al. (2020) and Kayastha, Stec et al. (2023). *Paramacrobotus areolatus* and *P. tonollii* from the USA are found at the base of the *areolatus* morphogroup clade and interestingly, *P. cf. klymenky* IT.048 from Italy was clustered with *P. bifrons* also from Italy, being *P. cf. klymenky* PT.006 from Portugal a sister lineage to that cluster.

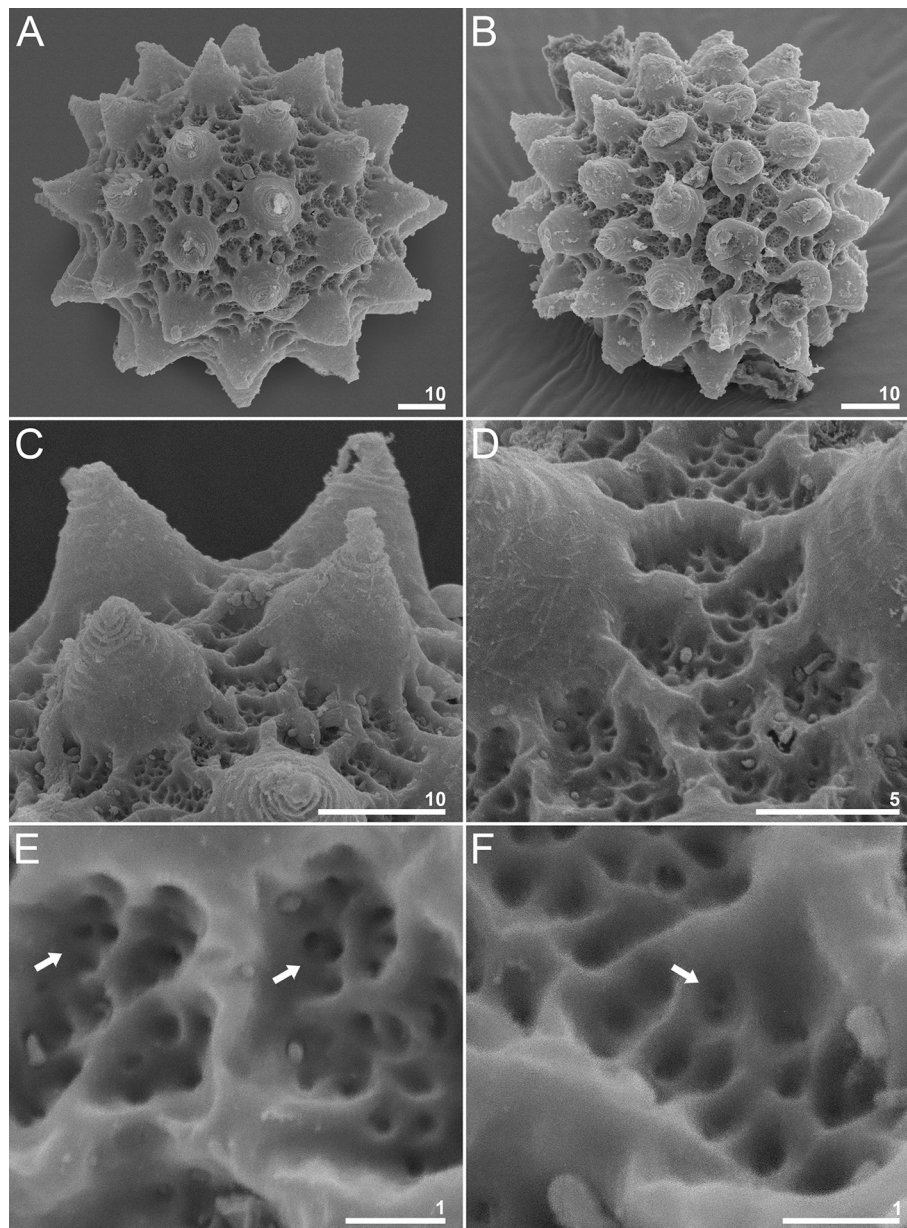


Figure 9. *Paramacrobiotus puma* sp. nov. Egg seen in SEM: A-B, chorion; C, processes; D, areolae between the processes; E-F, a closer look at the areolation between 2 processes; the arrows indicate the pores inside the areolae. Scale bars = μm .

Discussion

To date, 83 species of tardigrades have been reported for Mexico. Particularly for the genus *Milnesium*, 5 species have been previously reported: *Milnesium barbadosense* Meyer and Hinton, 2012 (Moreno-Talamantes et al., 2019), *M. cassandrae* Moreno-Talamantes, Roszkowska, García-Aranda, Flores-Maldonado and Kaczmarek, 2019 (Moreno-

Talamantes et al., 2019), *M. fridae* Moreno-Talamantes, León-Espinosa, García-Aranda, Flores-Maldonado and Kaczmarek, 2020 (Moreno-Talamantes et al., 2020), *M. sp.* (Moreno-Talamantes et al., 2020) and *M. tardigradum tardigradum* Doyère, 1840 (Schuster, 1971; Beasley, 1972; Kaczmarek et al., 2011; Moreno-Talamantes et al., 2019, 2020). However, currently, the geographical distribution of *Milnesium tardigradum* is restricted to Central and

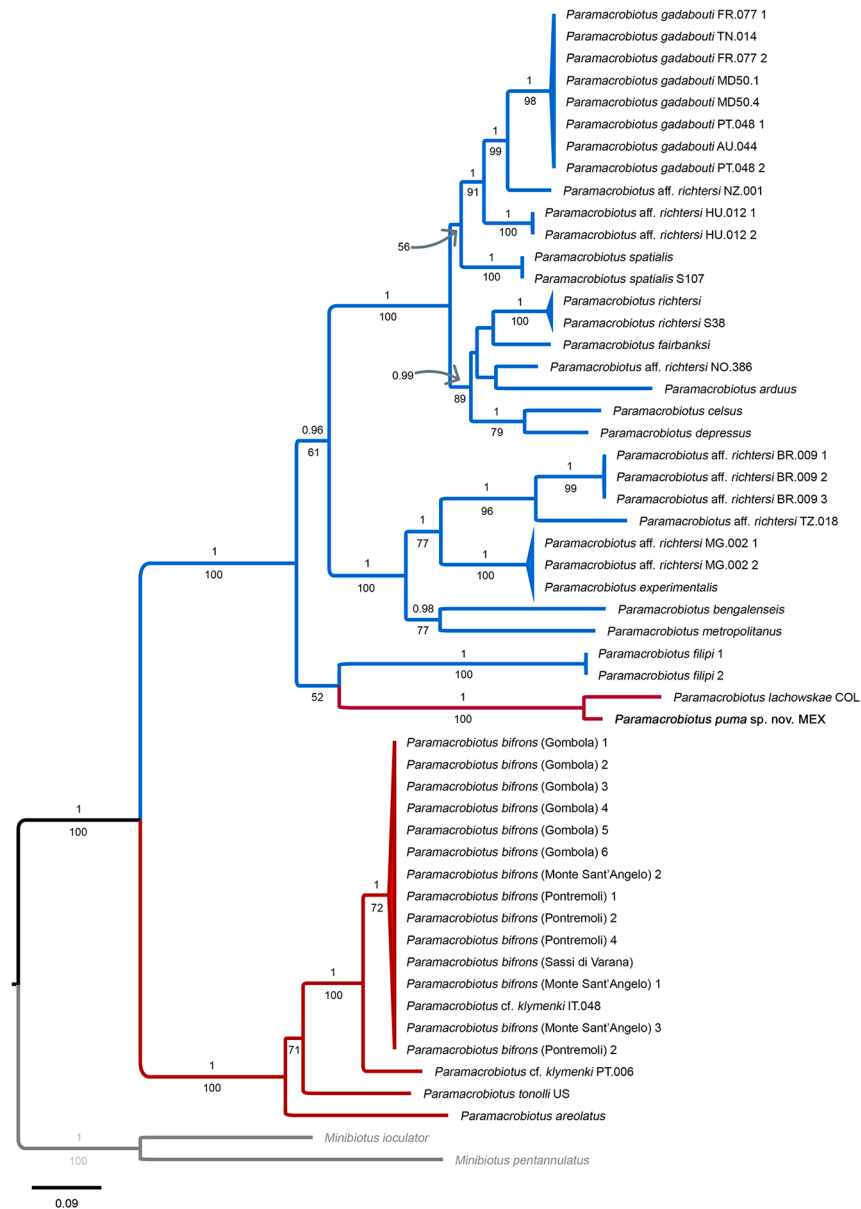


Figure 10. Phylogeny constructed from concatenated sequences of the genus *Paramacrobolus* (18S rRNA + 28S rRNA + ITS-2 + COI; Table 2). Numbers above branches indicate Bayesian posterior probabilities values (≥ 0.90), while number below branches indicate bootstrap support values (≥ 50). In agreement with previous phylogenies (Basu et al., 2023; Kayastha, Stec et al., 2023; Stec, Krzywański et al., 2020), taxa of the *richtersi* and *areolatus* morphogroups are indicated by blue and red branches, respectively. The outgroup is indicated in gray font. The new species is in bolded font. The scale bar represents substitutions per position.

Western Europe (Kaczmarek et al., 2011; Michalczyk et al., 2012a, b; Morek et al., 2018), so the records provided by Schuster (1971) and Beasley (1972) are doubtful and it is necessary to inspect the specimens collected in Mexico since they are probably a different species. *Milnesium*

longiungue and *M. cf. reductum* represent a new record for Mexico and the number of species of the genus *Milnesium* increases to 7.

Regarding the family Hypsibiidae, 4 subfamilies have been recorded: Diphasconinae, Hypsibiinae,

Itaquisconinae and Pilatobiinae (Schuster, 1971; Ramazzotti & Maucci, 1983; Moreno-Talamantes et al., 2019; Dueñas-Cedillo et al., 2020). For the subfamily Hypsibiinae, 4 species belonging to the genus *Hypsibius* are present in the country: *H. cf. convergens* recorded by Schuster (1971), *H. cf. microps* and *H. cf. pallidus* recorded by Dueñas-Cedillo et al. (2020) and *H. pallidus* Thulin, 1911 recorded by Ramazzotti and Maucci (1983), and with the record of *H. cf. dujardini*, there are 5 species recorded for the genus. And as for the subfamily Pilatobiinae, *Notahypsibius pallidoides* represents the first species of this genus recorded for Mexico.

Specifically for the genus *Paramacrobiotus*, *P. areolatus* (Murray, 1907) and *P. richtersi* (Murray, 1911) were recorded by Schuster (1971), being the only 2 species of the genus that are present in Mexico. However, these records should be considered doubtful, because although both species were previously considered cosmopolitan (Kaczmarek et al., 2011, 2017), they are currently within 2 morphogroups of closely related species and their distribution is very restricted. Currently, *Paramacrobiotus areolatus* is restricted to Svalbard and Greenland (Stec, Krzywański et al., 2020), and *P. richtersi* to Ireland and Finland (Kayastha, Mioduchowska et al., 2023). Furthermore, in addition to the diagrams showing the buccopharyngeal apparatus and the egg type, Schuster (1971), did not provide more details of the chorion or the egg ornamentation. Therefore, it is necessary to inspect the specimens registered in Mexico that were attributed to *P. areolatus* and *P. richtersi* by Schuster, since they are most likely different species. *Paramacrobiotus gadabouti* is a new record for Mexico, and with the description of *P. puma* sp. nov., there are 4 species of the genus recorded for the country.

Phylogenetic analysis. The genus *Paramacrobiotus* has been studied on several occasions. Guidetti et al. (2009), erected the genus by separating it from *Macrobiotus* Schultze, 1834. Subsequently, the redescription of *Paramacrobiotus richtersi*, the nominal taxon (Guidetti et al., 2019) and *P. areolatus* (Stec, Krzywański et al., 2020), contributed significantly by providing detailed morphological and molecular data from both species and several additional new species described. Recently, Basu et al. (2023) and Kayastha, Stec et al. (2023) described *Paramacrobiotus bengalenseis* and *P. gadabouti*, respectively, contributing to new phylogenetic hypotheses. Basu et al. (2023) demonstrated the monophyly of the *richtersi* group but found the *areolatus* group as paraphyletic, Kayastha, Stec et al. (2023) found the representatives of the *areolatus* group formed a paraphyletic group caused by *P. lachowskiae* which was clustered together with the *richtersi* morphogroup.

In the present study, we found a tree topology very similar to those previously published by Stec, Krzywański et al. (2020) and Kayastha, Stec et al. (2023), however, the *richtersi* group was recovered as paraphyletic and the *areolatus* group as polyphyletic (Fig. 10). As for *Paramacrobiotus puma* sp. nov., it was clustered together with *P. lachowskiae* from Colombia, both species being the only neotropical representatives within the *areolatus* morphogroup along the phylogenies. These results indicate that *Paramacrobiotus puma* sp. nov. is very close to *P. lachowskiae*, as seen in the genetic distances (p-distances), particularly for the ribosomal marker 28s rRNA, which proved to be a similar haplotype between both species (see results above). Nevertheless, morphologically, several differences are found between both species, such as the macroplacoid length sequence, the type of eggs and the egg processes shape (see differential diagnosis).

Finally, according to the phylogenetic tree obtained, we recovered *Paramacrobiotus cf. klymenky* IT.048 from Italy, clustered with *P. bifrons*, which indicates that they may represent closely related species. Also, *Paramacrobiotus cf. klymenky* PT.006 is recovered as a sister taxon of that cluster (Fig. 10). In our study, the p-distances between *Paramacrobiotus cf. klymenky* IT.048 and *P. bifrons*, showed to be low (between 0.53-2.72%) for the COI marker, but very variable regarding the ITS-2 marker (between 0.55-6.55%) (Supplementary material, SM2). Stec, Krzywański et al. (2020) studied *Paramacrobiotus cf. klymenky* IT.048 along with *P. cf. klymenky* PT.006, where despite being morphologically identical (suggesting they were a single species), both species presented discordant genetic distances between the ITS-2 and COI markers. Moreover, regardless of the genetic distance method used, for the COI marker they were different species and as for the ITS-2 marker, they turned out to be the same species. Nevertheless, although in the different phylogenies they are clustered together (Basu et al., 2023; Kayastha, Stec et al., 2023; Stec, Krzywański et al., 2020), whether the Italian and the Portuguese populations are different, or the same species is difficult to answer so far, since a greater number of studies with an integrative approach are still needed to gradually gain a better resolution within this genus.

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References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Anguas-Escalante, A., Jesús-Navarrete, A., Demilio, E., Pérez-Pech, W. A., & Goulberg, J. H. (2020). A new species of Tardigrada from a Caribbean reef lagoon, *Florarctus yucatanensis* sp. nov. (Halechiniscidae: Florarctinae). *Cahiers de Biologie Marine*, 61, 377–385. <https://doi.org/10.21411/CBM.A.CD1B185A>
- Basu, S., Babu, R., Siddique, A., & Purushothaman, J. (2023). Integrative description of *Paramacrobotus bengalensis* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae), a new limno-terrestrial tardigrade species from the state of West Bengal, India. *European Journal of Taxonomy*, 890, 23–48. <https://doi.org/10.5852/ejt.2023.890.2249>
- Beasley, C. W. (1972). Some tardigrades from Mexico. *Southwestern Naturalist*, 17, 21–29. <https://doi.org/10.2307/3669835>
- Beasley, C. W., Kaczmarek, Ł., & Michalczyk, Ł. (2008). *Doryphoribius mexicanus*, a new species of Tardigrada (Eutardigrada: Hypsibiidae) from Mexico (North America). *Proceedings of the Biological Society of Washington*, 121, 34–40. <https://doi.org/10.2988/07-30.1>
- Beasley, C. W., & Miller, W. R. (2007). Tardigrada of Xinjiang Uygur Autonomous Region, China. *Proceedings of the Tenth International Symposium on Tardigrada. Journal of Limnology*, 66 (Suppl. 1), 49–55. <https://doi.org/10.4081/jlimnol.2007.s1.49>
- Bertolani, R., Guidetti, R., Marchioro, T., Altiero, T., Rebecchi, L., & Cesari, M. (2014). Phylogeny of Eutardigrada: new molecular data and their morphological support lead to the identification of new evolutionary lineages. *Molecular Phylogenetics and Evolution*, 76, 110–126. <https://doi.org/10.1016/j.ympev.2014.03.006>
- Biserov, V. I. (1998). Tardigrades of the Caucasus with a taxonomic analysis of the genus *Ramazzottius* (Parachela: Hypsibiidae). *Zoologischer Anzeiger*, 236, 139–159.
- Brandoli, S., Cesari, M., Massa, E., Vecchi, M., Rebecchi, L., & Guidetti, R. (2024). Diverse eggs, diverse species? Production of two egg morphotypes in *Paramacrobotus bifrons*, a new eutardigrade species within the *areolatus* group. *The European Zoological Journal*, 91, 274–297. <https://doi.org/10.1080/24750263.2024.2317465>
- Casquet, J., Thebaud, C., & Gillespie, R. G. (2012). Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. *Molecular Ecology Resources*, 12, 136–141. <https://doi.org/10.1111/j.1755-0998.2011.03073.x>
- Claxton, S. K. (1998). A revision of the genus *Minibiotus* (Tardigrada: Macrobiotidae) with descriptions of eleven new species from Australia. *Records of the Australian Museum*, 50, 125–160. <https://doi.org/10.3853/j.0067-1975.50.1998.1276>
- Darriba, D., Posada, D., Kozlov, A., Stamatakis, A., Morel, B., & Flouri, T. (2020). ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution*, 37, 291–294. <https://doi.org/10.1093/molbev/msz189>
- Dastych, H. (1985). West Spitsbergen Tardigrada. *Acta Zoologica Cracoviensia*, 28, 169–214.
- Degma, P., & Guidetti, R. (2024). Actual checklist of Tardigrada species (2009–2024, 43th Edition: 01-07-2024). Available from <http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf> [accessed 10 March 2024]. https://dx.doi.org/10.25431/11380_1178608
- Degma, P., Michalczyk, Ł., & Kaczmarek, Ł. (2008). *Macrobiotus derkai*, a new species of Tardigrada (Eutardigrada, Macrobiotidae, *huziori* group) from the Colombian Andes (South America). *Zootaxa*, 1731, 1–23. <https://doi.org/10.11646/zootaxa.1731.1.1>
- Dueñas-Cedillo, A., Martínez-Méndez, E., García-Román, J., Armendáriz-Toledano, F., & Ruiz, E. A. (2020). Tardigrades from Iztaccíhuatl Volcano (Trans-Mexican Volcanic Belt), with the description of *Minibiotus citlalium* sp. nov. (Eutardigrada: Macrobiotidae). *Diversity*, 12, 271. <https://dx.doi.org/10.3390/d12070271>
- Dueñas-Cedillo, A., Venegas, I., García-Román, J., Jurado, E., Cuellar-Rodríguez, G., Villegas-Guzmán, G. A. et al. (2024). Towards an inventory of Mexican tardigrades (Tardigrada): a survey on the diversity of moss tardigrades with an emphasis in conifer forests from the Valley of Mexico Basin. *Check List*, 20, 471–498. <https://doi.org/10.15560/20.2.471>
- García-Román, J., Dueñas-Cedillo, A., Cervantes-Espinoza, M., Flores-Martínez, J. J., Vargas-Mendoza, C. F., Ruiz, E. A. et al. (2022). A strategy to provide a present and future scenario of Mexican biodiversity of Tardigrada. *Diversity*, 14, 280. <https://doi.org/10.3390/d14040280>

- Gąsiorek, P., Stec, D., Morek, W., & Michalczyk Ł. (2017). An integrative redescription of *Echiniscus testudo* (Doyère, 1840), the nominal taxon for the class Heterotardigrada (Ecdysozoa: Panarthropoda: Tardigrada). *Zoologischer Anzeiger*, 270, 107–122. <https://doi.org/10.1016/j.jcz.2017.09.006>
- Gąsiorek, P., Stec, D., Morek, W., & Michalczyk, Ł. (2018). An integrative redescription of *Hypsibius dujardini* (Doyère, 1840), the nominal taxon for Hypsibioidea (Tardigrada: Eutardigrada). *Zootaxa*, 4415, 45–75. <https://doi.org/10.11646/zootaxa.4415.1.2>
- Gąsiorek, P., Stec, D., Zawierucha, Z., Kristensen, R. M., & Michalczyk, Ł. (2018). Revision of *Testechiniscus* Kristensen, 1987 (Heterotardigrada: Echiniscidae) refutes the polar-temperate distribution of the genus. *Zootaxa*, 4472, 261–297. <https://doi.org/10.11646/zootaxa.4472.2.3>
- Glime, J. M. (2017). Tardigrade survival. Chapter 5-1. In J. M. Glime (Ed.), *Bryophyte ecology. Volume 2. Bryological Interaction*. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Accessed June 21, 2024 from: <http://digitalcommons.mtu.edu/bryophyte-ecology2/>
- Guidetti, R., & Bertolani, R. (2005). Tardigrade taxonomy: an updated checklist of the taxa and a list of characters for their identification. *Zootaxa*, 845, 1–46. <https://doi.org/10.11646/zootaxa.845.1.1>
- Guidetti, R., Cesari, M., Bertolani, R., Altiero, T., & Rebecchi, L. (2019). High diversity in species, reproductive modes and distribution within the *Paramacrobiotus richtersi* complex (Eutardigrada, Macrobiotidae). *Zoological Letters*, 5, 1–28. <https://doi.org/10.1186/s40851-018-0113-z>
- Guidetti, R., Cesari, M., Giovannini, I., Ebel, C., Förschler, M. I., Rebecchi, L. et al. (2022). Morphology and taxonomy of the genus *Ramazzottius* (Eutardigrada; Ramazzottiidae) with the integrative description of *Ramazzottius kretschmanni* sp. nov. *The European Zoological Journal*, 89, 346–370. <https://doi.org/10.1080/24750263.2022.2043468>
- Guidetti, R., Schill, R. O., Bertolani, R., Dandekar, T., & Wolf, M. (2009). New molecular data for tardigrade phylogeny, with the erection of *Paramacrobiotus* gen. nov. *Journal of Zoological Systematics and Evolutionary Research*, 47, 315–321. <https://doi.org/10.1111/j.1439-0469.2009.00526.x>
- Heinis, F. (1911). Beitrag zur Kenntnis der zentralamerikanischen Moosfauna. *Revue Suisse de Zoologie*, 19, 253–266.
- Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522. <https://doi.org/10.1093/molbev/msx281>
- Iharos, G. (1966). Neue Tardigraden-Arten aus Ungarn (Neuere Beiträge zur Kenntnis der Tardigraden Fauna Ungarns VI). *Acta Zoologica Hungarica*, 12, 111–122.
- Kaczmarek, Ł., & Michalczyk, Ł. (2017). The *Macrobiotus hufelandi* (Tardigrada) group revisited. *Zootaxa*, 4363, 101–123. <https://doi.org/10.11646/zootaxa.4363.1.4>
- Kaczmarek, Ł., Cytan, J., Zawierucha, K., Diduszko D., & Michalczyk, Ł. (2014). Tardigrades from Peru (South America), with descriptions of three new species of *Parachela*. *Zootaxa*, 3790, 357–379. <https://doi.org/10.11646/zootaxa.3790.2.5>
- Kaczmarek, Ł., Diduszko, D., & Michalczyk, Ł. (2011). New records of Mexican Tardigrada. *Revista Mexicana de Biodiversidad*, 82, 1324–1327. <https://doi.org/10.22201/ib.20078706e.2011.4.754>
- Kaczmarek, Ł., Gawlak, M., Bartels, P. J., Neslon, D. R., & Roszkowska, M. (2017). Revision of the genus *Paramacrobiotus* Guidetti et al., 2009 with the description of a new species, re-descriptions and a key. *Annales Zoologici*, 67, 627–656. <https://doi.org/10.3161/00034541ANZ2017.67.4.001>
- Kaczmarek, Ł., Michalczyk, Ł., & McInnes, S. J. (2014). Annotated zoogeography of non-marine Tardigrada. Part I: Central America. *Zootaxa*, 3763, 1–62. <https://doi.org/10.11646/zootaxa.3763.1.1>
- Kaczmarek, Ł., Michalczyk, Ł., & McInnes, S. J. (2015). Annotated zoogeography of non-marine Tardigrada. Part II: South America. *Zootaxa*, 3923, 1–107. <https://doi.org/10.11646/zootaxa.3923.1.1>
- Kaczmarek, Ł., Michalczyk, Ł., & McInnes, S. J. (2016). Annotated zoogeography of non-marine Tardigrada. Part III: North America and Greenland. *Zootaxa*, 4203, 1–249. <https://doi.org/10.11646/zootaxa.4203.1.1>
- Kaczmarek, Ł., Roszkowska, M., Poprawa, I., Janelt, K., Kmita, H., Gawlak, M. et al. (2020). Integrative description of bisexual *Paramacrobiotus experimentalis* sp. nov. (Macrobiotidae) from republic of Madagascar (Africa) with microbiome analysis. *Molecular Phylogenetics and Evolution*, 145, 106–730. <https://doi.org/10.1016/j.ympev.2019.106730>
- Kalyaanamoorthy, S., Minh, B., Wong, T., von Haeseler, A., & Jermini, L. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589. <https://doi.org/10.1038/nmeth.4285>
- Katoh K., Misawa, K., Kuma K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30, 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, 20, 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kayastha, P., Mioduchowska, M., Warguła, J., & Kaczmarek, Ł. (2023). A review on the genus *Paramacrobiotus* (Tardigrada) with a new diagnostic key. *Diversity*, 15, 977. <https://doi.org/10.3390/d15090977>
- Kayastha, P., Stec, D., Ślugocki, Ł., Gawlak, M., Mioduchowska, M., & Kaczmarek, Ł. (2023). Integrative taxonomy reveals new, widely distributed tardigrade species of the genus *Paramacrobiotus* (Eutardigrada: Macrobiotidae). *Scientific Reports*, 13, 2196. <https://doi.org/10.1038/s41598-023-28714-w>
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). Mega X: molecular evolutionary genetics analysis across

- computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- León-Espinosa, G. A., Marley, N., Moreno-Talamantes, A., Nelson, D., & Rodríguez-Almaraz, G. (2017). Biodiversity of tardigrades in Mexico. Accessed November 18, 2023 from: https://www.researchgate.net/publication/320597470_Biodiversity_of_Tardigrades_in_Mexico
- León-Espinosa, G. A., Moreno-Talamantes, A., & Rodríguez-Almaraz, G. A. (2019). Ositos de agua (Tardigrada) de México: los famosos desconocidos. *Biología y Sociedad*, 4, 61–70. <https://doi.org/10.29105/bys2.4-40>
- May, R. M. (1948). Nouveau genre et espèce de tardigrade du Mexique: *Hapломacrobíotus hermosillensis*. *Bulletin de la Société Zoologique de France*, 73, 95–97.
- McInnes, S. J. (1994). Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. *Journal of Natural History*, 28, 257–352. <https://doi.org/10.1080/00222939400770131>
- Michalczyk, L., & Kaczmarek, Ł. (2003). A description of the new tardigrade *Macrobiotus reinhardtii* (Eutardigrada, Macrobiotidae, *harmsworthi* group) with some remarks on the oral cavity armature within the genus *Macrobiotus* Schultze. *Zootaxa*, 331, 1–24. <https://doi.org/10.11646/zootaxa.331.1.1>
- Michalczyk, L., & Kaczmarek, Ł. (2006). *Macrobiotus huziori*, a new species of Tardigrada (Eutardigrada: Macrobiotidae) from Costa Rica (Central America). *Zootaxa*, 1169, 47–59. <https://doi.org/10.11646/zootaxa.1169.1.3>
- Michalczyk, L., & Kaczmarek, Ł. (2013). The Tardigrada register: a comprehensive online data repository for tardigrade taxonomy. *Journal of Limnology*, 72, 175–181. <https://doi.org/10.4081/jlimnol.2013.sl.e22>
- Michalczyk, L., Welnicz, W., Frohme, M., & Kaczmarek, Ł. (2012a). Redescriptions of three *Milnesium* Doyère, 1840 taxa (Tardigrada: Eutardigrada: Milnesiidae), including the nominal species for the genus. *Zootaxa*, 3154, 1–20. <https://doi.org/10.11646/zootaxa.3154.1.1>
- Michalczyk, L., Welnicz, W., Frohme, M., & Kaczmarek, Ł. (2012b). Corrigenda of Zootaxa, 3154: 1–20 Redescriptions of three *Milnesium* Doyère, 1840 taxa (Tardigrada: Eutardigrada: Milnesiidae), including the nominal species for the genus. *Zootaxa*, 3393, 66–68. <https://doi.org/10.11646/zootaxa.3393.1.6>
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D. S., Woodhams, M. D., von Haeseler, A. et al. (2020). IQ-TREE 2. New models and efficient methods for phylogenetic inference in the Genomic Era. *Molecular Biology and Evolution*, 37, 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Mironov, S. V., Dabert, J., & Dabert, M. (2012). A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit *Aegithalos caudatus* (Passeriformes: Aegithalidae): morphological description with DNA barcode data. *Zootaxa*, 3253, 54–61. <https://doi.org/10.11646/zootaxa.3253.1.2>
- Morek, W., & Michalczyk, L. (2020). First extensive multilocus phylogeny of the genus *Milnesium* (Tardigrada) reveals no congruence between genetic markers and morphological traits. *Zoological Journal of the Linnean Society*, 188, 681–693. <https://doi.org/10.1093/zoolinnean/zlz040>
- Morek, W., Stec, D., Gąsiorek, P., Surmacz, B., & Michalczyk, L. (2018). *Milnesium tardigradum* Doyère, 1840: The first integrative study of interpopulation variability in a tardigrade species. *Journal of Zoological Systematics and Evolutionary Research*, 57, 1–23. <https://doi.org/10.1111/jzs.12233>
- Moreno-Talamantes, A., & León-Espinosa, G. A. (2019). Nuevo registro de *Diaforobiotus islandicus* (Richters, 1904) (Eutardigrada: Richtersiidae) para México. *Árido-Ciencia*, 6, 5–12.
- Moreno-Talamantes, A., León-Espinosa, G. A., García-Aranda, M. A., Flores-Maldonado, J. J., & Kaczmarek, Ł. (2020). The genus *Milnesium* Doyère, 1840 in Mexico with description of a new species. *Annales Zoologici*, 70, 467–486. <https://doi.org/10.3161/00034541ANZ2020.70.4.001>
- Moreno-Talamantes, A., Roszkowska, M., García-Aranda, M. A., Flores-Maldonado, J. J., & Kaczmarek, Ł. (2019). Current knowledge on Mexican tardigrades with a description of *Milnesium cassandrae* sp. nov. (Eutardigrada: Milnesiidae) and discussion on the taxonomic value of dorsal pseudoplates in the genus *Milnesium* Doyère, 1840. *Zootaxa*, 4691, 501–524. <https://doi.org/10.11646/zootaxa.4691.5.5>
- Moreno-Talamantes, A., Roszkowska, M., Guayasamin, P. R., Flores, J. J., & Kaczmarek, Ł. (2015). First record of *Dactylobiotus parthenogeneticus* Bertolani, 1982 (Eutardigrada: Murrayidae) in Mexico. *Check List*, 11, 1723. <http://dx.doi.org/10.15560/11.4.1723>
- Murray, J. (1907). XXV. Arctic Tardigrada, collected by Wm. S. Bruce. *Earth Environ. Transactions of the Royal Society of Edinburgh, Earth Sciences*, 45, 669–681.
- Nelson, D. R., Bartels, P. J., & Guil, N. (2018). Tardigrade ecology. In R. O. Schill (Ed.), *Water bears: the biology of tardigrades* (pp. 163–210). Cham: Springer.
- Nelson, D., Guidetti, R., & Rebecchi, L. (2015). Chapter 17 - Phylum Tardigrada. In J. Thorp, & Rogers, C. (Eds.), *Thorp and Covich's freshwater invertebrates (Fourth edition)* (pp. 347–380). San Diego: Academic Press.
- Nelson, D., & Marley, N. J. (2000). The biology and ecology of lotic Tardigrada. *Freshwater Biology*, 44, 93–108. <https://doi.org/10.1046/j.1365-2427.2000.00586.x>
- Núñez, P. G., León-Espinosa, G. A., Vázquez, R., Peña-Salinas, M. E., Rodríguez-Almaraz, G. A., & Moreno-Talamantes, A. (2021). First tardigrade records from San Pedro Mártir, Baja California, Mexico. *Check List*, 17, 1131–1136. <https://doi.org/10.15560/17.4.1131>
- Palacio, P. J. L., & Guilbaud, M. N. (2015). Patrimonio natural de la Reserva Ecológica del Pedregal de San Ángel y áreas cercanas: sitios de interés geológico y geomorfológico al sur de la Cuenca de México. *Boletín de la Sociedad Geológica Mexicana*, 67, 227–244.

- Pérez-Pech, W. A., Anguas-Escalante, A., Cutz-Pool, L. Q., & Guidetti, R. (2017). *Doryphoribius chetumalensis* sp. nov. (Eutardigrada: Isohypsibiidae) a new tardigrade species discovered in an unusual habitat of urban areas of Mexico. *Zootaxa*, 4344, 345–356. <http://dx.doi.org/10.11646/zootaxa.4344.2.9>
- Pérez-Pech, W. A., Anguas-Escalante, A., De Jesús-Navarrete, A., & Hansen, J. G. (2018). Primer registro genérico de tardígrados marinos en costas de Quintana Roo, México. *Academia Journal*, 10, 1909–1912.
- Pérez-Pech, W. A., De Jesús-Navarrete, A., Demilio, E., Anguas-Escalante, A., & Hansen, J. G. (2020). Marine Tardigrada from the Mexican Caribbean with the description of *Styraconyx robertoi* sp. nov. (Arthrotardigrada: Styraconyxidae). *Zootaxa*, 4731, 492–508. <http://dx.doi.org/10.11646/zootaxa.4731.4.3>
- Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C. et al. (2008). Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution*, 48, 369–71. <http://dx.doi.org/10.1016/j.ympev.2008.03.024>
- Pilato, G. (1981). Analisi di nuovi caratteri nello studio degli Eutardigradi. *Animalia*, 8, 51–57.
- Pilato, G. (2000). *Macrobiotus centesimus*, new species of eutardigrade from the South America. *Bollettino delle Sedute della Accademia Gioenia di Scienze Naturali in Catania*, 33, 97–101.
- Pilato, G. (2006). Remarks on the *Macrobiotus polyopus* group, with the description of two new species (Eutardigrada, Macrobiotidae). *Zootaxa*, 1298, 37–47. <https://doi.org/10.11646/zootaxa.1298.1.4>
- Pilato, G., & Binda, M. G. (2010). Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa*, 2404, 1–54. <https://doi.org/10.11646/zootaxa.2404.1.1>
- Pilato, G., Kiosya, Y., Lisi, O., Inshina, V., & Biserov, V. (2011). Annotated list of Tardigrada records from Ukraine with the description of three new species. *Zootaxa*, 3123, 1–31. <https://doi.org/10.11646/zootaxa.3123.1.1>
- Pilato, G., Kiosya, Y., Lisi, O., & Sabella, G. (2012). New records of Eutardigrada from Belarus with the description of three new species. *Zootaxa*, 3179, 39–60. <https://doi.org/10.11646/zootaxa.3179.1.2>
- Pilato, G., & Lisi, O. (2006). Notes on some tardigrades from southern Mexico with description of three new species. *Zootaxa*, 1236, 53–68. <https://doi.org/10.11646/zootaxa.1236.1.4>
- Pilato, G., Sabella, G., & Lisi, O. (2016). Two new species of *Milnesium* (Tardigrada: Milnesiidae). *Zootaxa*, 4132, 575–587. <https://doi.org/10.11646/zootaxa.4132.4.9>
- Ramazzotti, G. (1956). Tre nuove specie di Tardigradi ed altre specie poco comuni. *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano*, 10, 284–291.
- Ramazzotti, G., & Maucci, W. (1983). Il Phylum Tardigrada. 3th Edition. *Memorie dell' Istituto Italiano di Idrobiologia*, 41, 1–1012.
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 901–904. <https://doi.org/doi:10.1093/sysbio/syy032>
- Rocha, A. M., González-Reyes, A. X., Ostertag, B., & Lisi, O. (2022). The genus *Milnesium* (Eutardigrada, Apochela, Milnesiidae) in Argentina: description of three new species and key to the species of South America. *European Journal of Taxonomy*, 822, 1–54. <https://doi.org/10.5852/ejt.2022.822.1807>
- Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Sands, C. J., McInnes, S. J., Marley, N. J., Goodall-Copestake, W. P., Convey, P., & Linse, K. (2008). Phylum Tardigrada: an “individual” approach. *Cladistics*, 24, 1–18. <http://dx.doi.org/10.1111/j.1096-0031.2008.00219.x>
- Schuster, R. O. (1971). Tardigrada from Barranca del Cobre, Sinaloa and Chihuahua, Mexico. *Proceedings of the Biological Society of Washington*, 84, 2130–224.
- Stec, D., Dudziak, M., & Michalczyk, Ł. (2020). Integrative descriptions of two new Macrobiotidae species (Tardigrada: Eutardigrada: Macrobiotidae) from French Guiana and Malaysian Borneo. *Zoological Studies*, 59, e23. <https://doi.org/10.6620/ZS.2020.59-23>
- Stec, D., Gąsiorek, P., Morek, W., Koszytła, P., Zawierucha, K., Michno, K. et al. (2016). Estimating optimal sample size for tardigrade morphometry. *Zoological Journal of the Linnean Society*, 178, 776–784. <https://doi.org/10.1111/zoj.12404>
- Stec, D., Kristensen, R. M., & Michalczyk, Ł. (2020). An integrative description of *Minibiotus ioculator* sp. nov. from the Republic of South Africa with notes on *Minibiotus pentannulatus* Londoño et al., 2017 (Tardigrada: Macrobiotidae). *Zoologischer Anzeiger*, 286, 117–134. <https://doi.org/10.1016/j.jcz.2020.03.007>
- Stec, D., Krzywański, Ł., Zawierucha, K., & Michalczyk, Ł. (2020). Untangling systematics of the *Paramacrobiotus areolatus* species complex by an integrative redescription of the nominal species for the group, with multilocus phylogeny and species delineation in the genus *Paramacrobiotus*. *Zoological Journal of the Linnean Society*, 188, 694–716. <https://doi.org/10.1093/zoolinnean/zlzl63>
- Stec, D., Morek, W., Gąsiorek, P., & Michalczyk, Ł. (2018). Unmasking hidden species diversity within the *Ramazzottius oberhaeuseri* complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela). *Systematics and Biodiversity*, 16, 357–376. <https://doi.org/10.1080/14772000.2018.1424267>

- Stec, D., Roszkowska, M., Kaczmarek, Ł., & Michalczyk, Ł. (2018). *Paramacrobiotus lachowskiae*, a new species of Tardigrada from Colombia (Eutardigrada: Parachela: Macrobiotidae). *New Zealand Journal of Zoology*, 45, 43–60. <https://doi.org/10.1080/03014223.2017.1354896>
- Stec, D., Smolak, R., Kaczmarek, Ł., & Michalczyk, Ł. (2015). An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya. *Zootaxa*, 4052, 501–526. <https://doi.org/10.11646/zootaxa.4052.5.1>
- Stec, D., Vecchi, M., Calhim, S., & Michalczyk, Ł. (2021). New multilocus phylogeny reorganises the family Macrobiotidae (Eutardigrada) and unveils complex morphological evolution of the *Macrobiotus hufelandi* group. *Molecular Phylogenetics and Evolution*, 160, 106987. <https://doi.org/10.1016/j.ympev.2020.106987>
- Stucky, B. J. (2012). SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *Journal of Biomolecular Techniques*, 23, 90–93. <https://doi.org/10.7171/jbt.12-2303-004>
- Sugiura, K., Matsumoto, M., & Kunieda, T. (2022). Description of a model tardigrade *Paramacrobiotus metropolitanus* sp. nov. (Eutardigrada) from Japan with a summary of its life history, reproduction and genomics. *Zootaxa*, 5134, 92–112. <https://doi.org/10.11646/zootaxa.5134.1.4>
- Tumanov, D. V. (2006). Five new species of the genus *Milnesium* (Tardigrada, Eutardigrada, Milnesiidae). *Zootaxa*, 1122, 1–23. <https://doi.org/10.11646/zootaxa.1122.1.1>
- Tumanov, D. V. (2020). Integrative redescription of *Hypsibius pallidoides* Pilato et al., 2011 (Eutardigrada: Hypsibioidea) with the erection of a new genus and discussion on the phylogeny of Hypsibiidae. *European Journal of Taxonomy*, 681, 1–37. <https://doi.org/10.5852/ejt.2020.681>
- Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, 27, 171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Vecchi, M., Stec, D., Vuori, T., Ryndov, S., Chartrain, J., & Calhim, S. (2022). *Macrobiotus naginae* sp. nov., a new xerophilous tardigrade species from Rokua Sand Dunes (Finland). *Zoological Studies*, 61, e22. <https://doi.org/10.6620/ZS.2022.61-22>
- Zeller, C. (2010). *Untersuchung der phylogenie von Tardigraden anhand der Genabschnitte 18S rDNA und Cytochrom c Oxidase Untereinheit 1 (COX I) (MSc. Thesis)*. Technical University of Applied Sciences Wildau. Wildau, Germany.