



# 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

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**Abstract.** From a large-scale sampling of limnoterrestrial tardigrades in the Valley of Mexico Basin, in the Huasteca Potosina, and Monarch Butterfly Sanctuary, Mexico, we develop an initial species list and determine the type of vegetation and substrates for most sampled species. In total 160 moss samples and over 2000 tardigrades were obtained, belonging to 17 genera, 35 putative species, one subspecies, of which eight could be accurately identified to species or subspecies, three to affinity, and 15 as morpho-species; the remaining samples were identified to genus, mainly due to the few specimens available and their preservation was not optimal. We provide novel records for one genus and two species from the country. Our estimates on five vegetation types and three substrate types indicates that our inventory is incomplete, and as many as twice the number of species may still be found in these ecosystems.

**Key words.** Accumulation curves, new records, tardigrade collection

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## INTRODUCTION

The Valley of Mexico Basin is an enclosed hydrographic unit located in the Trans-Mexican Volcanic Belt physiographic province. It extends approximately 110 km<sup>2</sup> from Xochimilco Lake to the semi-arid regions of Pachuca, Hidalgo. It was originated 700,000 years ago, when the Chichinautzin Mountains were formed (Imaz 1989). This region is among the most studied by botanists (e.g. Rzedowski and Rzedowski 2001) and zoologists. Zoological studies have mainly focused on vertebrates (e.g. Navarro-Frias et al. 2007; Ramirez-Bautista et al. 2009; Reséndiz-Cruz et al. 2017) and some invertebrate taxa, such as springtails (e.g. García-Gómez et al. 2010), several orders of insects (Morón and Márquez 2012; Hernández-Baz 2009), terrestrial mollusks (Naranjo-García and Olivera-Carrasco 2007), mites (Palacios-Vargas and Iglesias 2007), fleas (Acosta and Fernández 2007), and rotifers (Sarma et al. 2009). Studies focusing on meiofauna, such as the phylum Tardigrada Doyère, 1840, are scarce, despite the diversity of the fauna, which has importance in the functioning of ecosystems, as part of food webs, and in the transference of energy and nutrients (Guil et al. 2009). The phylum Tardigrada is comprised of hydrophilic micrometazoans, 50–1200 µm long, which inhabit terrestrial, marine, and freshwater habitats worldwide (Nelson 2001). In Mexico, the study of this phylum began with the pioneering work of Heinis (1911), and since then only five studies have recorded and described Mexican species during the rest of the 20th century (May 1948; Schuster 1971; Beasley 1972; Ramazzotti and Maucii 1983; Hoffman and Jimenez 1994). Eight more species were described and 75 were reported during the 21st century (García-Román et al. 2022); 15 of them were recorded within the Valley of Mexico Basin (Beasley 1972; Dueñas-Cedillo et al. 2020; García-Román et al. 2022). Over the last 10 years, the number of recorded species and new species has increased. The most recent analysis of the diversity of Mexican tardigrades had estimated that more than 290 species might be found in Mexico (García-Román



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et al. 2022). This highlights the importance of studying the alpha taxonomy of this taxon, as well as the need for the development of scientific collections of Tardigrada.

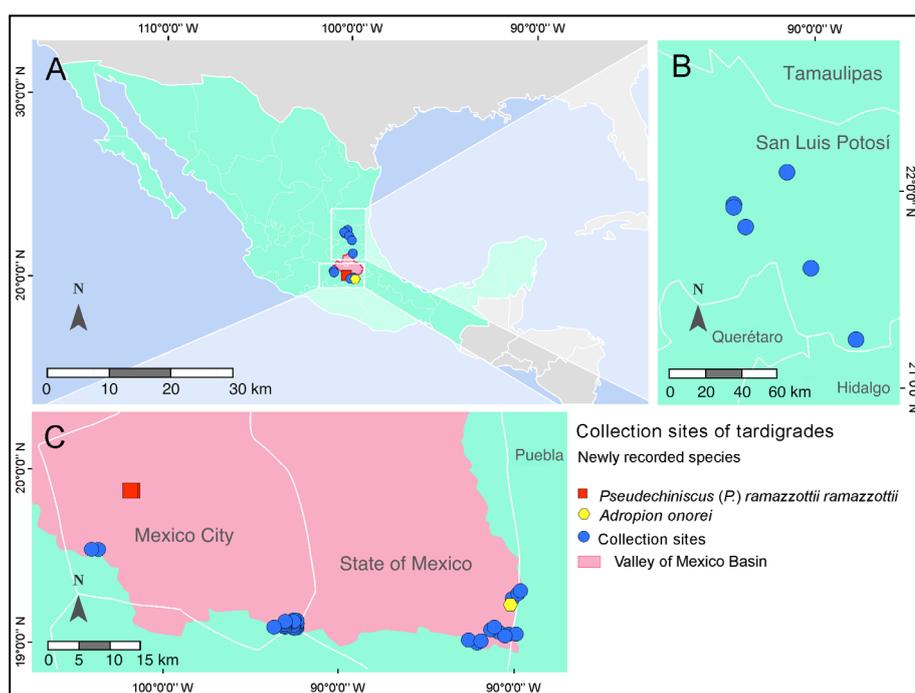
Thus, we present here the results of a large-scale sampling of the phylum Tardigrada in the Valley of Mexico Basin, as well as some surrounding areas in the Huasteca Potosina and the Monarch Butterfly Sanctuary, Mexico. The primary goals of this contribution are: (1) to develop an initial list of tardigrade species in the studied regions, (2) to evaluate our progress towards a complete list of species in Mexico, (3) to determine how vegetation and substrate types contribute to the assembly of tardigrade species, and (4) to present the species records in the context of their geographical distribution.

From 2,822 specimens, we identified 17 genera, eight species, 35 putative identifications, and one subspecies from Izta-Popo National Park, Milpa Alta, Bosque del Pedregal National Park, and from Cumbres del Ajusco National Park. We also provide a list of the taxa that are housed in the Collection of limnoterrestrial tardigrades in the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, and in the Laboratorio de Ecología, Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional. Both tardigrade collections house the specimens that support the results of Dueñas-Cedillo et al. (2020, 2021) and García-Román et al. (2022).

### METHODS

**Sampling.** Tardigrade specimens were obtained from 43 samples collected in 2010–2021 in the Valley of Mexico Basin (Mexico City, State of Mexico and Hidalgo), Huasteca Potosina (San Luis Potosí), and Monarch Butterfly Sanctuary (Michoacán) (Figure 1). Mosses were sampled in an elevation gradient, at altitudes of 657–4,500 m; they were growing on rock, bark, and soil in forests of *Pinus*, *Cupressus*, *Quercus*, and *Abies* spp., tropical rain forest, tundra, and alpine grassland (Table 1). The proportion of samples is the following: from Mexico City and State of Mexico, 81%, and from states of Michoacán, Hidalgo, and from San Luis Potosí, 19%. At each sampling site, different moss morphotypes were identified, and a 5 × 5 cm square was sampled for each morphotype. We recorded the collecting site, vegetation, substrate type (rock, bark, or soil), and habitat information (e.g. lighting, humidity, and growth type). We documented the type of specimens obtained in each sample (e.g. specimen, cyst, egg, and/or exuvia). The number of samples, geographical coordinates, altitude, vegetation type, substrate type, and geographical distribution of sample sites are presented in Table 1. We used the method of Dueñas-Cedillo et al. (2020) to obtain tardigrades from the moss samples. The specimens were mounted on microscope slides with Hoyer liquid or polyvinyl lactophenol and observed under a Zeiss Axioskop phase contrast microscope, with an Axiocam ERC 55 digital camera, and Olympus IX 71 inverted fluorescence microscope, with a Zeiss-Axiocam 503 digital camera. A subset of specimens was mounted for the scanning electron microscopy (SEM). Briefly, specimens for SEM were first boiled in absolute ethanol and transferred to cold absolute ethanol; this procedure was repeated three times, each for 5 min. Another boiling was done in absolute ethanol until complete evaporation. Finally, the specimens were mounted on metal plates and covered with gold for both SEM and preservation in the

**Figure 1.** Collection sites of this study. **A.** Sites within and around the Valley of Mexico Basin. **B.** Sites in San Luis Potosí state. **C.** Sites with newly recorded species in the Valley of Mexico Basin located in Bosque Pedregal National Park and Parque Nacional Izta-Popo.



**Table 1.** Locality, sample number, geographic coordinates, vegetation, and substrate type of mosses sampled collected.

Locality	# of moss samples	Latitude	Longitude	Elevation (m)	Vegetation type	Substrate type
Milpa Alta <sup>†</sup>	40	19°05'26.52"N to 19°06'10.10"N	98°57'33.60"W to 98°59'14.56"W	2230–3680	<i>Pinus</i> sp., <i>Cupressus</i> sp., <i>Quercus</i> sp. Forest, scrub, grassland	Rock, soil, tree bark
Bosque Pedregal National Park <sup>**</sup>	24	19° 17' 11.42"N to 19° 17' 50"N	99° 11'58.73"W to 99° 12' 40.87"W	2421–2439	<i>Pinus</i> sp., <i>Abies</i> sp., <i>Quercus</i> sp.	Rock
Cumbres del Ajusco National Park <sup>*§</sup>	9	19° 12' 23.7"N to 19° 12' 26.2"N	99° 15' 17"W to 99° 15' 17"W	3278	<i>Pinus hartwegii</i> , <i>Festuca amplissima</i> , <i>Muhlenbergia macroura</i>	Tree bark, soil
Izta-Popo National Park <sup>*¶</sup>	57	19° 04' 24"N to 19° 08' 42"N	98° 42' 48"W to 98° 38' 19"W	2700–4500	<i>Pinus</i> sp., <i>Cupressus</i> sp., <i>Quercus</i> sp. forest, alpine grassland	—
Monarch Butterfly Sanctuary, Michoacán	10	19° 34' 30"N	100° 16' 36"W	2700	<i>Pinus</i> , <i>Abies</i> sp. forest, <i>Asclepias</i> sp.	Tree bark
El Chico National Park	10	19° 04' 52"N	98° 39' 40"W	3613	<i>Pinus</i> , <i>Abies</i> sp. forest	—
Huasteca Potosina	10	21° 37' 16"N to 22° 05' 58"N	98° 05' 55"W to 99° 01' 10"W	127–657	Tropical rainforest	Tree bark, soil

\* Newly recorded species.

<sup>†</sup>For geographic coordinates of each sample, see Dueñas-Cedillo (2012).

<sup>\*\*</sup>For geographic coordinates of each sample, see Dueñas-Cedillo (2015).

<sup>§</sup>For geographic coordinates of each sample, see García-Román et al. (in review).

<sup>¶</sup>For geographic coordinates of each sample, see Dueñas-Cedillo et al. (2020).

collection. Specimens were examined in a Hitachi S-2469N scanning electron microscope.

**Taxonomy.** Taxonomic identifications were based on specialized literature, taxonomic keys, and/or revisions of families and genera. Taxonomically important structures were measured, whenever the orientations of body, claws, and buccopharyngeal apparatus were appropriate. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. The terminology used for the buccopharyngeal apparatus corresponds to that by Pilato and Binda (1998a) and Kaczmarek and Michalczyk (2017). The macroplacoid length sequence is presented according to Kaczmarek et al. (2014b). Hypsibioidea claws were measured using the protocols given by Beasley et al. (2008), while Macrobiotioidea claws were measured using the protocols given by Kaczmarek and Michalczyk (2017). The *pt* ratio is expressed as a percentage, and its value was always indicated in italics (Pilato 1981). Morphometric data were collected using the Excel template Echiniscoidea v. 1.4, Apochela v. 1.4, and Parachela v. 1.8, which are available from the Tardigrade Register (<http://www.tardigrada.net/register/submit.htm>) (Michalczyk and Kaczmarek 2013). The keys by Michalczyk et al. (2012a, 2012b) and taxonomic morphological characters by Morek et al. (2016) were used for the class Eutardigrada, order Apochela. The keys by Claxton (1998), Fontoura and Pilato (2007), Pilato and Binda (2010), and Kaczmarek and Michalczyk (2009, 2017) were used for the order Parachela, as well as the integrative study by Gąsiorek et al. (2018). Species of the *Diphascocon pingue* complex were identified using the key by Pilato and Binda (1999) and Fontoura and Pilato (2007). For the class Heterotardigrada, we followed the criteria of Ramazzotti and Maucci (1983) and Gąsiorek et al. (2019, 2021, 2023).

**Material collected.** The specimens mounted on microscope slides and those processed for SEM, integrated the Collection of limnoterrestrial tardigrades associated to Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, and Laboratorio de Ecología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México.

All specimens prepared for slides and SEM were deposited in the Collection of Mexican Limnoterrestrial Tardigrades. The collection integrates more than 2,800 slides (also stored in a digital database), with geographical and ecological data including type of vegetation and substrate, microhabitat, and collection site. According to the classification of Villaseñor (2015), which is modified from that of McGinley (1993), corresponds to level 8 on his scale, which correspond to specimens identified, and incorporated into a database. The collection also includes specimens in different stages of the tardigrade life cycle. The larger proportion is adult specimens, followed by exuviae, eggs, juveniles, and individuals in a cyst state.

**Data analysis.** To determine our contribution to the objective of constructing a complete list of tardigrade species in Mexico (based on the data presented here), three species-accumulation curves were estimated, one for total richness, another for vegetation type, and the third for substrate type, using R v. 4.3.2 software (R Core Team 2021) with the vegan package v. 2.6-4 (Oksanen et al. 2016). Species-richness estimates were calculated using the incidence-based coverage estimator (ICE) with the EstimateS v. 9.1 (Colwell 1997). Finally, the worldwide distribution of the new records for the studied area were mapped on the global biogeographic regionalization scheme by Morrone (2007).

## RESULTS

A total of 2822 tardigrades were mounted on slides, and 60 specimens prepared for SEM (30 specimens of *Minibiotus citlalium*, 20 specimens of *Macrobiotus* sp., and 10 specimens of *Adropion* sp.) (Figure 2). Of specimens mounted on slides, 86% of them were identified to class, 85% to order, 85% to family, 80% to genus, and 45% to species or subspecies. From the two taxonomic valid Classes in the phylum recovered, Eutardigrada had the most abundance of individuals ( $n = 1967$ ). The three tardigrade orders were found. The number of families found was seven and included some of the most common families of the phylum (Echiniscidae, Milnesiidae, Hypsibiidae, Doryphoribiidae, Ramazzottiidae, Isohypsibiidae and Macrobiotidae); the most abundant family in numbers of individuals was Macrobiotidae ( $n = 1502$ ). Seventeen genera were identified, of which *Minibiotus* was the most abundant ( $n = 300$ ). There are several genera (*Echiniscus* C.A.S. Schultze, 1840, *Astatumen* Pilato, 1997, *Itaquascon* de Barros, 1939, *Ramazzottius* Binda & Pilato, 1986, *Doryphoribius* Pilato, 1969, *Isohypsibius* Thulin, 1928, and *Calcarobiotus* Dastych, 1993) that were represented by fewer than five specimens.

Eight species and one subspecies were identified, and 35 putative species were determined. Of these putative species, 18 were given the status of “cf.” for tardigrades whose qualitative characters fit the description or redescription of a species but the quantitative data differ from the described ranges; additionally these lack egg or DNA barcodes (Stec et al. 2018). Three taxa were identified as “aff.” When some qualitative characters of specimens fit a species group (herein, the *Macrobiotus hufelandi* group and *Mesobiotus harmsworthi* group). Among the putative species are 15 morphotypes, which are based on qualitative characters, although the condition of our specimen(s) did not allow further taxonomic information to be obtained.

*Pseudechiniscus (Pseudechiniscus) ramazzottii ramazzottii* is reported for the first time from Mexico, and *Adropion onorei* (Pilato, Binda, Napolitano & Moncada, 2002) the first time from North America (Table 2). *Minibiotus citlalium* was the most abundant species, with 285 identified. *Pseudechiniscus (Meridioniscus) juanita* de Barros, 1939 is represented by only one specimen. Associated substrate, vegetation, and altitudinal data for the identified species are provided in Table 1.

The following is a complete genera, species, and putative species list with taxonomic definitions. Full data for the two newly reported species are also provided below.

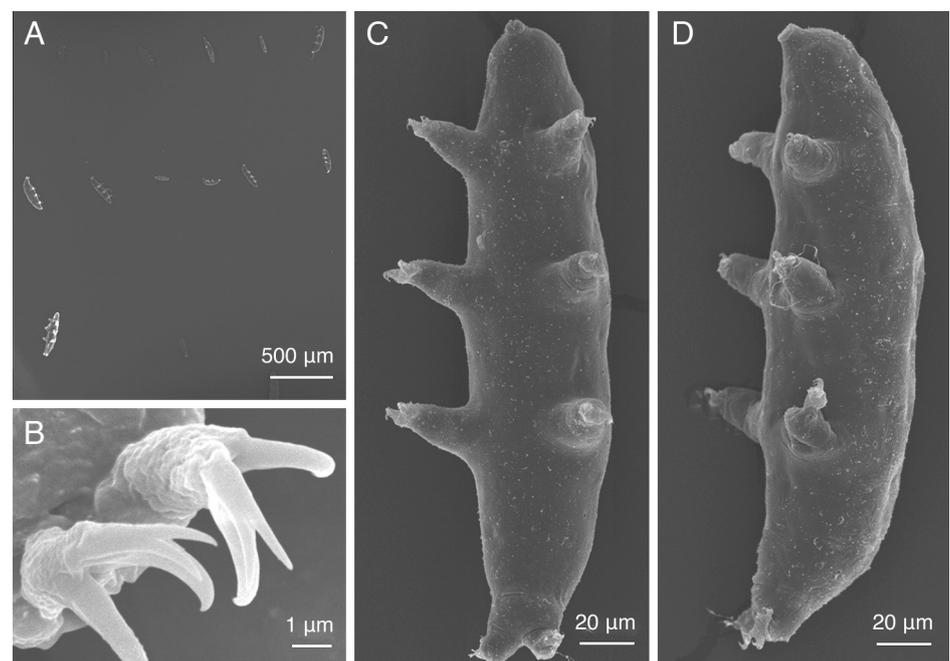
Phylum Tardigrada Doyère, 1840  
Class Heterotardigrada Marcus, 1927  
Order Echiniscoidea Richters, 1926  
Family Echiniscidae Thulin, 1928

### Genus *Claxtonia* Gąsiorek & Michalczyk, 2019

#### *Claxtonia* cf. *mauccii*

Our specimens match Gąsiorek et al.’s (2019d) description by *Claxtonia* (Figure 3A). We identified our material by the presence of cirri A, although sometimes it was found to be broken. In some specimens, it

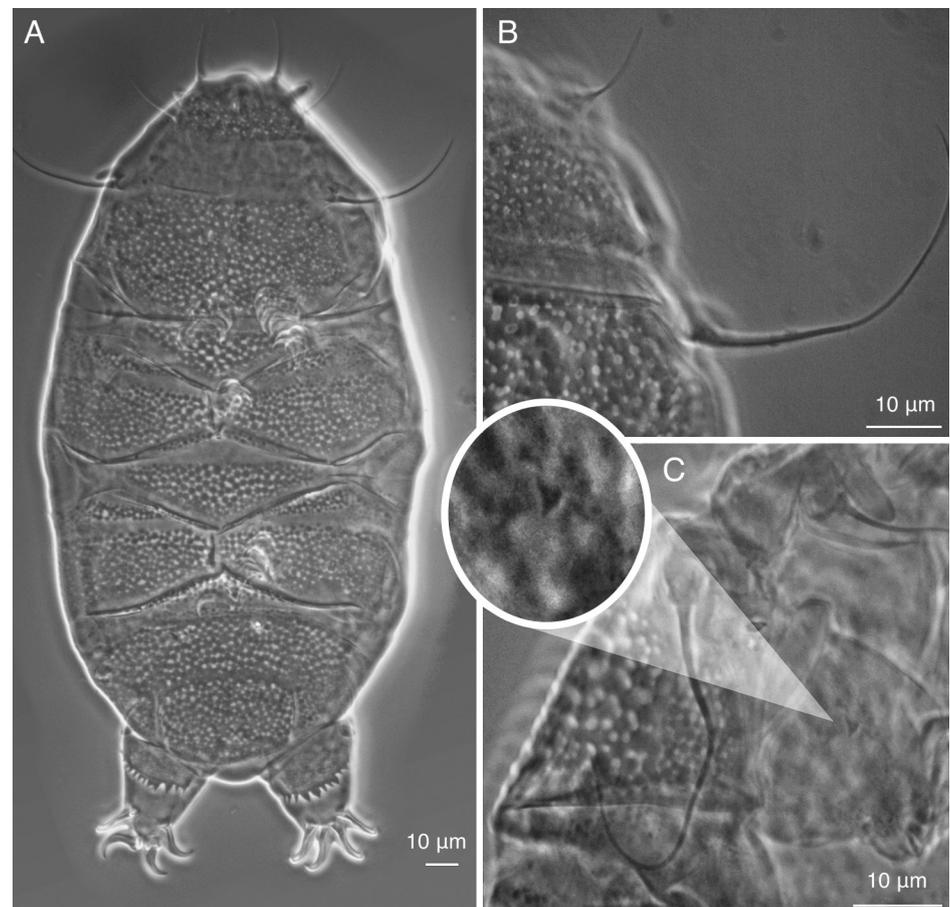
**Figure 2.** Scanning electron micrographs of *Minibiotus citlalium* and *Macrobiotus* sp. **A.** General view of the stub. **B.** Detail of the claws of *M. citlalium*. **C, D.** *Macrobiotus* sp.: **(C)** ventral view; **(D)** lateral view.



**Table 2.** Species list, including catalog number, collecting sites data and number of specimens.

Catalog number	Species	Collection site, substrate/habitat, elevation	No. of specimens
CNT1921	<i>Pseudechiniscus (Meridioniscus) juanita</i>	Bosque de Tlalpan National Park, <i>Grimmia pulla</i> on rock; <i>Quercus</i> forest; 2400 m alt.	1
CNT1918–CNT1920	<i>Pseudechiniscus (Pseudechiniscus) ramazzottii ramazzottii</i>	Bosque de Tlalpan National Park, <i>Grimmia pulla</i> on rock, <i>Quercus</i> forest, 2400 m alt.	3
CNT2603–CNT2638	<i>Adropion onorei</i>	Cumbres del Ajusco National Park, moss on rock, alpine grassland; 3900 m alt. Izta-Popo Nacional Park; moss on bark; <i>Abies religiosa</i> forest; 3498 m alt. Ibid.; moss on bark <i>Pinus hartwegii</i> forest, 3700 m to 3957 m alt.	35
CNT958–CNT963	<i>Diphascon mitrense</i>	Izta-Popo Nacional Park; moss on bark; <i>Abies religiosa</i> forest; 3498 m alt.	5
CNT967–CNT978	<i>Diphascon pingue</i>	Izta-Popo Nacional Park; moss on bark; <i>Abies religiosa</i> forest; 3498 m alt.	11
CNT1074–CNT1167	<i>Pilatobius nodulosus</i> (Ramazzotti, 1957)	Izta-Popo Nacional Park; moss on bark and soil; <i>Abies religiosa</i> and <i>Pinus hartwegii</i> forest; 3500–3800 m alt.	153
CNT593–CNT877	<i>Minibiotus citlalium</i> Dueñas-Cedillo & García-Román, 2020	Izta-Popo Nacional Park, moss on bark, soil, and rock; mixed, <i>Abies religiosa</i> , <i>Pinus hartwegii</i> forest, scrub, and tundra alpine; 2700–4500 m alt.	285
CNT878–CNT970	<i>Minibiotus sidereus</i> Pilato, Binda & Lisi, 2003	Izta-Popo Nacional Park; moss on bark and soil; mixed, <i>Abies religiosa</i> , and <i>Pinus hartwegii</i> forest; 2700–3800 m alt.	93

**Figure 3.** *Claxtonia cf. mauccii*. **A.** Habitus in dorsal view. **B.** Detail of cephalic region and cirri **A. C.** Detail of small spine on first pair of legs.



was not longer than 30% of the body length (Figure 3B). Based on the presence of a small spine on the first pair of legs (Figure 3C), a terminal faceted plate, and the absence of the third median plate (Figure 3A), as well as quantitative characters (Ramazzotti 1956; Ramazzotti and Maucci 1983), we assigned specimens to this species.

Within *Claxtonia*, species delimitation is a complicated, mainly due to problems with descriptions, intraspecific and ontogenetic variability, and the lack of DNA barcodes (Gąsiorek et al. 2023). Therefore, we cannot be certain that our specimens represent *C. maucii*, and we use “cf.” here.

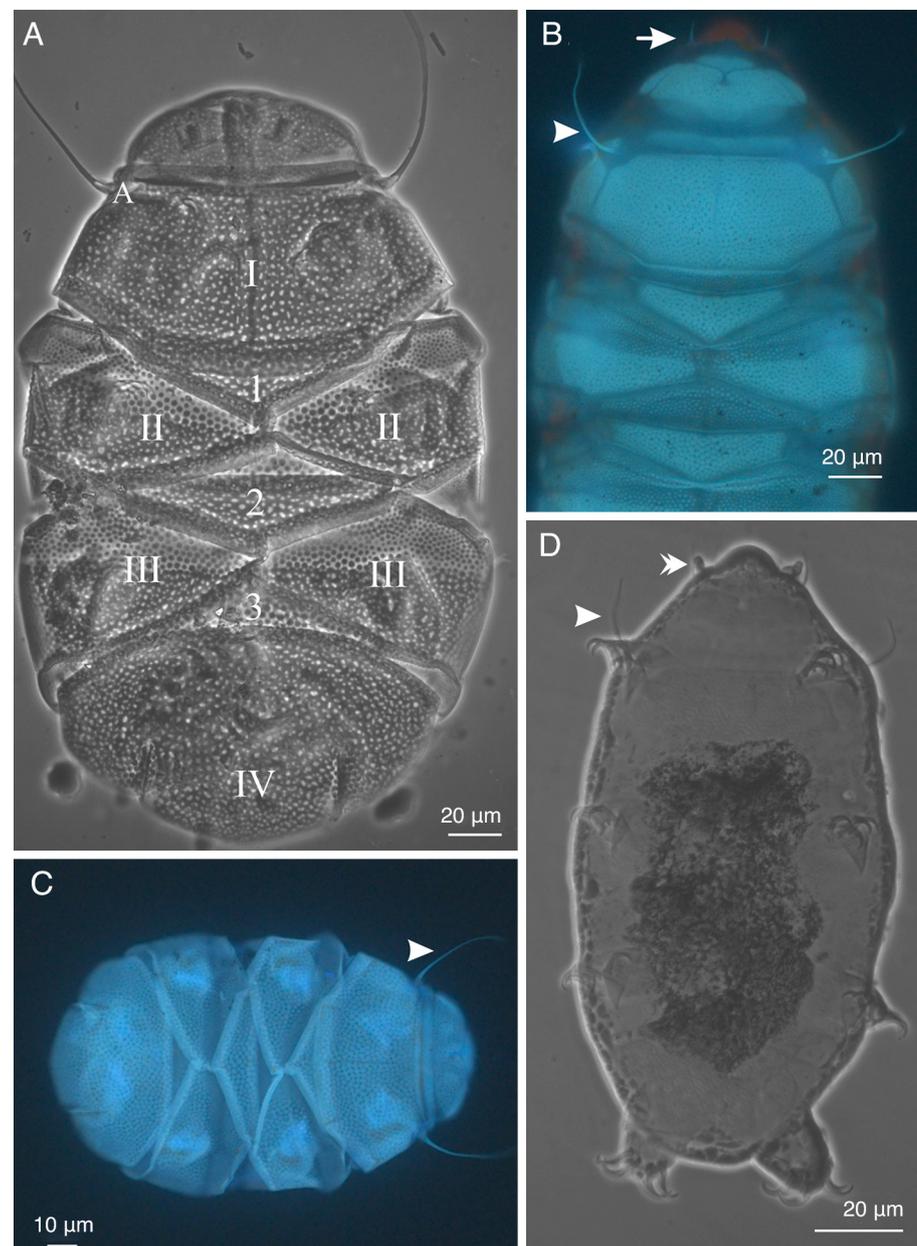
#### Genus *Echiniscus* C.A.S. Schultze, 1840

Echiniscidae with armature consisting of cephalic plate, scapular plate (I), first median plate (I), first paired plates (II), second median plate (2), second paired plates (III), third median plate (3; often lacking), terminal plate (IV, Figure 4A–D), internal cirri and cirri A (Figure 4B), internal buccal cirri (Figure 4B), cirri A (Figure 4B–D), and cephalic papillae (Figure 4D; Ramazzotti and Maucci 1983, 1983).

#### *Pseudechiniscus (Pseudechiniscus) ramazzottii ramazzottii* Maucci, 1952

**New record.** MEXICO – MEXICO CITY · Bosque del Pedregal National Park; 19°17'42.7"N, 099°12'10.3"W; 2421 m alt.; 03.III.2013; A. Dueñas-Cedillo leg.; 3 adults; on *Grimmia pulla* on rock, *Quercus* forest; CNT1918-CNT1920.

**Figure 4.** *Echiniscus*. **A.** Habitus in dorsal view: scapular plate (I), first median plate (I), first paired plates (II), second median plate (2), second paired plates (III), third median plate (3), terminal plate (IV) **B.** Detail of cephalic region showing internal buccal cirri (white arrow), cirri A (arrowhead). **C.** Detail dorsal cuticle showing cirri A (arrowhead). **D.** Detail of cephalic region showing cirri A (arrowhead) and cephalic papillae (double arrowhead).



**Identification.** The specimens match the definition of the genus *Pseudechiniscus* by Gąsiorek et al. (2021). We identified the presence of the pseudosegmental plate IV (Figure 5A,B). We noted pseudo-hemispherical cephalic papillae, which are attached to the laterally head surface and indicative of the subgenus *Pseudechiniscus* (Gąsiorek et al. 2021). The pseudosegmental plate had two triangular lobes (Ramazzotti and Maucci 1983; Figure 5A,B).

**Remarks.** *Pseudechiniscus (P.) ramazzottii ramazzottii* was described from Italy by Maucci (1952). It is a rare species, known from only a few localities in Italy, Hungary, Russia, Japan, USA (Alabama and Wisconsin), and Peru (McInnes 1994; Kaczmarek et al. 2016; Figure 6).

#### *Pseudechiniscus (Pseudechiniscus) cf. suillus* (Ehrenberg, 1853)

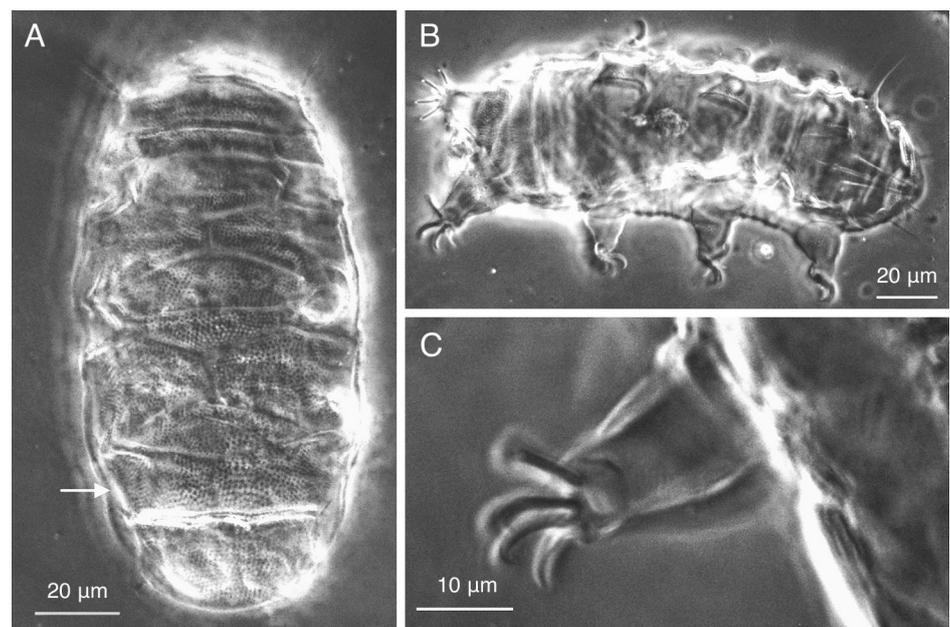
Our specimens match the definition of the genus definition *sensu* Ramazzotti and Maucci (1983) and Grobys et al. (2020). We identified pseudochiniscids with cephalic internal and external cirri, and spherical or slightly elongated cephalic papillae (secondary clava), and only lateral cirrus A present and with a finger-like clava (primary clava) near its base. Dorsal sculpturing is of the *Pseudechiniscus* type, which consists of endocuticular pillars and usually lacks pores.

The *Pseudechiniscus suillus–facettalis* species complex is one of the most difficult to identify, and unfortunately due to the poor condition of our specimens, we could not corroborate our tentative identification using the most recent redescription of *P. suillus sensu* Grobys et al. (2020).

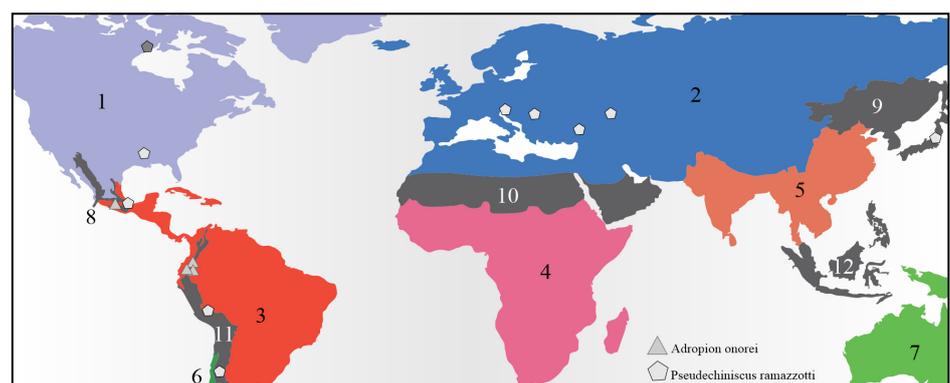
#### *Pseudechiniscus (Meridioniscus) juanita* de Barros, 1939

The specimens match Pilato and Lisi's (2006) definition of this species, which was based on animals from Chiapas, Mexico. We noted that the cuticular plates with dots less spaced than on the cephalic plate, and on the scapular plate they are smaller and more numerous than on the other plates.

**Figure 5.** *Pseudechiniscus (Pseudechiniscus) ramazzottii ramazzottii*. **A.** Habitus in dorsal view, showing pseudosegmental plate (white arrow). **B.** Detail, in dorsal view. **C.** Detail of claws II.



**Figure 6.** Worldwide distribution of *Adropion onorei* and *Pseudechiniscus (Pseudechiniscus) ramazzottii ramazzottii* with biogeographical regions shown: 1 = Nearctic region, 2 = Palearctic region, 3 = Neotropical region, 4 = Ethiopian region, 5 = Oriental region, 6 = Andean region, 7 = Australian region, 8 = Mexican transition zone, 9 = Chinese transition zone, 10 = Saharo-Arabian transition zone, 11 = South American transition zone, 12 = Indo-Malayan transition zone. Map taken from Morrone et al. 2023.



***Pseudechiniscus (Meridioniscus) novaezeelandiae* group, morphospecies 1**

The specimens match the definitions of the genus by Ramazzotti and Maucci (1983) and Grobys et al. (2020). We identified this morphospecies by its dactyloid cephalic papillae, which are attached to the body cuticle only at their bases. Striae, usually large and well developed, are present. Dorsal median plates I and II are divided transversely; the pseudosegmental plate has lobes in the form of spines, which are sometimes bifurcated. In this morphospecies, we observed that the terminal lobes of the pseudosegmental plate form sharp spines.

***Pseudechiniscus (Meridioniscus) novaezeelandiae* group, morphospecies 2**

The specimens match the definition of the genus *sensu* Ramazzotti and Maucci (1983) and Grobys et al. (2020). We identified this morphospecies by its dactyloid cephalic papillae, which are attached to the body cuticle only at their bases. Striae, usually large and well developed, present. Dorsal median plates I and II are divided transversely, and the pseudosegmental plate has lobes in the form of spines, which are sometimes bifurcated. In this morphospecies, we found that the pseudosegmental plate has terminal conical lobes, with the apexes of these lobes seemingly bifurcated.

Class Eutardigrada Richters, 1926

Order Apochela Schuster, Nelson, Grigarick & Christenberry, 1980

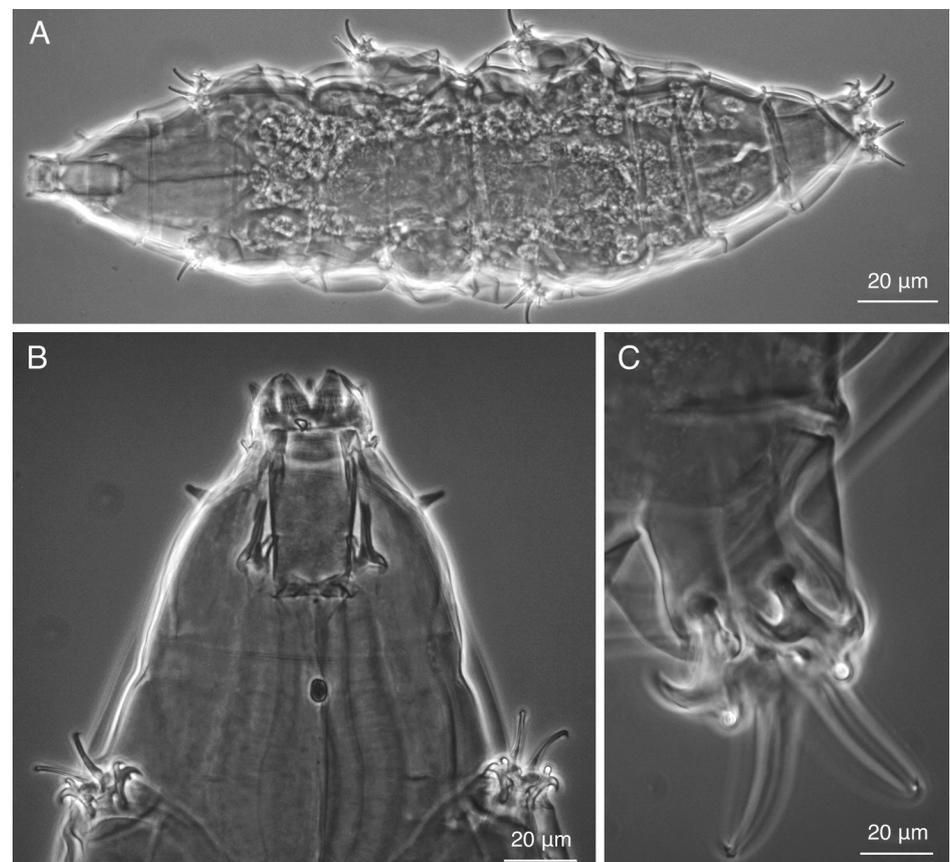
Family Milnesiidae Ramazzotti, 1962

**Genus *Milnesium* Doyère, 1840**

Eutardigrades (Figure 7A–C) with six cephalic papillae and six buccal lamellae; buccal tube short and wide, pharynx without placoids (Figure 7B). The principal branch and the secondary branch of the claws are completely divided; the second is bifid or tripartite (Figure 7C) (Pilato and Binda 2010).

In our *Milnesium* specimens, the cuticle is smooth, there are six peribuccal lamellae, and claw configuration is 3-3, 3-3.

**Figure 7.** *Milnesium*. **A.** Habitus in dorsal view. **B.** Detail of bucco pharyngeal apparatus. **C.** Detail of claws IV.



Order Parachela Schuster, Nelson, Grigarick and Christenberry, 1980  
 Superfamily Hypsibiodea Pilato, 1969  
 Family Hypsibiidae Pilato, 1969  
 Subfamily Diphasconinae Dastych, 1992

### Genus *Diphascon* Plate, 1888

Cuticle smooth (Figure 8A). Bucco-pharyngeal apparatus of the *Diphascon* type (Figure 8B). A “drop shaped” cuticular thickening may be present between the buccal tube and the pharyngeal tube (Figure 8B). Six peribuccal lobes are present. Pharyngeal apophyses may be present or absent, and placoids are present. Stylet furcae are typically shaped. Claws are of the *Hypsibius* type (Figures 8B). Lunules are either present or absent. The eggs are smooth and laid in the exuvium (Pilato and Binda 2010).

### *Diphascon* cf. *chilenense* Plate, 1888

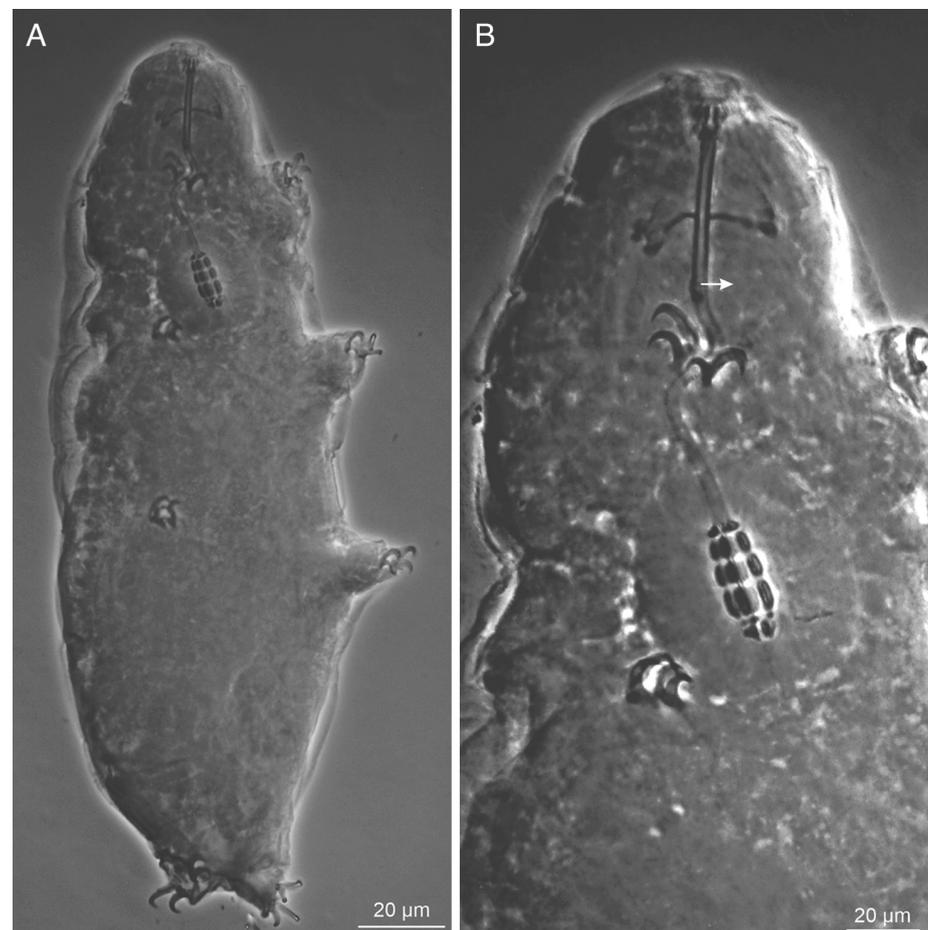
*Nomen dubium* according to (Dastych 2015)

Buccal tube 20  $\mu\text{m}$  long, and stylet support insertion points at 10.2  $\mu\text{m}$  (*pt* 51) on the buccal tube length. Pharyngeal bulb with three macroplacoids, microplacoid, and septulum; the macroplacoids are almost equal in length: M1 = 2.14  $\mu\text{m}$  (*pt* 10.7), M2 = 2.3 (*pt* 11.5), M3 = 2.4 (*pt* 12). Macroplacoid line 7.1  $\mu\text{m}$  (*pt* 35.4). Placoid line 9  $\mu\text{m}$  (*pt* 45). Claws are of the *Hypsibius* type. Morphological and morphometric characters conform to the definition of this species by Pilato and Binda (1998b).

### *Diphascon* cf. *claxtonae*

Buccal tube 18.7  $\mu\text{m}$  long; stylet support insertion points at 10.9  $\mu\text{m}$  (*pt* 60.6) on the buccal tube length. Pharyngeal bulb with three macroplacoids, microplacoid and septulum. Macroplacoid length sequence  $1 \geq 2 < 3$ ; lengths of macroplacoids: M1 = 2.9–3.0  $\mu\text{m}$  (*pt* 15.5–15.8), M2 = 2.7–2.9  $\mu\text{m}$  (*pt* 14.4–15.3), M3 = 3.8–4.0  $\mu\text{m}$  (*pt* 20.3–21.1), microplacoid and septulum. Macroplacoid line 9.3–10.0  $\mu\text{m}$  (*pt* 49.7–52.6). Placoid line 11.6–12.0  $\mu\text{m}$  (*pt* 62–63.2). Claws of the *Hypsibius* type. The morphological and morphometric characters conform to the description by Pilato and Binda (1998a), as well as to the keys by Pilato and Binda (1999) and Fontoura and Pilato (2007). This species belongs to the *D. pingue* species complex (Fontoura and Pilato 2007).

**Figure 8.** *Diphascon* **A.** Habitus in dorsal view. **B.** Detail of bucco-pharyngeal apparatus showing “drop shaped” cuticular thickening at white arrow.



***Diphascon cf. dastychi***

Buccal tube 20.9–22.0 µm long, and stylet support insertion point at 12.5–12.8 µm (*pt* 58.2–59.8) on the buccal tube length. Pharyngeal bulb with three macroplacoids, microplacoid, and septulum; macroplacoid length sequence 1<2<3; macroplacoid lengths: M1 = 3.5–3.7 µm (*pt* 16.7–16.8), M2 = 3.5–4.0 µm (*pt* 16.7–18.2), M3 = 4.5–5.0 µm (*pt* 21.5–22.7), microplacoid and septulum. Macroplacoid line 11.7–13.5 µm (*pt* 56–58). Placoid line 13.0–15.0 µm (*pt* 62.2–68.2). Claws are of the *Hypsibius* type. The morphological and morphometric characters conform to the description and keys by Pilato and Binda (1999) and Fontoura and Pilato (2007). This species belongs to the *D. pingue* species complex (Fontoura and Pilato 2007).

***Diphascon cf. faialense***

Buccal tube 18.1–18.5 µm long, stylet support insertion points at 10.5–10.8 µm (*pt* 58.0–58.4) on the buccal tube length. Pharyngeal bulb with three macroplacoids (length sequence 1>2<3) M1 = 2.6–2.9 µm (*pt* 14.4–15.7), M2 = 2.4–2.9 µm (*pt* 13.3–15.7), M3 = 3.8–4.0 µm (*pt* 21.0–21.6), microplacoid and septulum. Macroplacoid line 9.1–9.9 µm (*pt* 50.3–53.5). Placoid line 11.7–12.0 µm (*pt* 64.6–64.9). Claws of the *Hypsibius* type. The morphological and morphometric characters conform to the description and keys given by Pilato and Binda (1999), and Fontoura and Pilato (2007). This species belongs to the *D. pingue* species complex (Fontoura and Pilato 2007). Taxon is not illustrated.

***Diphascon mitrense* Pilato, Binda & Qualtieri, 1999**

Buccal tube 19.4–20.7 µm long, stylet support insertion points at 11.0–12.0 µm (*pt* 56.7–58.0) on the buccal tube length. Pharyngeal bulb with three macroplacoids (length sequence 1>2<3) M1 = 2.4–3.0 µm (*pt* 12.9–14.5), M2 = 1.9–2.2 µm (*pt* 9.8–10.6), M3 = 2.6–3.5 µm (*pt* 13.4–16.9), microplacoid and septulum. Macroplacoid line 7.3–8.6 µm (*pt* 37.6–41.5). Placoid line 9.7–11.7 µm (*pt* 50.0–56.5). Claws of the *Hypsibius* type. Morphological and morphometric characters conform to the description and keys given by Pilato and Binda (1999), and Fontoura and Pilato (2007). This species belongs to the *D. pingue* species complex (Fontoura and Pilato 2007).

***Diphascon pingue* (Marcus, 1936)**

Buccal tube 19.0–16.0 µm long, stylet support insertion points at 8.4–9.0 µm (*pt* 56.4–56.3) on the buccal tube length. Pharyngeal bulb with three macroplacoids (length sequence 1>2<3) M1 = 2.1–2.5 µm (*pt* 14.1–15.6), M2 = 2.3–2.8 µm (*pt* 15.4–17.5), M3 = 2.6–3.0 µm (*pt* 17.4–18.8), microplacoid and septulum. Macroplacoid line 6.7–7.2 µm (*pt* 45.0). Placoid line 8.7–10.0 µm (*pt* 58.4–62.5). Claws of the *Hypsibius* type. Except for the stylet muscle support, the morphological and morphometric characters conform to the description of Pilato and Binda (1998), as well as to the keys of Pilato and Binda (1999), and Fontoura and Pilato (2007). This species belongs to the *D. pingue* species complex (Fontoura and Pilato 2007).

***Diphascon cf. pinguiforme***

Buccal tube 18.4–19.0 µm long, stylet support insertion point at 10.4–11.0 µm (*pt* 56.5–57.9) on the buccal tube length. Pharyngeal bulb with three macroplacoids (length sequence 1>2<3) M1 = 3.4–3.5 µm (*pt* 18.5), M2 = 3.1–3.5 µm (*pt* 16.8–18.4), M3 = 4.4–4.5 µm (*pt* 23.7–23.9), microplacoid and septulum. Macroplacoid line 11.4–11.5 µm (*pt* 60.5–62.0). Placoid line 12.5–14.0 µm (*pt* 67.9–73.7). Claws of the *Hypsibius* type. Morphological and morphometric characters conform to the description of Pilato and Binda (1998b). Taxon is not illustrated.

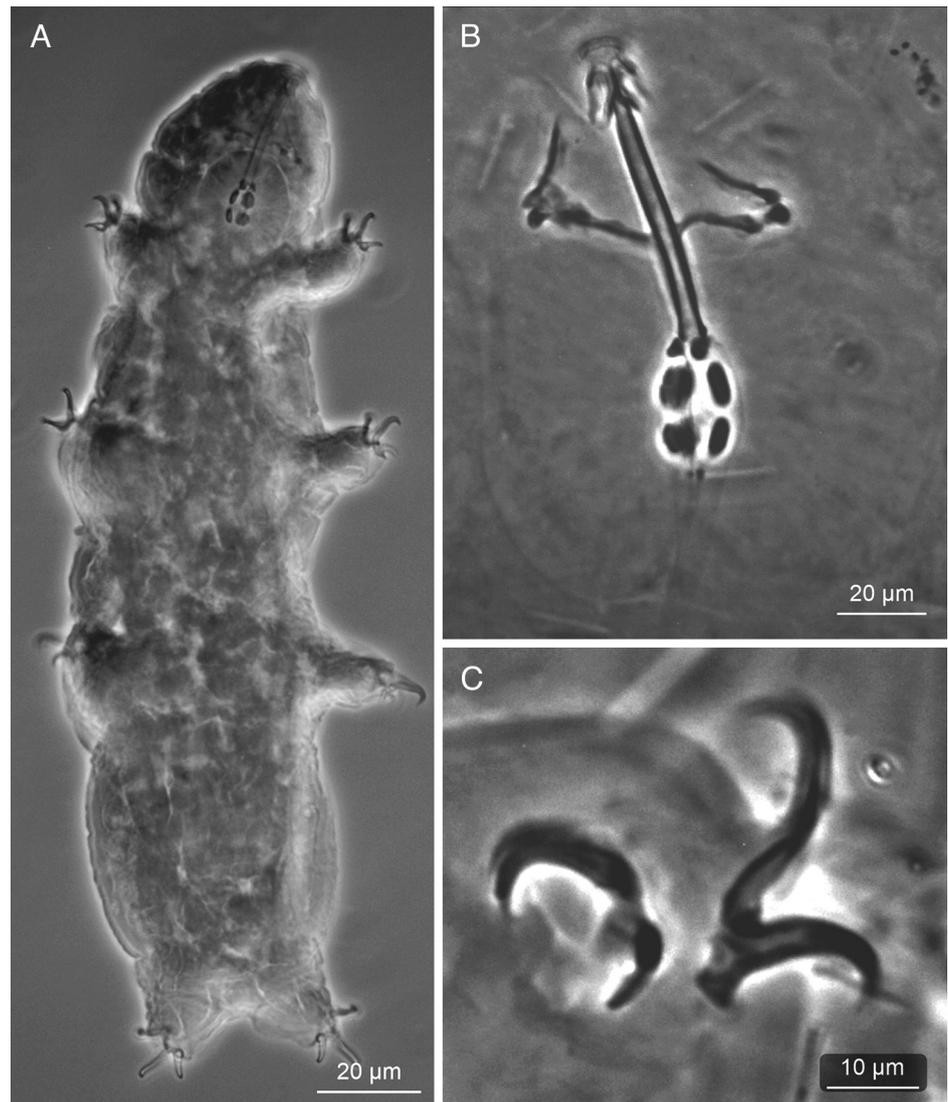
***Diphascon cf. victoriae***

Buccal tube 23.5–24 µm long, stylet support insertion point at 14.0–15.0 µm (*pt* 59.6–62.5) on the buccal tube length. Pharyngeal bulb with three macroplacoids (length sequence 1<2<3) M1 = 3.7–4.0 µm (*pt* 15.7–16.7), M2 = 3.7–5.0 µm (*pt* 15.7–20.8), M3 = 5.5–6.5 µm (*pt* 23.4–27.1), microplacoid and septulum. Macroplacoid line 14.0–15.0 µm (*pt* 59.6–62.5). Placoid line 16.0–16.5 µm (*pt* 68.1–68.8). Claws of the *Hypsibius* type. The morphological and morphometric characters conform to the description and keys given by Pilato and Binda (1999), and Fontoura and Pilato (2007). This species belongs to the *D. pingue* species complex (Fontoura and Pilato 2007).

Subfamily Hypsibiinae Pilato, 1969

**Genus *Hypsibius* Ehrenberg, 1848**

Cuticle transparent (Figure 9A). Buccal apparatus is of the *Hypsibius* type (Figure 9B). Six peribuccal lobes are present. Pharyngeal apophyses and placoids present. The furca of the stylet has the typical shape. Claws are of the *Hypsibius* type. Lunules are absent in the known species (Figure 9C). Eggs are smooth and laid in the exuvium, except in one or two species (Pilato and Binda 2010).



**Figure 9.** *Hypsibius* **A.** Habitus in dorsal view. **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws III.

#### ***Hypsibius cf. dujardini***

Buccal tube 23 µm long. Stylet supports inserted about 60.8 µm on the buccal tube. Pharyngeal bulb with two macroplacoids (length sequence 1<2) and a septulum (*pt* 6.8). Claws are of the *Hypsibius* type. Base of external claws II–III 3.5 µm long, primary branch 6.8 µm long, and secondary branch 4.7 µm long. Base of posterior claw 2.8 µm long, primary branch 9.0 µm, and secondary branch 5.2 µm. There is a cuticular bar between the posterior and anterior claw.

This species was recently redescribed by Gasiorek et al. (2018). The morphological and morphometric characteristics of our specimens conform to the redescription and key by Gasiorek et al (2018).

#### ***Hypsibius cf. exemplaris***

Buccal tube 25 µm long; stylet supports inserted on the buccal tube at >65.5% of its length. Claws are of the *Hypsibius* type.

We identified this species using the key by Gasiorek et al. (2018). However, we found few specimens.

#### ***Hypsibius cf. pedrottii***

Buccal tube 15.7–24.7 µm long; stylet supports inserted at 61.1–66.2% of the buccal tube length. Pharyngeal bulb with two macroplacoids (length sequence 1>2), of which the first macroplacoid is constricted and measures 2.0–4.2 µm long (*pt* 11.6–20.2). The second macroplacoid is 1.8–3.2 µm long (*pt* 8.9–13.9). The macroplacoid line measures 4.6–8.6 µm long (*pt* 26.8–38.6). Claws are of the *Hypsibius* type.

The morphometric characters of our specimens fit the description of this species by Bertolani et al. (1987). However, this species has only been recorded in Italy and its presence in Mexico must be confirmed.

***Hypsibius cf. microps***

Buccal tube 17.1–21.7  $\mu\text{m}$  long; stylet supports inserted at 58–61.9% of the buccal tube length. Pharyngeal bulb with two macropilacoids (length sequence 1>2) and without constrictions. Macropilacoid measurements are MI: 1.8–2.4  $\mu\text{m}$  long (*pt* 10.4–13.1); MII: 1.7–2.2  $\mu\text{m}$  (*pt* 8.5–12.6). Claws are of the *Hypsibius* type.

Morphological characteristics of our specimens conform to the redescription of this species by Kaczmarek and Michalczyk (2009); however, some morphometric characteristics differ.

***Hypsibius cf. pallidus***

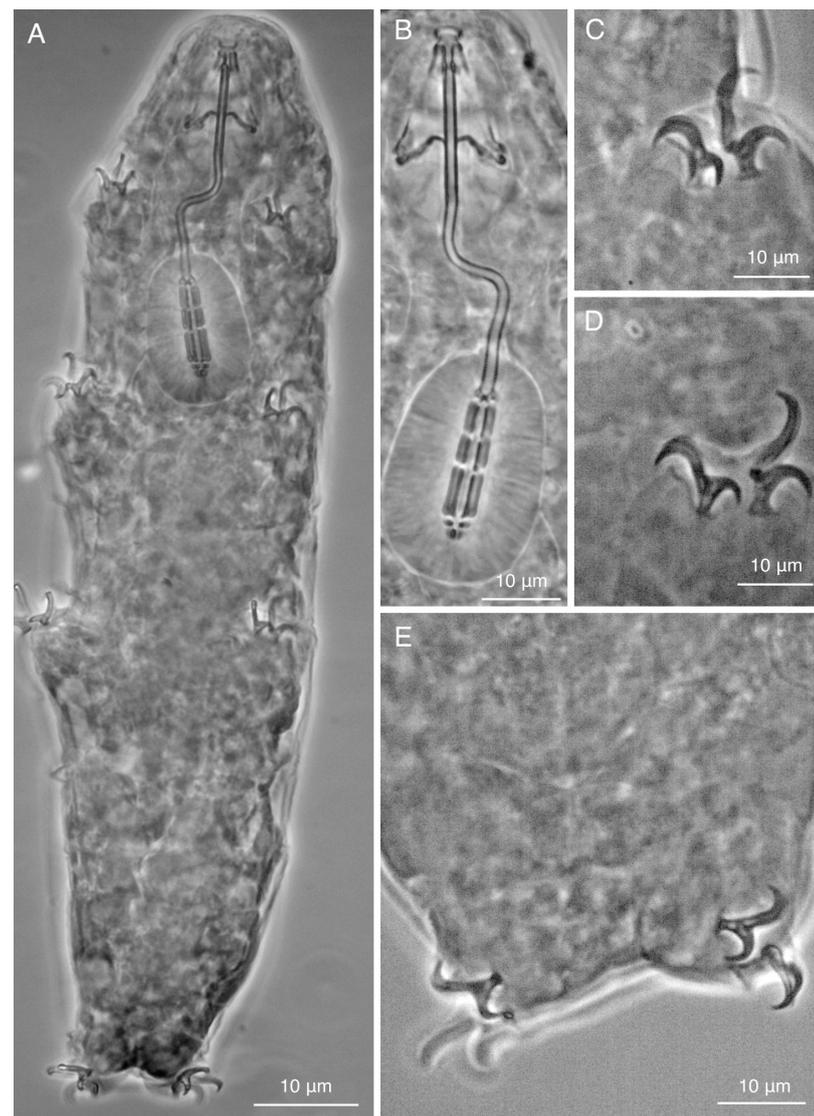
The bucco-pharyngeal apparatus is of the *Hypsibius* type. Average length of the buccal tube is 23.29  $\mu\text{m}$  long; stylet supports are inserted at 51.6–58.1% of the buccal tube length. Pharyngeal bulb with two macropilacoids (length sequence 1>2) and without constrictions. Claws are of the *Hypsibius* type. Morphological characteristics of our specimens conform to the redescription of the species by Kaczmarek and Michalczyk (2009). However, some morphometric characteristics differ from the redescription.

Subfamily Itaquasconinae Bartoš in Rudescu, 1964

***Adropion onorei* (Pilato, Binda, Napolitano & Moncada, 2002)**

**New records.** MEXICO – STATE OF MEXICO • Amecameca, Izta Popo National Park; 19°05'14"N, 098°40'03"W; 3498 m alt.; 23.I.2018; F. Armendariz-Toledano leg.; 12 adults; on moss on bark, *Abies religiosa* forest; CNT2603-CNT2614 • Amecameca, Izta Popo National Park; 19°05'06"N, 098°38'49"W, 3700 m alt.; 22.II.2018; J. García-Román leg.; 5 adults; on moss on bark, *Pinus hartwegii* forest; CNT2615-CNT2619 • Amecameca, Izta Popo National Park; 19°07'31"N, 098°39'11"W, 3957 m alt.; 25.III.2018, A. Dueñas-Cedillo

**Figure 10.** *Adropion onorei*. **A.** Habitus in dorsal view. **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws I, showing cuticular bar near base of inner claws. **D.** Detail of claw III, showing cuticular bar near base of inner claw. **E.** Detail of claws IV.



leg; 17 adults; moss on bark *Pinus hartwegii* forest; CNT2620-CNT2637 – MEXICO CITY · Magdalena Contreras, Cumbres del Ajusco National Park; 19°12'26"N, 099°15'32"W; 3900 m alt.; 23.VIII.2019; J. Gacía-Román leg.; 1 adult; on moss on bark, alpine grassland CNT2638.

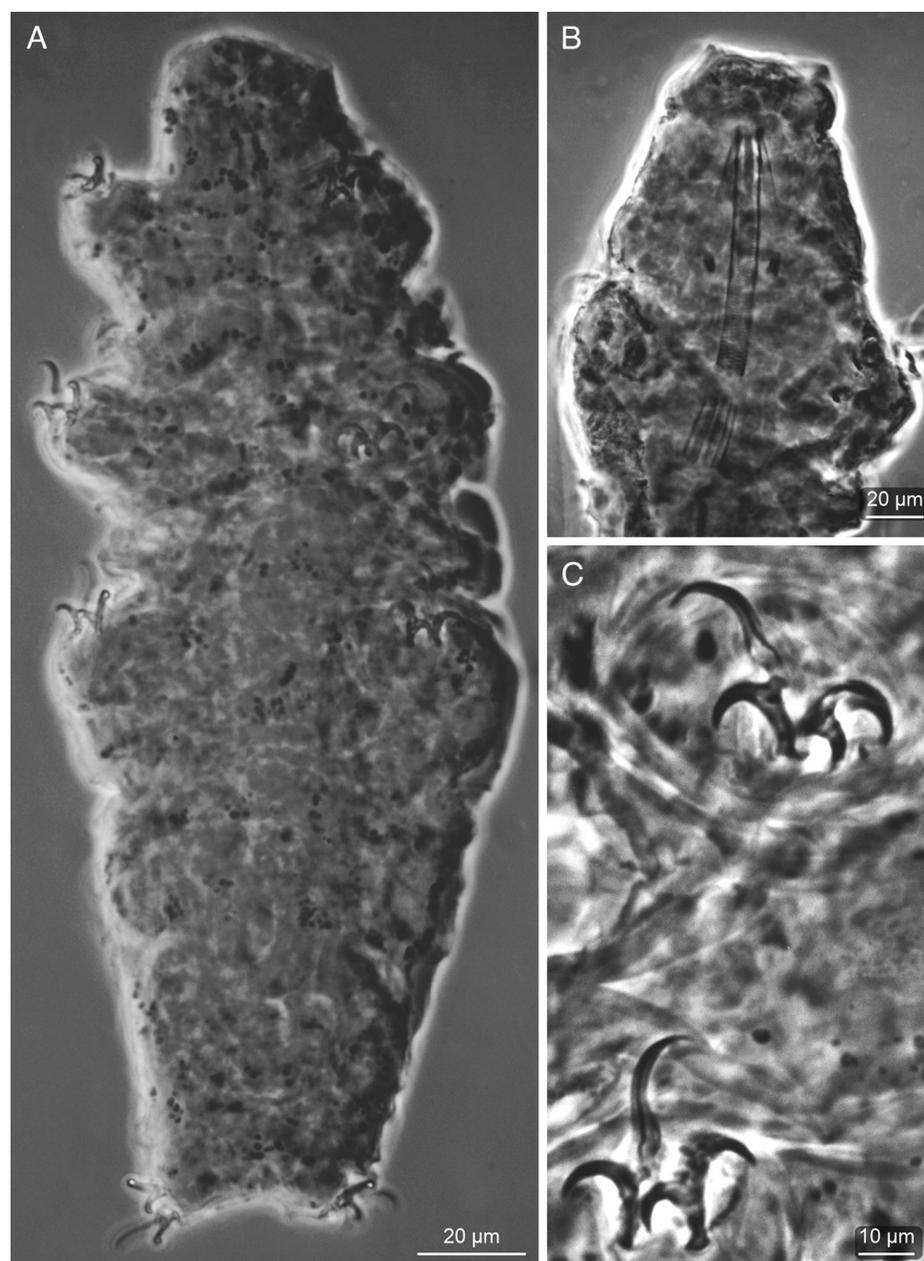
**Identification.** Our specimens match the description by Pilato et al. (2002). We noted the presence of three macroplacoids ( $2 > 1 < 3$ ), microplacoid, and septulum. Cuticular bars are present near the inner claw on the first three pairs of legs, and the base of the rear claw is expanded (Figure 10). There are no spines on the claw bases, except in two individuals that showed inconspicuous crenation.

**Remarks.** *Adropion onorei* was described from Ecuador (Pilato et al. 2002) but has not been found since. Our specimens are the first recorded from Mexico and North America and greatly expands this species' distribution (Figure 6).

#### Genus *Astatumen* Pilato, 1997

The bucco-pharyngeal apparatus is of the *Astatumen* type (Figure 11A, B). There is no cuticular thickening present between the buccal tube and the pharyngeal tube in the known species. Pharyngeal apophyses are absent. There are no placoids, or only one undivided placoid present. The stylet furcae are very small and of the *Astatumen* type. Claws are of the *Hypsibius* type (Figure 11C). Lunules are absent (Figure 11C). Eggs smooth and laid in the exuvium (Pilato and Binda 2010).

**Figure 11.** *Astatumen* **A.** Habitus **B.** Detail of bucco pharyngeal apparatus. **C.** Detail of claws II.



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

**Genus *Degmion* Gąsiorek, Morek & Michalczy, 2023**

The cuticle is sculptured in the caudal body, and anterior portions have weaker sculpturing or are smooth. The buccal tube is followed by an annulated pharyngeal tube (Figure 12A, B), and there is a drop-like thickening between them. The pharyngeal bulb is roundish or slightly oval and always containing two macroplacoids which are similar in length and in rows that look as parentheses; a septulum is present. Pseudolunulae below claws I–IV may be present, but only basal cuticular bars may be present below claw IV (Gąsiorek et al. 2023).

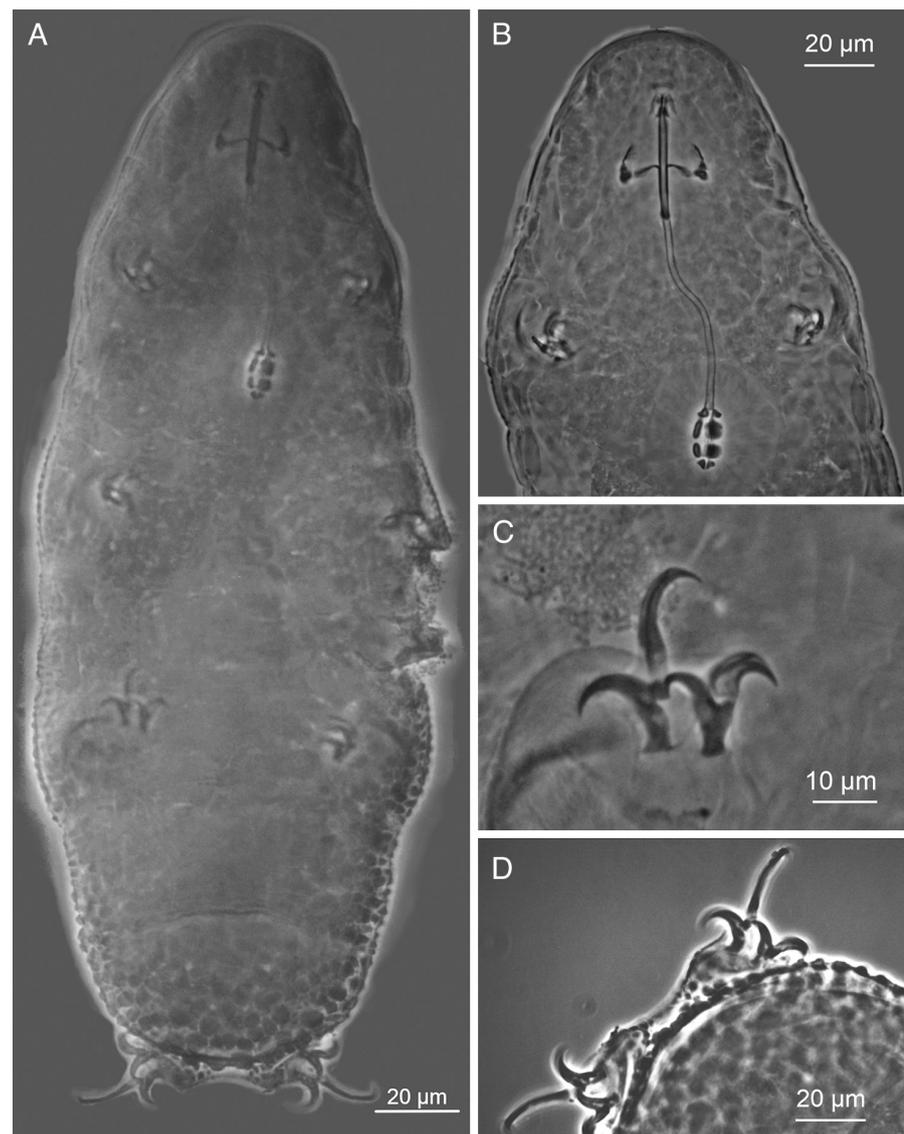
***Degmion nodulosus* (Ramazzotti, 1957)**

The cuticle is ornamented by irregularly shaped tubercles (Figure 12A, B). The first segment is usually smooth or with granulation, the second segment usually has small polygons that increase in size toward the caudal region (Figure 12A, D). The claws are distinct in size and shape in all pairs of claws; in the fourth pair, the posterior claw has a long, slender primary branch (Figure 12C, D). Our specimens fit the taxonomic definition of this species as given by Ramazzotti and Maucci (1983).

***Degmion* cf. *rugocaudatus***

The cuticle in the caudal region of the dorsum is granulated, and the larger granules are formed from minuscule rounded platelets; this granulated zone never reaches the 3rd pair of legs (Ramazzotti and Maucci 1983).

**Figure 12.** *Degmion nodulosus*. **A.** Habitus **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws II. **D.** Detail of claws IV.



***Pilatobius* Bertolani, Guidetti, Marchioro, Altiero, Rebecchi & Cesari, 2014**

Diagnosis amended by Gąsiorek, Morek & Michalczyk (2023)

The cuticle is uniformly sculptured throughout the dorsal body; dorsolateral gibbositities may be present (Gąsiorek et al. 2023). The buccal tube is followed by an annulated pharyngeal tube, and there is a drop like thickening between them. The pharyngeal bulb is roundish or slightly oval and always contains two macroplacoids of similar length and in rows that look like parentheses; there is a septulum (Bertolani et al. 2014). Pseudolunulae below claws I–IV may be present; cuticular bars of various types (internal, anterior, posterior, and orthogonal) are present (Gąsiorek et al. 2023).

***Pilatobius* cf. *rugosus***

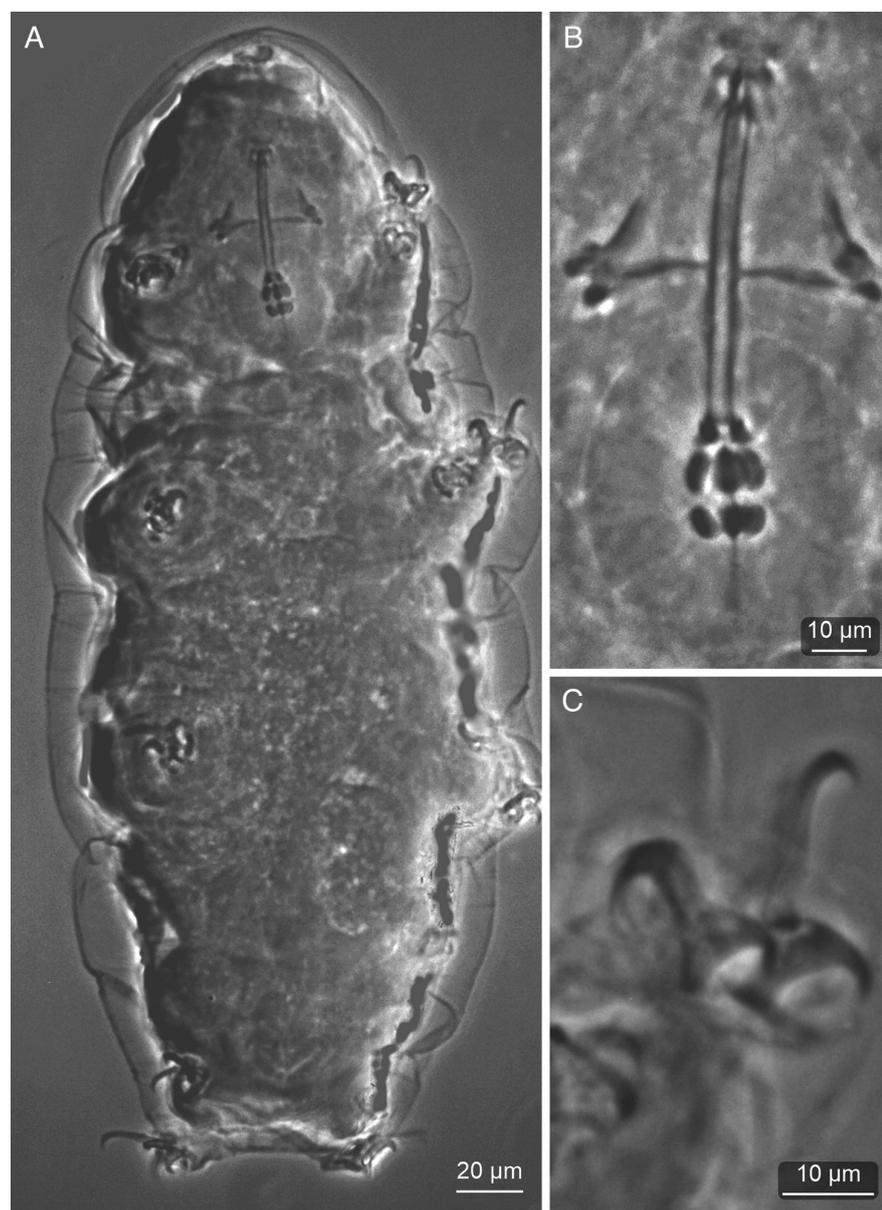
The cuticle is covered with an extremely fine granulation composed of regularly arranged granules, which become somewhat larger and more distinct in the caudal dorsum, posterior to the third pair of legs (Ramazzotti and Maucci 1983).

Family Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008

**Genus *Ramazzottius* Binda & Pilato, 1986**

The bucco-pharyngeal apparatus is of the *Hypsibius* type, *Ramazzottius* variant (Figure 13A, B). Pharyngeal apophyses and placoids are present (Figure 13B). The stylet furcae are typically shaped. Claws are of the

**Figure 13.** *Ramazzottius*. **A.** Habitus **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws II.



*Ramazzottius* type (Figure 13C). Very small, reduced lunules are present. Eggs are laid freely, and the egg-shell bears processes (Pilato and Binda 2010).

Superfamily Isohypsibioidea Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008  
Family Doryphoribiidae Gąsiorek, Stec, Morek & Michalczyk, 2019

#### Genus *Doryphoribius* Pilato, 1969

The bucco-pharyngeal apparatus is of *Doryphoribius* type (Figure 14A, B). Six peribuccal lobes present. The peribuccal papulae are difficult to see. Pharyngeal apophyses and placoids are present. The stylet furcae are typically shaped. The two claws on each leg are differ slightly from each other. Lunules are reduced or absent. Eggs are smooth and laid in the exuvium (Pilato and Binda 2010).

Family Isohypsibiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008.

#### Genus *Isohypsibius* Thulin, 1928

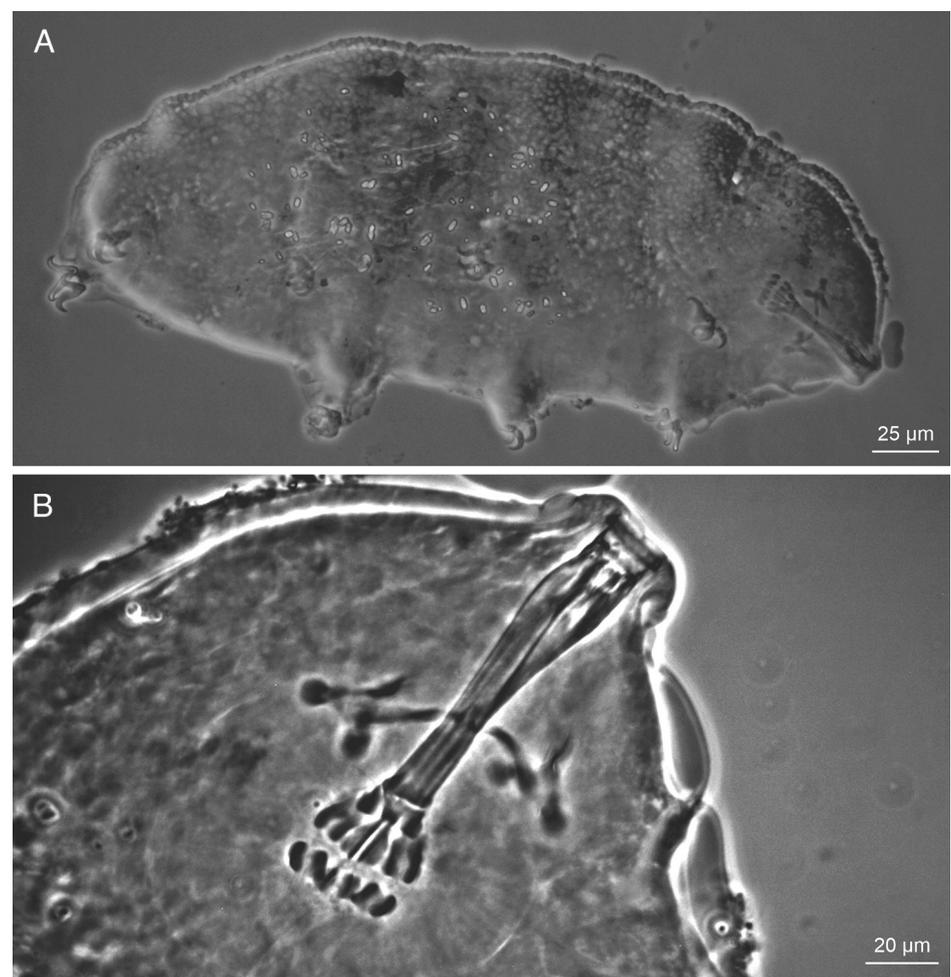
The bucco-pharyngeal apparatus is of the *Isohypsibius* type, *Isohypsibius* variant. Six peribuccal lobes are present. Pharyngeal apophyses and placoids are present. Claws are of the *Isohypsibius* type, and diplo-claws on the same leg slightly differ in size and shape. The stylet furcae are typically shaped. Lunules are present or absent. Eggs are smooth and laid in the exuvium, but the eggs of many *Isohypsibius* species are undescribed (Pilato and Binda 2010).

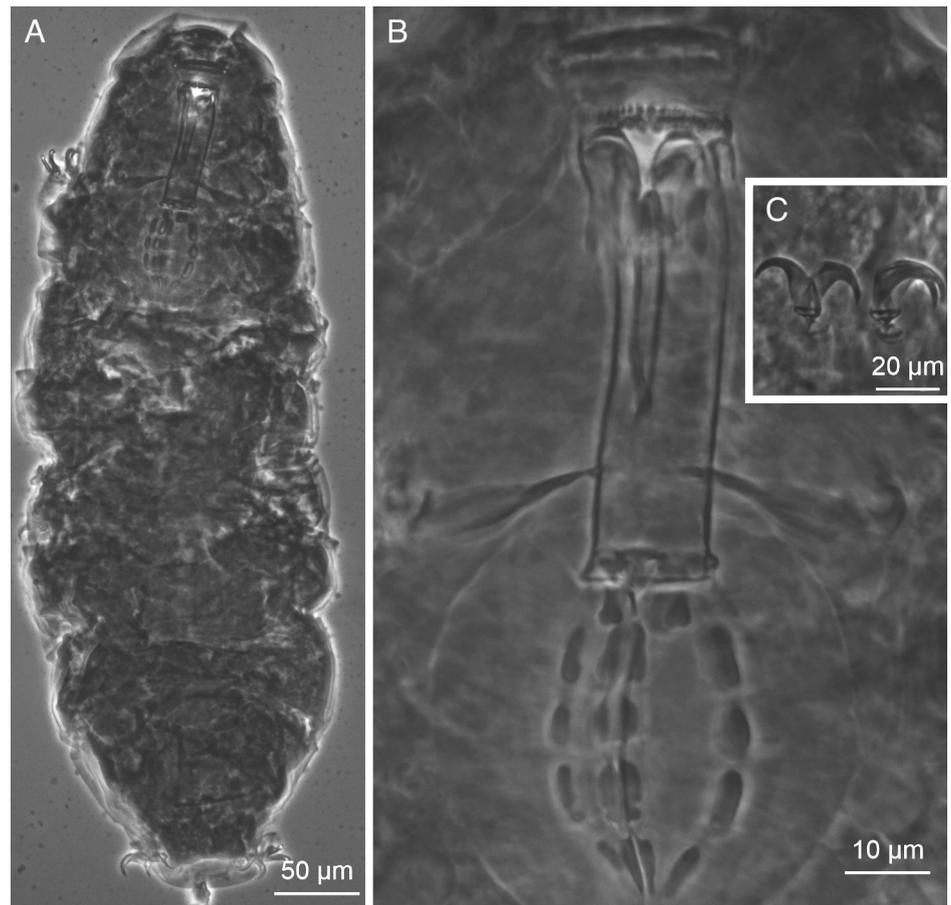
Superfamily Macrobiotioidea Thulin, 1928  
Family Macrobiotidae Thulin, 1928

#### Genus *Calcarobiotus* Dastych, 1993

The bucco-pharyngeal apparatus is of the *Macrobiotus* type (Figure 15A, B; rigid buccal tube with a ventral

**Figure 14.** *Doryphoribius*. **A.** Habitus in lateral view. **B.** Detail of bucco-pharyngeal apparatus.





**Figure 15.** *Calcarobiotus* **A.** Habitus **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws II.

strengthening bar). Claws are of *Calcarobiotus* type, with a thin stalk evident, a wide, transverse septum dividing the basal portion from the rest of the claw, a wide basal portion of the claw with a large distal part, with or without spurs (Figure 15A, C); main and secondary branches, at least on the first pairs of legs, similar and almost symmetrical respect to median plate of the claw. Eggs are laid free (Guidetti and Bertolani, 2001).

#### **Genus *Calcarobiotus*, subgenus *Discrepunguis* Guidetti & Bertolani, 2001**

Hind claws differ in shape to claws on the first three pairs of legs, and showing a small basal portion, a long common tract, and short secondary branches (Guidetti and Bertolani 2001).

#### **Genus *Macrobotus* C.A.S. Schultze, 1834**

The bucco-pharyngeal apparatus is of the *Macrobotus* type, *Macrobotus* variant (Figure 16A, B). Pharyngeal apophyses and placoids are present. Stylet furcae are typically shaped. Claws are of the *hufelandi* type. Lunules are present. Eggs are laid freely, and the eggshell bears processes, but two exceptions are known (Pilato and Binda 2010).

#### ***Macrobotus* aff. *lissostomus***

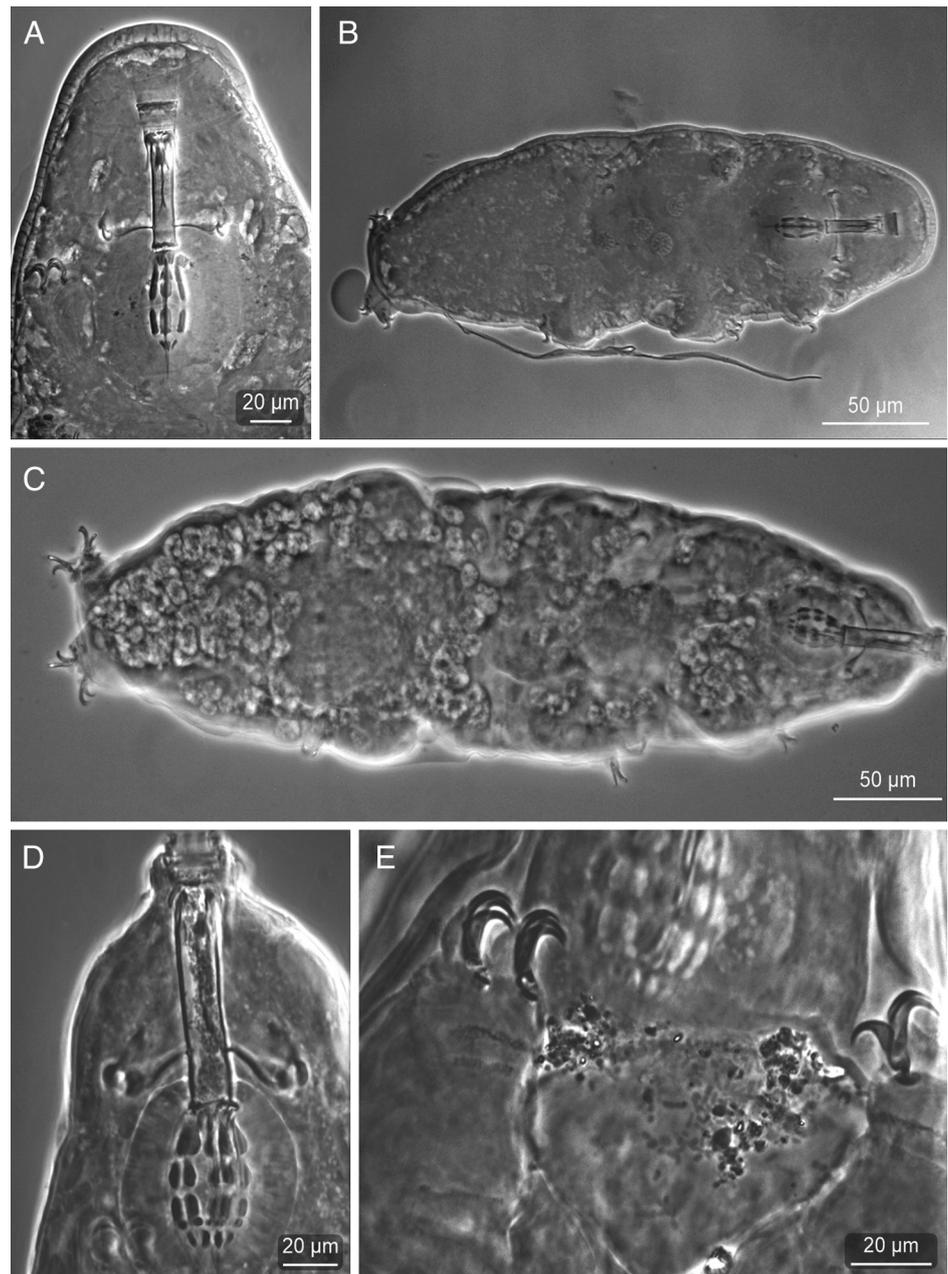
Specimens without teeth in the oral cavity under light microscopy, so based on the four types of oral cavity armature recognized in the *Macrobotus hufelandi* group (Kaczmarek and Michalczyk 2017); the oral cavity of our specimens is of the *lissostomus* type.

#### ***Macrobotus* aff. *patagonicus***

Our specimens present the second and third bands of teeth (seen under the light microscope), and these bands of teeth corresponding to the *patagonicus* type cavity armature *sensu* (Kaczmarek and Michalczyk 2017).

#### **Genus *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016**

The cuticle is without pores (Figure 16C). The mouth ring bears 10 peribuccal lamellae; there is a rigid buccal tube, three roundish macroplacoids arranged along a curved line, and the microplacoid is clearly



**Figure 16. A, B.** *Macrobiotus*: (A) detail of bucco-pharyngeal apparatus; (B) habitus. **C–E.** *Mesobiotus*: (C) habitus; (D) detail of the bucco-pharyngeal apparatus; (E) detail of claws I.

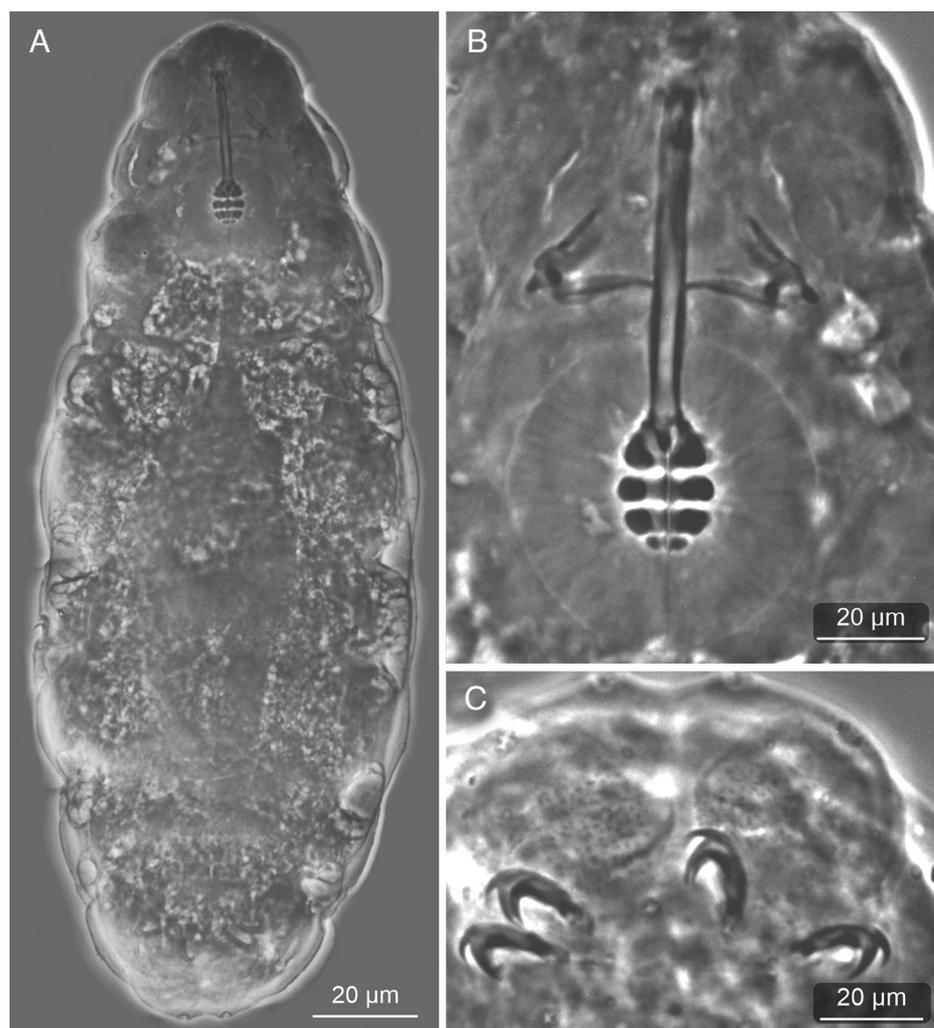
close (less than its length) to the third macroplacoid (Figure 16D). Claws are of the Y-type (Figure 16E). Eggs are laid freely and characterized by their conical or hemispherical processes, which generally have pointed tips (Vecchi et al. 2016).

#### ***Mesobiotus* aff. *harmsworthi***

The bucco-pharyngeal apparatus is of the *Macrobiotus* type (Figure 16D), with the ventral lamina and 10 peribuccal lamellae. The mouth is antero-ventral in position. The oral cavity armature is well developed and composed of three bands of teeth. The pharyngeal bulb is spherical, with triangular apophyses, three rod-shaped macroplacoids, and a triangular microplacoid (length sequence  $1 > 2 < 3$ ). Claws are of the *Mesobiotus* type (Kaczmarek et al. 2018). However, eggs of our specimens were not found to corroborate the identification.

#### **Genus *Minibiotus* R.O. Schuster, 1980**

Ten peribuccal papulae are present, but there are no peribuccal lamellae. Mouth antero-ventral in position; teeth in oral cavity absent or strongly reduced (Figure 17A, B). Buccal tube short, rigid, and narrow ( $\leq 12\%$  of buccal tube length); wall thickened below point of insertion of stylet supports; stylet supports inserted at 73%



**Figure 17.** *Minibiotus*. **A.** Habitus. **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws IV.

or less of buccal tube length; ventral support short (62% or less of buccal tube length) and macroplacoid row length 42% or less of buccal tube length. Claws of *Macrobiotus* type (Figure 17C) (Claxton 1998).

#### ***Minibiotus* cf. *intermedius* Plate, 1888**

The body cuticle is smooth, without pores and granulation; granulation on the pair of legs IV is hardly visible. The buccal tube has an anterior and a posterior bend and is slightly thicker below the stylet insertion point. The pharyngeal bulb has three granular macroplacoids and a small microplacoid (length sequence  $1 > 2 \leq 3$ ; Claxton 1998; Kaczmarek et al. 2022). However, eggs of our specimens were not found to corroborate the identification.

#### ***Minibiotus* *cittalium* Dueñas-Cedillo & García-Román, 2020**

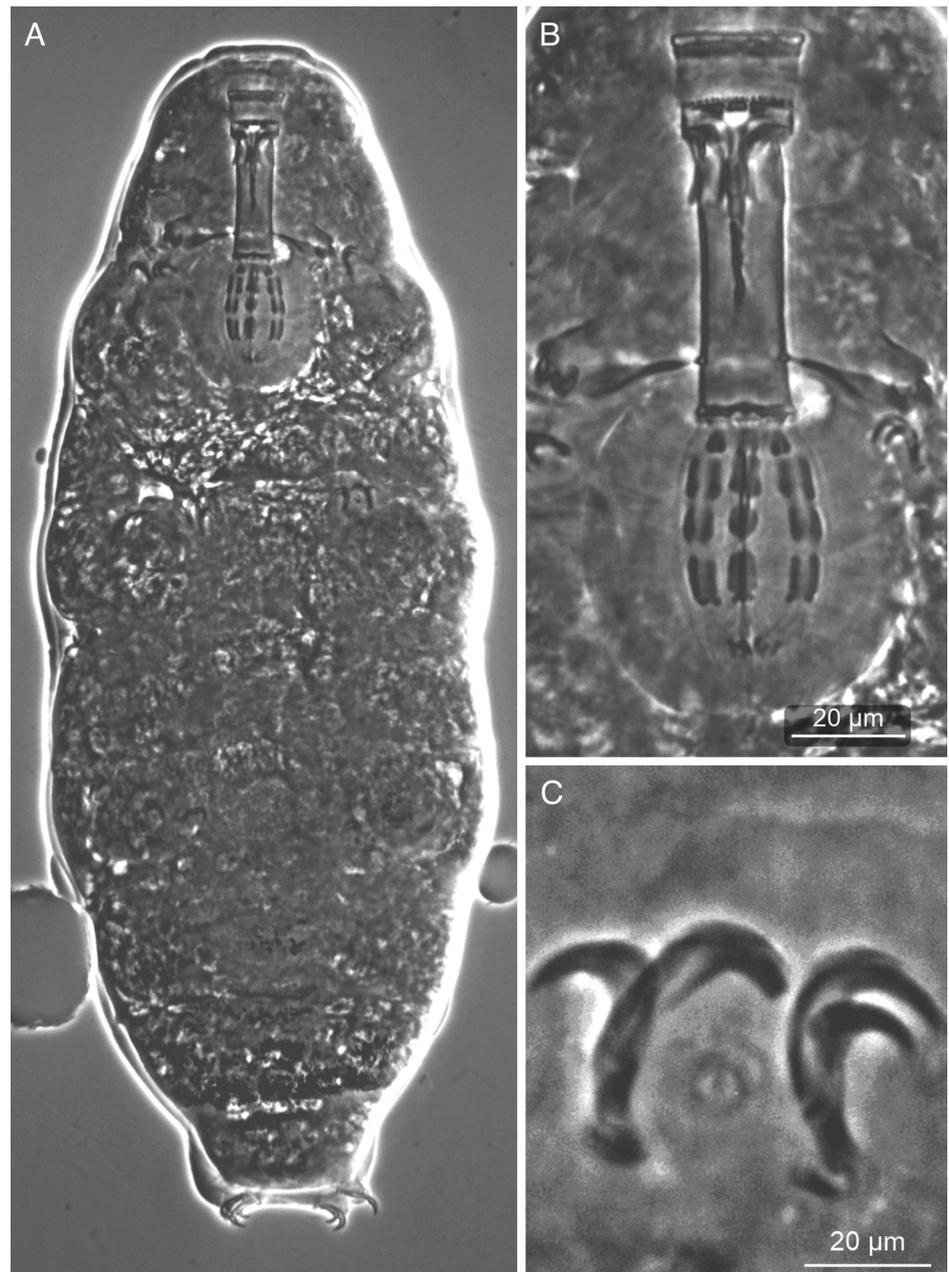
All our specimens show the characters of *M. cittalium* as proposed by Dueñas-Cedillo et al. (2020).

#### ***Minibiotus* *sidereus* Pilato, Binda & Lisi, 2003**

All collected specimens show the characters of *M. sidereus* as proposed by Pilato and Lisi (2003). The cuticle has three types of pores, circular, elliptical, and star-shaped.

#### **Genus *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009**

The pharyngeal bulb has apophyses and three rod-shaped macroplacoids; the microplacoid, if present, is separated from the third macroplacoid by more than its length. Stylet furcae are typically shaped (Figure 18A). The cuticle is without pores. The bucco-pharyngeal apparatus is of the *Macrobiotus* type, *Macrobiotus* variant (Figure 18B). Claws are of the *hufelandi* type (Figure 18C). Eggs are laid freely and have conical processes; the eggshell is areolated (Pilato and Binda 2010).

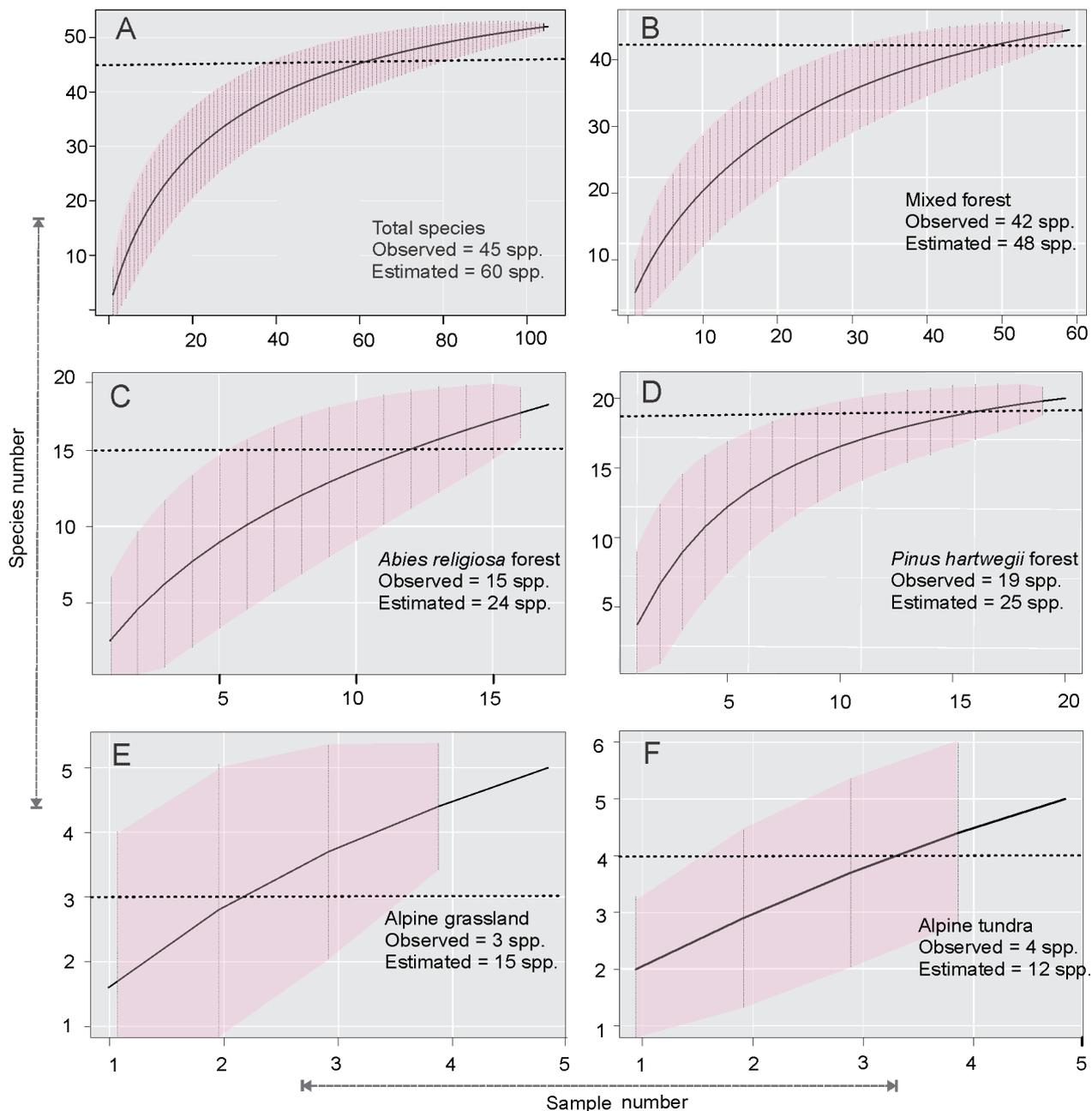


**Figure 18.** *Paramacrobotus*. **A.** Habitus. **B.** Detail of bucco-pharyngeal apparatus. **C.** Detail of claws II.

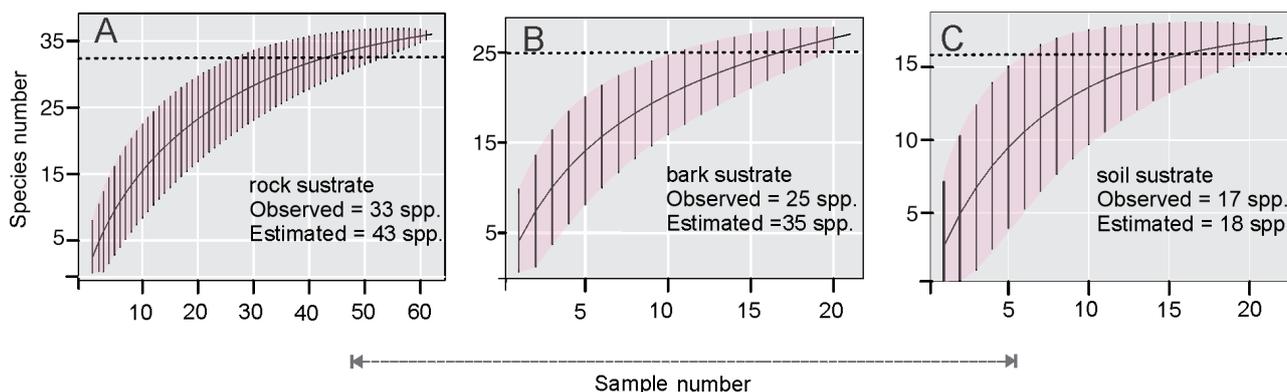
Most of our tardigrade specimens are from coniferous forests (73%), which also includes mixed forest, and fewer are from tropical forest (6%) and alpine grassland (7%). Regarding substrate type, 48% of our specimens were obtained from bark and 27% from rock. Adults make up 94% of the specimens, exuvia make up 5%, and cysts and eggs only 1%.

The total species-accumulation curve, including data from all samples, shows that the curve does not reach the asymptote ( $Obs_{spp} = 45$  spp.,  $Est_{spp} = 60$  spp.; Figure 19A). On the type of vegetation, we observed that in mixed forests ( $Obs_{spp} = 42$ ,  $Est_{spp} = 48$ ; Figure 19B) the asymptote is almost reached. Conversely, in the *Abies* forest ( $Obs_{spp} = 15$ ,  $Est_{spp} = 24$ , Figure 19C), and *Pinus hartwegii* forest ( $Obs_{spp} = 19$ ,  $Est_{spp} = 25$ , Figure 19D) the taxa recorded are close to the total number of species according to the Clench models. In the alpine grassland ( $Obs_{spp} = 5$ ,  $Est_{spp} = 10$ , Figure 19E) and alpine tundra ( $Obs_{spp} = 5$ ,  $Est_{spp} = 12$ ; Figure 19F), the species-accumulation curve estimates that only approximately half of the species in these three vegetation types have been found.

The numbers of species recorded living on rock ( $Obs_{spp} = 33$ ,  $Est_{spp} = 43$ ; Figure 20A) and bark ( $Obs_{spp} = 25$ ,  $Est_{spp} = 35$ ; Figure 20B) do not reach the estimated species number. Conversely, the species-accumulation curve of the moss and of soil samples is closer to the asymptote ( $Obs_{spp} = 17$ ,  $Est_{spp} = 18$ , Figure 20C).



**Figure 19.** Accumulation curves for different landscapes (vegetation types) indicating observed and estimated species numbers. **A.** Total species number identified in this study. **B.** Mixed forest. **C.** *Abies religiosa* forest. **D.** *Pinus hartwegii* forest. **E.** Alpine grassland. **F.** Alpine tundra. Dotted lines indicate number of the species observed.



**Figure 20.** Accumulation curves for different substrates indicating observed and estimated species numbers. **A.** Rock. **B.** Bark. **C.** Soil. Dotted lines indicate the number of species observed.

## DISCUSSION

Biological inventories are a useful tool for conservation and provide credible, verifiable data to meet the needs of basic taxonomy and systematics (Wheeler 1995). Taxon-targeted inventories allow for the rationalization of regional biotic inventories, while increasing taxonomic coverage and spatial resolution. Such inventories allow for a deeper understanding of ecological patterns, distribution, and discovery of cryptic diversity (Kremen 1994). Our study intensively sampled more than 40 localities in the Valley of Mexico Basin, focusing on the phylum Tardigrada. Among the eight fully identified species, we provide the first records for a genus and two new species from Mexico. Our species-accumulation estimates from five vegetation types and three substrate types indicate that our inventory is incomplete, and as many as twice the number of species may be present in some ecosystems than what is currently known.

**Taxonomic identification.** A major factor that promotes species inventories is the amount of taxonomic information available to in the group being studied (Wheeler 1995). Based on the current taxonomic information available, we were able to confidently identify about 20% of the morphological species found. We could not fully identify the remaining approximately 80% because: 1) identification in some species complexes requires integrative methods (i.e. intraspecific variance analysis both with morphological and genetic data (Bertolani et al. 2010), 2) variation of continuous traits of in many specimens were outside the known range of variation for known species, and 3) in some instances we had few specimens and their preservation was not optimal for taxonomic inference. Our results suggest that a considerable proportion of species diversity within our study area is cryptic, and it is currently impossible to diagnose, unless methodologies with higher taxonomic resolution are used.

The first century of the study of tardigrades, focused on alpha taxonomy, inventorying, naming, and describing species (Guidetti and Bertolani 2005), and during this stage, morphological characters allowed for several species hypotheses to be generated. However, at present many of the species are now known to be species complexes (Michalczyk et al. 2012b; Kaczmarek and Michalczyk 2017, Kaczmarek et al. 2018), and integrated molecular, ecological, and distributional data are now necessary to delimit species (Gąsiorek et al. 2019, 2021, 2023).

**Newly recorded species.** Our new records of *Pseudechiniscus (Pseudechiniscus) ramazzottii ramazzottii*, *Adropion onorei*, and *Claxtonia* are based on hypothesis criteria given by the authors of those species, and our identification of these species from Mexico, these records increase the number of genera to 43 and species to 77 in the country (García-Román et al. 2022).

García-Román et al (2022) determined that 10% of the Mexican tardigrade diversity displays pattern of distribution such as American, Nearctic, or Neotropical. Our records of *A. onorei* and *Claxtonia* add additional evidence that some tardigrade species have shown distribution patterns *Adropion onorei* was described from Ecuador (Pilato et al. 2002) within the South American transition zone, a region where Neotropical and Andean biotic elements coexist (Morrone 2007). Our new record of this species from the Valley of Mexico Basin is the northernmost occurrence known and is also from a transition zone. This supporting a wider distribution pattern in the Neotropical region, such as is known for six species in Mexico: *Pseudechiniscus juanita*, *Diphascion mitrense* Pilato, Binda & Qualtieri, 1999, *Macrobotus kazmierskii* Kaczmarek & Michalczyk, 2009, *Macrobotus kirghizicus* Tumanov, 2005, *Minibiotus sidereus* Pilato, Binda & Lisi, 2003, and *Doryphoribius quadrituberculatus* Kaczmarek & Michalczyk, 2004.

The genus *Claxtonia* was recently proposed and its diagnosis amended (Gąsiorek et al. 2023; Degma et al. 2021), and the 16 currently recognized species are known from nine countries (McInnes 1994; Kaczmarek et al. 2016; Gąsiorek et al. 2023). Of the valid species, *C. wendtii* (Richters, 1903) has been recorded from central North America, and *C. aliquantilla* (Grigarick, Schuster & Nelson, 1983) and *C. marginopora* (Grigarick, Schuster & Nelson, 1983) from northern South America (Venezuela). Our records support that the center of the American continent, the Mexican Transition Zone, also presents conditions for the existence of this genus.

Half of the Mexican tardigrade fauna (50%) consists of widely distributed species distributed in more than three biogeographic regions (García Román et al. 2022). *Pseudechiniscus (P.) ramazzottii* is one such species. This species was described from Italy by Maucci (1952) and has since been recorded from the United States, Canada, Peru, Italy, Hungary, Russia, and Japan (McInnes 1994; Kaczmarek et al. 2016). It would be worth exploring if these records are of a single specie with a disjunct distribution, as known for other Tardigrada, or if some these records belong to different species (Gąsiorek et al. 2021).

The distribution of the species recently recorded in our study suggest that the Mexican Transition Zone is important for modeling the distribution patterns of tardigrades in the Americas, whose definition has been based on the distribution of macrofauna (Halfpeter and Morrone 2017). In this work, considering the tardigrade records in perspective concerning the biogeographical regionalization of Mexico allowed us to recognize distribution patterns that are shared with numerous biotic elements already recorded in the area (*Adropion onorei* with Neotropical distribution, and *Pseudechiniscus (P.) ramazzottii ramazzottii* with widely distribution).

**Completeness of the Inventory.** Our study is part of the current National Inventory of Mexican Tardigrades and includes the collection, taxonomy, and curation undertaken by us for the last 11 years. Specimens were

obtained from various geographic regions, vegetation types, and substrates, which allowed us to document vegetation types and substrates of our tardigrade records. Our species-accumulation curves show that the number of observed species is close to the expected species estimates in mixed forests (Figure 17, in the remaining ecosystems from 6 (in the *Pinus hartwegii* forest) to 12 species (in the alpine grassland) are yet to be discovered (Figure 17). Our results are congruent with those reported by García-Román et al. (2022), who calculated that 74%, or as many as 290 species, of Mexico's tardigrade fauna has yet to be documented. These estimates should be taken conservatively because they do not include the most extensive ecosystems, such as forests and xerophytic shrublands, which together account for almost 50% of Mexico's surface area (SEMARNAT 2002). Xerophytic shrublands have the greatest potential for the discovery of new species due to the xeric capabilities of tardigrades (Glime 2017).

Little is known about interspecific relationships and biotic interactions that are involved in the process of habitat colonization, establishment, and trophic structure of tardigrade communities (Nelson et al. 2018). Our results show that tardigrade species may coexist. For example, both *Claxtonia cf. mauccii* and *Adropion onorei* were recorded from Iztaccihuatl Volcano at 3030–4007 m elevation. These species were sympatric at two collection sites at this elevation, living in moss on bark in an *Abies religiosa* forest (3,498 m), and in a *Pinus hartwegii* forest (3700 m). Based on their bucco-pharyngeal apparatus and their feeding habits, we hypothesize that *C. cf. mauccii* and *A. onorei* may constitute the primary consumers (herbivores) and decomposers in their communities, respectively (Guidetti et al. 2012). Coexistence and trophic interactions of apparent sympatric species have been reported by several authors (Sanchez-Moreno et al. 2008; Guil and Sanchez-Moreno 2013). However, these observed patterns should not remain descriptive. They should be evaluated under a macroecological approach at different spatial and temporal scales and considering all chains that make up the food webs and the links within these chains, as well as the trophic level to which the links belong (Rodríguez et al. 2017; Abarca-Arenas et al. 2022).

**Tardigrada in Mexico.** One of the constraints on studying tardigrade fauna of Mexico is general lack of collection data with the specimens available in collections. Specimens collected during 20th century were transferred out of the country to foreign collections, leaving the country without type specimens or reference specimens of described species.

No new collections were made until 2015 (Moreno-Talamantes et al. 2015), but since 2017 several researchers have focused on documenting the tardigrade diversity in various regions of Mexico and have generated new records and described new species (Pérez-Pech et al. 2017, 2020a, 2020b; León-Espinosa et al. 2019; Moreno-Talamantes and León-Espinosa 2019; Moreno-Talamantes et al. 2019, 2020; Anguas-Escalante et al. 2020; Dueñas-Cedillo et al. 2020; Núñez et al. 2021).

Despite recent increases in knowledge of the Tardigrada in Mexico, the need for a scientific collection that meets current conservation standards has not yet been addressed. The lack of scientific collections has limited the study of these animals. Tardigrade research in Mexico is best supported by collections of specimens and slides, which are integrated into departmental laboratories or larger collections that house them. However, corresponding catalog numbers have not been reported in the literature. Now that the present collection has been established, work now focuses on continuing to sample the tardigrade fauna of Mexico, consolidating the collection, and addressing research questions derived from the taxonomic and biological information associated with the collection's specimens.

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## ADDITIONAL INFORMATION

### Conflict of interest

The authors declare that no competing interests exist.

### Ethical statement

No ethical statement is reported.

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### Author contributions

Conceptualization: FAT, ADC. Data curation: ADC, IV. Formal analysis: FAT, IV, ADC. Funding acquisition: CFVM, GAVG, EAR, FAT. Investigation: ADC, FAT. Methodology: FAT, IV, JGR, ADC. Resources: CFVM, GAVG, EAR, FAT. Supervision: FAT, EJ, EAR. Visualization: JGR. Project administration: FAT, EAR, GCR. Software: IV, JGR. Validation: ADC, FAT. Writing – original draft: ADC, FAT. Writing – review and editing: ADC, FAT, JGR, CFVM, EAR, EJ.

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### Data availability

All data that support the findings of this study are available in the Collection of limnoterrestrial tardigrades in the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, and in the Laboratorio de Ecología, Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional.

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