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ECOSYSTEMS

New species and records of tardigrades from a biological repository collection from the Sierra Nevada de Santa Marta, Colombia

OSCAR LISI, ANISBETH DAZA, ROSANA LONDOÑO & SIGMER QUIROGA

Abstract: The Sierra Nevada de Santa Marta (SNSM) is a mountain range in northern Colombia, recognized for its high biological richness. To date, the existence of 27 species of tardigrades is known for this particular region. Morphological and morphometric analysis of 51 specimens, found in 16 samples of bryophytes and lichens collected at the SNSM, and deposited in the tardigrade collection of the "Centro de Colecciones Científicas de la Universidad del Magdalena" between 2011 and 2016 was carried out. The species *Mixibius gibbosus* **sp. nov.** is described based on the following main traits: presence of gibbosities (up to date never reported for any species of the genus *Mixibius*), isodiametric tubercles and relevant morphometric differences with respect to the most similar congeneric species. Additionally, the species *Diphascon pingue pingue sensu lato*, *Hypsibius* cf. *allisoni* and *Adropion onorei* are, for the first time, recorded for Colombia and the latter is recorded for the first time out of Ecuador. All records are new for the SNSM.

Key words: Biological collections, Eutardigrada, *Mixibius*, morphometry, Tardigrada, water bears.

INTRODUCTION

In the last decade, the number of scientific publications with descriptions of species and records of tardigrades has increased markedly in South America. In accordance with the new taxonomic novelties of the recent years (Degma et al. 2009-2020), up to date, a total of 250 species of limnoterrestrial tardigrades have been recorded; more in detail, 125 species are known for Argentina, 36 for Bolivia, 68 for Brazil, 67 for Chile, 59 for Colombia, 40 for Ecuador (including Galápagos Island), 9 for Paraguay, 30 for Peru, 1 for Suriname, 27 for Uruguay and 21 for Venezuela (McInnes 1994, Meyer 2013, Caicedo et al. 2014, Kaczmarek et al. 2015, 2017, Roszkowska et al. 2015, 2016, 2017, 2018, 2019, Londoño et al. 2015, 2017, Melo et al. 2015, Daza et al. 2017, Barros 2020, Stec et al. 2018, Lisi et al. 2017, 2019, 2020). Of these, 108 have been described as new species from type material collected in this part of the world; 20 in the last ten years (Table I).

Colombia is one of the countries in which knowledge of the biodiversity of tardigrades has increased most rapidly, going from 30 known species in 2009 to 59 by 2020 (Caicedo et al. 2014, Lisi et al. 2014a, 2017, 2019, 2020, Melo et al. 2015, Stec et al. 2018, Daza et al. 2017, Londoño et al. 2015, 2017). 9 species (*Bryodelphax kristenseni, Crenubiotus revelator, Doryphoribius rosanae, Itaquascon pilatoi, Meplitumen aluna, Milnesium kogui, Minibiotus pentannulatus, Paramacrobiotus lachowskae, Pam. sagani*) have been described, and 18 have been recorded, for

Table I. New limnoterrestrial tardigrade species described from South America in the last ten years.

Country	Species
Argentina	*Macrobiotus kristenseni* Guidetti, Peluffo, Rocha, Cesari & Moly de Peluffo, 2013
	Mesobiotus pseudoblocki Roszkowska, Stec, Ciobanu & Kaczmarek, 2016
	Milnesium argentinum Roszkowska, Ostrowska & Kaczmarek, 2015
	Milnesium beatae Roszkowska, Ostrowska & Kaczmarek, 2015
	Minibiotus pseudostellarus Roszkowska, Stec, Ciobanu & Kaczmarek, 2016
Colombia	*Bryodelphax kristenseni* Lisi Daza, Londoño & Quiroga, 2017
	Crenubiotus revelator Lisi, Londoño & Quiroga, 2020
	Doryphoribius rosanae Daza, Caicedo, Lisi & Quiroga, 2017
	Itaquascon pilatoi Lisi, Londoño & Quiroga, 2014
	Meplitumen aluna Lisi, Daza, Londoño, Quiroga & Pilato, 2019
	Milnesium kogui Londoño, Daza, Caicedo, Quiroga & Kaczmarek, 2015
	Minibiotus pentannulatus Londoño, Daza, Lisi & Quiroga, 2017
	Paramacrobiotus lachowskae Stec, Roszkowska, Kaczmarek & Michalczyk, 2018
	Paramacrobiotus sagani Daza, Caicedo, Lisi & Quiroga, 2017
Ecuador	*Doryphoribius amazzonicus* Lisi, 2011
	Mesobiotus romani Roszkowska, Stec, Gawlak & Kaczmarek, 2018
	Paramacrobiotus spinosus Kaczmarek, Gawlak, Bartels, Nelson & Roszkowska, 2017
Peru	*Isohypsibius condorcanquii* Kaczmarek, Cytan, Zawierucha, Diduszko & Michalczyk, 2014
	Macrobiotus pisacensis Kaczmarek, Cytan, Zawierucha, Diduszko & Michalczyk, 2014
	Paramacrobiotus intii Kaczmarek, Cytan, Zawierucha, Diduszko & Michalczyk, 2014

Colombia from a relatively small area in the north of the country known as Sierra Nevada de Santa Marta (SNSM), which is a small mountain range of about 17.000 km^2, isolated from the Andes, characterized by its high biological richness and endemism (Viloria 2005, Vásquez-V & Serrano-G 2009). About 11.350 specimens of tardigrades collected in this region between 2011 and 2017, have been carefully mounted and deposited in the Biological Collection of the "Centro de Colecciones Científicas de la Universidad del Magdalena" (CCC), and some of this material is still under study. As a result of reviewing some of the material in this collection, the aim of the present study is to describe a new species of the genus *Mixibius* Pilato, 1992 and to add new species records of *Adropion* Pilato, 1987, *Diphascon* Plate, 1888, *Fractonotus* Pilato, 1998 and *Hypsibius* Ehrenberg, 1848 genera, found in bryophytes and lichens collected in the north-western side of the SNSM. Only three additional species of *Mixibius* have been recorded for South America: *Mixibius fueginus* Pilato & Binda, 1996 and *Mixibius saracenus* (Pilato, 1973) for Argentina and *Mixibius ornatus* Pilato, Binda, Napolitano & Moncada, 2002 for Ecuador.

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MATERIALS AND METHODS

This survey was based on a morphological and morphometric analysis of tardigrade specimens

deposited in the "Centro de Colecciones Científicas de la Universidad del Magdalena", Santa Marta, Colombia, under the catalogue acronym CBUMAG:TAR. The material examined was collected from bryophyte and lichen samples between 2011 and 2016 by Anisbeth Daza, Martín Caicedo, Paula Sepúlveda, Rosana Londoño and Sigmer Quiroga, from two localities (San Lorenzo and Medium basin of Garupal River) in the Sierra Nevada de Santa Marta, Colombia, from 538 and 2,565 m a.s.l. (Table II). The specimens are mounted and preserved on microscope slides, some in Hoyer's medium and others in PVA mounting media (Elvanol and a lacto-phenol solution BioQuip Products Inc. catalog # 6371A).

The photographic and morphometric work for the Colombian material was carried out using a Phase Contrast Microscope (PCM) Zeiss Axiolab A1 with an adapted digital camera Zeiss AxioCam ERc5s and the software Zeiss Zen 2.3 (Blue edition). Instead, the photomicrographs of the material deposited in the Pilato and Binda collection (University of Catania) were made using a Leica PCM equipped with "Cannon S40" digital camera. The drawing was made using Adobe Illustrator CC2017. Imaging processing and plate assembling were performed with Adobe Photoshop CC2017 and CS5.

The structures were measured only if they were undamaged and their orientation appeared suitable. All measurements are given in micrometres (µm). The animal body length was measured from the anterior margin to the end of the body excluding the hind legs. The buccal tube length and the level of the stylet support insertion on the buccal tube were measured according to Pilato (1981) as well as the calculation of the *pt* ratio (ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage), all *pt* values are provided in italics. The claw length was measured according to the methodology proposed by Beasley et al. (2008), and for correct comparisons with species described in the past, we measured also the entire claw length according to Pilato et al. (1982). Morphometric data were processed using the Order Parachela ver. 1.7 template, with some modifications, available from the Tardigrada Register (Michalczyk & Kaczmarek 2013, www.tardigrada.net/register). The configuration and number of gibbosities follow the system proposed by Michalczyk & Kaczmarek (2010).

Identification, using literature and specimen comparison, was based on morphological characters. Taxonomy follows Degma et al. (2009-2020). Literature used for the identification included: Pilato & Binda (2010) and Gąsiorek et al. (2019a) for genus identification, Ramazzotti & Maucci (1983) for provisional diagnosis of some species or species groups, and, for definitive species identification, taxonomic keys and/or original descriptions of several species (Richters 1902, Marcus 1936, Horning et al. 1978, Pilato & Binda 1997/1998, 1998, 1999, Pilato et al. 1999, 2002, 2004, 2010, Biserov 1999, Kaczmarek & Michalczyk 2004, Li & Li 2008, Gąsiorek et al. 2019a, b, Lisi 2011, Lisi et al. 2014a, b). The abbreviations of genus names are according to Perry et al. (2019).

For comparison, we have examined specimens of: Material deposited in the CCC (Santa Marta, Magdalena, Colombia): holotype and paratypes of *Ita. pilatoi* (slide Nos. CBUMAG:TAR:00074, 00076 and 00079). Material deposited in the Pilato and Binda Collection (Catania, Italy): holotype and paratypes of *Dor. amazzonicus* (slide Nos. 4828 and 5422), *Mix. ornatus* Pilato, Binda, Napolitano & Moncada, 2002 (slide Nos. 4770, 5046-5048), *Mix. parvus* Lisi, Sabella & Pilato, 2014 (slide Nos. 5536 and 5537), *Mix. schnurae* Pilato, Lisi & Binda, 2010 (slide Nos. 5419 and 5420), and *Mix. sutirae* Pilato, Binda & Lisi 2004 (slide Nos. 4986-4893); specimens of

Table II. Information about samples from which all studied specimens had been extracted, and collection slides in which they were deposited. CBUMAG = Centro de Colecciones Científicas de la Universidad del Magdalena. "m a.s.l." = meters above sea level.

Sample	Locality	Geographic coordinates	Altitude (m a.s.l.)	Microhabitat	Tardigrade species	Slide numbers CBUMAG	Specimens	Total specimens
1	San Lorenzo	11° 06'30.7'' N 74° 03'33.4'' W	2322	Moss on soil: *Sphagnum meridense*	*Adropion onorei*	326	5	9
						328	3	
						329	1	
2	San Lorenzo	11° 06'39.8'' N 74° 03'19.0'' W	2265	Lichens on tree trunk: *Parmotrema* sp. *Usnea* sp.	*Adropion onorei*	358	1	1
					Doryphoribius amazzonicus	358	1	1
3	San Lorenzo	11° 06'41.8'' N 74° 03'33.2'' W	2207	Lichen on tree trunk: *Pseudocyphellaria aurata*	*Diphascon pingue pingue sensu lato*	364	2	2
4	San Lorenzo	11° 06'29.5'' N 74° 04'16.3'' W	2066	Moss on soil: *Sphagnum meridense*	*Doryphoribius amazzonicus*	366	1	1
5	San Lorenzo	11° 06'29.5'' N 74° 04'16.3'' W	2066	Bryophytes on concrete: -Mosses: *Macromitrium* sp. *Thuidium peruvianum* -Liverworts: *Cephalozia* sp. *Lejeunea* sp. *Plagiochila* sp.	*Diphascon* sp., *pingue* group	371	1	1
					Diphascon pingue pingue sensu lato	367	1	5
						368	1	
						369	1	
						370	2	
					Hypsibius cf. *allisoni*	370	1	1
6	San Lorenzo	11° 06'27.7'' N 74° 04'16.3'' W	2082	Bryophytes and lichens on tree trunk: -Moss: *Campylopus reflexisetus* -Liverwort: *Frullania ericoides* -Lichens: *Parmotrema* sp. *Hypotrachyna* sp. *Usnea* sp.	*Fractonotus* sp.	372	1	1
7	San Lorenzo	11° 06'16.9'' N 74° 03'31.2'' W	2517	Lichens on tree trunk: *Hypotrachyna* sp. *Parmotrema* sp. *Usnea* sp.	*Adropion onorei*	513	1	1
					Mixibius gibbosus **sp. nov.**	511	1	4
						512	1	
						515	1	
						519	1	

Table II. Continuation.

8	San Lorenzo	11°06'16.9'' N 74°03'31.2'' W	2517	Lichens on tree trunk: *Heterodermia* sp. *Hypotrachyna* sp. *Parmotrema* sp.	*Mixibius gibbosus* **sp. nov.**	546	1	1
9	San Lorenzo	11°06'29.5'' N 74°03'34.2'' W	2319	Lichens on tree trunk: *Heterodermia* sp. *Hypotrachyna* sp. *Yoshimuriella* sp.	*Mixibius gibbosus* **sp. nov.**	613	4	4
					Hypsibius cf. *allisoni*	613	1	1
					Itaquascon cf. *pilatoi*	614	1	1
10	San Lorenzo	11°06'29.5'' N 74°03'34.2'' W	2319	Lichen on tree trunk: *Sticta* cf. *laciniata*	*Fractonotus* sp.	615	3	3
					Dianea sattleri sensu lato	615	1	3
						616	2	
					Diphascon pingue pingue sensu lato	615	3	3
11	San Lorenzo	11°06'29.5'' N 74°03'34.2'' W	2319	Moss on tree trunk: *Prionodon densus*	*Hypsibius* cf. *allisoni*	618	1	1
12	San Lorenzo	11°06'29.5'' N 74°03'34.2'' W	2319	Lichens on tree trunk: *Heterodermia* sp. *Hypotrachyna* sp. *Parmotrema* sp. *Sticta* sp. *Usnea* sp.	*Dianea sattleri sensu lato*	627	1	1
13	San Lorenzo	11°06'42.6'' N 74°03'32.4'' W	2213	Bryophyte on tree trunk: *Plagiothecium* sp.	*Dianea sattleri sensu lato*	631	1	1
14	San Lorenzo	11°06'21.2'' N 74°03'18.6'' W	2565	Bryophytes and lichens on tree trunk: -Mosses: *Daltonia marginata* *Zygodon reinwardtii* -Lichens: *Heterodermia* sp. *Hypotrachyna* sp. *Usnea* sp.	*Diphascon pingue pingue sensu lato*	653	2	2
15	San Lorenzo	11°06'21.2'' N 74°03'18.6'' W	2565	Bryophyte and lichens on tree trunk: -Moss: *Macromitrium aureum* -Lichens: *Heterodermia* sp. *Parmotrema* sp. *Sticta* sp.	*Diphascon pingue pingue sensu lato*	663	1	2
						668	1	
16	Río Garupal	10°14'01.0'' N 73°48'18.4'' W	538	Bryophyte on tree trunk: *Plagiochila* sp.	*Doryphoribius amazzonicus*	130	1	1

Dip. pingue pingue Marcus, 1936 from Alps (slide Nos. 1495-6, 1986, 1605-6, 2353, 2373, 2710-2) and of *Hys. allisoni* Horning, Schuster & Grigarick, 1978 from Tierra del Fuego (slide Nos. 4391-9, 4403, 4407, 4428), the diagnosis of these two latter taxa is due to Prof. G. Pilato (G. Pilato pers. comm.) before he deposited the material in the collection.

RESULTS

A total of 51 individuals, found in 16 samples, were reviewed. The collecting data and microhabitat composition are shown in Table II.

Taxonomic account

Class: Eutardigrada Richters, 1926
Order: Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Superfamily: Hypsibioidea Pilato, 1969
Family: Hypsibiidae Pilato, 1969
Subfamily: Diphasconinae Dastych, 1992
Genus: *Diphascon* Plate, 1888

***Diphascon* sp., *pingue* group**

Material examined. 1 specimen in total: sample 5, CBUMAG:TAR:00371.

Remarks. The only specimen found allowed to notice the characters of the *pingue* group and some differences with respect to *Dip. pingue sensu stricto*; however, attribution to another precise, or new, species of said group was not possible. Until now, no species of the *pingue* group has been reported for Colombia.

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

Material examined. 14 specimens in total: sample 3, CBUMAG:TAR:00364 (2 specimens); sample 5, 00367 (1 specimen), 00368 (1 specimen), 00369 (1 specimen), 00370 (2 specimens); sample 10, 00615 (3 specimens); sample 14, 00653 (2 specimens); sample 15 00663 (1 specimen), 00668 (1 specimen).

Remarks. This is the first record of this species for Colombia. The material fits rather well the description of the species and subspecies, but according to Kaczmarek et al. (2015), with whom we agree, "the presence *Diphascon pingue sensu stricto* in South America is questionable and requires verification", therefore we preferred not to report this taxon as *sensu stricto*.

Subfamily: Hypsibiinae Pilato, 1969

Genus: *Hypsibius* Ehrenberg, 1848

Hypsibius* cf. *allisoni

Material examined. 3 specimens in total: sample 5, CBUMAG:TAR:00370 (1 specimen); sample 9, 00613 (1 specimen); sample 11, 00618 (1 specimen).

Remarks. The species was reported for other South American countries (Argentina, Chile and Ecuador) but never for Colombia; our material fits the qualitative characters of the species originally described by Horning et al. (1978), however, the *locus typicus* is in New Zealand, and our small sample size together with the quality of the material prevent from the necessary very careful examination for sure species diagnosis (also according to Kaczmarek et al. 2015), therefore we preferred not reporting our species as surely belonging to *Hys. allisoni sensu stricto*.

Subfamily: Itaquasconinae Bartoš in Rudescu, 1964

Genus: *Adropion* Pilato, 1987

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

Material examined. 11 specimens in total: sample 1, CBUMAG:TAR:00326 (5 specimens), 00328 (3 specimens), 00329 (1 specimen); sample 2, 00358 (1 specimen); sample 7, 00513 (1 specimen).

Remarks. The species was until now reported only from Ecuador (Pilato et al. 2002, 2004) and represents a new record for Colombia.

Genus: *Itaquascon* de Barros, 1939

Itaquascon* cf. *pilatoi

Material examined. 1 specimen in total: sample 9, CBUMAG:TAR:00614.

Remarks. Our specimen is most similar to *Ita. pilatoi* originally described by Lisi et al. (2014a), but it has wider buccal tube (*pt* external width 15.7 *vs* 10.1-11.0), more robust claws and higher ratio of internal/external (or anterior/posterior) claw lengths on each leg: for example, the anterior/posterior ratio of claws of legs IV is about 71% *vs* 58.3% - 61.4%. The body length is 343.2 µm *vs* 235 - 396 µm in *Ita. pilatoi*, therefore, differences due to allometry are excluded. It may represent a new species, but having found only one specimen prevents from ascertaining this possibility.

Incerta subfamilia

Genus: *Mixibius* Pilato, 1992

***Mixibius gibbosus* sp. nov.** (Figs. 1-5, Table III)
ZooBank Life Science Identifier (LSID) - urn:lsid:zoobank.org:act:D4CDBC59-C268-441E-8654-8B860EF9ED2D

Material examined. 9 specimens in total: sample 9, holotype (CBUMAG:TAR:00613-4) and 3 paratypes (00613), one of which in simplex state; sample 7, 4 paratypes (00511, 00512, 00515, 00519); sample 8, 1 paratype (00546) (Table II). These samples were similar because shared the following genera of lichens: *Hypotrachyna* sp., *Parmotrema* sp. and *Heterodermia* sp.

Type locality. San Lorenzo, Sierra Nevada de Santa Marta, Magdalena, Colombia, 11°06'16.9'' N 74°03'31.2'' W, 2517 m a.s.l., and 11°06'16.9'' N 74°03'31.2'' W, 2319 m a.s.l. (Table II).

Species diagnosis. Colourless, eye-spots present, cuticle with gibbosities, not always evident, gibbosity formula probably VII:3/4-3/4-3/4-3/4-3/4-3/4-3/4 (it is doubtful whether it might be present, additionally, a very first row of only median gibbosities at neck level, considered absent in the given formula); row I at the level of legs I; the three rows II, IV and VI, placed respectively between legs I and II, II and III, III and IV, show lateral gibbosities divided into an anterior and a posterior portion. Dorsal and lateral cuticle with evident tubercles variously shaped but always isodiametric (not elongated). Bucco-pharyngeal apparatus of the *Mixibius* type: buccal tube rigid, without ventral lamina and with hook-shaped apophysis for the insertion of the stylet muscles (AISM) slightly asymmetrical with respect to the frontal plane, followed caudally by thickenings; buccal armature seemingly absent; AISM robust; stylet supports inserted rather anteriorly on the buccal tube (*pt* 59.3 - 63.2); pharyngeal bulb with apophyses and two rod-shaped macroplacoids; microplacoid and septulum absent. External claws of the *Isohypsibius* type, internal claw of modified *Isohypsibius* type, as typical of *Mixibius*; accessory points and pseudolunulae present, no cuticular bars on the legs. Eggs not found.

Species description. Body size about 128-162 µm, colourless, eye-spots present. Dorsal cuticle with gibbosities, more evident in specimens with not well distended body. Gibbosity formula probably VII:3/4-3/4-3/4-3/4-3/4-3/4-3/4 (Fig. 1); there might be present, additionally, a very first row of only median gibbosities at neck level, but the available material does not allow to ascertain this detail and we considered

this possible gibbosities absent in the given formula; for this reason, we put a question mark in Fig. 1. Row I placed at the level of legs I. The uncertainty in the gibbosity number of all rows is due to the dorso-medial gibbosities, which are in the shape of a more or less wide structure that can appear undivided medially (counting as a unique gibbosity; Fig. 2a, b), or divided (counting as a couple of gibbosities; Fig. 2c, d), but it is not clear whether this depends on real variability, or just on the visibility of the medial, longitudinal groove (drawn as an interrupted line in Fig. 1) dividing the two dorso-medial gibbosities. The dorso-medial gibbosities of rows I-VI are similarly developed and shaped. The dorso-lateral gibbosities of rows I, III, V (in correspondence to legs I, II and III respectively) are relatively evident and more typically shaped, rounded (Fig. 1); instead, in rows II, IV, VI, the dorso-lateral gibbosities are less evident, and curiously doubled, being divided into a more anterior and a more posterior sub-gibbosity by a transverse groove on the external side (Fig. 1 and Fig. 2a, b, d arrowheads). In particular, row VI shows smaller dorso-lateral gibbosities, making more difficult their observation. Row VII has all gibbosities smaller, but morphologically similar to rows I, III, V (in particular, with dorso-lateral gibbosities undivided).

Dorsal and lateral cuticle with evident tubercles (Fig. 2), triangular, quadrangular, polygonal or rounded but always isodiametric; such tubercles are rather uniformly sized and give a leopard-spotted appearance; no cuticular roughness.

Bucco-pharyngeal apparatus (Fig. 3a-e) of the *Mixibius* type: buccal tube rigid, without ventral lamina and with hook-shaped AISM slightly asymmetrical with respect to the frontal plane, with the dorsal more prominent and rounded (Fig. 3c); AISM appear robust in dorso-ventral view (Fig. 3d, e); one additional,

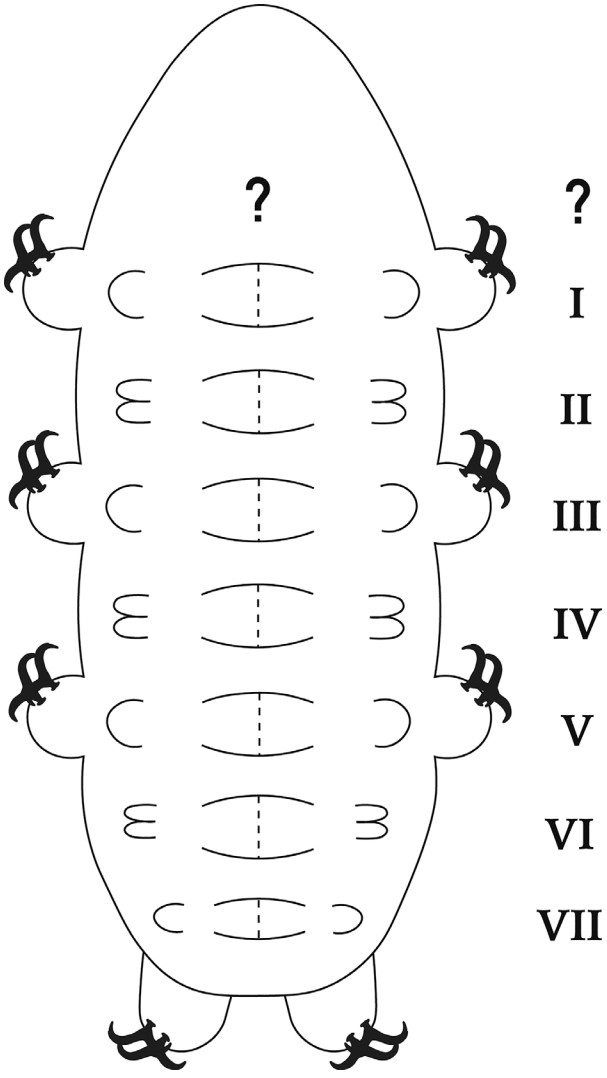

Figure 1. Schematic drawing showing the gibbosity shape and arrangement. The question marks indicate the doubt about presence or absence of a very first medial gibbosity. Roman numbers indicate the row numbers of the surely present gibbosities.

flat thickening posterior to each AISM (Fig. 3c). No peribuccal papulae, buccal teeth or buccal crests visible.

Stylet supports inserted on the buccal tube at 59.3-63.2 percent of its length; pharyngeal bulb with apophyses and two rod-shaped macroplacoids (Fig. 3b); the first, longer, with central constriction, the second without any constriction. Microplacoid and septulum absent.

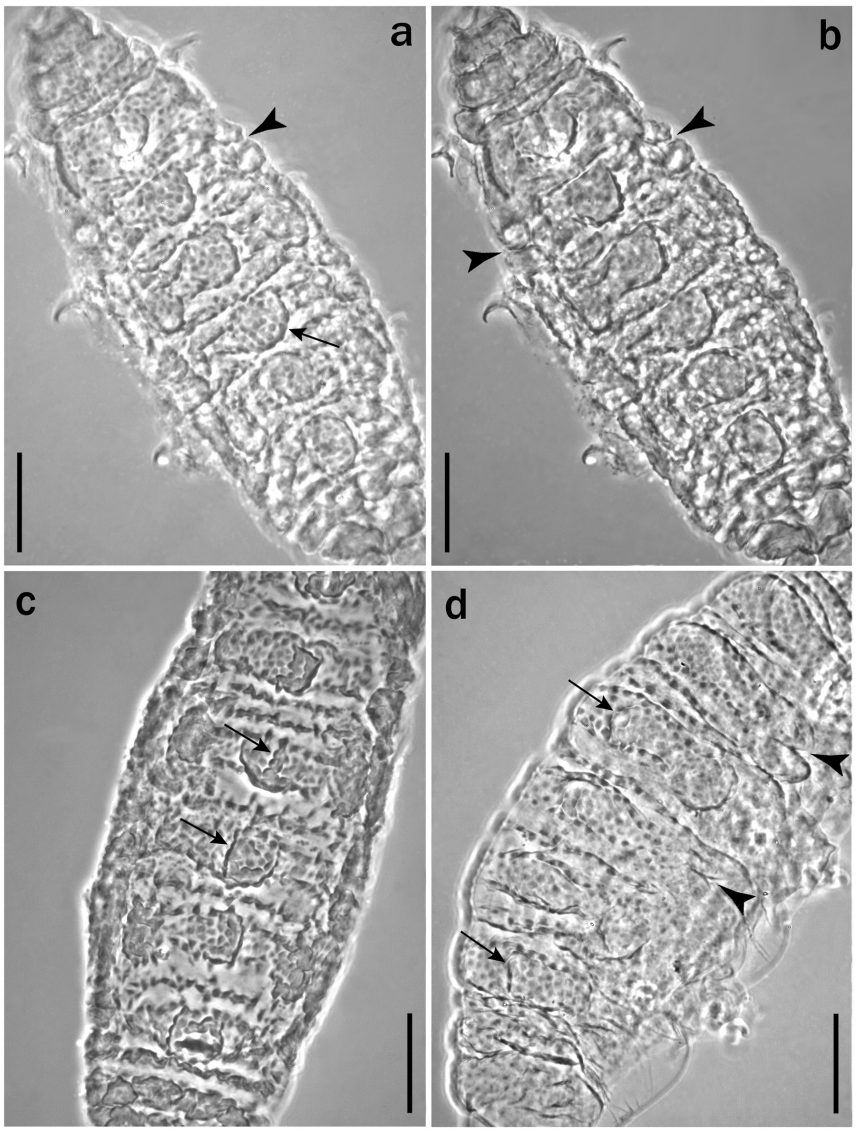

Figure 2. Cuticular structures of *Mixibius gibbosus* sp. nov. (a) General appearance of the dorsal cuticle of a paratype (slide No. 00613-8); the arrow indicates one of the undivided medial gibbosities, the arrowhead indicates the transverse division of one lateral gibbosity of row II; the cuticular tubercles giving a leopard-spotted appearance are also visible. **(b)** Same paratype as in (a), in a different focal plane; both lateral gibbosities of row II show the transverse division (arrowheads). **(c)** dorsal cuticle of the holotype (slide No. 00613-4); the arrows indicate two medial gibbosities with central longitudinal division. **(d)** dorsal and lateral cuticle of a paratype (slide No. 00511-4); the arrows indicate the central longitudinal division of two medial gibbosities, the arrowheads indicate the transverse division of lateral gibbosities of rows II and IV. Scale bars are all 20 µm.

Claws, moderately robust, typical of the genus (Fig. 4a-d): external of *Isohypsibius* type, internal of modified *Isohypsibius* type as typical of the genus; accessory points (Fig. 4b, d) present on main branches. All claw bases with pseudolunulae, the internal claws of legs I-III difficult to see, more evident on external claws of the same legs and on anterior claws of legs IV, and very evident on posterior claws of legs IV (Fig. 4d). No cuticular bars on the legs.

Morphometric analysis is provided in Table III. Eggs not found.

Etymology: the specific epithet "gibbosus" is a Latin adjective meaning "provided with gibbosity".

Differential diagnosis. This is the first *Mixibius* species described with gibbosities, and for this character it differs from all known congeneric. One might wonder whether this species might have been described as an *Isohypsibius* (in the old meaning of the genus) in the past, but *Mix. gibbosus* **sp. nov.** differs from all described species formerly attributed to the genus *Isohypsibius* with gibbosities (now belonging to the genera *Ursulinius* Gąsiorek, Stec,

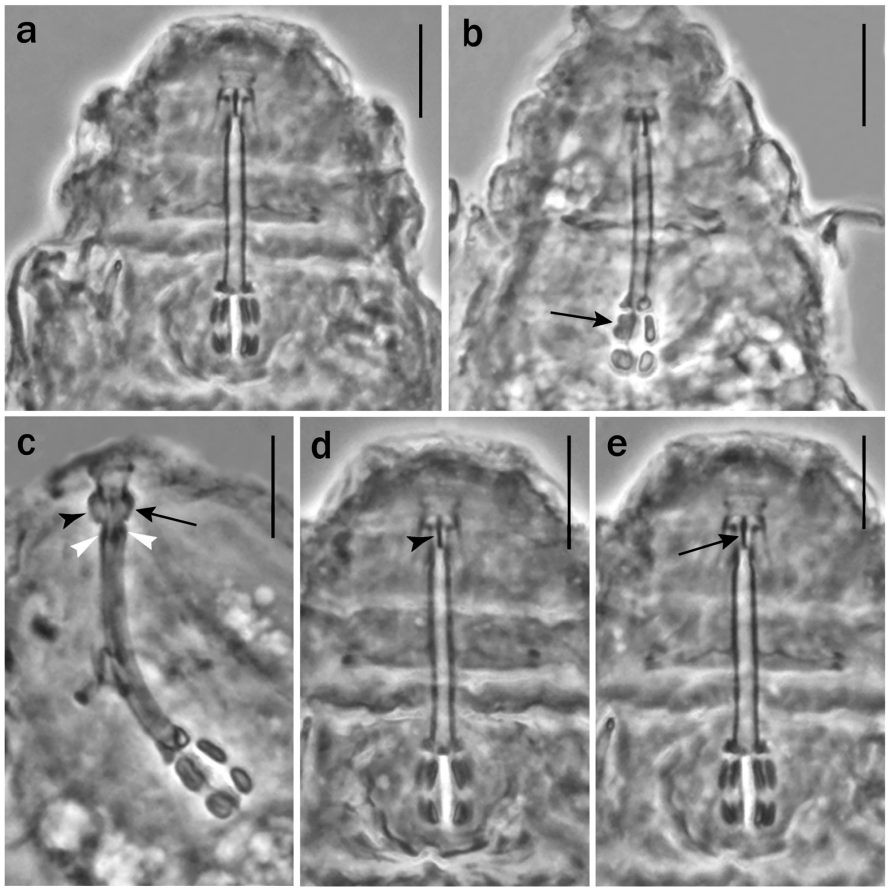

Figure 3. Bucco-pharyngeal apparatus of *Mixibius gibbosus* **sp. nov. (a)** general view of the holotype (slide No. 00613-4). **(b)** general view of a paratype (slide No. 00613-8); the black arrow indicates the central constriction of the first macroplacoid. **(c)** lateral view of a paratype (slide No. 00546-4) showing the apophyses for the insertion of the stylet muscles (AISM) typical of the genus: dorsal (black arrowhead), ventral (black arrow), posterior thickenings (white arrowheads). **(d)** dorsal focal plane showing the relative AISM of the holotype (arrowhead). **(e)** ventral focal plane showing the relative AISM of the holotype (arrow). Scale bars are all 10 µm.

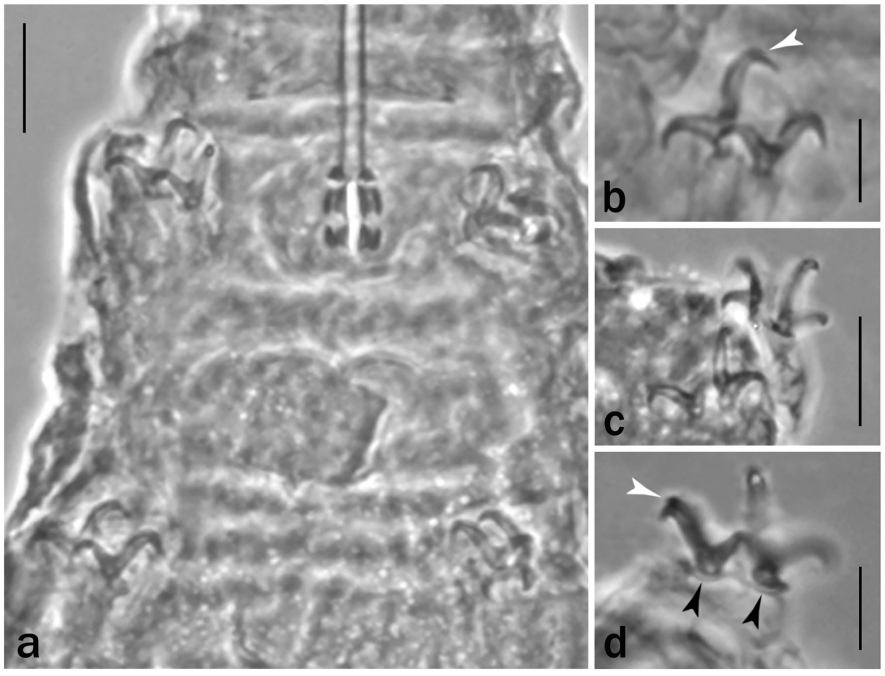

Figure 4. Claws of *Mixibius gibbosus* **sp. nov. (a)** claws of legs I and II of the holotype (slide No. 00613-4). **(b)** claws of legs II of a paratype (slide No. 00512-4); the white arrowhead indicates an accessory point. **(c)** claws of legs IV of a paratype (slide No. 00515-4). **(d)** claws of legs IV of another paratype (slide No. 00613-8); the white arrowheads indicate the pseudolunulae. Scale bars are 10 µm in (a) and (c), and 5 µm in (b) and (d).

Table III. Morphometric analysis of the holotype and paratypes of *Mixibius gibbosus* sp. nov.; for correct comparison with some past species descriptions, also the entire length of claws is indicated; *pt* values are in italics (the *pt* index is the ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage (Pilato 1981)); N = number of specimens measured for the given morphometric character. SD = standard deviation.

CHARACTER	N	RANGE						MEAN		SD		Holotype	
		µm			*pt*			µm	*pt*	µm	*pt*	µm	*pt*
Body length	5	128.0	–	161.7	*643.2*	–	*812.0*	143.7	*741.3*	12.9	*66.3*	161.7	*792.6*
Buccopharyngeal tube													
Buccal tube length	6	18.4	–	20.4		–		19.4	–	0.8	–	20.4	–
Stylet support insertion point	6	11.4	–	12.5	*59.3*	–	*63.2*	12.0	*61.7*	0.4	*1.5*	12.1	*59.3*
Buccal tube external width	5	2.0	–	2.1	*10.1*	–	*10.8*	2.1	*10.5*	0.1	*0.3*	2.1	*10.3*
Buccal tube internal width	4	1.1	–	1.3	*5.6*	–	*7.0*	1.2	*6.1*	0.1	*0.6*	1.2	*5.9*
Placoid lengths													
Macroplacoid 1	5	3.1	–	3.3	*15.9*	–	*17.3*	3.2	*16.5*	0.1	*0.5*	3.3	*16.2*
Macroplacoid 2	5	2.1	–	2.6	*10.6*	–	*13.1*	2.4	*12.0*	0.2	*1.0*	2.6	*12.7*
Macroplacoid row	5	6.0	–	6.3	*30.2*	–	*33.5*	6.2	*31.4*	0.1	*1.3*	6.3	*30.9*
Claw 1 heights													
External total	5	6.6	–	8.0	*35.7*	–	*40.8*	7.5	*38.8*	0.5	*2.0*	8.0	*39.2*
External base	1	2.9	–	2.9	*14.2*	–	*14.2*	2.9	*14.2*	?	?	2.9	*14.2*
External primary branch	1	5.4	–	5.4	*26.5*	–	*26.5*	5.4	*26.5*	?	?	5.4	*26.5*
External secondary branch	1	3.8	–	3.8	*18.6*	–	*18.6*	3.8	*18.6*	?	?	3.8	*18.6*
External base/primary branch percentual ratio	1	53.7	–	53.7		–		53.7	–	?	–	53.7	–
Internal total	4	5.5	–	5.7	*28.2*	–	*30.8*	5.6	*29.4*	0.1	*1.2*	?	?
Internal base	2	2.3	–	2.3	*11.6*	–	*11.8*	2.3	*11.7*	0.0	*0.1*	?	?
Internal primary branch	5	3.8	–	4.3	*19.5*	–	*22.2*	4.0	*20.6*	0.2	*1.1*	4.3	*21.1*
Internal secondary branch	4	2.8	–	3.6	*15.1*	–	*17.6*	3.2	*16.5*	0.3	*1.1*	3.6	*17.6*
Internal base/primary branch percentual ratio	2	59.0	–	60.5		–		59.8	–	1.1	–	?	–
Claw 2 heights													
External total	4	7.3	–	7.9	*37.4*	–	*41.3*	7.6	*39.1*	0.3	*1.8*	7.7	*37.7*
External base	4	2.7	–	3.3	*13.6*	–	*16.4*	3.0	*15.2*	0.3	*1.3*	3.3	*16.2*
External primary branch	4	4.9	–	5.3	*25.0*	–	*26.8*	5.1	*25.9*	0.2	*0.9*	5.1	*25.0*
External secondary branch	4	3.5	–	4.3	*17.7*	–	*21.1*	3.8	*19.3*	0.4	*1.4*	4.3	*21.1*
External base/primary branch percentual ratio	4	50.9	–	65.3		–		59.0	–	7.1	–	64.7	–
Internal total	1	6.7	–	6.7	*32.8*	–	*32.8*	6.7	*32.8*	?	?	6.7	*32.8*
Internal base	1	3.1	–	3.1	*15.2*	–	*15.2*	3.1	*15.2*	?	?	3.1	*15.2*
Internal primary branch	4	3.8	–	4.2	*20.6*	–	*21.1*	4.0	*20.8*	0.2	*0.3*	4.2	*20.6*
Internal secondary branch	4	2.8	–	3.6	*15.1*	–	*18.5*	3.2	*16.5*	0.4	*1.4*	3.3	*16.2*

Table III. Continuation.

Internal base/primary branch percentual ratio	1	73.8	–	73.8		–		73.8	–	?	–	73.8	–
Claw 3 heights													
External total	3	6.4	–	7.7	*34.2*	–	*37.7*	7.0	*35.5*	0.7	*2.0*	7.7	*37.7*
External base	3	2.7	–	3.0	*13.6*	–	*14.9*	2.9	*14.4*	0.2	*0.7*	3.0	*14.7*
External primary branch	3	4.8	–	5.6	*24.1*	–	*27.5*	5.2	*25.8*	0.4	*1.7*	5.6	*27.5*
External secondary branch	4	3.4	–	4.0	*17.1*	–	*20.5*	3.7	*18.5*	0.3	*1.5*	3.8	*18.6*
External base/primary branch percentual ratio	2	53.6	–	56.3		–		54.9	–	1.9	–	53.6	–
Internal total	4	5.3	–	6.4	*26.6*	–	*31.9*	5.7	*29.2*	0.5	*2.9*	6.4	*31.4*
Internal base	4	2.0	–	2.5	*10.1*	–	*12.6*	2.3	*11.7*	0.2	*1.2*	2.4	*11.8*
Internal primary branch	4	3.4	–	4.5	*17.1*	–	*22.1*	4.0	*20.1*	0.5	*2.2*	4.5	*22.1*
Internal secondary branch	3	3.1	–	3.8	*16.8*	–	*18.6*	3.4	*17.5*	0.4	*1.0*	3.8	*18.6*
Internal base/primary branch percentual ratio	4	53.3	–	62.5		–		58.4	–	3.8	–	53.3	–
Claw 4 heights													
Anterior total	2	5.4	–	5.5	*27.8*	–	*29.2*	5.5	*28.5*	0.1	*1.0*	?	?
Anterior base	2	1.9	–	2.8	*10.3*	–	*14.1*	2.4	*12.2*	0.6	*2.7*	?	?
Anterior primary branch	5	4.0	–	4.6	*20.1*	–	*22.5*	4.1	*21.1*	0.3	*1.0*	4.6	*22.5*
Anterior secondary branch	4	2.7	–	3.4	*13.6*	–	*16.7*	3.0	*15.5*	0.3	*1.4*	3.4	*16.7*
Anterior base/primary branch percentual ratio	2	47.5	–	68.3		–		57.9	–	14.7	–	?	–
Posterior total	3	7.3	–	8.2	*39.5*	–	*42.1*	7.9	*40.8*	0.5	*1.3*	?	?
Posterior base	3	2.9	–	3.3	*14.6*	–	*16.9*	3.0	*15.8*	0.2	*1.2*	?	?
Posterior primary branch	6	4.8	–	5.6	*24.1*	–	*27.6*	5.0	*25.9*	0.3	*1.5*	5.6	*27.5*
Posterior secondary branch	4	3.5	–	3.9	*17.9*	–	*19.6*	3.7	*19.0*	0.2	*0.7*	3.9	*19.1*
Posterior base/primary branch percentual ratio	3	59.2	–	68.8		–		62.8	–	5.2	–	?	–

Morek & Michalczyk, 2019 and *Dianea* Gąsiorek, Stec, Morek & Michalczyk, 2019) in having some lateral gibbosities transversally divided, and in a unique gibbosity formula (both in the case the questioned neck gibbosities were present, and absent). We here compare *Mix. gibbosus* **sp. nov.** with the congeneric species having cuticular ornamentation (*Mixibius ninguidus* Biserov, 1999, *Mix. ornatus* Pilato, Binda, Napolitano & Moncada, 2002, *Mix. parvus* Lisi, Sabella & Pilato, 2014, *Mix. schnurae* Pilato, Lisi & Binda, 2010, *Mix. sutirae* Pilato, Binda & Lisi, 2004 and *Mix. tibetanus* H. Li & X. Li, 2008), thus excluding those with smooth cuticle (*Mix. felix* Pilato, Sabella, D'Urso & Lisi, 2017, *Mix. fueginus* Pilato & Binda, 1996, *Mix. pilatoi* L. Wang, 2009 and *Mix. saracenus* (Pilato, 1973)).

Apart from the presence of cuticular gibbosities, *Mixibius gibbosus* **sp. nov.** also differs from *Mix. tibetanus* and *Mix. parvus* in having two macroplacoids and no microplacoid (three macroplacoids and a microplacoid in those two species); more in detail, the new species also differs from *Mix. tibetanus* in having a different cuticular sculpture. Li & Li (2008) wrote in the description of the holotype

of this species, "Dorsal cuticle with many small tubercules differing in shape and size (maximum diameter of 3.4 mm; Fig. 12)"; however, in Fig. 12 of Li & Li (2008), the cuticle of the holotype of *Mix. tibetanus* shows a totally different appearance with respect to that of *Mixibius gibbosus* **sp. nov.**, so that we think those authors mistaken a reticular sculpturing (which we think to see in that image) for tubercles. *Mix. gibbosus* **sp. nov.** also differs from *Mix. parvus*, again, in the different cuticular sculpturing, since in the latter species the cuticle is roughly wrinkled without forming tubercles.

The most similar species is *Mixibius ornatus* (Fig. 5a, b), from Ecuador, thus resulting the closest to the new species also from the geographic point of view. A similarity with *Mix. gibbosus* **sp. nov.**, apart from the presence of cuticular tubercles, is also the tendency in *Mix. ornatus* to outline, in some portions of the cuticle, areas which seem to foreshadow the formation of cuticular gibbosities (Fig. 5a), but such areas are not prominent enough to be considered, in our opinion, true cuticular gibbosities and, as a matter of facts, in specimens with well distended cuticle they become invisible. Besides, such apparent "gibbosities" of some specimens are lower in number than in the new species (it is not possible to provide a formula of the arrangement of said "gibbosities" of *Mix. ornatus* due to the difference in their visibility among the specimens). *Mix. gibbosus* **sp. nov.** also differs from *Mix. ornatus* in having different cuticular tubercles: bigger, more evident, better outlined end with more regular shape (always isodiametric) in the new species, while in *Mix. ornatus* they are smaller, more numerous, more irregular (also elongated) and variously sized; actually, our observations on the type material of this species revealed a different appearance than in the new species (which shows a leopard-spotted ornamentation): in *Mix. ornatus* it is possible to speak of a general rugosity of the cuticle which outlines those irregular tubercles (Fig. 5a). In addition, *Mix. gibbosus* **sp. nov.** differs from *Mix. ornatus* in having stouter claws (compare Fig. 4a-d with Fig. 5b). As regards morphometric differences (Table IV), the new species differs from *Mix. ornatus* in having (for all comparisons: the first range value refers to the new species, the second to the compared species): slightly wider buccal tube (*pt 10.1-10.8 vs 7.7-9.4*), more anterior stylet supports (*pt 59.3-63.2 vs 69.1-70.5*), longer placoid row (*pt 30.2-33.5 vs 23.6-27.9*), longer first macroplacoid (*pt 15.9-17.3 vs 12.7-15.2*), shorter claws (*pt* II-III external *34.2-41.3 vs 46.5* holotype; *pt* II-III internal *26.4-32.8 vs 36.7* holotype; *pt* IV anterior *27.8-29.2 vs 35.9-42.8*; *pt* IV posterior *39.4-42.1 vs 46.6-52.8*).

Mixibius gibbosus **sp. nov.** differs from *Mix. sutirae*, in having different cuticular tubercles: bigger, more evident, better outlined end with more regular shape (always isodiametric) in the new species, while in *Mix. sutirae,* like in *Mix. ornatus*, they are smaller, more numerous, more irregular (also elongated) and variously sized; also, for *Mix. sutirae*, our observations on the type material revealed a general rugosity of the cuticle which outlines those irregular tubercles (Fig. 5c) while the new species, shows a more regular leopard-spotted ornamentation. Buccal crests not visible in the new species *vs* visible, though described as "very thin", in *Mix. sutirae*. The new species has also stouter claws (compare Fig. 4a-d with Fig. 5d). As regards morphometric differences (Table IV), the new species differs from *Mix. sutirae* in having (for all comparisons: the first range value refers to the new species, the second to the compared species): more cephalic stylet supports (*pt 59.3-63.2 vs 70.5-70.8*) and shorter claws (*pt* II-III internal *26.4-32.8 vs 39.1-39.9* which were the only measurable claws in this species).

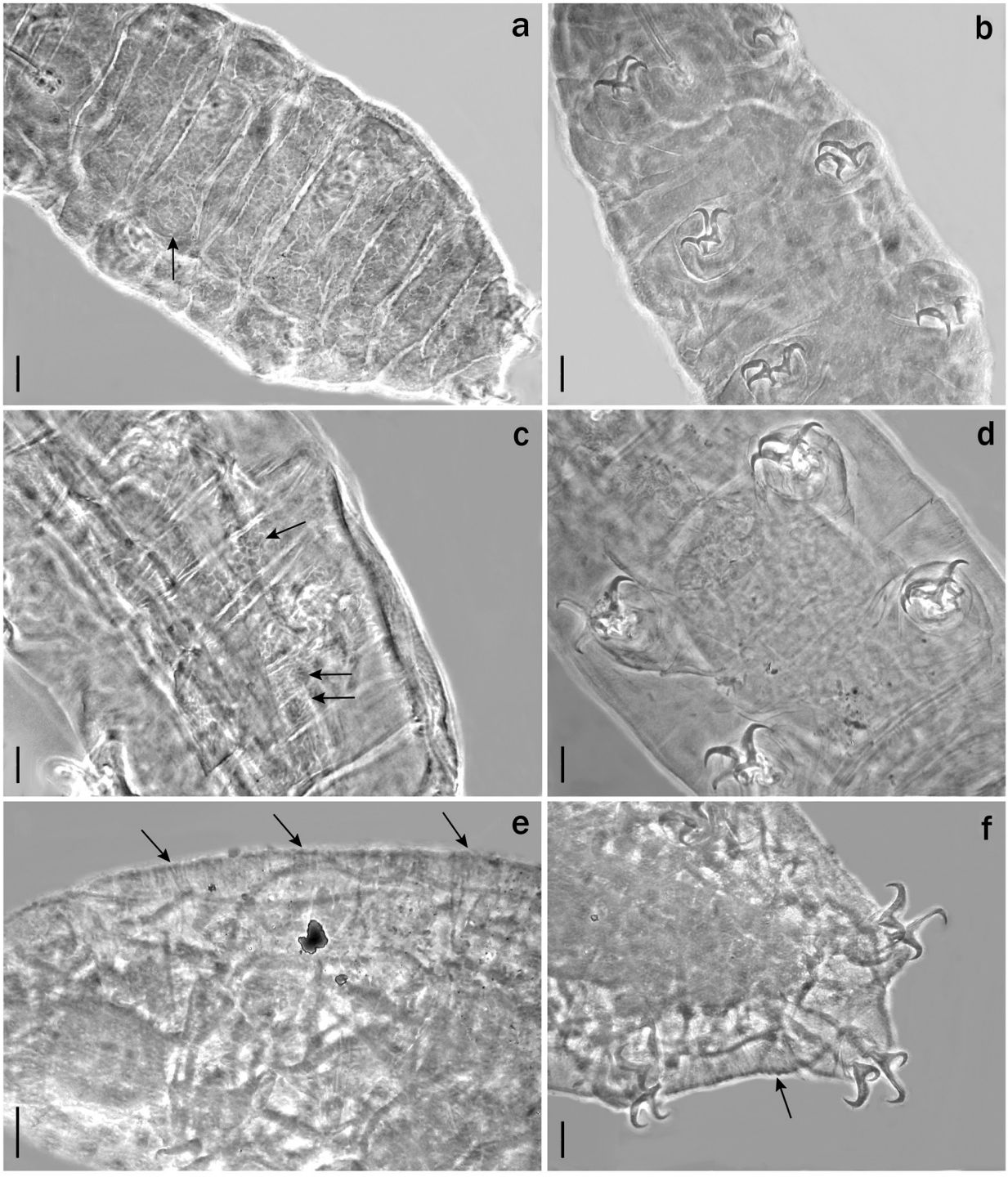

Figure 5. Holotypes of the species most similar to *Mixibius gibbosus* **sp. nov. (a-b)** *Mixibius ornatus* (slide No. 4770): **(a)** dorsal cuticle (the arrow indicates a weak gibbosity-like structure); **(b)** claws. **(c-d)** *Mixibius sutirae* (slide No. 4990): **(c)** dorsal cuticle (the arrows indicate some groups of tubercles); **(d)** claws. **(e-f)** *Mixibius schnurae* (slide No. 5420): **(e)** dorsal cuticle (the arrows indicate some bands of rugosity); **(f)** caudal end of the body showing claws of legs IV, and some tubercles (arrow). All material deposited in the Pilato and Binda collection (University of Catania). Scale bars are all 10 µm.

Mixibius gibbosus **sp. nov.** differs from *Mix. ninguidus* by a different type of cuticular ornamentation, which in *Mix. ninguidus* consists in of just a general rugosity and slight lateral granulation; besides, the new species has slightly stouter claws (compare Fig. 4a-d in the present paper with Fig. 3c, d in Biserov 1999). As regards morphometric differences (Table IV), the *pt* index ranges here presented about *Mix. ninguidus* were calculated from Biserov (1999, Table 3), where only mean and standard error of said *pt* indices were provided. The new species differs from *Mix. ninguidus* in having (for all comparisons: the first range value refers to the new species, the second to the compared species): slightly smaller inner buccal tube width (*pt 5.6-7.0 vs* about *7.4-8.4*), shorter placoid row (*pt 30.2-33.5 vs* about *38.5-41.7*), shorter first macroplacoid (*pt 15.9-17.3 vs* about *19.2-21.0*), shorter posterior claws of legs IV (*pt 39.4-42.1 vs* about *47.6-51.0*).

Order: Isohypsibioidea Guil, Jørgensen & Kristensen, 2019
Superfamily: Isohypsibioidea Sands, McInnes, Marley, Goodal-Copestake, Convey & Linse, 2008
Family: Doryphoribiidae Gąsiorek, Stec, Morek & Michalczyk, 2019
Genus: *Doryphoribius* Pilato, 1969

***Doryphoribius amazzonicus* Lisi, 2011**

Material examined. 3 specimens in total: sample 2, CBUMAG:TAR:00358 (1 specimen); sample 4, 00366 (1 specimen), sample 16, 00130 (1 specimen).

Remarks. The species was previously reported for Colombia, for the same locality (San Lorenzo) in the SNSM by Lisi et al. (2014a), herein it is recorded for Medium basin of Garupal River, also in the SNSM.

Table IV. Morphometric comparison of *Mixibius gibbosus* sp. nov. with the most similar species. The numbers in brackets indicate how many specimens were measured. For correct comparison with some past species, claw measurements refer to the entire claw, and the values relative to claws II and III are joined together. The *pt* index ranges about *Mix. ninguidus* were calculated from Biserov (1999, Table 3). The *pt* index is the ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage (Pilato 1981).

	***M. gibbosus* sp. nov.**	***M. ornatus* [7]**	***M. sutirae* [2]**	***M. shnurae* [3]**	***M. ninguidus* [8]**
Body length (µm)	128 - 162 [5]	170 - 222	230 - 252	230 - 247	190 - 250
Buccal tube length (µm)	18.4 - 20.4 [6]	21.8 - 28.1	30.7 - 31.3	26.6 - 30.1	24.0 - 27.0
Buccal tube external width (*pt*)	*10.1 - 10.8* [5]	*7.7 - 9.4*	*9.1 - 9.6*	*11.1 - 12.4*	-
Buccal tube internal width (*pt*)	*5.6 - 7.0* [5]	-	-	-	*7.4 - 8.4*
Stylet support insertion point (*pt*)	*59.3 - 63.2* [6]	*69.1 - 70.5*	*70.5 - 70.8*	*62.8 - 64.3*	about *62.6 - 63.6*
Placoid row (*pt*)	*30.2 - 33.5* [5]	*23.6 - 27.9*	*26.7 - 28.4*	*35.3 - 38.5*	about *38.5 - 41.7*
First placoid (*pt*)	*15.9 - 17.3* [5]	*12.7 - 15.2*	*14.3 - 14.4*	*19.2 - 22.3*	about *19.2 - 21.8*
Second placoid (*pt*)	*10.6 - 13.3* [5]	*9.5 - 11.0*	*10.1 - 10.5*	*11.7 - 12.9*	about *13.4 - 15.0*
External claw I (*pt*)	*35.7 - 40.8* [5]	-	-	-	-
Internal claw I (*pt*)	*28.2 - 30.8* [4]	-	-	-	-
External claw II-III (*pt*)	*34.2 - 41.3* [6]	*46.5* (holotype)	-	*50.0 - 55.3*	-
Internal claw II-III (*pt*)	*26.4 - 32.8* [4]	*36.7* (holotype)	*39.1 - 39.9*	*41.9 - 47.7*	-
Anterior claw IV (*pt*)	*27.8 - 29.2* [2]	*35.9 - 42.8*	-	*46.8 - 48.2*	-
Posterior claw IV (*pt*)	*39.4 - 42.1* [3]	*46.6 - 52.8*	-	*60.7 - 62.5*	about *47.6 - 51.0*

Family: Isohypsibiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008
Genus: *Dianea* Gąsiorek, Stec, Morek & Michalczyk, 2019

Dianea sattleri* (Richters, 1902) *sensu lato

Material examined. 5 specimens in total: sample 10, CBUMAG:TAR:00615 (1 specimen), 00616 (2 specimens); sample 12, 00627 (1 specimen); sample 13, 00631 (1 specimen).

Remarks. The species was previously reported for Colombia (for the same locality in the SNSM) by Lisi et al. (2014a). However, the *locus typicus* is in Germany and we agree with Kaczmarek et al. (2015) in considering doubtful all South American reports of this species; we therefore consider both the present new finding and the previous of 2014 (Lisi et al. 2014a) as *sensu lato*.

Genus: *Fractonotus* Pilato, 1998

***Fractonotus* sp.**

Material examined. 4 specimens in total: sample 6, CBUMAG:TAR:00372 (1 specimen); sample 10, 00615 (3 specimens).

Remarks. The small sample size and the quality of the material prevented from sure diagnosis. The genus was already known for South America with *Fra. caelatus sensu lato* (Marcus, 1928) from Ecuador, and *Fra. verrucosus sensu lato* (Richters, 1900) from Brazil and Colombia (Kaczmarek et al. 2015, Gąsiorek et al. 2019b); our material looks more similar to the former species.

DISCUSSION

Biological collections represent an invaluable scientific resource, but their use beyond the taxonomy, depends on the detailed identification of the deposited specimens. In some collections, biological material is consigned as a result of large-scale research and sometimes, for reasons of lack of time, much of this material cannot be identified with high taxonomic resolution. The review of biological collections is a necessity, and this important work must be done carefully to increase our understanding of biodiversity. Regarding the collection of tardigrades in the "Centro de Colecciones Científicas de la Universidad del Magdalena", it possesses approximately 11.350 specimens, of which 11% have been identified up to species. The present work shows that material not yet identified, hides taxonomic novelties; this revision increases the number of known species from 59 to 64 for Colombia. It is worth noting that counting *Mixibius gibbosus* **sp. nov.**, a total of 10 species have been described as new to science from type material collected in the SNSM; except for *Pam. lachowskae*, the type material is found in the "Centro de Colecciones Científicas de la Universidad del Magdalena".

The new species is interesting also from the morphological and taxonomic point of view because until now no *Mixibius* species was known having cuticular gibbosities, and some of these structures show also peculiar morphological characteristics (*i.e.* the curious transverse division of lateral gibbosities of rows II, IV and VI).

Despite not providing DNA sequences due to the characteristics of the study material (previously prepared and deposited in a collection), the morphological analyses carried out, allow us to reveal information that can be used later by other researchers and represents an important contribution to the knowledge of the biodiversity of Colombia, providing additional evidence of the high biological richness of the SNSM.

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OSCAR LISI[1,2]
https://orcid.org/0000-0001-5495-2742

ANISBETH DAZA[2]
https://orcid.org/0000-0002-0847-4724

ROSANA LONDOÑO[2]
https://orcid.org/0000-0002-8688-9247

SIGMER QUIROGA[2,3]
https://orcid.org/0000-0002-3321-1360

[1]Università di Catania, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Via Androne 81, 95124 Catania, Italy

[2]Universidad del Magdalena, Grupo de Investigación en Manejo y Conservación de Fauna, Flora y Ecosistemas Estratégicos Neotropicales MIKU, Carrera 32 # 22-08, Santa Marta, Colombia

[3]Universidad del Magdalena, Facultad de Ciencias Básicas, Programa de Biología, Carrera 32 # 22-08, Santa Marta, Colombia

Correspondence to: **Oscar Lisi**
E-mail: olisi@unict.it

Author contributions

Each author contributed significantly to the development of the study; species identification and description were conducted in equal cooperation regime. Lisi O. revised the material deposited in the Pilato and Binda Collection (Catania, Italy). Daza A., Londoño R. and Quiroga S. revised the material deposited in the Centro de Colecciones Científicas de la Universidad del Magdalena (CCC) (Santa Marta, Magdalena, Colombia). All the authors made up the plates, worked in the manuscript and approved the final version.