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The genus *Milnesium* (Eutardigrada, Apochela, Milnesiidae) in Argentina: description of three new species and key to the species of South America

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Abstract. A revision of microscope slides deposited between 2014 and 2017 in the collection of Rocha and Doma (National University of La Pampa, Argentina) revealed three new species of the genus *Milnesium* Doyère, 1840: *M. pelufforum* sp. nov., *M. irenae* sp. nov. and *M. quiranae* sp. nov. *Milnesium pelufforum* sp. nov. is mostly characterized by ten transverse bands of sculptured cuticle and pseudoplates (the first band, until now, never detected in the genus), six peribuccal lamellae and claw configuration [2-2]-[2-2] in young or [2-3]-[3-2] in senior specimens. *Milnesium irenae* sp. nov. is mostly characterized by complex cuticular ornamentation including a fine reticulation different from the typical one in the genus; it also has pseudoplates, six peribuccal lamellae, medioventral peribuccal papilla reduced, stylets, their furcae and supports very developed, and claw configuration [2-3]-[2-2]. *Milnesium quiranae* sp. nov. is mostly characterized by smooth cuticle, six peribuccal lamellae, and claw configuration [3-3]-[3-3]; with growing, the medioventral peribuccal papilla reduces and the buccal tube becomes wider. With the present contribution the genus *Milnesium* now has 48 valid species, and the number of described limno-terrestrial tardigrade species from South America has risen to 11, including 8 from Argentina with 5 from Salta and La Pampa province.

Keywords. Tardigrades, taxonomy, Neotropical region, Salta, Santa Rosa, ontogenetic change.

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Introduction

The genus *Milnesium* Doyère, 1840 had been considered monospecific for 62 years (from 1928 to 1990), and its only species *Milnesium tardigradum* Doyère, 1840 was regarded as rather variable and cosmopolitan. Starting in 1990 (Binda & Pilato 1990), authors started describing new species of the genus, each one less variable and not cosmopolitan. Since 2000, the rate of description of new species of the genus has increased remarkably, and Michalczyk *et al*. (2012a, 2012b) redescribed the nominal species questioning its presumed cosmopolitism. Descriptions of new species continued, so that today 45 species are attributed to the genus (excluding the subspecies *Milnesium tardigradum trispinosa* Rahm, 1931 and the species *M. dujiangensis* Yang, 2003, according to Morek *et al.* 2016 and Suzuki 2016).

Based on the superficial morphology of the dorso-lateral cuticle, two species groups were established: the *tardigradum* group and the *granulatum* group, with smooth and sculptured cuticles, respectively (Michalczyk *et al*. 2012a, 2012b). Such groups, from the phylogenetic point of view, have been recently questioned (Morek *et al*. 2016), and even invalidated (Morek & Michalczyk 2020), but they can still be practical for purely morphological comparison between species.

Of the 45 known species of *Milnesium*, 8 (17.8 %) are South American. Claps & Rossi (1984) made the first record of *Milnesium* in Argentina, for Salta Province. To date, Argentina has 5 recorded species of *Milnesium* (11.1% of the global total) but the South American tardigrade fauna is still poorly known (e.g., González Reyes *et al.* 2020). In this paper, we describe three new species of *Milnesium* from two regions of Argentina, one region in the North (Salta) and the other in the South-central area (La Pampa).

Material and methods

This contribution is part of an ongoing review work on the Rocha and Doma tardigradological collection (Department of Natural Sciences of the National University of La Pampa, Argentina, deposited between 2014 and 2017). About 85 slides were examined, of which 26 were selected because containing three new species of the genus *Milnesium* described in the present paper.

The material had been extracted from lichen and moss samples growing on sidewalk trees, taken from the cities of Salta and Santa Rosa. Samples were taken from trees of similar size (about 5 m tall) at 1.3 m height from the trunk surface facing the road. The samples were collected in May 2014 (Salta) and September 2017 (Santa Rosa), and were stored in paper bags at room temperature. For processing, they were hydrated in a plastic sieve (1.1 mm mesh), then transferred 24 h later to Petri dishes filled with mineral water for examination, using a stereoscopic microscope. Tardigrades and exuviae were separated with a micropipette. Active tardigrades were killed by placing them in a heater at about 60°C, or else using hot water (80°C). The material was mounted on slides with polyvinyl-lactophenol medium.

Tardigrades were identified using phase contrast microscopy (PCM; Leica DM 500), equipped with a digital camera (ICC 50 HD). Detection of pseudoplates, in all three new species described in the present paper, and assessment of their configuration was performed using a fluorescence microscope (FM; Carl Zeiss Palm MicroBean realease 4.8 and digital camera AxioCam 506 color).

Pseudoplate arrangement formulas are given following Moreno-Talamantes *et al.* (2019) (whose system, in turn, was based on that proposed by Michalczyk & Kaczmarek (2010) for the numbering of gibbosities in species of *Doryphoribius* and *Isohypsibius*); such formulas consist of a sequence of

Roman numbers, indicating the pseudoplate rows from the most anterior (I) to the most posterior (IX in species of *Milnesium* according to Moreno-Talamantes *et al.* 2019), and each Roman number (i.e., row of pseudoplates) is followed by an Arabic number indicating the number of pseudoplates of the indicated row. However, in the present work it was necessary to add a pseudoplate row (the most cephalic, never detected until now) with respect to the nine indicated by Moreno-Talamantes *et al.* (2019), as explained in the Discussion and shown in Table 3.

Morphometric data were obtained using the AxioVision software SE64, given in micrometers (μm) and handled with the Excel template "Apochela" (ver. 1.4) from the tardigrade Register (Michalczyk & Kaczmarek 2013); the Excel files with the complete datasets of measurements are provided as supplementary material. Student *t*-tests (one-side tests) for statistical significance of differences between species morphometry (only when ranges of the given characters of the two compared species overlapped) were perfomed through Microsoft Office Excel software and the results are reported in Tables 4–5, 7, 11 (relative to the various differential diagnoses); when ranges did not overlap, they are reported directly in the text of the differential diagnoses. The buccal tube was measured according to Michalczyk *et al*. (2012a, 2012b) and claws according to Tumanov (2006). Percentage ratios between the length of the structure considered and that of the buccal tube (*pt*) were calculated following Pilato (1981) and *pt* ranges are indicated in italics within square brackets. Claw configuration is given according to Michalczyk *et al.* (2012a, 2012b).

Species diagnoses were performed using original species descriptions, taxonomic keys and other useful pieces of literature: Binda & Pilato 1990; Maucci 1991; Pilato *et al*. 2002, 2016; Kaczmarek *et al*. 2004, 2012; Tumanov 2006; Kaczmarek & Michalczyk 2007; Meyer & Hinton 2010, 2012; Michalczyk *et al*. 2012a, 2012b; Meyer *et al*. 2013; Bartels *et al*. 2014; Ciobanu *et al.* 2015; Meyer 2015; Morek *et al*. 2016, 2019a, 2019b, 2020a; Pilato & Lisi 2016; Moreno-Talamantes *et al*. 2019, 2020; Surmacz *et al*. 2019 and Sugiura *et al*. 2020.

For comparison with our material, the following type specimens from the Pilato and Binda Collection (Museum of the Section of Animal Biology, Department of Biological, Geological and Environmental Sciences, University of Catania) were examined: holotype and paratypes of *Milnesium brachyungue* Binda & Pilato, 1990 (slides Nos 3940–49), *M. minutum* Pilato & Lisi, 2016 (slide No. 4127), *M. reticulatum* Pilato, Binda & Lisi, 2002 (slides Nos 4851–4860), *M. sandrae* Pilato & Lisi, 2016 (slide No. 4290) and *M. tumanovi* Pilato, Sabella & Lisi, 2016 (slides Nos 3904, 3916). Paratypes of *M. almatyense* Tumanov, 2006 (slide No. 5106), *M. antarcticum* Tumanov, 2006 (slide No. 5104), *M. asiaticum* Tumanov, 2006 (slide No. 5105) *M. beasleyi* Kaczmarek, Jakubowska & Michalczyk, 2012 (slide No. 5518), and *M. longiungue* Tumanov, 2006 (slide No. 5103) were also examined.

Having worked with already mounted slides, determination of exact life stage was not possible; we separated, when ontogenetic change was observed (i.e., in *Milnesium pelufforum* sp. nov. and *Milnesium quiranae* sp. nov.), smaller from larger specimens according to the morphological and morphometric differences between the differentiable groups. For confirmation, we applied the method proposed by Surmacz *et al*. (2020), and the results perfectly corresponded to our grouping of specimens: in *Milnesium pelufforum*, two groups were identified, with the smaller specimens called 'young' (hatchlings or hatchlings plus second instar), and the larger called 'senior' (from second or third instar on); in *Milnesium quiranae*, three groups were identified, called 'young' (hatchlings or hatchlings plus second instar), 'intermediate' (second or third instar) and 'senior' (from third or fourth instar on) (Supp. file 7). Regarding *Milnesium irenae* sp. nov., instead, none of the approaches gave the possibility to separate specimen groups. The three graphs obtained through said analysis, representing the clustering or its absence for each species, are provided in supplementary materials.

In preparing differential diagnoses we made the following decisions: 1) referring to the classical two morphological groups of the genus *Milnesium* (*tardigradum* and *granulatum*) for morphological convenience; very recently (Morek *et al*. 2016, 2021; Morek & Michalczyk 2020), the phylogenetic non-significance of those two groups was pointed out, but Morek & Michalczyk (2020) proposed new phylogenetic groups each with a variable morphology, thus not usable for the aims of the present paper. 2) For species with ontogenetic change, we prepared separate differential diagnoses for young and senior specimens. 3) In morphometric comparisons, when only a single value of a metric character, instead of a range, is reported for a compared species, this reflects the only information available from the literature. 4) When the original description of a compared species made no distinction between the measurements of external and internal claws of legs I–III, or of anterior and posterior claws of legs IV, we provide equivalent measurement indication for our new species for more direct comparison.

Institutional acronyms

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

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

Results

Taxonomic account

Phylum Tardigrada Doyère, 1840
Class Eutardigrada Richters, 1926
Order Apochela Schuster, Nelson, Grigarick & Christenberry, 1980
Family Milnesiidae Ramazzotti, 1962
Genus *Milnesium* Doyère, 1840

Milnesium pelufforum sp. nov.
urn:lsid:zoobank.org:act:0DA71F31-0FA3-4F27-91EC-CC028C5AC808
Figs 1–12, Tables 1–5

Diagnosis

Ten transverse bands, better defined in senior specimens, of sculptured cuticle consisting of dimples forming a reticular pattern. Dimples, larger in young than in senior specimens, often showing some internal structure; ten rows of pseudoplates also present, better outlined in senior specimens, formula CP: I:1; II:4; III:6; IV:6; V:10; VI:10; VII:10; VIII:12; IX:12; X:4. Pseudoplate rows coincide with sculptured bands but sculpturing is not limited only on pseudoplates.

Six peribuccal lamellae, six peribuccal papillae equal in size, two lateral cephalic papillae. Buccal tube nearly cylindrical, wider in senior specimens; claw configuration [2-2]-[2-2] in young, [2-3]-[3-2] in senior specimens (but with very small basal spurs, especially on legs IV).

Etymology

The new species is dedicated to Maria Cristina Moly de Peluffo and Julio Ricardo Peluffo, the first researchers of tardigrades from the National University of La Pampa, Argentina.

Material examined

Holotype

ARGENTINA • ♀; Salta Province, Salta City; 24°47′18″ S, 65°24′38″ W, 1150 m a.s.l.; 2 May 2014; Rocha-Doma leg.; moss and lichens from trees; MCNS Tar.000021(3).

Paratypes

ARGENTINA • 2 ♀♀; same collection data as for holotype; MCNS Tar.000021(2), Tar.000021(4) • 4 ♀♀; same collection data as for holotype; UNICT 5898(1) to 5898(4) • 17 ♀♀; same collection data as for holotype; UNLPam 389(1) to 391(3), 454(1) to 454(5), 465(1) to 465(4), 456(4) to 456(5), 503(1) to 503(3).

Morphological description

Body length from 216 µm to 620 µm, reddish colour before mounting, eyes present (habitus in Figs 1, 3, 8). Dorsal and dorsolateral cuticle with variously shaped dimples (depressions) (Figs 1–3, 6, 11) forming a reticular pattern, arranged in ten transverse bands (as a tendency in young, as a rule in senior specimens), one band on each of the ten subsegments of the body (Figs 1, 3, 11) including the very first, cephalic one; dimples often showing internal structures of variable appearance (Figs 2, 6, 11), but further investigations are required to ascertain whether apparent presence or absence, and shape, of such internal structures is just due to matter of focus under the microscope and orientation of the structures in the preparation; dimples vary in size but not in shape or details of internal structure, between young and senior specimens. Pseudoplates, better outlined in senior specimens, present (Figs 4–5, 9–10), also arranged in ten transverse bands (again, including the very first, cephalic subsegment); pseudoplate rows correspond with the sculptured bands but the sculpturing is not limited only on pseudoplates; pseudoplate formula is CP: I:1; II:4; III:6; IV:6; V:10; VI:10; VII:10; VIII:12; IX:12; X:4 (based on senior specimens). Row I is situated at the level of the buccal tube and has only one medial pseudoplate, difficult to see, more or less rectangular, laying transversally, with rounded angles. Row II, situated just anteriorly to legs I, has four pseudoplates: two medial, about trapezoidal, touching in the central line along their longer side, and two separate lateral, about rectangular, laying obliquely and difficult to see. Row III, situated in line with legs I, has six pseudoplates: four central arranged in two pairs, all four connected, with the two anterior, difficult to see, about rectangular, and the two caudal, triangular, pointing backwards; two lateral pseudoplates, about ellyptical, laying obliquely and difficult to see. Row IV, situated between legs I and II, has six pseudoplates: the four medial arranged in two pairs, transversally elongated, connected, forming a unique about rectangular structure; each of these four pseudoplates vaguely rectangular but with the transverse line dividing the anterior and posterior couple not straight; two rounded lateral pseudoplates, difficult to see. Row V, situated in line with legs II, has ten pseudoplates: the six medial arranged in three pairs, transversally elongated, connected, forming a unique about rectangular/trapezoidal structure; each of these six pseudoplates vaguely rectangular but with the two transverse lines dividing the adjacent anterior/posterior pairs of pseudoplates not straight; lateral to that central complex, on each side, two separate pseudoplates, difficult to see, with the mid-lateral less developed and vaguely in the shape of a curved trapezium, while the most lateral is more developed and vaguely quadrangular. Row VI, situated between legs II and III, has ten pseudoplates: two medial rectangular, sided laterally and very elongated transversally; lateral to them, on each side, four separate pseudoplates difficult to see, arranged in a quadrangle. Row VII, situated in line with legs III, has ten pseudoplates: the six medial arranged in three pairs, transversally elongated, connected, forming a unique about rectangular structure; each of these six pseudoplates vaguely rectangular but with the two transverse lines dividing the adjacent anterior/posterior pairs of pseudoplates not straight; lateral to that central complex, on each side, two separate pseudoplates, with the mid-lateral less developed and vaguely in the shape of a curved trapezium, while the most lateral is more developed and vaguely quadrangular. Row VIII, situated just posterior to legs III, has twelve pseudoplates with the four medial arranged in two pairs, transversally elongated, connected, forming a unique about rectangular structure; each of these four pseudoplates vaguely rectangular but with the two anterior well visible and not thin, while the two posterior difficult to see and very thin; lateral to that central complex, on each side, four separate pseudoplates arranged in a quadrangle: the two mid-lateral elongated transversally, the two very lateral vaguely quadrangular; besides, the two anterior pseudoplates of the tetrad difficult to see, while the two posterior better visible. Row IX, situated between legs III and IV, has twelve pseudoplates with a central aggregation of 10 pseudoplates forming a complex pattern (see Fig. 10), and two lateral single pseudoplates about quadrangular, aligned mid-posteriorly. Row X, situated just anterior to legs IV, has four about quadrangular pseudoplates, sided and aligned in a single row transversally.

Cuticular grooves present dorsally, but this and other details of the cuticular ornamentation vary depending on life stage (see below).

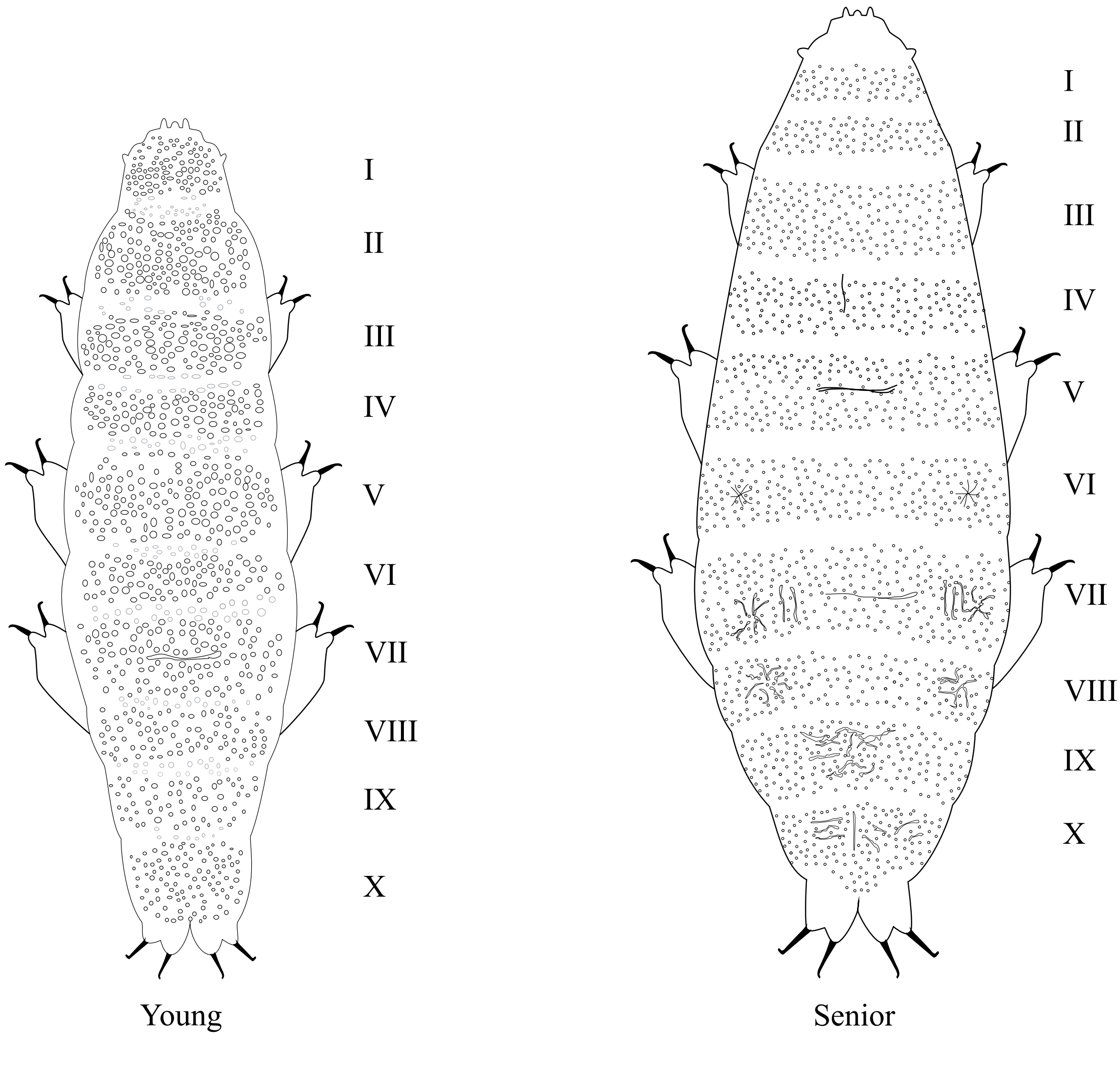

Fig. 1. *Milnesium pelufforum* sp. nov. Schematic drawing of a young and a senior specimen showing the ten sculptured bands.

Six peribuccal lamellae and six peribuccal papillae plus two lateral papillae present. Buccal tube (Figs 7A, 12A) nearly cylindrical (posterior/anterior width ratio 82–100%), more slender in young specimens; *pt* of the stylet support insertion point on the buccal tube [*65.0–73.8*].

Claws of the *Milnesium* type with configuration [2-2]-[2-2] in young (Fig. 7B–C), [2-3]-[3-2] in senior specimens (Fig. 12B–D) but with very small basal spurs where present, in particular on legs IV where they are just a little spine (Fig. 12B–D, white arrows); claws stout, secondary branches with basal thickenings ('lunulae'; Figs 7C and 12C–D, white arrowheads), primary branches with small accessory points (Figs 7B and 12B–C, black arrows); cuticular bars present on legs I–III (Figs 7B and 12B–C, black arrowheads); percentual ratio of secondary branches with respect to primary branches for each claw couple higher for legs I, slightly lower for legs II–III and more significantly lower for legs IV (Tables 1 and 2).

More detailed description is given in the following paragraphs separating young and senior specimens.

Young specimens (probably hatchlings: 216–305 µm; Figs 1, 3–7, Table 1, Supp. file 1)
Cuticular dimples larger, especially in proportion to the body size (about 2–3.5 µm); dimples tend to form ten transverse bands almost touching one another and connected, at least in some areas, by dimples appearing less evident (Fig. 1). Pseudoplates less developed and less distinct from one another in each row than is senior specimens (Figs 4–5). Few simple, short, cuticular depressions can be present on some segments, tending to lay transversally, the most developed is constantly present at the level of legs III in the shape of a transverse groove (Figs 1, 3, 6, white arrowheads).

Buccal tube (Fig. 7A) more slender than in senior specimens (*pt* of standard width [*43.7–52.1*]).

Claw configuration [2-2]-[2-2] (Fig. 7B–C); percentual ratio of secondary branches with respect to primary branches for each claw couple with a less marked difference (than in senior specimens) between legs I and legs IV (Table 1): 71–90% for claws I, 66–86% for claws IV, difference is 4–5%.

Senior specimens (probably from second instar on: 390–620 µm; Figs 1, 8–12, Table 2, Supp. file 2)
Cuticular dimples smaller than in young specimens (about 1–2 µm), especially in proportion to the body size; ten clear transverse bands of dimples, more spaced from one another than in young specimens and separated by smooth cuticle (Figs 1, 11). Starting with legs II in about 50% of the specimens, with legs III in the rest of them, irregular, usually branched cuticular depressions are present, tending to lay

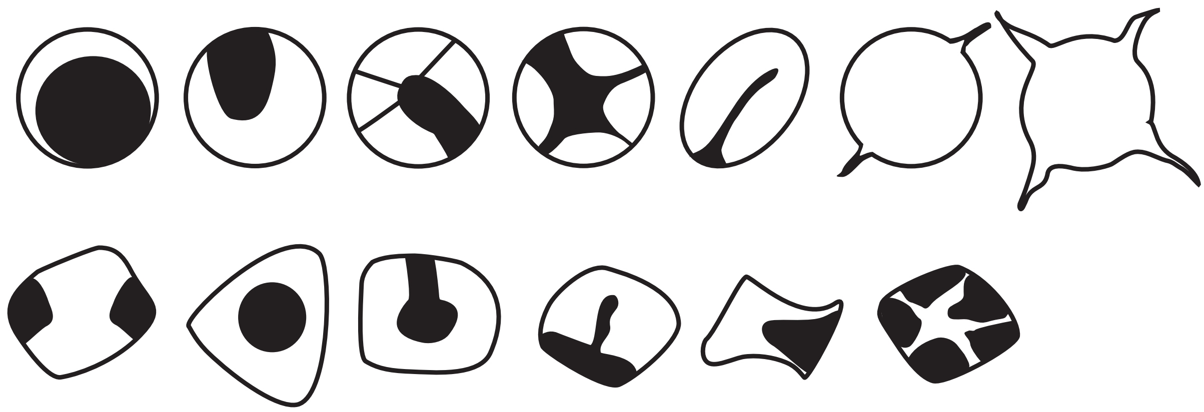

Fig. 2. *Milnesium pelufforum* sp. nov. Schematic drawing of cuticular dimples showing their shapes and different appearances of internal structures.

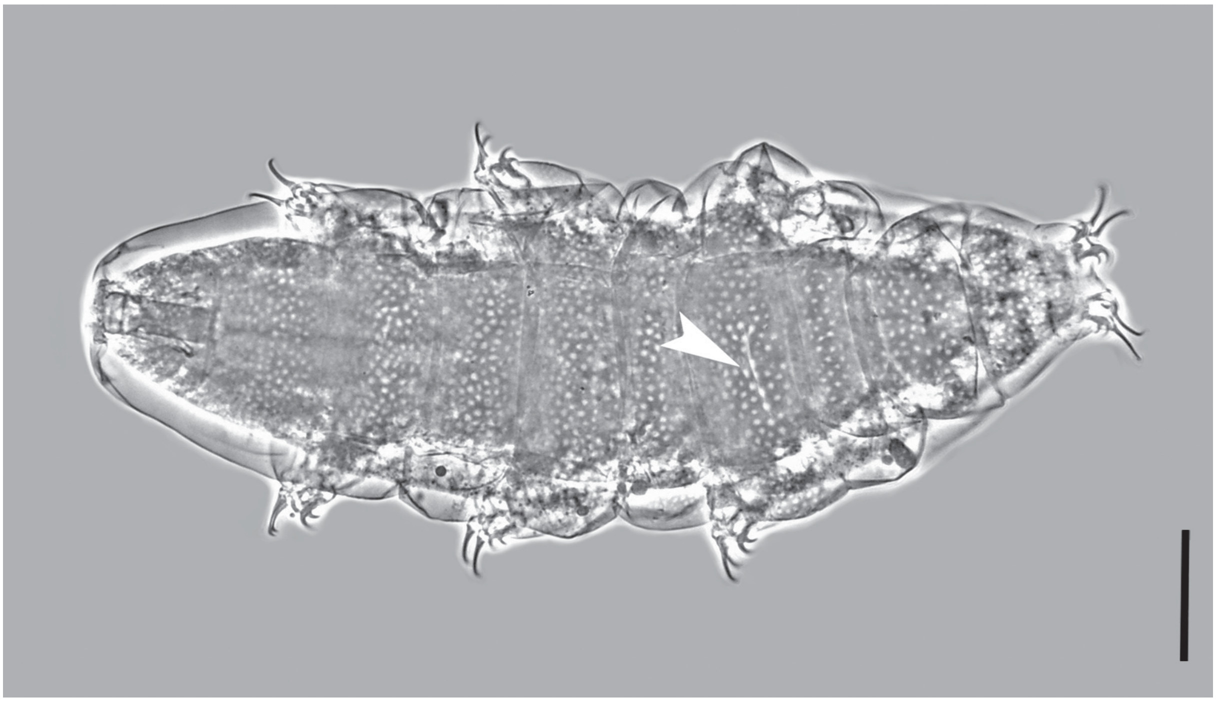

Fig. 3. *Milnesium pelufforum* sp. nov., paratype, young (slide No. 5898 (Pilato and Binda Collection)). Habitus. The arrowhead indicates the dorsal cuticular groove constantly present in young specimens at the level of legs III. Scale bar = 30 µm.

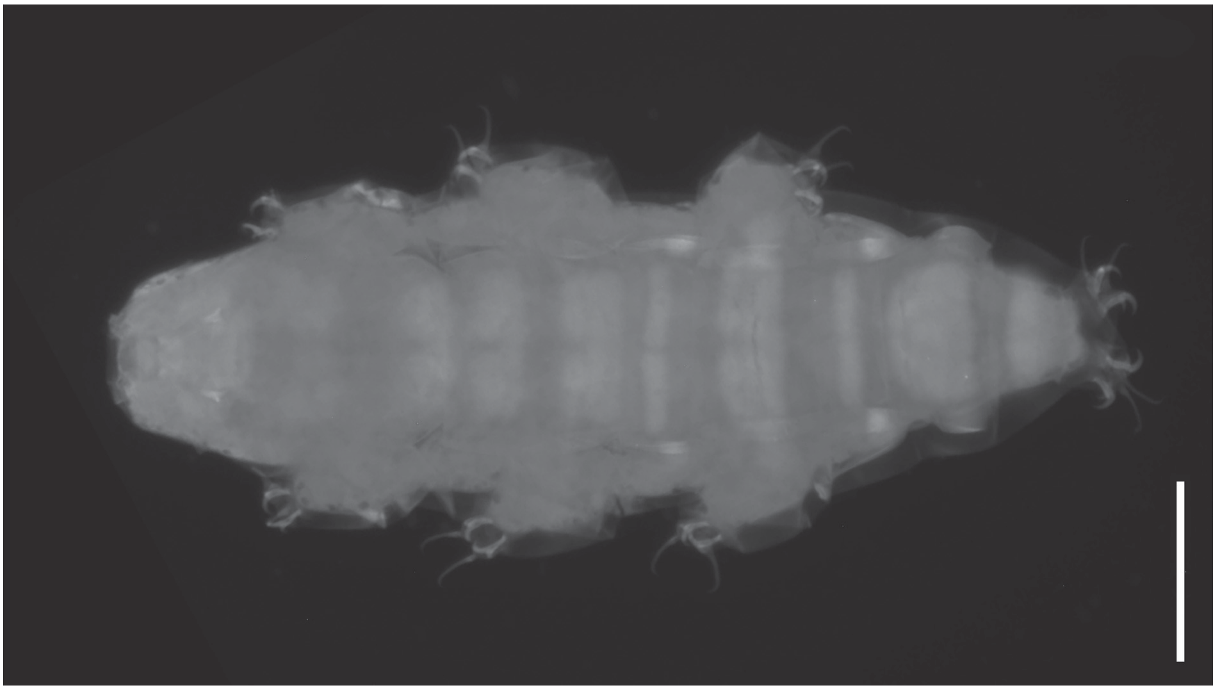

Fig. 4. *Milnesium pelufforum* sp. nov., paratype, young (slide No. UNLPam 389-1) under UV fluorescence microscope, showing various pseudoplates. Scale bar = 50 µm.

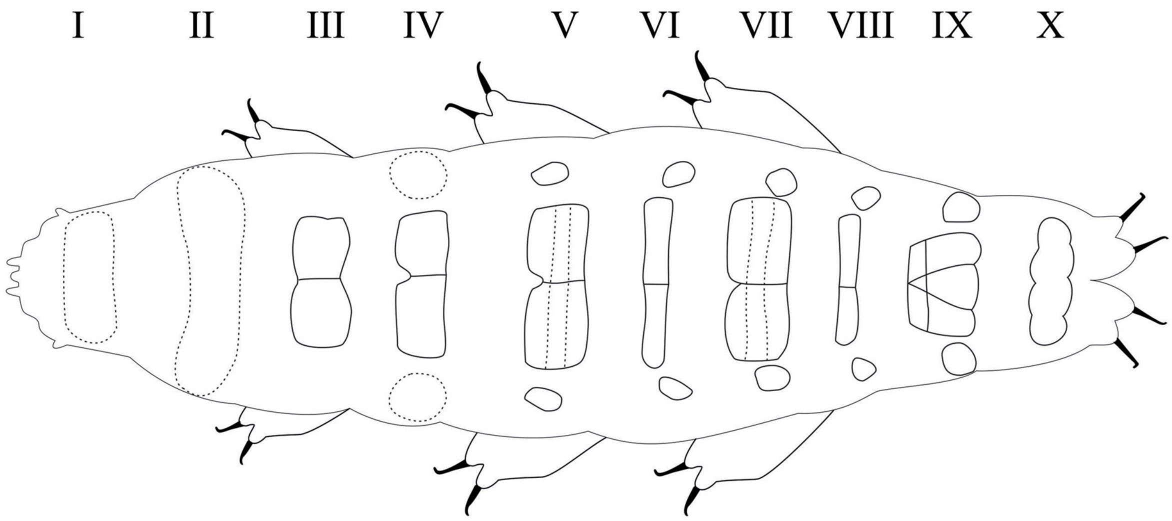

Fig. 5. Semi-schematic drawing of pseudoplate configuration in the young specimens of *Milnesium pelufforum* sp. nov.

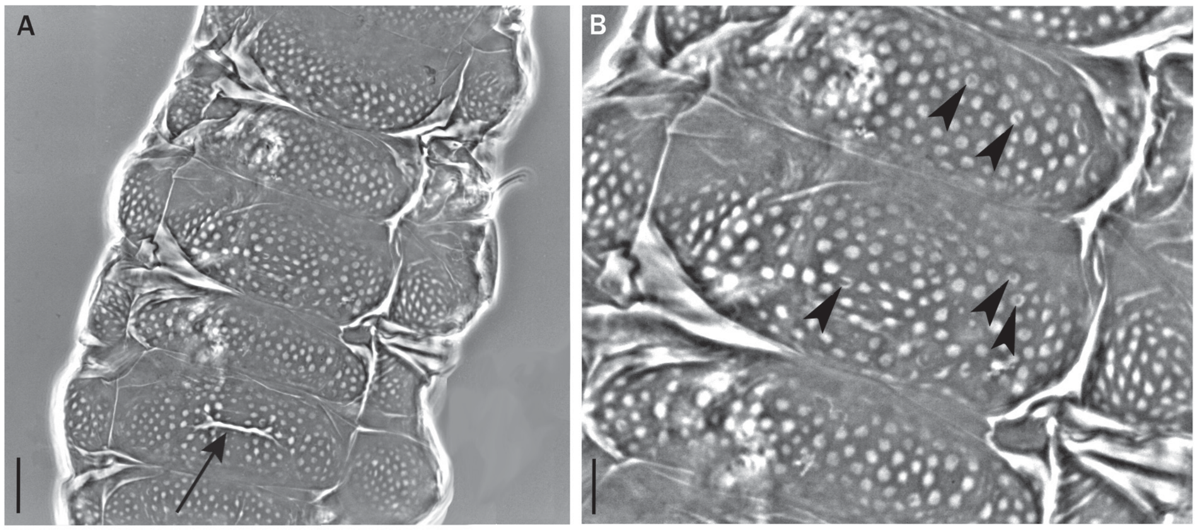

Fig. 6. *Milnesium pelufforum* sp. nov., paratype, young (slide No. 5898 (Pilato and Binda Collection)). Details of the cuticular ornamentation. **A**. General view of the dorsal cuticle showing several bands of sculptured cuticle and the cuticular groove at the level of legs III (arrow). **B**. Magnification of a portion of dorsal cuticle shown in A, better showing the cuticular dimples, some with evident internal structure (arrowheads), and others with more or less out of focus internal structure (the internal of the dimples is not bright, e.g., the dimples in the centre of the photo). Scale bars: A = 20 µm; B = 10 µm.

transversally or obliquely, more conspicuous on the caudal segments and all in general more developed and complex than in young specimens (Figs 1, 8, 11).

Buccal tube (Fig. 12A) stouter than in young specimens (*pt* of standard width [*55.2–64.0*]).

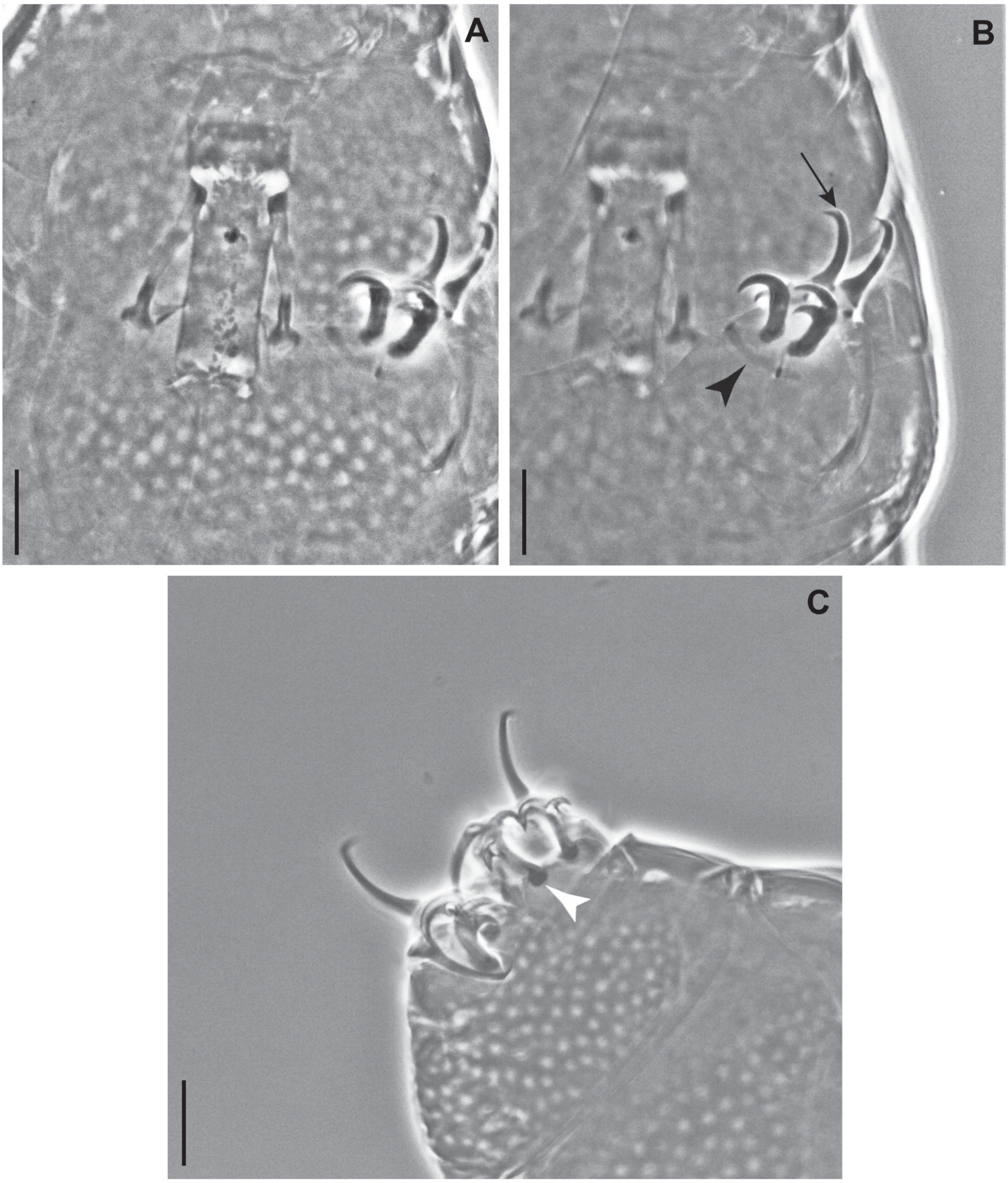

Fig. 7. *Milnesium pelufforum* sp. nov., paratype, young (slide No. UNLPam 503-1). Buccal tube and claws. **A**. Buccal tube and related structures. **B**. Claws of legs I; the black arrow indicates the accessory points, the black arrowhead indicates the leg cuticular bar. **C**. Claws of legs IV; the white arrowhead indicates a 'lunule'. Scale bars = 10 µm.

Claw configuration [2-3]-[3-2] (Fig. 12B–D); if these senior specimens include already the second instar, this would indicate early claw configuration change. Basal spurs of internal secondary branches I–III very small, and those of anterior secondary branches IV reduced to a little spine (Fig. 12B–D, white arrows); secondary branches with basal thickenings ('lunulae') which are larger on legs IV (Fig. 12C–D, white arrowheads); percentual ratio of secondary branches with respect to primary branches for each claw couple with a more marked difference between legs I and legs IV (Table2): 87–99% for claws I, 72–86% for claws IV, difference is 13–15%.

Remarks

It was not possible to examine under SEM the (already mounted) studied material, therefore we considered the bright spots forming the reticular pattern visible under PCM as 'dimples' (or depressions), and not true pseudopores (as defined by Morek *et al*. 2020a) as a consequence of an interpretation, also suggested by one anonymous reviewer. Such structures perfectly correspond in size, spatial distribution, and general appearance, to what can be seen in PCM images of many other species descriptions, in which the structures have been determined using SEM; true pseudopores are, instead, far smaller and usually more scattered and less visible. *Milnesium pelufforum* sp. nov. is the first species of the genus described with cuticular dimples that show some internal structure and form the reticulation arranged in ten transverse bands, as well as pseudoplates. The presence of ten transverse rows/bands of both structures, instead of a maximum of nine (the rule until now in *Milnesium*) is the result of their presence in the new species also on the very first, cephalic, subsegment, where they were not found (or noticed) in the other species until now; this requires an update in the indication of the pseudoplate formula of all species of *Milnesium* with pseudoplates (see Discussion and Table 3). Morphometric data are given in Tables 1 (young specimens) and 2 (senior specimens); in Tables 4 and 5 (for senior and young specimens respectively) the statistically significant differences (through Student *t*-tests) of overlapping *pt* ranges of claw heights between the new species and the similar ones.

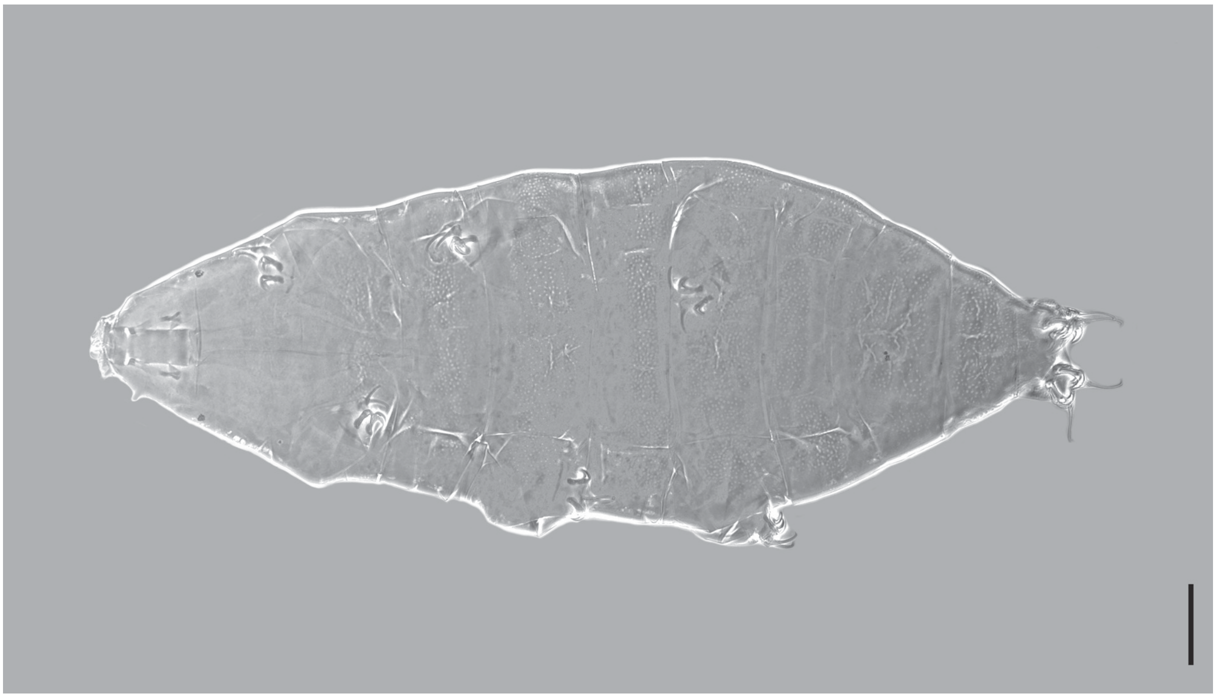

Fig. 8. *Milnesium pelufforum* sp. nov., holotype, ♀ (slide No. MCNS tar. 000021-3). Habitus. Scale bar = 50 µm.

Table 1 (continued on next page). Measurements (in µm) and *pt* values of selected morphological structures of young specimens of *Milnesium pelufforum* sp. nov. mounted in polyvinyl-lactophenol medium. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: N = number of specimens or structures measured; SD = standard deviation.

Character	N	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	12	216 – 305	*1080 – 1615*	259	*1262*	28	*160*
Peribuccal papillae length	2	3.1 – 3.2	*14.7 – 15.1*	3.1	*14.9*	0.1	*0.3*
Lateral papillae length	8	2.9 – 3.3	*14.6 – 15.9*	3.1	*15.1*	0.1	*0.6*
Buccal tube							
Length	12	17.9 – 21.7	–	20.5	–	1.0	–
Stylet support insertion point	12	13.0 – 15.7	*65.3 – 73.8*	14.7	*71.6*	0.7	*2.3*
Anterior width	12	9.6 – 12.1	*46.6 – 55.9*	10.8	*52.6*	0.7	*2.5*
Standard width	12	9.2 – 10.7	*43.7 – 52.1*	9.7	*47.5*	0.5	*2.5*
Posterior width	12	9.1 – 10.2	*45.4 – 53.7*	9.7	*47.5*	0.3	*2.3*
Standard width/length ratio	12	44% – 52%	–	47%	–	3%	–
Posterior/anterior width ratio	12	82% – 99%	–	91%	–	5%	–
Claw 1 lengths							
External primary branch	11	9.5 – 11.2	*45.0 – 53.7*	10.2	*49.9*	0.5	*2.7*
External base + secondary branch	11	7.9 – 9.1	*37.9 – 47.4*	8.4	*41.2*	0.4	*2.4*
External branches length ratio	10	77% – 90%	–	83%		5%	
Internal primary branch	10	9.1 – 10.3	*43.8 – 52.5*	9.8	*48.1*	0.4	*2.9*
Internal base + secondary branch	11	7.3 – 9.1	*35.9 – 44.7*	8.0	*38.9*	0.5	*2.5*
Internal branches length ratio	9	71% – 89%	–	81%		5%	
Claw 2 lengths							
External primary branch	11	9.8 – 11.6	*46.1 – 55.9*	10.6	*51.6*	0.5	*3.2*
External base + secondary branch	12	7.9 – 9.2	*38.2 – 45.6*	8.4	*40.7*	0.4	*2.2*
External branches length ratio	11	74% – 92%	–	80%		5%	
Internal primary branch	11	9.7 – 11.0	*46.0 – 56.5*	10.3	*50.3*	0.4	*3.1*
Internal base + secondary branch	12	7.3 – 9.0	*34.8 – 42.0*	7.9	*38.3*	0.6	*2.2*
Internal branches length ratio	11	69% – 86%	–	77%		5%	
Claw 3 lengths							
External primary branch	11	9.8 – 11.4	*46.4 – 57.0*	10.6	*52.0*	0.5	*3.2*
External base + secondary branch	10	7.5 – 9.4	*37.7 – 45.0*	8.4	*41.1*	0.6	*2.3*
External branches length ratio	10	73% – 85%	–	80%		4%	
Internal primary branch	12	9.9 – 10.7	*47.9 – 57.9*	10.3	*50.4*	0.3	*2.7*
Internal base + secondary branch	9	7.2 – 8.7	*35.7 – 40.6*	7.9	*38.3*	0.6	*2.1*
Internal branches length ratio	9	69% – 82%	–	76%		4%	

Table 1 (continued). Measurements (in µm) and *pt* values of selected morphological structures of young specimens of *Milnesium pelufforum* sp. nov. mounted in polyvinyl-lactophenol medium.

Character	N	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Claw 3 lengths							
Anterior primary branch	9	10.8– 12.4	*50.9 – 60.5*	11.7	*56.2*	0.6	*3.3*
Anterior base + secondary branch	12	7.8– 9.7	*37.5 – 45.8*	8.5	*41.3*	0.6	*2.6*
Anterior branches length ratio	9	66%– 84%	–	75%		5%	
Posterior primary branch	9	11.4– 12.7	*54.2 – 63.0*	12.3	*59.0*	0.4	*2.9*
Posterior base + secondary branch	11	7.9– 10.3	*37.5 – 47.6*	9.1	*43.7*	0.7	*3.0*
Posterior branches length ratio	9	67%– 86%	–	75%		5%	

Differential diagnosis

Based on the presence of dimples on the dorsal cuticle, *Milnesium pelufforum* sp. nov. can be similar to many species of the old *granulatum* group (Michalczyk *et al.* 2012a, 2012b), including both species with true dimples ascertained through SEM, and others in which the value of the bright spots visible under PCM (whether dimples or pseudopores) on the cuticle is still to be verified, but the appearance under PCM can be similar to the cuticular sculpturing of *Milnesium pelufforum*. The new species differs from all of them (indeed, from all congeneric species, according to their descriptions/redescriptions) by the presence of ten transverse bands of sculptured cuticle and rows of pseudoplates, and the presence of dimple internal structures.

We here provide separate differential diagnoses for young and senior specimens of the new species, due to the ontogenetic change, limiting the comparisons to species with the same claw configurations.

Senior specimens of *Milnesium pelufforum* sp. nov. can be similar to those of *M. beasleyi* Kaczmarek, Jakubowska & Michalczyk, 2012, *M. cassandrae* Moreno-Talamantes, Roszkowska, García-Aranda, Flores-Maldonado & Kaczmarek, 2019, *M. krzysztofi* Kaczmarek & Michalczyk, 2007, *M. lagniappe* Meyer, Hinton & Dupré, 2013, *M. reticulatum* Pilato, Binda & Lisi, 2002 and *M. pacificum* Sugiura, Minato, Matsumoto & Suzuki, 2020 by having a more or less similar cuticular ornamentation (seen under PCM) and claw configuration [2-3]-[3-2], but the senior specimens of the new species differ from them as follows:

1. *Milnesium beasleyi* only known from the type locality in Turkey, by different body colour: reddish in *M. pelufforum* sp. nov. vs yellow in *M. beasleyi*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs no band arrangement described in *M. beasleyi*; dimple diameter of about 1–2 µm in *M. pelufforum* vs 0.1–0.4 µm in *M. beasleyi*; presence of pseudoplates in *M. pelufforum* vs not reported in *M. beasleyi*; statistically significant lower *pt* of the lateral papillae, [*13.8–19.9*, mean *16.0*] in *M. pelufforum* vs [*19.6–23.7*, mean *21.5*] in *M. beasleyi* (t_{11} = -10.06, $p < 0.001$); different buccal tube width: higher *pt* of standard width [*55.2–64.0*] in *M. pelufforum* vs [*31.2–39.8*] in *M. beasleyi*; statistically significant differences about *pt* of several claw heights (Table 4).

2. *Milnesium cassandrae* (adults) only known from the terra typica (Mexico; Moreno-Talamantes *et al.* 2019, 2020), by different body colour: reddish in *M. pelufforum* sp. nov. vs white or transparent with light yellow brownish tones before fixation in *M. cassandrae*; sculptured cuticle with dimples

Table 2 (continued on next page). Measurements (in µm) and *pt* values of selected morphological structures of senior specimens of *Milnesium pelufforum* sp. nov. mounted in polyvinyl-lactophenol medium. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: N = number of specimens or structures measured; SD = standard deviation.

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	12	390–620	*1334–2087*	526	*1629*	78	*206*	576	*1624*
Peribuccal papillae length	8	6.1–8.8	*19.5–25.9*	7.5	*22.9*	1.0	*2.6*	8.4	*23.7*
Lateral papillae length	11	4.0–6.0	*13.8–19.9*	5.2	*16.0*	0.7	*1.7*	5.3	*14.8*
Buccal tube									
Length	12	26.1–36.6	–	32.3	–	3.2	–	35.5	–
Stylet support insertion point	12	18.1–23.8	*65.0–69.4*	21.6	*67.0*	1.8	*1.9*	23.4	*66.1*
Anterior width	12	16.0–23.2	*58.1–66.3*	20.1	*62.0*	2.3	*2.9*	22.5	*63.3*
Standard width	12	15.2–22.8	*55.2–64.0*	19.1	*59.0*	2.2	*3.3*	20.6	*58.2*
Posterior width	12	15.4–22.8	*56.0–65.0*	19.6	*60.5*	2.3	*3.6*	22.2	*62.5*
Standard width/length ratio	12	55%–64%	–	59%	–	3%	–	58%	–
Posterior/anterior width ratio	12	91%–100%	–	97%	–	2%	–	99%	–
Claw 1 lengths									
External primary branch	11	12.1–15.8	*41.8–48.9*	14.5	*45.1*	1.4	*2.7*	15.4	*43.3*
External base + secondary branch	10	11.0–15.7	*37.4–46.2*	13.5	*42.0*	1.5	*3.5*	13.5	*38.2*
External branches length ratio	9	87%–98%	–	92%		3%		88%	
Internal primary branch	9	11.6–15.0	*39.1–46.8*	13.6	*42.9*	1.3	*3.1*	?	*?*
Internal base + secondary branch	11	11.0–15.3	*36.0–43.0*	12.8	*39.8*	1.4	*2.8*	13.2	*37.1*
Internal spur	7	2.6–4.0	*7.6–12.8*	3.4	*10.1*	0.5	*1.9*	2.8	*7.9*
Internal branches length ratio	8	90%–99%	–	94%		3%		–	
Claw 2 lengths									
External primary branch	10	12.2–18.0	*46.7–55.2*	16.1	*50.0*	1.9	*3.5*	17.3	*48.8*
External base + secondary branch	12	10.9–16.2	*37.7–47.6*	14.1	*43.7*	1.6	*3.2*	14.7	*41.5*
External branches length ratio	10	81%–95%	–	88%		4%		85%	
Internal primary branch	9	12.1–17.3	*43.6–52.2*	15.0	*47.9*	1.7	*3.3*	16.4	*46.4*
Internal base + secondary branch	9	11.4–15.3	*37.6–45.0*	13.7	*41.4*	1.2	*3.0*	15.1	*42.6*
Internal spur	7	2.8–4.9	*10.7–15.9*	4.0	*12.6*	0.6	*1.9*	?	*?*
Internal branches length ratio	7	82%–92%	–	86%		3%		92%	
Claw 3 lengths									
External primary branch	9	12.4–18.4	*46.6–56.0*	15.8	*50.0*	2.0	*3.8*	16.9	*47.7*
External base + secondary branch	8	11.4–14.9	*38.6–47.8*	13.4	*43.0*	1.3	*2.8*	14.9	*42.0*
External branches length ratio	8	79%–92%	–	86%		4%		88%	
Internal primary branch	9	11.7–16.8	*42.8–52.8*	14.8	*46.6*	1.8	*3.9*	15.4	*43.4*
Internal base + secondary branch	8	11.9–14.5	*35.3–44.0*	13.0	*39.7*	0.8	*3.3*	13.1	*36.9*
Internal spur	8	3.0–5.0	*10.4–16.2*	3.9	*12.4*	0.7	*2.0*	4.0	*11.3*
Internal branches length ratio	7	80%–86%	–	83%		2%		85%	

Table 2 (continued). Measurements (in µm) and *pt* values of selected morphological structures of senior specimens of *Milnesium pelufforum* sp. nov. mounted in polyvinyl-lactophenol medium.

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Claw 4 lengths									
Anterior primary branch	11	16.3–22.8	*58.1 – 67.3*	19.8	*61.6*	2.0	*3.2*	22.8	*64.3*
Anterior base + secondary branch	11	11.8–18.0	*42.4 – 52.4*	15.0	*46.7*	1.9	*3.2*	16.3	*46.0*
Anterior spur	8	2.0–3.9	*6.1 – 13.3*	2.7	*8.3*	0.8	*2.7*	2.2	*6.2*
Anterior branches length ratio	11	72%–86%	–	76%		4%		72%	
Posterior primary branch	10	17.7–23.0	*61.1 – 70.0*	20.8	*64.7*	2.0	*3.5*	21.9	*61.8*
Posterior base + secondary branch	12	12.8–18.1	*44.2 – 53.4*	15.9	*49.2*	1.8	*3.2*	17.5	*49.3*
Posterior branches length ratio	10	72%–80%		75%		3%		80%	

arranged in ten bands in *M. pelufforum* vs sparsely distributed, not forming bands or reticular design, in *M. cassandrae*; presence of ten pseudoplate rows in *M. pelufforum* vs nine rows in *M. cassandrae*, and with different number in each row (Table 3); statistically significant larger dimple diameter, 1–2 µm in *M. pelufforum* vs 0.6–1.4 µm in *M.cassandrae* (t_{13} = -18.02, $p < 0.001$); statistically significant higher *pt* of stylet support insertion point, [*65.0–69.4*, mean *67.0*] in *M.pelufforum* vs [58.7–67.6, mean *63.5*] in *M. cassandrae* (t_{12} = 3.33, $p < 0.01$); statistically significant higher buccal tube posterior/anterior width ratio, 91–100%, mean 97% in *M. pelufforum* vs 81–96%, mean 89% in *M. cassandrae* (t_{12} = 4.39, $p < 0.001$); statistically significant differences about *pt* of some claw heights (Table 4).

3. *Milnesium krzysztofi* known from Costa Rica (type locality), Perú (Kaczmarek *et al*. 2014) and Colombia (Lisi *et al.* 2014; Londoño *et al.* 2015; Melo *et al.* 2015), by different body colour: reddish in *M. pelufforum* sp. nov. vs white or transparent in *M. krzysztofi*; eyes present in *M. pelufforum* vs absent in *M. krzysztofi*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs not forming bands in *M. krysztofi*; different number of pseudoplates, not reported in *M. krzysztofi*, but visible in Kaczmarek & Michalczyk (2007: figs 2–7) in were it is possible to see at least six rows with different number/arrangement of pseudoplates; buccal tube nearly cylindrical in *M. pelufforum* vs funnel-shaped in *M.krzysztofi*: anterior and posterior width of the buccal tube of this species not available from description but the difference is visually evident comparing Fig. 12A of the present paper with Kaczmarek & Michalczyk (2007: fig. 12); different buccal tube width: higher *pt* of standard width [*55.2–64.0*] in *M. pelufforum* vs [*33.1–38.4*], in *M.krzysztofi*; statistically significant differences about *pt* of several claw heights (Table 4).

4. *Milnesium lagniappe* only known from the type locality in USA, by different body colour: reddish in *M. pelufforum* sp. nov. vs white or transparent in *M. lagniappe*; eyes visible in *M. pelufforum* vs not visible in *M. lagniappe*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs arranged in nine bands in *M. lagniappe*; different number/arrangement of pseudoplates, not reported in *M. lagniappe*, but visible in Meyer *et al.* (2013: fig. 1a-b) where it is possible to see at least seven rows different from the correspondent 10 rows of *M. pelufforum*; statistically significant larger dimple diameter, 1–2 µm in *M. pelufforum* vs 0.7–1.3 µm in *M. lagniappe* (t_{13} = 9,01, $p < 0.001$); statistically significant lower *pt* of the peribuccal papillae, [*19.5–25.9*, mean *22.9*] in *M. pelufforum* vs [*22.7–34.7*, mean *28.0*] in *M. lagniappe* (t_8 = 4.32, $p < 0.001$); statistically significant lower *pt* of the lateral papillae: [*13.8–19.9*, mean *16.0*] in *M. pelufforum* vs [*16.9–30.5*, mean *23.2*] in *M. lagniappe* (t_{11} = 3.79, $p < 0.001$); lower *pt* of stylet support insertion point [*65.0–69.4*] in *M. pelufforum* vs [*69.7–73.4*] in

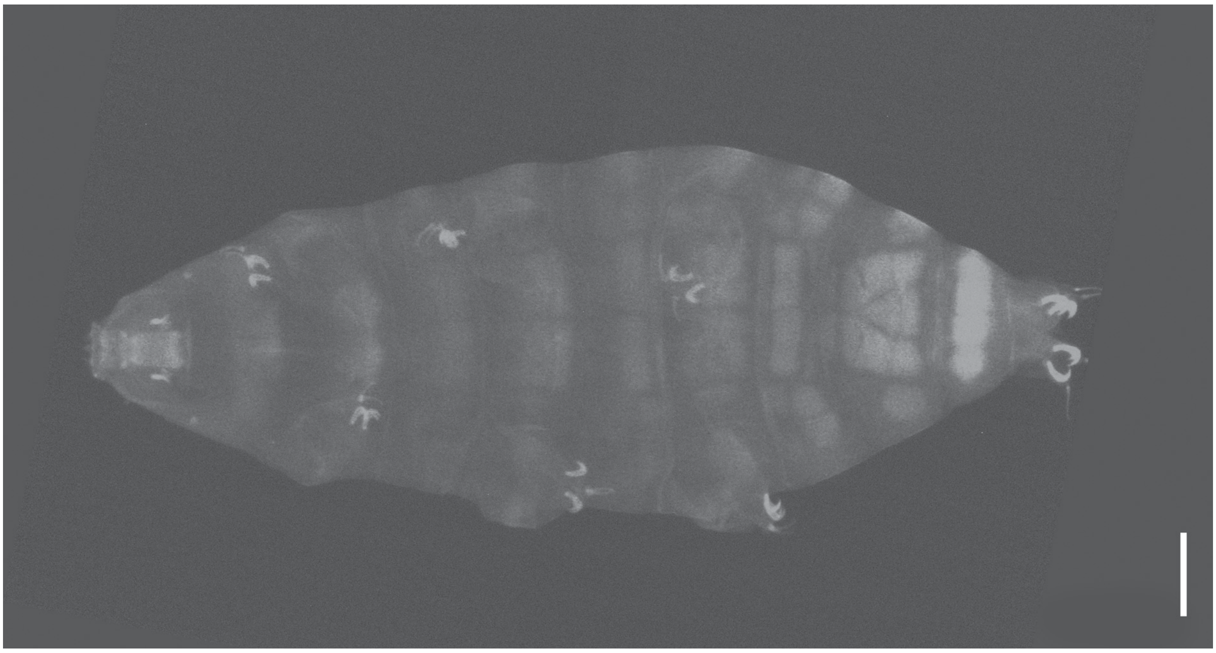

Fig. 9. *Milnesium pelufforum* sp. nov., holotype, ♀ (slide No. MCNS tar. 000021-3), under UV fluorescence microscope, showing various pseudoplates. Scale bar = 50 µm.

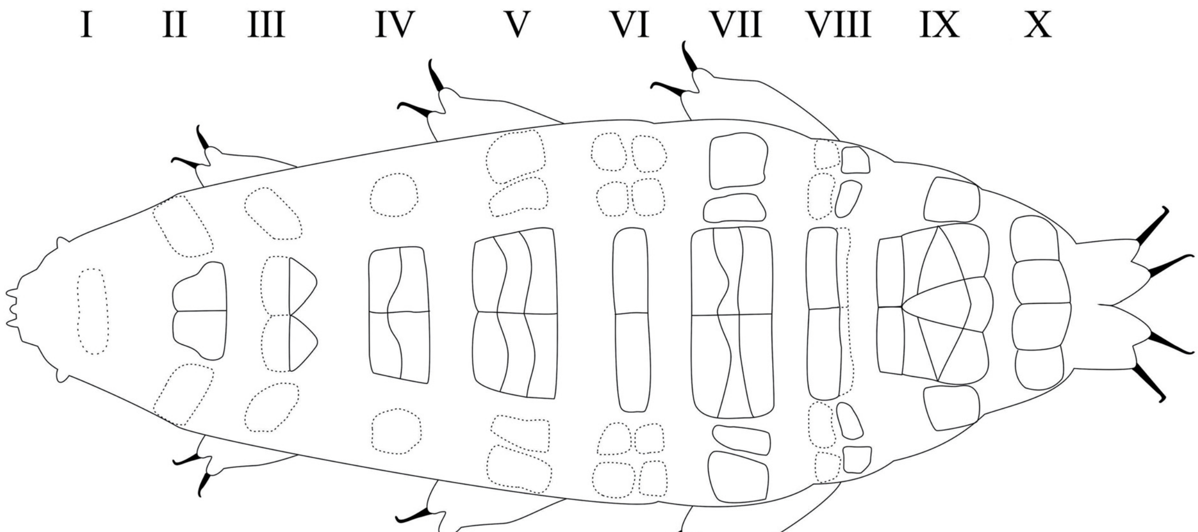

Fig. 10. Semi-schematic drawing of pseudoplate configuration in the senior specimens of *Milnesium pelufforum* sp. nov.

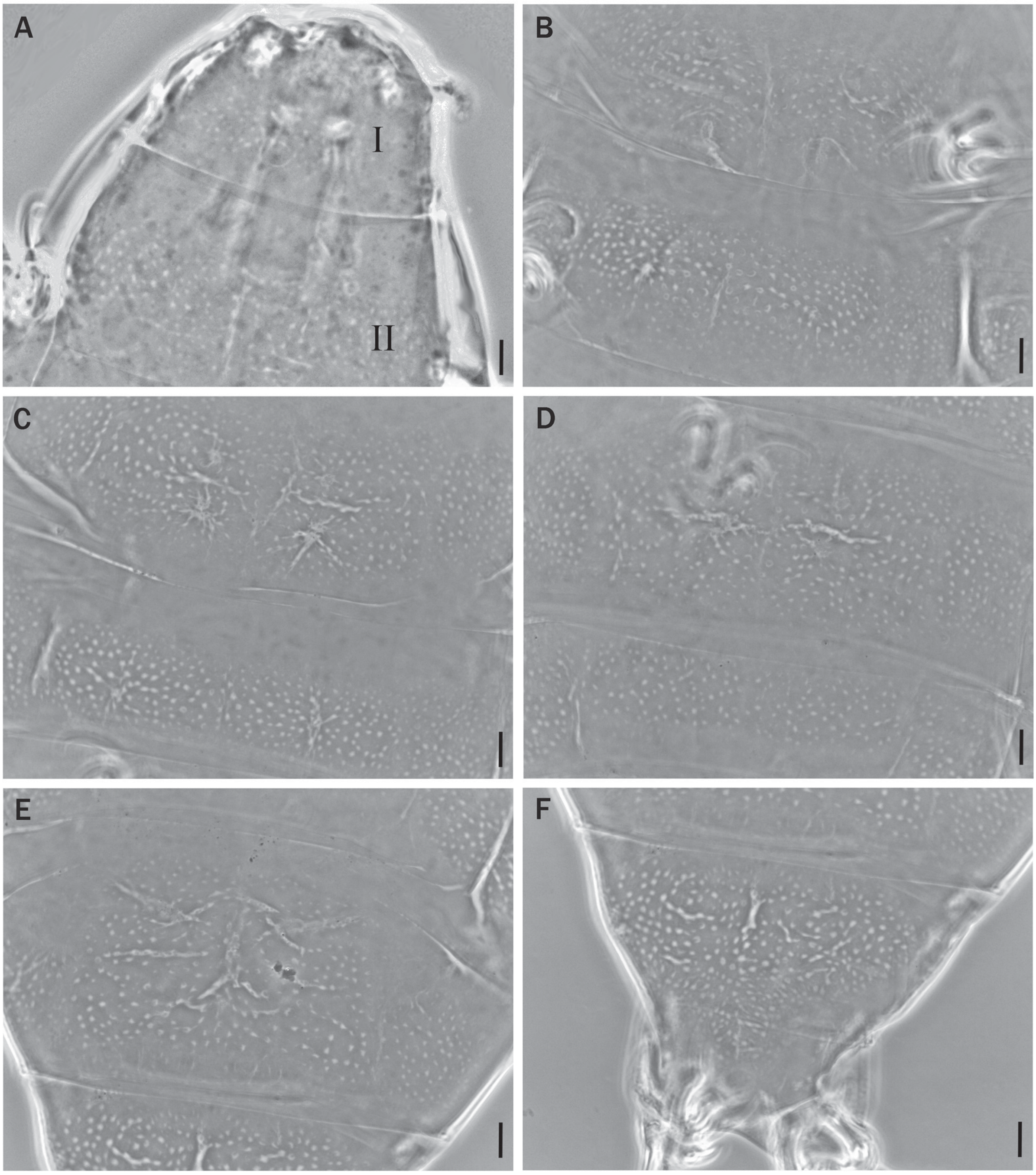

Fig. 11. *Milnesium pelufforum* sp. nov., holotype, ♀ (slide No. MCNS tar. 000021-3). Overview of the ten bands of cuticular dimples from the head (above) to the caudal end of the body (below) **A**. First and second bands (roman numbers). **B**. Third and fourth bands (the fourth also shows in the centre several dimples with internal structures). **C**. Fifth and sixth bands. **D**. Seventh and eighth bands (the seventh also shows in the centre some dimples with internal structures). **E**. Ninth band. **F**. Tenth band. In all pictures (A–F) cuticular grooves are also visible. Scale bars = 10 μm.

M. lagniappe; different buccal tube width: lower *pt* of standard width [*55.2–64.0*] in *M. pelufforum* vs [*63.4–77.9*] in *M. lagniappe*; statistically significant differences about *pt* of several claw heights (Table 4).

5. *Milnesium reticulatum* only known from the type locality in the Seychelles islands, by different body colour: reddish in *M. pelufforum* sp. nov. vs transparent in *M. reticulatum*; different number of peribuccal lamellae: six in *M. pelufforum* vs four in *M. reticulatum*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs nine in *M. reticulatum*; presence of pseudoplates in *M. pelufforum* vs not reported in *M. reticulatum*; higher *pt* buccal tube standard width, [*55.2–64.0*] in *M. pelufforum* vs [*30.4–37.4*] in *M. reticulatum*; higher *pt* of many claw lengths: external primary and secondary branches of leg II, [*46.7–55.2*] and [*37.7–47.6*] in *M. pelufforum* vs [*35.6–38.8*] and [*26.8–29.6*] respectively in *M. reticulatum*; internal primary and secondary branches II [*43.6–52.2*] and [*37.6–45.0*] in *M. pelufforum* vs [*33.2–36.6*] and [*26.0–27.1*] respectively in *M. reticulatum*; external primary and secondary branches III [*46.6–56.0*] and [*38.6–47.8*] in *M. pelufforum* vs [*35.6*] and [*26.8–

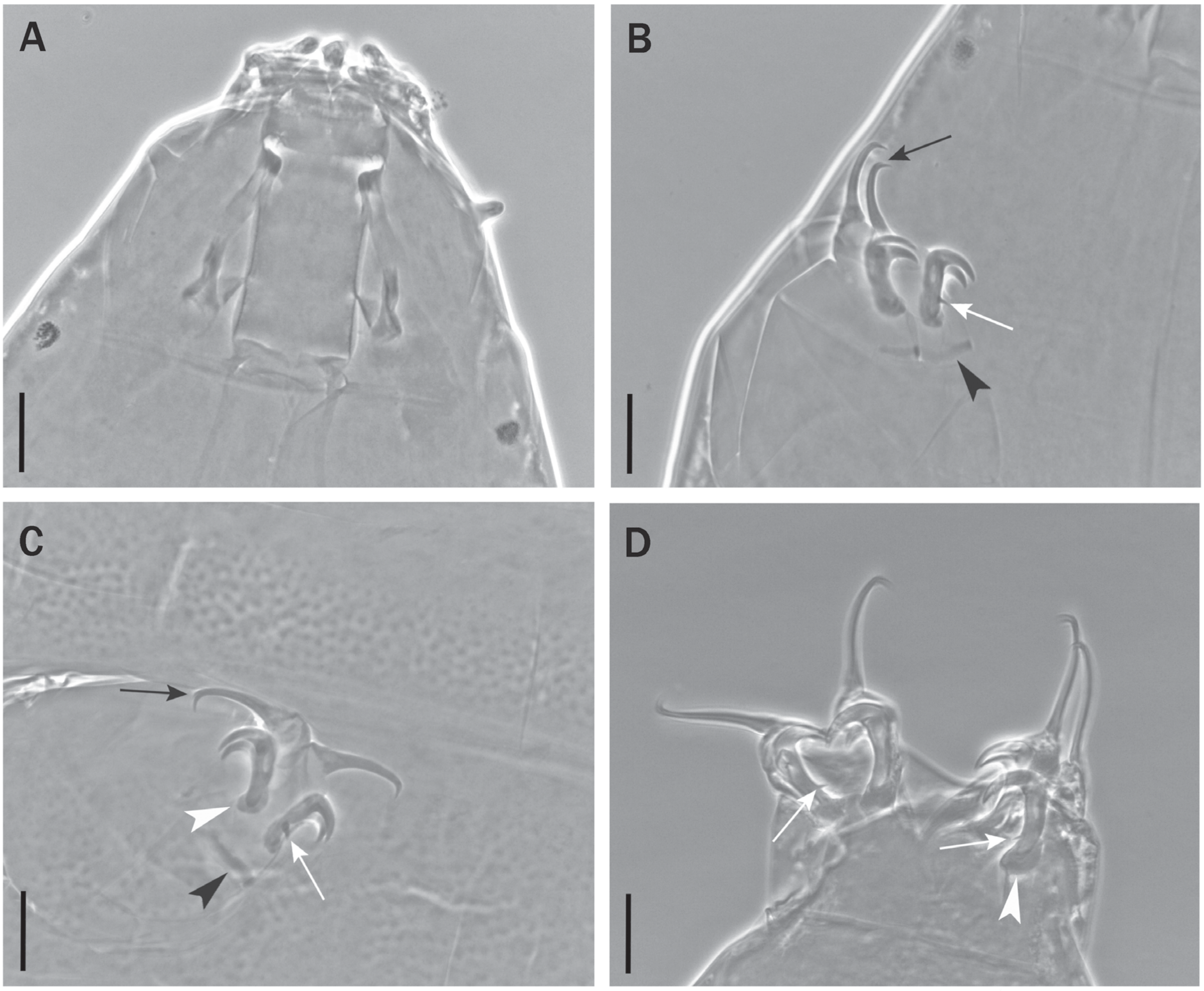

Fig. 12. *Milnesium pelufforum* sp. nov., holotype, ♀ (slide No. MCNS tar. 000021-3). Cephalic region and claws. **A**. Cephalic region with the buccal tube and related structures in focus. **B**. Claws of legs I. **C**. Claws of legs III. **D**. Claws of legs IV. In B–D the black arrows indicate the accessory points, the white arrows indicate the basal spurs, the white arrowheads indicate the 'lunulae', the black arrowheads indicate the leg cuticular bars. Scale bars = 10 µm.

29.6] in respectively *M. reticulatum*; internal primary and secondary branches of leg III [*42.8–52.8*] and [*35.3–44.0*] in *M. pelufforum* vs [*33.2*] and [*26.0–27.1*] respectively in *M. reticulatum*; anterior primary and secondary branch IV [*58.1–67.3*] and [*42.4–52.4*] in *M. pelufforum* vs [*37.9–39.7*] and [*29.2–33.0*] respectively in *M. reticulatum*; posterior primary and secondary branches branch IV [*61.1–70.0*] and [*44.2–53.4*] in *M. pelufforum* vs [*41.7–44.3*] and [*29.2–35.0*] respectively in *M. reticulatum*.

6. *Milnesium pacificum* only known from the terra typica in Japan, by different body colour: reddish in *M. pelufforum* sp. nov. vs creamy withe, transparent or with three brownish longitudinal stripes in *M. pacificum*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs no band arrangement in *M. pacificum*; dimple diameter about 1–2 µm in *M. pelufforum* vs about 0.50–0.65 µm in *M. pacificum*; presence of ten rows of pseudoplates in *M. pelufforum* vs nine rows in *M. pacificum*; statistically significant higher *pt* of the peribuccal papillae [*19.5–25.9*, mean *22.9*] in *M. pelufforum* vs [*16.3–22.4*, mean *18.7*] in *M. pacificum* (t_8 = 5.09, p < 0.001); different buccal tube width: higher *pt* of standard width [*55.2–64.0*] in *M. pelufforum* vs [*33.0–40.8*] in *M. pacificum*; statistically significant higher *pt* of stylet support insertion point [*65.0–69.4*, mean *67.0*] in *M. pelufforum* vs [*57.1–67.8*, mean *62.2*] in *M. pacificum* (t_{12} = 4.54, p < 0.001); statistically significant differences about *pt* of most claw heights (Table 4).

Young specimens of *Milnesium pelufforum* sp. nov. can be similar to four species, *M. cassandrae* Moreno-Talamantes, Roszkowska, García-Aranda, Flores-Maldonado & Kaczmarek, 2019, *M. katarzynae* Kaczmarek, Michalczyk & Beasley, 2004, *M. pacificum* Sugiura, Minato, Matsumoto & Suzuki, 2020 and *M. variefidum* Morek, Gąsiorek, Stec, Blagden & Michalczyk, 2016, due to a more or less similar cuticular ornamentation and claw configuration [2-2]-[2-2]. Young specimens of *M. pelufforum* differ from them as follows:

1. *Milnesium cassandrae* (hatchlings and youngs) only known from the terra typica (Mexico; Moreno-Talamantes *et al.* 2019, 2020), by different body colour: reddish in *M. pelufforum* nov. sp. vs white or transparent with light yellow brownish tones before fixation in *M. cassandrae*; presence of ten rows of pseudoplates, better developed, in *M. pelufforum* vs nine rows poorly developed in *M. cassandrae*; eyes present in *M. pelufforum* vs absent in hatchling and youngs of *M. cassandrae*; different buccal tube width: higher *pt* of standard width [*43.7–52.1*] in *M. pelufforum* vs [*28.2–39.4*] in *M. cassandrae*; statistically significant higher *pt* of stylet support insertion point [*65.3–73.8*, mean *71.6*] in *M. pelufforum* vs [*56.5–70.5*, mean *66.3*] in *M. cassandrae* (t_{12} = 4.38, p < 0.005); statistically significant differences about *pt* of almost all claw heights (Table 5).

2. *Milnesium katarzynae* known from China, Sichuan Province (type locality), Costa Rica (Kaczmarek *et al.* 2014) and Colombia (Caicedo *et al*. 2014; Londoño *et al.* 2015; Melo *et al.* 2015), by different body colour: reddish in *M. pelufforum* sp. nov. vs white in *M. katarzynae*; eyes present in *M. pelufforum* vs absent of *M. katarzynae*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs no band arrangement in *M. katarzynae*; dimple diameter of about 2–3.5 µm in young specimens of *M. pelufforum* vs about 0.5–1.5 µm in *M. katarzynae*; pseudoplates present in *M. pelufforum* vs not reported in *M. katarzynae*; different buccal tube width: higher *pt* of standard width [*43.7–52.1*] in *M. pelufforum* vs [*21.7– 26.6*] in *M. katarzynae*; statistically significant higher *pt* of stylet support insertion point [*65.3–73.8*, mean *71.6*] in *M. pelufforum* vs [*73.3–78.3*, mean *75.8*] in *M. katarzynae* (t_{12} = -6.13, p < 0.001); different *pt* of external primary and secondary branches I, [*41.8–48.9*] and [*37.4–46.2*] in *M. pelufforum* vs [*40.0–40.7*] and [*26.6–26.7*] respectively in *M. katarzynae*; external primary and secondary branches II [*46.7–55.2*] and [*37.7–47.6*] vs [*40.0–40.7*] and [*26.7–28.3*] respectively in *M. katarzynae*; external primary and secondary branches III [*46.6–56.0*] and [*38.6–47.8*] vs [*40.7–41.8*] and [*28.3*] respectively in *M. katarzynae*, anterior primary and secondary branches IV [*58.1–67.3*] and [*42.4–52.4*] vs [*43.5–43.8*] and [*26.7–28.3*] respectively in *M. katarzynae.*

Table 3 (continued on next page). Information about pseudoplates for all species that possess them (including some new reports). The correspondence of the new row indication (present paper) and the previous by Moreno-Talamantes *et al.* (2019) is furnished. "-" indicates no pseudoplate reported for that row; "?" indicates unclear state.

Level along the body	Anterior		Leg I	Between I–II	Leg II	Between II–III	Leg III	Behind leg III	Posterior		Reference
Rows (present paper)	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	
Rows (Moreno-Talamantes *et al.* 2019)		(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)	
M. pelufforum sp. nov.	1	4	6	6	10	10	10	12	12	4	Present paper: formula Figs 5, 10 (schematic drawings) Figs 4, 9 (UV fluorescence)
M. irenae sp. nov.	–	4	4	8	10	8	10	8	12	4	Present paper: formula Fig. 15 (schematic drawing) Fig. 14 (UV fluorescence)
M almatyense Morek *et al.* 2020b	–	?	?	4	4	2	4	4	10	1	Morek *et al.* 2020b: Fig. 2a (PCM) Fig. 2b (schematic drawing) Fig. 3a–d, e (SEM)
M. berladnicorum Morek *et al.* 2016	–	1?	1?	2?	2	2	6	4	8	2	Morek *et al.* 2016: Fig. 6a (schematic drawing) Description
M. cassandrae Moreno-Talamantes *et al.* 2019	–	4	2	4	10	6	10	8	10	2	Moreno-Talamantes *et al.* 2019: formula Fig. 1a (PCM) Fig. 3 (UV fluorescence) Fig. 4 (schematic drawing)_
M eurystomun Morek *et al.* 2020a	–	2	2	2	4	2	4	4	10	4	Morek *et al.* 2020a: Fig. 8 (PCM) Fig. 9 (schematic drawing) Description
M variefidum Morek *et al.* 2016	–	–	–	2	6	2	6	4	10	2	Morek *et al.* 2016: Fig. 2a (PCM) Fig. 2b–c (SEM) Fig. 2d (schematic drawing) Description

Table 3 (continued). Information about pseudoplates for all species that possess them (including some new reports). The correspondence of the new row indication (present paper) and the previous by Moreno-Talamantes *et al.* (2019) is furnished. "-" indicates no pseudoplate reported for that row; "?" indicates unclear state.

Level along the body	Anterior	Leg I	Between I–II	Leg II	Between II–III	Leg III	Behind leg III	Posterior	Reference
M. beatae Tibbs *et al.* 2016	Pseudoplates reported but without formula/drawing								Tibbs *et al.* 2016: Fig. 1b–d (UV fluorescence)
M. pacificum Sugiura *et al.* 2020	Pseudoplates reported but without formula/drawing								Sugiura *et al.* 2020: Fig. 6a–b (PCM) Fig. 6a'–b'(UV fluorescence)
M. alpigenum Morek *et al.* 2019b	Pseudoplates reported but without formula/drawing								Morek *et al.* 2019b: Fig. 1b (SEM), 1D (PCM)
M. inceptum Morek *et al.* 2019b	Pseudoplates reported but without formula/drawing								Morek *et al.* 2019b: Fig. 2d (PCM), 2E (SEM)
M. krysztofi Kaczmarek & Michalczyk 2007	Pseudoplates not reported but visible in figures								Kaczmarek & Michalczyk 2007: Figs 2–7 (SEM, PCM)
M. reductum Morek *et al.* 2020b	Pseudoplates reported but without formula/drawing								Morek *et al.* 2020b: Only row IX– Fig. 4d (SEM)
M. dornensis Ciobanu *et al*. 2015	Pseudoplates reported but without formula/drawing								Ciobanu *et al.* 2015: Only row IX – Fig. 4c, e (PCM, SEM)
M. lagniappe Meyer *et al.* 2013	Pseudoplates not reported but visible in one figure								Meyer *et al.* 2013: Fig. 1a (PCM)

Table 4 (continued on next page). Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of claw heights between senior specimens of *Milnesium pelufforum* sp. nov. and the similar species.

Species Character	***M. pelufforum* sp. nov.**	***M. beasleayi***	***M. cassandrae***	***M. krzysztofi***	***M. lagniappe***	***M. pacificum***
Claw/Leg I						
External primary branch	*41.8–48.9 (45.1)*				47.2–59.7 (53.3) t_{11} = -4.45, $p < 0.001$	
External secondary branch	37.4–46.2 (42.0)	33.2–38.7 (35.7) t_{10} = 5.20, $p < 0.001$				
Internal primary branch	39.1–46.8 (42.9)	39.2–44.0 (41.9) t_9 = 2.59, $p < 0.05$			46.3–56.3 (50.5) t_9 = -4.68, $p < 0.001$	34.4–48.1 (40.4) t_9 = 2.13, $p < 0.05$
Internal secondary branch	36.0–43.0 (39.8)			34.2–36.4 (35.5) t_{11} = 4.41, $p < 0.001$		
Claw/Leg II						
External primary branch	46.7–55.2 (50.0)				51.7–63.5 (57.9) t_{10} = -3.92, $p < 0.001$	36.8–52.7 (46.9) t_{10} = 2.10, $p < 0.05$
External secondary branch	37.7–47.6 (43.7)	32.1–39.8 (36.8) t_{12} = 5.25, $p < 0.001$				35.5–43.6 (39.5) t_{12} = 2.05, $p < 0.05$
Internal primary branch	43.6–52.2 (47.9)		37.8–55.6 (44.0) t_9 = 2.10, $p < 0.05$	37.4–47.2 (43.7) t_9 = 2.73, $p < 0.05$	48.8–62.1 (54.3) t_9 = -4.87, $p < 0.001$	
Internal secondary branch	37.6–45.0 (41.4)			34.3–38.0 (35.9) t_7 = 4.75, $p < 0.001$		32.4–42.1 (36.8) t_7 = 5.56, $p < 0.001$
Claw/Leg III						
External primary branch	46.6–56.0 (50.0)				49.7–63.0 (56.1) t_9 = -4.69, $p < 0.001$	

Table 4 (continued). Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of claw heights between senior specimens of *Milnesium pelufforum* sp. nov. and the similar species.

Species Character	***M. pelufforum*** sp. nov.	***M. beasleayi***	***M. cassandrae***	***M. krzysztofi***	***M. lagniappe***	***M. pacificum***
External secondary branch	38.6–47.8 (43.0)	33.8–39.7 (37.4) t_8 = 6.47, $p < 0.001$				33.2–42.3 (38.6) t_8 = 2.40, $p < 0.05$
Internal primary branch	42.8–52.8 (46.6)	45.5–51.5 (48.0) t_9=13.59, $p < 0.001$			50.0–60.1 (55.2) t_9 = -8.23, $p < 0.001$	
Internal secondary branch	35.3–44.0 (39.7)	32.7–36.9 (35.2) t_8 = 5.11, $p < 0.001$		31.9–35.8 (43.3) t_8 = 4.96, $p < 0.001$	39.1–48.3 (42.6) t_8 = -2.71, $p < 0.01$	37.6–51.3 (43.0) t_8 = –2.80, $p < 0.01$
Claw/Leg IV						
Anterior primary branch	58.1–67.3 (61.6)				62.9–74.0 (69.0) t_{11}=5.20, $p < 0.001$	41.6–60.3 (50.6) t_{11} = 4.43, $p < 0.001$
Anterior secondary branch	42.4–52.4 (46.7)		34.8–51.8 (41.3) t_{11} = 2.11, $p < 0.05$			32.8–47.1 (42.1) t_{11} = 3.29, $p < 0.01$
Posterior primary branch	61.1–70.0 (64.7)	59.0–64.2 (60.8) t_{10} = 3.33, $p < 0.01$			66.1–76.6 (70.9) t_{10} = -5.19, $p < 0.001$	41.1–61.1 (53.1) t_{10} = 4.37, $p < 0.001$
Posterior secondary branch	44.2–53.4 (49.2)	39.7–46.7 (42.8) t_{12} = 4.99 $p < 0.001$	37.2–53.2 (43.6) t_{12} = 2.45, $p < 0.05$	39.0–43.7–(40.7) t_{12} = 6.74, $p < 0.001$		

Table 5. Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of claw heights between young specimens of *Milnesium pelufforum* sp. nov. and the similar species.

Species / Character	***M. pelufforum*** sp. nov.	***M. cassandrae***	***M. pacificum***	***M. variefidum***
Claw/Leg I				
External primary branch	*45.0–53.7* *(49.9)*	*35.1–52.8* (*42.9*) $T_9=3.44, p<0.01$		
External secondary branch	*37.9–47.4* (*41.2*)	*30.1–39.7* (*35.1*) $T_{10}=4.8, p<0.001$		
Internal primary branch	*43.8–52.5* (*48.1*)	35.8–48.4 (42.1) $T_9=3.91, p<0.005$		32.8–45.1 (38.9) $T_{10}=4.69, p<0.001$
Internal secondary branch	*35.9–44.7* (*38.9*)	30.2–39.6 (34.3) $T_{10}=3.81, p<0.005$	*23.7–37.4* (*32.5*) $T_{10}=3.48, p<0.005$	
Claw/Leg II				
External primary branch	*46.1–55.9* (*51.6*)		*29.6–52.1* (*41.4*) $T_{10}=3.45, p<0.005$	38.0–50.9 (43.7) $T_{10}=4.25$ $p<0.001$
External secondary branch	*38.2–45.6* (*40.7*)	*30.9–40.9* (*36.3*) $T_{11}=3.94, p<0.001$		27.6–39.7 (33.8) $T_{11}=4.65, p<0.001$
Internal primary branch	*46.0–56.5* (*50.3*)	*37.4–53.5* (*43.3*) $T_{10}=3.14, p<0.005$		
Internal secondary branch	*34.8–42.0* (*38.3*)	29.9–40.9 (34.7) $T_{11}=1.91, p<0.05$		25.9–38.1 (32.0) $T_{11}=2.93, p<0.01$
Claw/Leg III				
External primary branch	*46.4–57.0* (52.0)	*39.4–48.9* (*44.7*) $T_{10}=5.78, p<0.001$		38.8–50.3 (44.4) $T_{10}=3.87$ $p<0.001$
External secondary branch	*37.7–44.0* (*41.1*)	*31.3–38.5* (*35.1*) $T_9=6.93, p<0.001$		25.9–39.0 (33.3) $T_9=3.39, p<0.005$
Internal primary branch	*47.9–57.9* (*50.4*)	39.0–47.2 (44.4) $T_{11}=4.48, p<0.001$	*31.0–50.9* (*44.7*) $T_{11}=-2.93, p<0.01$	
Internal secondary branch	*35.7–40.6* (*38.3*)	30.4–40.0 (34.2) $T_{18}=3.48, p<0.001$		25.5–37.2 (32.0) $T_8=3.81, p<0.005$
Claw/Leg IV				
Anterior primary branch	*50.9–60.5* (*56.2*)	*41.5–54.9* (*49.7*) $T_8=2.73, p<0.01$	*40.0–56.2* (*48.7*) $T_8=2.77, p<0.001$	
Anterior secondary branch	*37.5–45.8* (*41.3*)	*26.1–40.7* (*34.4*) $T_{10}=3.37, p<0.005$		30.6–42.2 (35.2) $T_{10}=2.54, p<0.01$
Posterior primary branch	*54.2–63.0* (*59.0*)	*45.8–56.7* (*52.3*) $T_{11}=3.99, p<0.001$		
Posterior secondary branch	*37.5–47.6* (*43.7*)	*30.6–40.9* (*36.1*) $T_{10}=5.65, p<0.001$		27.5–41.0 (36.2) $T_{10}=3.63, p<0.005$

3. *Milnesium pacificum* (hatchlings) only known from the terra typica in Japan, by different body colour: reddish in *M. pelufforum* sp. nov. vs creamy white, transparent or with three brownish longitudinal stripes in *M. pacificum*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs no band arrangement in *M. pacificum*; dimple diameter about 2–3.5 µm in *M. pelufforum* vs about 0.61–0.82 µm in *M. pacificum*; shorter peribuccal papillae, *pt* [*14.7–15.1*] in *M. pelufforum* vs [*15.8–20.7*] in *M. pacificum*; longer lateral papillae, *pt* [*14.6–15.9*] in *M. pelufforum* sp. nov. vs [*12.1–14.2*] in

M. pacificum; different buccal tube width: higher *pt* of standard width [*43.7–52.1*] in *M. pelufforum* vs [*30.1–39.4*] in *M. pacificum*; statistically significant higher *pt* of stylet support insertion point [*65.3–73.8*, mean *71.6*] in *M. pelufforum* vs [*58.7–69.2*, mean *63.9*] in *M. pacificum* (t_{12} = 5.18 $p < 0.001$); statistically significant differences about *pt* of many claw heights (Table 5).

4. *Milnesium variefidum* (hatchlings and youngs) only known from the type locality in Scotland, by the body colour: reddish in *M. pelufforum* sp. nov. vs yellowish before fixation and transparent afterwards in *M. variefidum*; sculptured cuticle with dimples arranged in ten bands in *M. pelufforum* vs cuticle appearing smooth but with faint pseudopores, not arranged in bands, in *M. variefidum*; presence of ten pseudoplate rows better visible and outlined in *M. pelufforum* vs seven rows of pseudoplates only occasionally visible or poorly developed in *M. variefidum* (Table 3); different buccal tube width: *pt* of standard width [*43.7–52.1*] in *M. pelufforum* vs [*22.1–33.8*] in *M. variefidum*; different *pt* ranges of claw heights: external primary and secondary branch I [*45.0–53.7*] and [*37.9–47.4*] in *M. pelufforum* vs [*33.7–44.7*] and [*27.7–35.8*] in *M. variefidum* respectively; internal secondary branch I [*35.9–44.7*] in *M. pelufforum* vs [*28.2–35.4*] in *M. variefidum*; internal primary branch II and III [*46.0–56.5*] and [*47.9–57.9*] in *M. pelufforum* vs [*33.9–45.6*] and [*34.2–47.2*] in *M. variefidum* respectively; statistically significant differences about *pt* of most of the other claw heights (Table 5).

Milnesium irenae sp. nov.
urn:lsid:zoobank.org:act:E52817A9-9080-427A-B4DF-AD4C58C52352
Figs 13–18, Tables 3, 6–7

Diagnosis

Peculiar, complex cuticular ornamentation including cuticular grooves, branched rugosity and a true, very fine, reticular design appearing different from that of the congeneric species with reticulated cuticle. Nine rows of pseudoplates present, formula: CP: I:0; II:4; III:4; IV:8; V:10; VI:8; VII:10; VIII:8; IX:12; X:4. Six peribuccal lamellae present, six peribuccal papillae with the medioventral reduced, two lateral cephalic papillae; buccal tube slightly funnel-shaped; stylets, their furcae and supports very developed. Claw configuration [2-3]-[2-2]; internal secondary branches of legs I–III with long basal spurs directed towards, almost touching the claw base. Ontogenetic change not observed in the available material, but hatchlings are probably lacking.

Etymology

We dedicate the new species to the researcher Irene Luisa Doma.

Material examined

Holotype

ARGENTINA • ♀; La Pampa Province, Santa Rosa City; 36º37′13″ S, 64º17′26″ W; about 177 m a.s.l.; 17 Sep. 2017; Rocha-Doma leg; moss and lichens from trees; UNLPam 1657(1).

Paratypes

ARGENTINA • 1 ♀; same collection data as for holotype; MCNS Tar.000023(2) • 4 ♀♀; same collection data as for holotype; UNICT 5899(1) to 5899(4) • 13 ♀♀; same collection data as for holotype; UNLPam 1367(1), 1430(1) to 1430(3), 1599(1), 1599(3), 1603(1), 1603(3), 1603(4), 1656(1) to 1656(4).

Morphological description

Body reddish up to 664 µm long (habitus in Fig. 13); large eyes present. Cuticle with complex sculpture (Fig. 16): on the dorsal surface of trunk segments (starting with legs I in very few specimens, with legs II in some, and with legs III in the majority of them), clearly visible cuticular grooves (Fig. 16A–B, D)

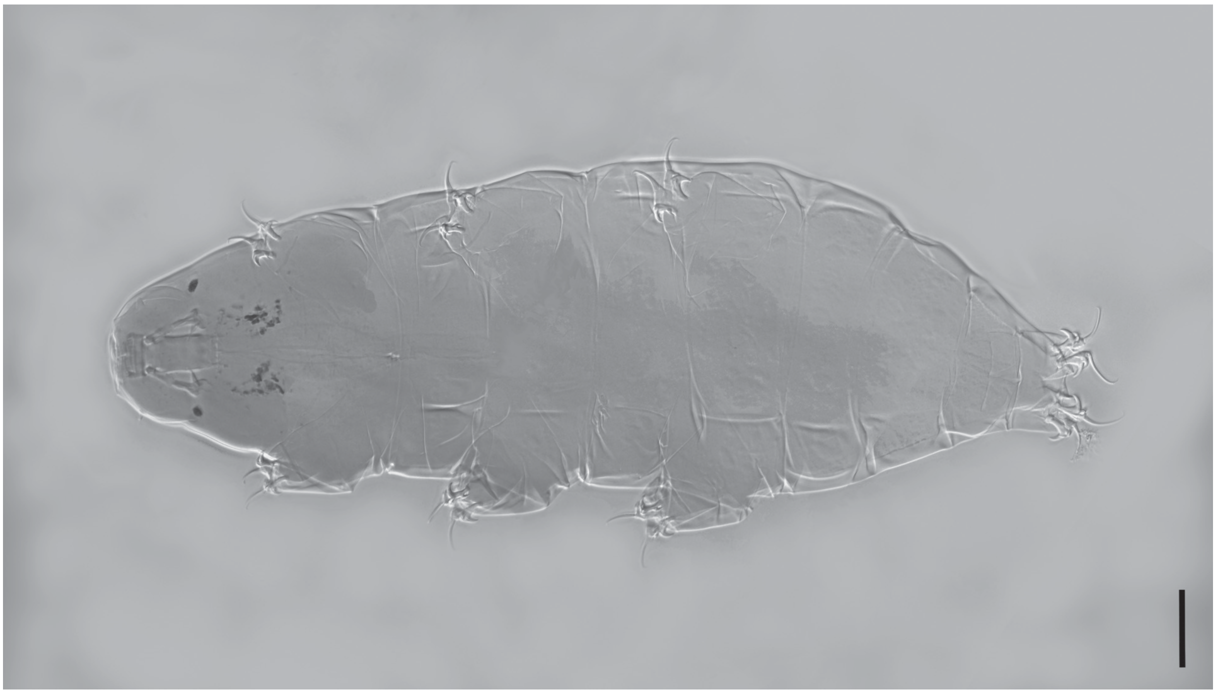

Fig. 13. *Milnesium irenae* sp. nov., holotype, ♀ (slide No. UNLPam 1657-1). Habitus. Scale bar = 50 µm.

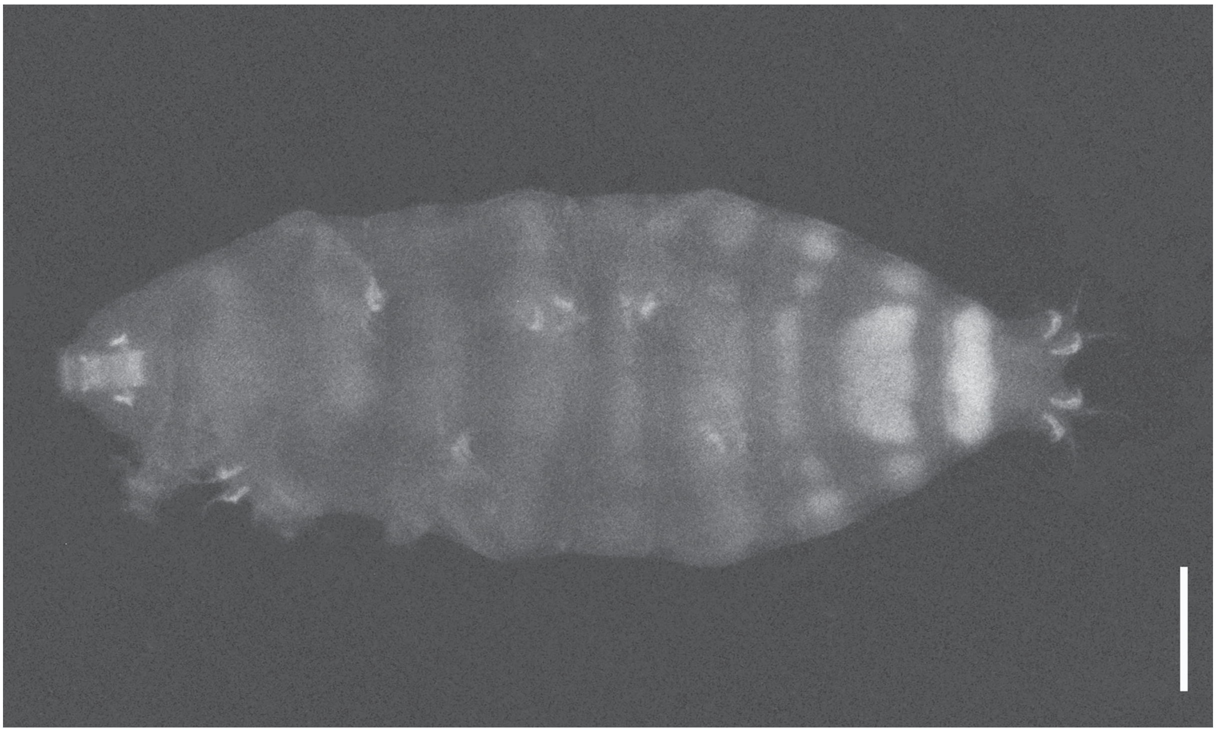

Fig. 14. *Milnesium irenae* sp. nov., paratype, ♀ (slide No. UNLPam 1430-1). View under UV fluorescence microscope, showing various pseudoplates. Scale bar = 50 µm.

are present, some developing from cuticular invaginations for muscle attachments (Fig. 16B arrows); the grooves form, more peripherally, a rugosity made of wrinkles that sometimes outline on some areas a network (Fig. 16A–B arrowheads) visible as bright crossing lines delimiting dark elongated 'dots'. The wrinkles gradually become interrupted and disappear, giving way to a reticular design (Fig. 16) consisting of a fine-scale mesh, elongated in a few areas, isodiametric in the rest with a mesh diameter of about 0.5–1.0 µm. This reticule made of isodiametric mesh, though difficult to see, is quite spread on the cuticle, also on more cephalic segments where the other elements of the cuticular sculpture are absent: on all specimens it is visible at least starting with legs II, but in some it starts with legs I. This reticule, made of a delicate mesh, has a rather different appearance from the more common reticular pattern, resulting from close dimples, of many other species of *Milnesium*; in *M. irenae* sp. nov. it looks instead more similar to the more irregular reticulations present in Parachela Schuster, Nelson, Grigarick & Christenberry, 1980, such as, for example, several species of Isohypsibiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008 (e.g., *Dianea sattleri* (Richters, 1902) and *Ursulinius pappi* (Iharos, 1966)) and Doryphoribiidae Gąsiorek, Stec, Morek & Michalczyk, 2019 (e.g., *Doryphoribius bindae* Lisi, 2011 and *Grevenius kotovae* (Tumanov, 2003)). Pseudoplates present, arranged in 9 rows, formula: CP: I:0; II:4; III:4; IV:8; V:10; VI:8; VII:10; VIII:8; IX:12; X:4 (Figs 14–15).

Row I is absent; row II, situated anteriorly to legs I, has four pseudoplates, all difficult to see: two medial, about quadrangular, touching in the central line, and two lateral, about trapezoidal, laying obliquely. Row III, situated in line with legs I, has four central pseudoplates arranged in two pairs, connected, with two anterior about rectangular, and two caudal, vaguely triangular pseudoplates. Row IV, situated between legs I and II, has eight pseudoplates: the four medial arranged in two pairs, transversally elongated, connected, forming a unique about rectangular structure; each of these four pseudoplates is also about rectangular but with the transverse line dividing the two anterior from the two posterior pseudoplates weakly outlined; two about rectangular pseudoplates, just lateral to the central complex, longitudinally elongated, plus two more lateral, bigger but difficult to see, about trapezoidal/ellyptical laying obliquely and slightly more anteriorly. Row V, situated in line with legs II, has ten pseudoplates: the six medial arranged in three pairs (the most cephalic difficult to see), transversally elongated, connected, forming

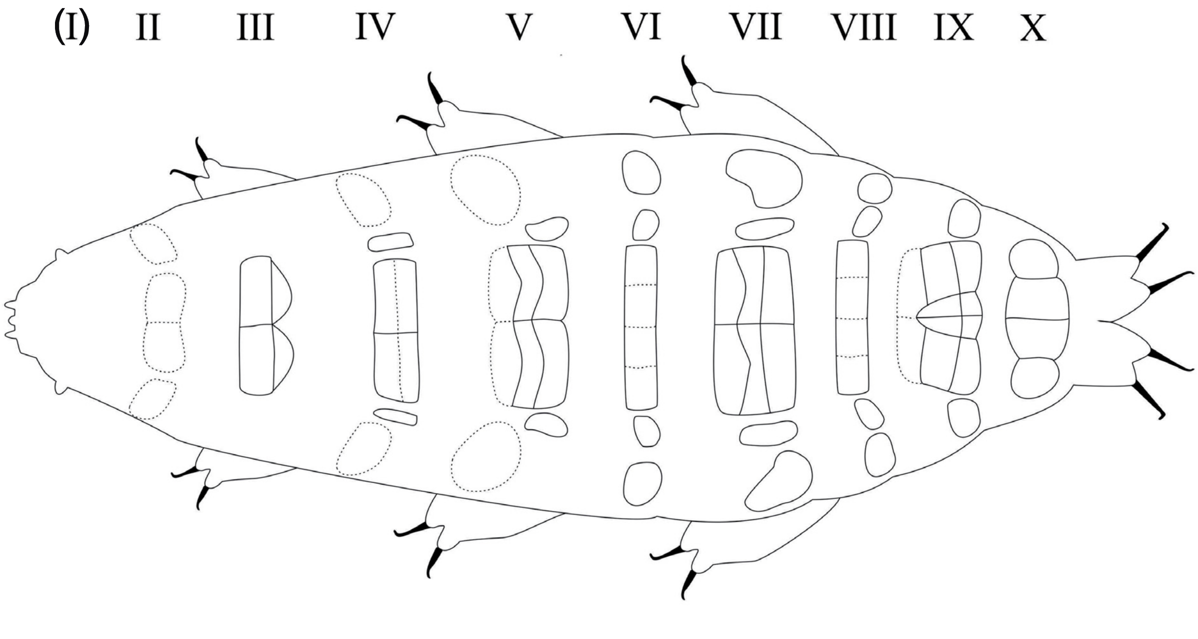

Fig. 15. Semi-schematic drawing of pseudoplate configuration in *Milnesium irenae* sp. nov.

a unique about rectangular structure; each these six pseudoplates vaguely rectangular but with the two transverse lines dividing the adjacent anterior/posterior pairs of pseudoplates not straight; two comma-shaped pseudoplates, just lateral to the central complex and aligned posteriorly, plus two more lateral pseudoplates, laying more anteriorly, bigger and about oval but difficult to see. Row VI, situated between legs II and III, has eight pseudoplates aligned in a single transverse row, with the four medial ones connected laterally and thus forming a unique wide rectangle (but with the longitudinal lines dividing the four pseudoplates weakly outlined); lateral to that central complex, two separate pseudoplates on each side, with the mid-lateral about oval, and the most lateral, bigger, more or less rounded/oval. Row VII, situated in line with legs III, has ten pseudoplates: the six medial arranged in three pairs, transversally elongated, connected, forming a unique about rectangular structure; each of these six pseudoplates vaguely rectangular but with the two transverse lines dividing the three pairs not straight; laterally, on each side, a longitudinally elongated pseudoplate, and a more lateral, bigger, about bean-shaped. Row

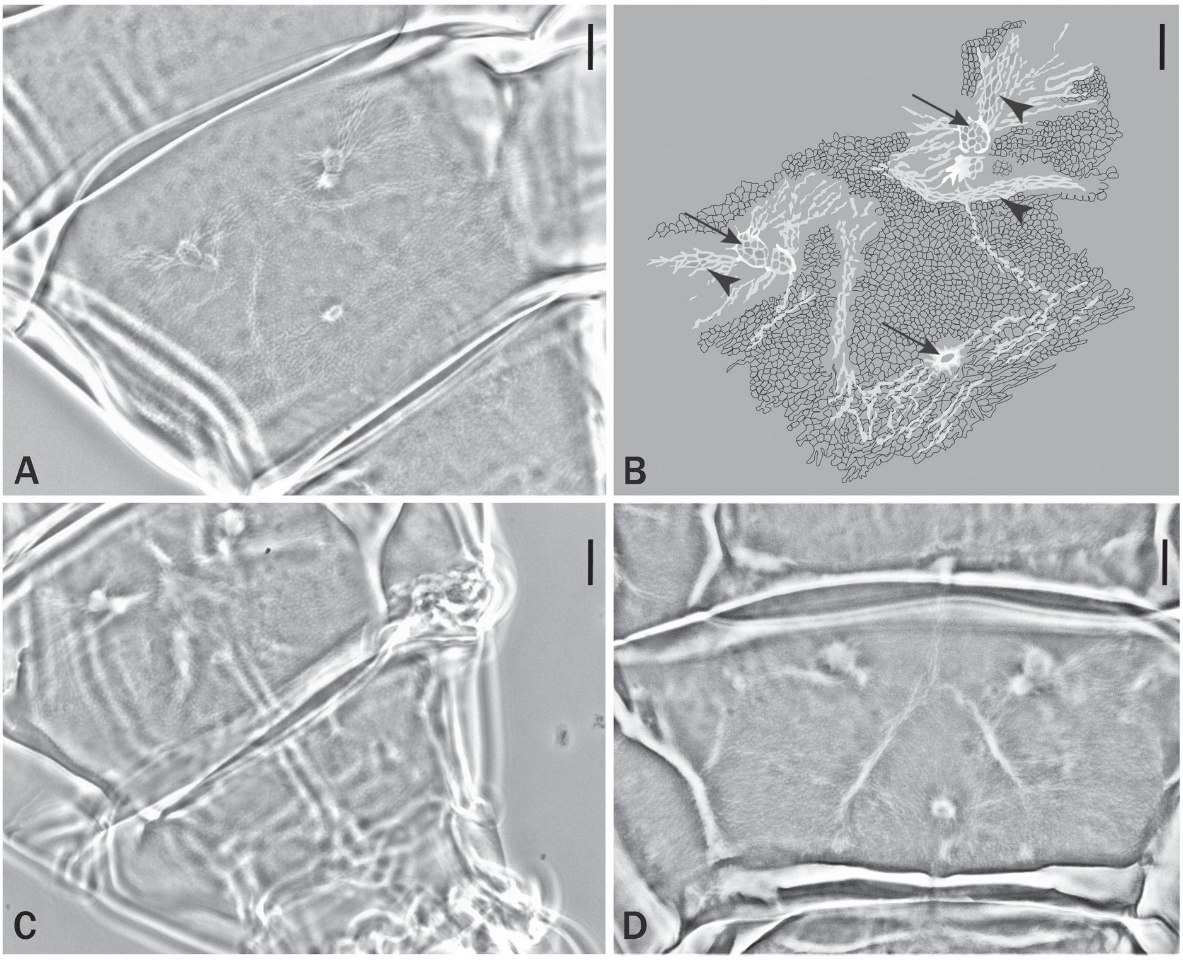

Fig. 16. *Milnesium irenae* sp. nov. **A–B**. Paratype, ♀ (slide No. UNLPam 1367-1). **C**. Paratype, ♀ (slide No. MCNS tar. 000023-2). **D**. Paratype, ♀ (slide No. UNICT 5899). Details of the cuticular ornamentation of the caudal segments (where it is more evident). **A**. Muscular attachments, grooves and rugosity arrangement, and, partially, the reticular pattern, are visible. **B**. Schematic drawing based on A, showing all components of the ornamentation: muscular attachments (arrows), grooves and rugosity system (in light grey) which forms crossings in some areas (arrowheads), reticular pattern. **C**. The reticular pattern is well visible. **D**. All cuticular sculpture components as in A are shown in another paratype. Scale bars = 10 µm.

VIII, situated just caudally to legs III, has eight pseudoplates aligned in a single transverse row, with the four medial connected laterally and thus forming a unique wide rectangle (but with the longitudinal lines dividing the four pseudoplates weakly outlined); lateral to that central complex, aligned with its posterior margin, two separate pseudoplates on each side, with the mid-lateral one about oval, and the most lateral one, bigger, more or less rounded/oval. Row IX, situated between legs III and IV, has twelve pseudoplates with a median aggregation of 10 pseudoplates (the more cephalic pair difficult to see) forming a complex pattern (see Fig. 15), and two lateral single pseudoplates about quadrangular/rounded, aligned posteriorly. Row X, situated just anterior to legs IV, has four pseudoplates, sided and aligned in a single row transversally, with the medial ones about quadrangular, the lateral ones about rounded.

Six peribuccal lamellae present, and six peribuccal papillae plus two lateral papillae present; medio-ventral peribuccal papilla reduced, (Fig. 17B arrow; *pt* of such papilla [*7.3–10.4*] vs [*14.6–20.8*] of the other peribuccal papillae). Buccal tube (Fig. 17) not perfectly cylindrical, slightly wider anteriorly (posterior/

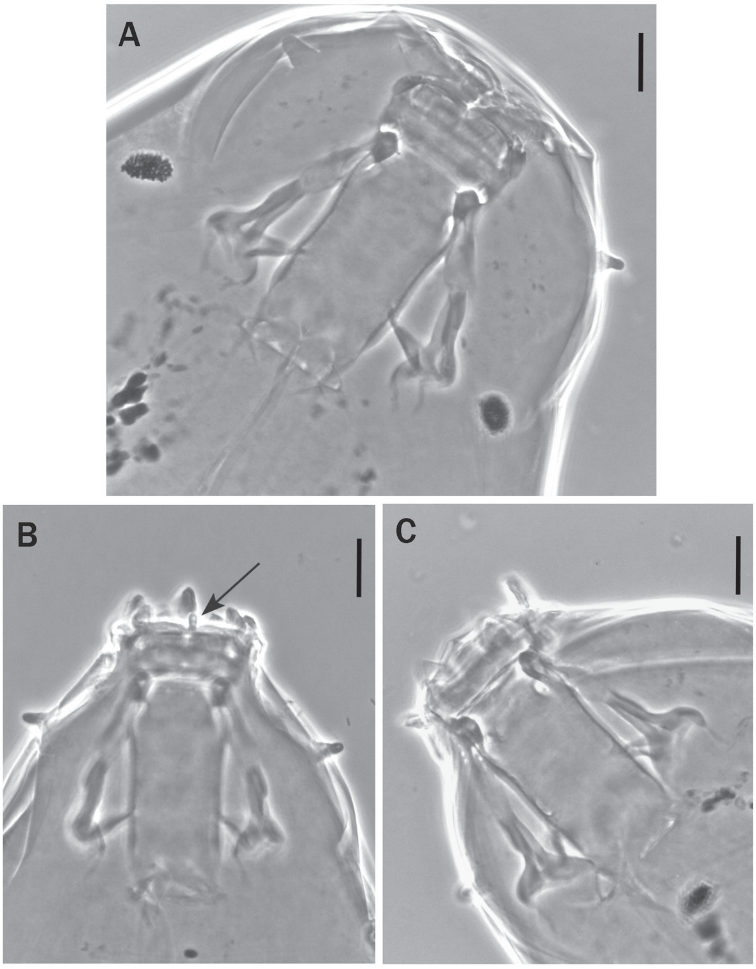

Fig. 17. *Milnesium irenae* sp. nov. **A**. Holotype, ♀ (slide No. UNLPam 1657-1). **B**. Paratype, ♀ (slide No. UNLPam 1599-3). **C**. Paratype, ♀ (slide No. UNLPam 1656-3). Cephalic region. **A**. Buccal tube and the noticeable development of the stylets, their furcae and supports. **B**. The reduced medio-ventral peribuccal papilla (arrow) in a paratype. **C**. Another paratype showing the same structures as in A, illustrating clearly the whale-tail shaped stylet furcae; the peribuccal lamellae are partially visible. Scale bars = 10 µm.

Table 6 (continued on next page). Measurements (in µm) and *pt* values of selected morphological structures of specimens of *Milnesium irenae* sp. nov. mounted in polyvinyl-lactophenol medium. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: N = number of specimens or structures measured; SD = standard deviation.

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	24	349 – 664	*1190 – 1614*	524	*1417*	83	*135*	638	*1571*
Peribuccal papillae length	9	5.3 – 8.3	*14.6 – 20.8*	6.6	*17.9*	1.0	*1.8*	?	*?*
Ventral peribuccal papillae length	6	2.9 – 4.2	*7.3 – 10.4*	3.3	*8.7*	0.5	*1.3*	?	*?*
Lateral papillae length	21	4.0 – 7.0	*11.6 – 17.6*	5.5	*15.0*	0.9	*1.3*	6.5	*16.0*
Buccal tube									
Length	24	25.5 – 42.4	–	36.9	–	4.1	–	40.6	–
Stylet support insertion point	24	18.1 – 30.5	*68.9 – 74.9*	26.7	*72.2*	3.2	*1.4*	30.4	*74.9*
Anterior width	24	13.3 – 24.6	*50.7 – 60.6*	20.4	*55.0*	2.9	*2.9*	24.6	*60.6*
Standard width	24	12.0 – 23.1	*46.1 – 56.9*	18.8	*50.6*	2.9	*3.3*	23.1	*56.9*
Posterior width	24	10.7 – 20.9	*41.4 – 51.5*	16.8	*45.2*	2.5	*2.7*	20.9	*51.5*
Standard width/length ratio	24	46% – 57%	–	50%	–	3%	–	57%	–
Posterior/anterior width ratio	24	78% – 88%	–	82%	–	3%	–	85%	–
Claw 1 lengths									
External primary branch	22	11.0 – 18.3	*38.7 – 47.1*	15.9	*43.0*	2.0	*2.3*	18.3	*45.1*
External base + secondary branch	22	10.3 – 16.0	*34.1 – 42.6*	14.0	*38.1*	1.6	*2.6*	15.9	*39.2*
External branches length ratio	19	82% – 94%	–	89%	–	3%	–	87%	–
Internal primary branch	22	11.0 – 17.8	*35.3 – 44.7*	15.1	*40.6*	2.0	*3.0*	17.8	*43.8*
Internal base + secondary branch	22	9.5 – 15.5	*32.7 – 41.3*	13.5	*36.2*	1.5	*2.6*	14.6	*36.0*
Internal spur	14	4.1 – 6.5	*11.4 – 16.2*	5.0	*13.3*	0.6	*1.2*	5.4	*13.3*
Internal branches length ratio	20	79% – 96%	–	90%	–	5%	–	82%	–
Claw 2 lengths									
External primary branch	24	13.5 – 20.7	*43.0 – 52.9*	17.8	*48.3*	2.0	*2.8*	20.3	*50.0*
External base + secondary branch	23	10.6 – 16.8	*35.4 – 44.7*	14.6	*39.6*	1.8	*2.6*	16.3	*40.1*
External branches length ratio	22	73% – 95%	–	83%	–	5%	–	80%	–
Internal primary branch	22	12.8 – 19.9	*40.7 – 50.8*	17.2	*45.9*	1.9	*2.9*	19.2	*47.3*
Internal base + secondary branch	22	10.1 – 16.6	*34.2 – 43.0*	14.2	*38.0*	1.6	*2.6*	14.9	*36.7*
Internal spur	19	4.2 – 6.9	*12.5 – 18.3*	5.8	*15.3*	0.8	*1.7*	6.8	*16.7*
Internal branches length ratio	20	77% – 93%	–	83%	–	4%	–	78%	–
Claw 3 lengths									
External primary branch	24	13.5 – 22.0	*44.8 – 55.9*	18.6	*50.3*	2.3	*2.9*	22.0	*54.2*
External base + secondary branch	23	10.5 – 18.2	*35.7 – 44.9*	14.9	*40.4*	1.9	*2.5*	18.2	*44.8*
External branches length ratio	22	75% – 89%	–	81%	–	4%	–	83%	–
Internal primary branch	23	13.2 – 20.5	*42.2 – 52.3*	17.5	*47.6*	2.0	*3.3*	20.1	*49.5*
Internal base + secondary branch	23	10.3 – 16.7	*34.2 – 44.4*	14.4	*38.4*	1.6	*2.6*	15.4	*37.9*
Internal spur	20	4.4 – 6.8	*12.0 – 17.8*	5.7	*15.2*	0.7	*1.6*	6.0	*14.8*
Internal branches length ratio	21	72% – 93%	–	81%	–	5%	–	77%	–

Table 6 (continued). Measurements (in µm) and *pt* values of selected morphological structures of specimens of *Milnesium irenae* sp. nov. mounted in polyvinyl-lactophenol medium.

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Claw 4 lengths									
Anterior primary branch	22	17.5 – 25.4	*55.2 – 64.2*	22.4	*60.0*	2.2	*3.2*	22.4	*55.2*
Anterior base + secondary branch	24	11.0 – 19.2	*39.8 – 49.0*	16.0	*43.4*	2.0	*2.7*	18.6	*45.8*
Anterior branches length ratio	21	63% – 83%	–	72%	–	5%	–	83%	–
Posterior primary branch	23	17.0 – 27.2	*59.4 – 68.4*	23.8	*63.6*	2.5	*2.9*	27.2	*67.0*
Posterior base + secondary branch	24	12.0 – 19.7	*40.6 – 50.4*	16.8	*45.5*	2.2	*2.8*	19.4	*47.8*
Posterior branches length ratio	22	65% – 78%	–	71%	–	3%	–	71%	–

anterior width ratio 78–88%); stylets very robust, with very developed furcae, whale-tail shaped, and with supports which after their insertion on the buccal tube becoming gradually wider, assuming overall a triangular shape (Fig. 17A, C); *pt* of stylet support insertion point on the buccal tube length [*68.9–74.9*].

Claws of the *Milnesium* type with configuration [2-3]-[2-2] (Fig. 18), rather robust, in particular the secondary branches; primary branches of legs IV more slender than on legs I–III (compare in Fig. 18A–B with C–D). Percentual ratio of secondary branches with respect to primary branches for each couple higher on legs I (79–96%), slightly lower on legs II–III (72–95%) and definitely lower on legs IV (63–83%). Internal secondary branches of legs I–III with long basal spurs which form an acute angle inferiorly and have tip nearly reaching the claw base (Fig. 18A). Secondary branches with basal thickenings (‘lunulae’, Fig. 18A, C white arrowheads), primary branches with very thin, short, accessory points (Fig. 18B black arrow); long and thick cuticular bars present under the claws I–III (Fig. 18A black arrowhead). Tiny cuticular tubercles, often difficult to see, present on all legs, more visible on legs IV (Fig. 18C–D white arrows).

Remarks

Milnesium irenae sp. nov. is the only described species of the genus with this form of complex cuticular sculpture, and with reticular pattern more similar to some Parachela than to other congeneric species with ‘reticulated’ cuticle. According to the literature, other congeneric species have a ‘reticule’ actually made of dimples close to one another, while in *M. irenae* the reticule mesh is not given by dimples. The medioventral peribuccal papilla is reduced (comments in Discussion). We found specimens with body length from 349 µm up to 664 µm (presumably, from second instar on) without remarkable differences between the smallest and the largest; therefore, possible ontogenetic change is unknown in the passage from hatchlings to second instar, while seems to be absent from second to third instar. Morphometric data are given in Table 6 and Supp. file 3; in Table 7 the statistically significant differences (through Student *t*-tests) of overlapping *pt* ranges of claw heights between the new species and the similar ones.

Differential diagnosis

Milnesium irenae sp. nov., due to its unusual cuticular sculpture, differs from all known species of the genus, though morphologically it has more affinity with some species of the old *granulatum* group. In addition, the characters of the stylets, their furcae and supports have peculiarities which differentiate it from many, if not all, congeneric species (the characters of those structures have not been described in detail in all past species descriptions). Here, we differentiate the new species from those having the same claw configuration [2-3]-[2-2] at least in some life stages, plus some kind of cuticular sculpture. These species are: *M. almatyense*

Tumanov, 2006 (youngs and seniors), *M. berladnicorum* Ciobanu, Zawierucha, Moglan & Kaczmarek, 2014, and adults of *M. variefidum* Morek, Gąsiorek, Stec, Blagden & Michalczyk, 2016.

Milnesium irenae sp. nov. differs from them as follows:

1. *Milnesium almatyense* known from Kazakhstan (type locality), Kyrgyz Republic (Morek *et al.* 2020b), by different body colour: reddish in *M.irenae* sp. nov. vs white in *M. almatyense*; eyes present in *M. irenae* vs absent of *M. almatyense*; different and more complex cuticular sculpture in *M. irenae* (with tiny mesh, long, branched grooves, and tubercles) equal in specimens of all sizes found vs simpler (with delicate reticulation only in hatchlings, while only pseudopores in juveniles and adults) in *M. almatyense*; nine rows of pseudoplates in *M. irenae* vs eight in *M. almatyense* with different number of pseudoplates in each correspondent row (Table 3); higher *pt* of buccal tube standard width, [*46.1–56.9*] in *M. irenae* vs [*26.2–33.1*] in *M. almatyense*; higher buccal tube standard width/length ratio, 46–57% in *M. irenae* vs 26–33% in *M. almatyense*.

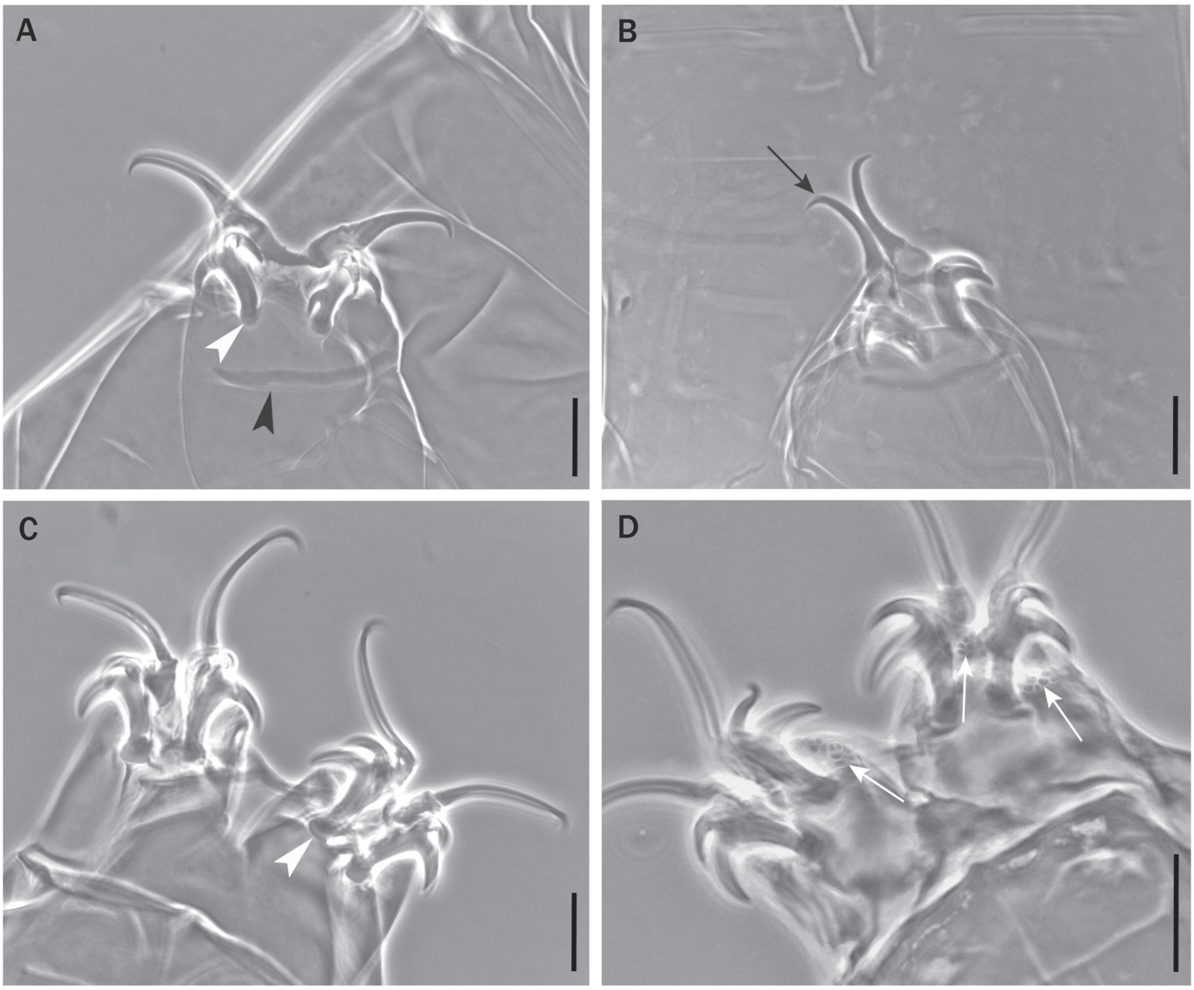

Fig. 18. *Milnesium irenae* sp. nov. **A, C**. Holotype, ♀ (slide No. UNLPam 1657-1). **B**. Paratype, ♀ (UNLPam 1430-3). **D**. Paratype, ♀ (slide No. UNLPam 1603-3). Claws and leg characters. **A**. Leg II; the white arrowhead indicates a lunule, the black arrowhead indicates the leg cuticular bar. **B**. Legs II; the black arrow indicates primary branch accessory points. **C**. Legs IV; the white arrowhead indicates a lunule. **D**. Legs IV, magnified; the white arrows indicate some tubercles. Scale bars = 10 µm.

Table 7. Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of claw heights between *Milnesium irenae* sp. nov. and the similar species.

Species **Character**	***M. irenae*** **sp. nov.**	***M. variefidum***
Claw/Leg I		
External primary branch	38.7–47.1 (43.1)	33.7–44.7 (40.0) $t_{21}=2.20$, $p<0.05$
External secondary branch	34.1–42.6 (38.0)	27.7–35.8 (31.8) $t_{23}=6.00$, $p<0.001$
Internal secondary branch	32.7–41.3 (36.2)	28.2–35.4 (31.7) $t_{22}=4.50$, $p<0.001$
Claw/Leg II		
External primary branch	43.0–52.9 (48.2)	38.0–50.9 (43.7) $t_{25}=3.56$, $p<0.001$
External secondary branch	35.4–44.7 (39.5)	27.6–39.7 (33.8) $t_{23}=4.29$, $p<0.001$
Internal primary branch	40.7–50.8 (45.9)	33.9–45.6 (40.6) $t_{21}=3.93$, $p<0.001$
Internal secondary branch	33.0–43.0 (37.8)	25.9–38.1 (32.0) $t_{22}=4.64$, $p<0.001$
Claw/Leg III		
External primary branch	44.8–55.9 (50.9)	38.8–50.3 (44.4) $t_{24}=4.60$, $p<0.001$
External secondary branch	35.7–44.9 (40.3)	25.9–39.0 (33.3) $t_{23}=5.68$, $p<0.001$
Internal primary branch	42.2–52.3 (47.7)	34.2–47.2 (41.0) $t_{23}=4.39$, $p<0.001$
Internal secondary branch	34.2–44.4 (38.3)	25.5–37.2 (32.0) $t_{23}=4.93$, $p<0.001$
Claw/Leg IV		
Anterior primary branch	55.1–64.2 (59.8)	46.6–61.0 (52.1) $t_{22}=3.28$, $p<0,01$
Anterior secondary branch	39.8–49.0 (43.3)	30.6–42.2 (35.2) $t_{24}=4.69$, $p<0.001$
Posterior primary branch	59.4–68.4 (63.6)	35.9–62.4 (53.1) $t_{22}=4.53$, $p<0.001$
Posterior secondary branch	40.6–50.4 (45.5)	27.5–41.0 (36.2) $t_{23}=7.51$, $p<0.001$

2. *Milnesium berladnicorum* only known from the type locality in Romania, by different body colour: reddish in *M. irenae* sp. nov. vs brownish in *M. berladnicorum*; different and more complex cuticular sculpture in *M. irenae* (with tiny mesh, long, branched grooves, and tubercles) vs simpler (with just bright spots, not ascertained whether either dimples or pseudopores) in *M. berladnicorum*; different number of pseudoplates in each correspondent row (Table 3); statistically significant higher buccal tube posterior/anterior width ratio, 78–88%, mean 82%, in *M. irenae* vs 69–79%, mean 73%, in adults of *M. berladnicorum*; ($t_{25} = 8.09$, $p < 0.001$).

3. *Milnesium variefidum* only known from the type locality in Scotland, by different body colour: reddish in *M. irenae* sp. nov. vs white or transparent in *M. variefidum*; eyes present in *M. irenae* vs absent in *M. variefidum*; different cuticular ornamentation, with tiny mesh, long, branched grooves, and tubercles in *M. irenae* vs with scattered pseudopores, minute wrinkles and only some short grooves in *M. variefidum*; presence of nine rows of pseudoplates in *M. irenae* vs seven rows in *M. variefidum* with different number of pseudoplates in each correspondent row (Table 3); statistically significant differences about *pt* of almost all claw heights (Table 7).

Milnesium quiranae sp. nov.
urn:lsid:zoobank.org:act:DDC4D209-B7E0-411A-8D83-0E086CB44D04
Figs 19–21, Tables 8–11

Diagnosis

Smooth cuticle, six peribuccal lamellae, six peribuccal papillae with the medioventral reducing with growing, two lateral cephalic papillae. Buccal tube nearly cylindrical in all life stages, becoming wider with growing. Claws with configuration [3-3]-[3-3] in all life stages.

Etymology

The new species is dedicated to the researcher Estela Maris Quirán, who published important contributions on the scientific knowledge of invertebrates in La Pampa, Argentina.

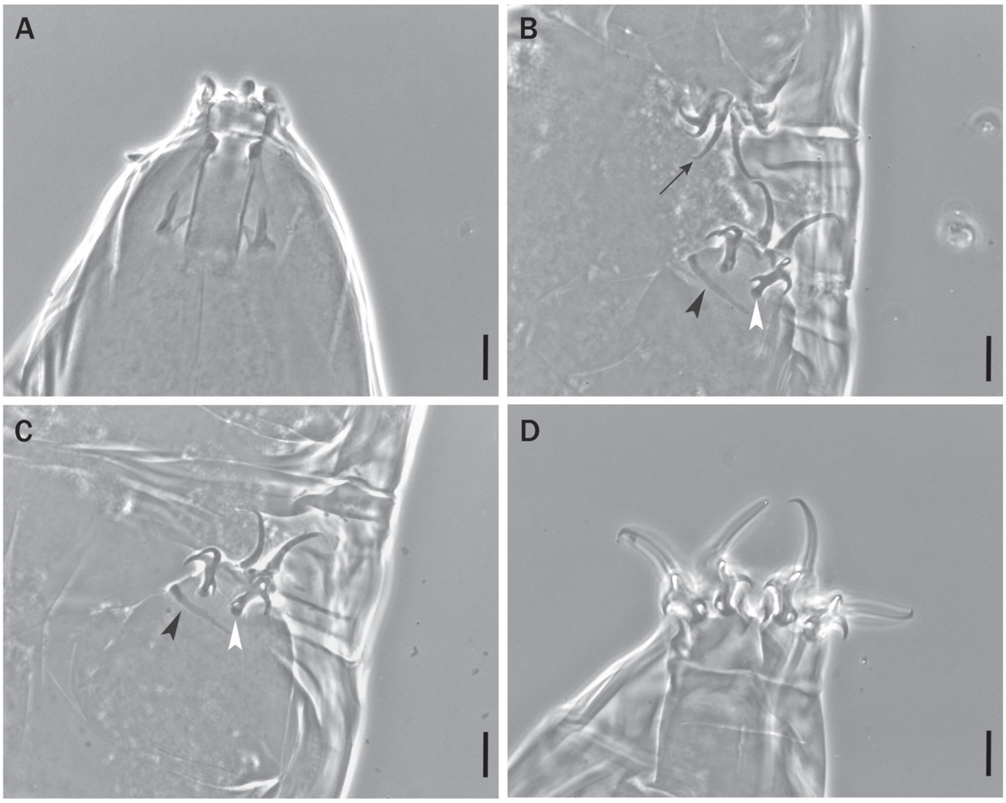

Fig. 19. *Milnesium quiranae* sp. nov., paratype, ♀ (slide No. UNLPam 566-1). Buccal tube and claws of a young. **A**. Buccal tube and related structures. **B**. Legs I and II; the black arrow indicates the accessory points, the black arrowhead indicates the leg cuticular bar, the white arrowhead indicates a lunule. **C**. Leg III; the black and the white arrowheads indicate the same as in B. **D**. Legs IV with their claws. Scale bars = 10 µm.

Material examined

Holotype

ARGENTINA • ♀; Salta Province, Salta City; 24°47′18″ S, 65°24′38″ W; 1150 m a.s.l.; 2 May 2014; Rocha-Doma leg.; moss and lichens from trees; MCNS Tar.000024(3).

Paratypes

ARGENTINA • 2 ♀♀; same collection data as for holotype; MCNS Tar.000025(3), Tar.000025(4) • 4 ♀♀; same collection data as for holotype; UNICT 5900(1) to 5900(4) • 21 ♀♀; same collection data as for holotype; UNLPam 558(1) to 558(3), 558(5), 560(1), 560(3), 560(4), 564(4), 565(1), 565(4), 565(5), 566(1) to 566(4), 570(1), 573(5), 576(4), 576(5), 586(1), 586(2).

Morphological description

Body length up to 770 µm (habitus in Fig. 20A), reddish colour before mounting, eyes present. Cuticle smooth (Fig. 20B–C) without dimples, wrinkles, pseudopores, reticulations, pseudoplates or gibbosities.

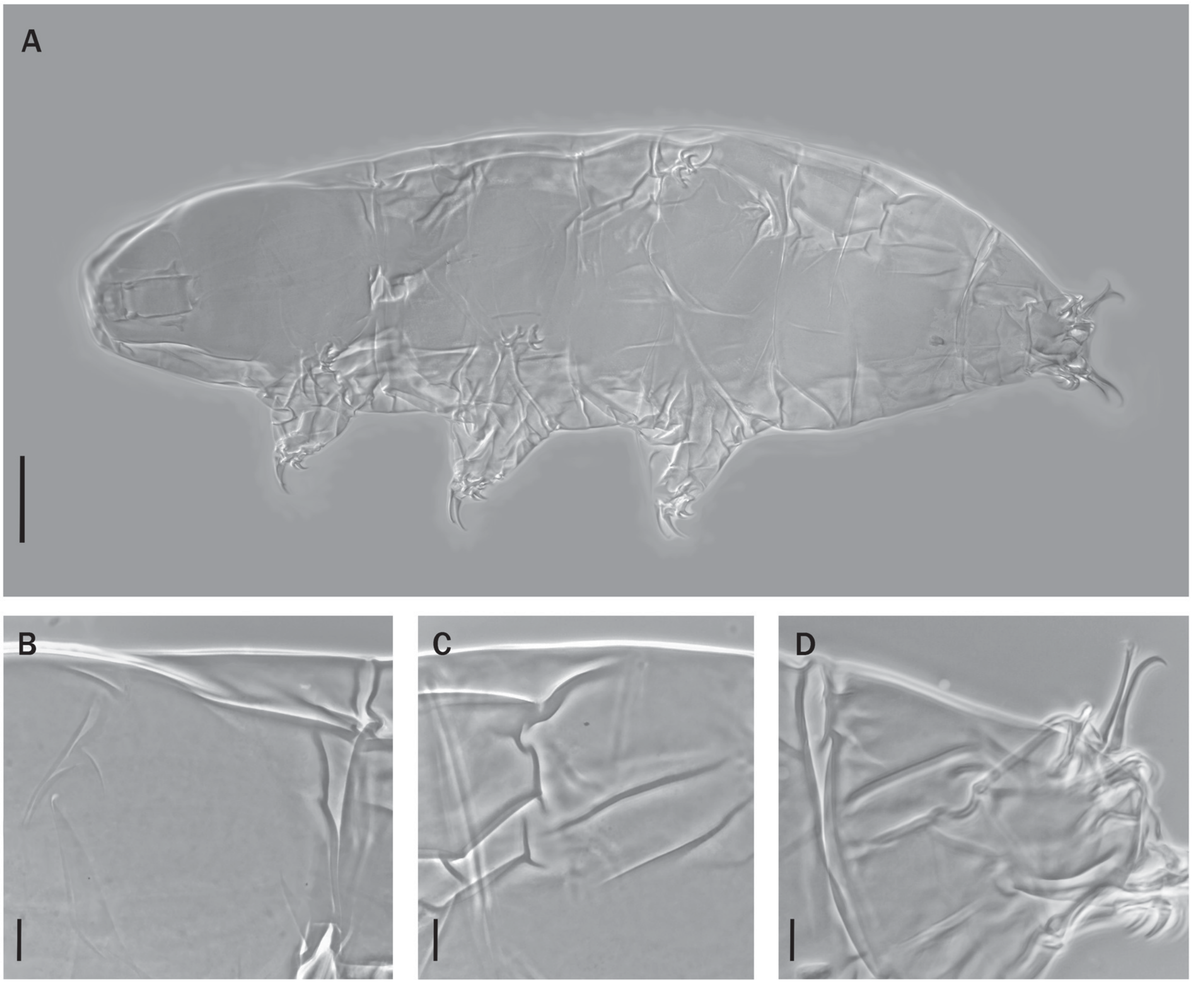

Fig. 20. *Milnesium quiranae* sp. nov., holotype, ♀ (slide No. MCNS Tar.000024-3). **A**. Habitus. **B**. Smooth cuticle at the level of legs I. **C**. Smooth cuticle between legs III and IV. **D**. Smooth cuticle of the caudal end of the body. Scale bars: A = 50 µm; B–D = 10 µm.

Table 8 (continued on next page). Measurements (in µm) and *pt* values of selected morphological structures of young specimens of *Milnesium quiranae* sp. nov. mounted in polyvinyl-lactophenol medium. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: N = number of specimens or structures measured; SD = standard deviation.

Character	N	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	19	270–460	*1112 – 1499*	380	*1322*	61	*108*
Peribuccal papillae length	13	4.6–7.4	*15.5 – 23.9*	5.6	*20.6*	0.9	*2.0*
Lateral papillae length	16	4.0–6.6	*12.6 – 20.6*	5.1	*17.9*	0.9	*2.0*
Buccal tube							
Length	19	23.0–36.5	–	28.8	–	4.7	–
Stylet support insertion point	19	16.3–25.4	*67.4 – 75.1*	20.2	*70.2*	2.9	*2.1*
Anterior width	19	10.4–18.2	*44.0 – 52.6*	13.8	*47.7*	2.7	*2.5*
Standard width	19	10.1–18.8	*43.2 – 51.5*	13.6	*46.9*	2.9	*3.0*
Posterior width	19	9.0–16.6	*36.5 – 45.5*	12.1	*41.8*	2.4	*3.0*
Standard width/length ratio	19	43%–52%	–	47%	–	3%	–
Posterior/anterior width ratio	19	80%–96%	–	88%	–	4%	–
Claw 1 heights							
External primary branch	19	10.8–15.0	*39.8 – 49.8*	12.7	*44.4*	1.4	*3.5*
External base + secondary branch	17	8.6–12.9	*31.2 – 40.6*	10.5	*36.4*	1.4	*2.6*
External spur	7	2.9–4.0	*12.5 – 16.6*	3.6	*13.6*	0.5	*1.5*
External branches length ratio	17	75%–91%	–	82%	–	5%	–
Internal primary branch	19	10.0–14.0	*37.9 – 47.0*	11.9	*41.8*	1.3	*3.2*
Internal base + secondary branch	18	8.4–12.5	*30.1 – 37.8*	9.9	*35.0*	1.2	*2.7*
Internal spur	11	3.7–6.1	*13.8 – 19.4*	4.8	*16.3*	0.9	*1.6*
Internal branches length ratio	18	76%–95%	–	83%	–	5%	–
Claw 2 heights							
External primary branch	19	11.1–16.8	*45.0 – 54.5*	14.1	*49.3*	1.5	*3.6*
External base + secondary branch	16	9.0–12.7	*33.5 – 43.3*	10.9	*37.4*	1.3	*2.8*
External spur	6	3.3–5.0	*10.0 – 16.4*	4.1	*14.1*	0.8	*2.4*
External branches length ratio	16	68%–81%	–	76%	–	4%	–
Internal primary branch	19	10.6–16.2	*43.3 – 52.8*	13.6	*47.6*	1.6	*3.5*
Internal base + secondary branch	19	8.4–12.2	*32.0 – 41.3*	10.3	*36.0*	1.2	*2.7*
Internal spur	12	3.4–6.4	*14.9 – 19.9*	5.3	*17.4*	0.9	*1.4*
Internal branches length ratio	19	70%–82%	–	76%	–	4%	–
Claw 3 heights							
External primary branch	17	12.1–17.6	*43.3 – 53.1*	14.1	*48.4*	1.6	*3.5*
External base + secondary branch	18	9.0–12.2	*32.9 – 43.3*	10.9	*37.8*	1.1	*3.3*
External spur	8	3.0–5.0	*12.6 – 16.4*	4.1	*14.7*	0.8	*1.5*
External branches length ratio	16	68%–83%	–	78%	–	4%	–
Internal primary branch	18	11.7–16.2	*43.0 – 52.6*	13.6	*47.3*	1.5	*3.5*
Internal base + secondary branch	19	8.6–12.0	*30.1 – 40.8*	10.2	*35.8*	1.1	*3.4*

Table 8 (continued). Measurements (in µm) and *pt* values of selected morphological structures of young specimens of *Milnesium quiranae* sp. nov. mounted in polyvinyl-lactophenol medium.

Character	N	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Internal spur	12	3.7–7.1	*12.8 – 19.6*	5.3	*17.8*	1.1	*1.9*
Internal branches length ratio	18	66%–81%	–	75%	–	4%	–
Claw 4 heights							
Anterior primary branch	19	13.0–19.0	*47.4 – 58.1*	15.4	*53.7*	2.0	*3.6*
Anterior base + secondary branch	19	9.7–13.6	*35.8 – 45.5*	11.6	*40.4*	1.4	*2.8*
Anterior spur	15	4.0–6.8	*13.4 – 21.3*	5.3	*17.6*	1.1	*2.8*
Anterior branches length ratio	19	69%–82%	–	75%	–	4%	–
Posterior primary branch	19	12.6–20.0	*50.3 – 61.5*	16.2	*56.4*	2.4	*3.6*
Posterior base + secondary branch	19	10.0–14.3	*37.4 – 47.2*	12.2	*42.6*	1.5	*3.1*
Posterior spur	12	3.2–7.0	*12.1 – 19.2*	4.4	*15.7*	1.1	*2.4*
Posterior branches length ratio	19	68%–86%	–	76%	–	5%	–

Six peribuccal lamellae and six peribuccal plus two lateral papillae present; medio-ventral peribuccal papilla reduced in senior specimens (Fig. 21B black arrowhead). Buccal tube (Figs 19A, 21A) from slightly funnel-shaped to almost cylindrical (posterior/anterior width ratio 80–98%), wider in senior specimens; *pt* of stylet support insertion point on the buccal tube [*65.5–75.1*]. Stylet furcae relatively large (Figs 19A, 21A).

Claws (Figs 19B–D, 21C–E) of the *Milnesium* type with configuration [3-3]-[3-3]. Secondary branches of legs IV more robust than on legs I–III (this difference is less marked in senior specimens; compare Fig. 19B–C with 19D, and Fig. 21C–D with 21E); basal spurs of internal claws of legs I–III and anterior claws of legs IV more developed than external I–III and posterior IV (Tables 8 and 9), but this difference may not be visible depending on claw position. Secondary branches with basal thickenings ('lunulae', Figs 19B–C, 21E, white arrowheads), primary branches with very small accessory points (Figs 19B, 21C, black arrows); cuticular bars present on legs I–III (Figs 19C, 21C, black arrowheads). Percentual ratio of secondary branches with respect to primary branches for each couple slightly higher for legs I than for all other legs (for all specimens collectively, legs I have 66–95% vs legs II–IV 62–86%; Tables 8–10); the ratio for all legs is on average slightly lower in senior than in young specimens (for all legs collectively, young specimens have 66–95% vs 63–87% in senior; compare Table 8 with Table 9).

Young specimens (hatchlings or hatchlings plus second instar: 270–460 µm; Fig. 19, Table 8, Supp. file 4)

Medioventral peribuccal papilla similar in size to the others; buccal tube (Fig. 19A) more slender (*pt* of buccal tube standard width [*43.2–51.5*]).

Intermediate specimens (probably second or third instar: 476–568 µm; Table 10, Supp. file 6)

These specimens show intermediate metric characters between young and senior.

Senior specimens (probably from third or fourth instar on: 585–770 µm; Figs 20–21, Table 9, Supp. file 5)

Medio-ventral peribuccal papilla reduced (Fig. 21B black arrowhead; *pt* of such papilla [*9.8–12.5*] vs [*22.6–31.6*] of the other peribuccal papillae); buccal tube wider (*pt* of buccal tube standard width [*59.1–67.9*]; Fig. 21A).

Remarks

The medioventral peribuccal papilla is reduced in senior specimens (comments in Discussion). Morphometric data are given in Table 8 for young, Table 9 for senior, Table 10 for intermediate specimens; in Table 11 the statistically significant differences (through Student *t*-test) of overlapping *pt* ranges of claw heights between the new species and the similar ones.

Fig. 21. Buccal tube, peribuccal papillae and claws of senior specimens of *Milnesium quiranae* sp. nov. **A, C–E**. Holotype, ♀ (slide No. MCNS Tar.000024-3). **B**. Paratype, ♀ (slide No. UNLPam 560-1). **A**. Buccal tube and related structures. **B**. Reduced medioventral peribuccal papilla (black arrowhead). **C**. Leg II with its claws; the black arrow indicates the accessory points, the black arrowhead indicates the leg cuticular bar. **D**. Leg III with its claws. **E**. Legs IV with their claws, the white arrowhead indicates a lunule. Scale bars = 10 µm.

Differential diagnosis

Based on having smooth cuticle, *M. quiranae* sp. nov. belongs to the old *tardigradum* group (Michalczyk *et al*. 2012a, 2012b). The new species, lacking pseudopores and pseudoplates, and having three points on the secondary branches of all claws [3-3]-[3-3] and six peribuccal lamellae, is similar to *M. antarcticum* Tumanov, 2006; *M. asiaticum* Tumanov, 2006; *M. barbadosense* Meyer & Hinton, 2012; *M. bohleberi* Bartels, Nelson, Kaczmarek & Michalczyk, 2014; *M. brachyungue* Binda & Pilato, 1990; *M. burgessi* Schlabach, Donaldson, Hobelman, Miller & Lowman, 2018; *M. eurystomum* Maucci, 1991 (emended by Morek *et al.* 2020a); *M. longiungue* Tumanov, 2006; *M. minutum* Pilato & Lisi, 2016; *M. pseudotardigradum* Surmacz, Morek & Michalczyk, 2019 *M. sandrae* Pilato & Lisi, 2016; *M. shilohae* Meyer, 2015; *M. swansoni* Young, Chappell, Miller & Lowman, 2016; *M. tumanovi* Pilato, Sabella & Lisi, 2016; *M. validum* Pilato, Sabella, D'Urso & Lisi, 2017; *M. zsalakoae* Meyer & Hinton, 2010.

For more precise comparisons, we first compare only senior specimens of the new species with all mentioned similar species, apart from *M. minutum*, which was very probably described based on young specimens and is therefore compared with the young of *M. quiranae* sp. nov.; senior specimens of *M. quiranae* differ from:

1. *Milnesium antarcticum*, only known from the type locality in Antarctica, by different body colour: reddish in *M. quiranae* sp. nov. vs reddish-brown in *M. antarcticum*; different buccal tube width: higher *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*35.4–43.9*] in *M. antarcticum*; different *pt* of many claw heights: external primary and secondary branches I [*45.6–54.9*] and [*34.2–41.8*] in *M. quiranae* vs [*34.0–43.2*] and [*22.9–28.4*] respectively in *M. antarcticum*; external primary and secondary branches III [*49.3–58.3*] and [*36.2–43.1*] in *M. quiranae* vs [*38.5–45.7*] and [*18.9–21.1*] respectively in *M. antarcticum*; posterior primary and secondary branches IV [60.1–69.7] and [*44.0–51.7*] in *M. quiranae* vs [*49.8–55-9*] and [*28.0–34.3*] respectively in *M. antarcticum*.

2. *Milnesium asiaticum* only known from the type locality in Kyrgyz Republic (Central Asia), by different body colour: reddish in *M. quiranae* sp. nov. vs slightly reddish or white in *M. asiaticum*; higher *pt* of buccal tube standard width [*59.1–67.9*] in *M. quiranae* vs [*30.0–41.6*] in *M. asiaticum*; higher *pt* of stylet supports insertion point [*67.5–73.6*] in *M. quiranae* vs [*63.9–66.9*] in *M. asiaticum*. Statistically significant differences about *pt* of several claw heights (Table 11).

3. *Milnesium barbadosense* known from the type locality in Barbados Islands (Caribbean Sea) and from Mexico (Moreno-Talamantes *et al.* 2019, 2020); by different body colour: reddish in *M. quiranae* sp. nov. vs white or transparent in *M. barbadosense*; eyes present in *M. quiranae* vs absent in *M. barbadosense*; different buccal tube width: higher *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*27.2–49.7*] in *M. barbadosense*; statistically significant differences about *pt* of many claw heights (Table 11).

4. *Milnesium bohleberi*, only known from the type locality in USA, by different body colour: reddish in *M. quiranae* sp. nov. vs white or transparent in *M. bohleberi*; eyes present in *M. quiranae* vs absent in *M. bohleberi*; statistically significant higher *pt* of the peribuccal papillae [*22.6–31.6*, mean *25.8*] in *M. quiranae* vs [*27.2–32.3*, mean *30.1*] in *M. bohleberi* (t_7 = -4.42, $p < 0.005$); statistically significant differences about *pt* of buccal tube anterior width [*57.9–66.1*, mean *62.5*] in *M. quiranae* vs [*63.4–74.7*, mean *68.1*] in *M. bohleberi* (t_{15} = -5.12, $p < 0.001$); statistically significant differences about *pt* of several claw heights (Table 11).

5. *Milnesium brachyungue*, known from Chile (type locality) and Colombia (Londoño *et al.* 2015); by different body colour: reddish in *M. quiranae* sp. nov. vs transparent in *M. brachyungue*; different buccal tube width: higher *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*37.0*] in *M. brachyungue*;

Table 9 (continued on next page). Measurements (in µm) and *pt* values of selected morphological structures of senior specimens of *Milnesium quiranae* sp. nov. mounted in polyvinyl-lactophenol medium. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: N = number of specimens or structures measured; SD = standard deviation.

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Body length	14	585 – 770	*1453 – 1871*	675	*1628*	55	*119*	585	*1453*
Peribuccal papillae length	6	9.2 – 13.0	*22.6 – 31.6*	10.8	*26.1*	1.6	*3.6*	?	*?*
Ventral peribuccal papillae length	4	4.2 – 5.0	*9.8 – 12.5*	4.8	*11.5*	0.4	*1.2*	?	*?*
Lateral papillae length	14	7.2 – 11.2	*18.8 – 27.2*	8.9	*21.5*	1.0	*2.3*	8.3	*20.6*
Buccal tube									
Length	14	38.3 – 43.2	–	41.4	–	1.5	–	40.3	–
Stylet support insertion point	14	26.6 – 30.5	*67.5 – 73.6*	29.2	*70.4*	1.1	*1.6*	29.0	*72.0*
Anterior width	14	23.8 – 28.1	*57.9 – 66.1*	25.9	*62.4*	1.2	*2.9*	26.6	*66.1*
Standard width	14	24.3 – 29.1	*59.1 – 67.9*	26.8	*64.7*	1.5	*3.2*	24.7	*61.4*
Posterior width	14	21.2 – 26.0	*52.3 – 61.6*	23.9	*57.6*	1.5	*3.3*	21.2	*52.7*
Standard width/length ratio	14	59% – 68%	–	65%	–	3%	–	61%	–
Posterior/anterior width ratio	14	80% – 97%	–	92%	–	4%	–	80%	–
Claw 1 heights									
External primary branch	14	18.6 – 22.6	*45.6 – 54.9*	20.4	*49.2*	1.1	*2.9*	18.6	*46.2*
External base + secondary branch	14	13.8 – 17.5	*34.2 – 41.8*	16.3	*39.4*	1.1	*2.1*	13.8	*34.2*
External spur	11	4.6 – 6.8	*10.6 – 16.5*	5.4	*12.9*	0.7	*1.7*	5.0	*12.4*
External branches length ratio	14	74% – 87%	–	80%	–	4%	–	74%	–
Internal primary branch	14	17.6 – 21.4	*43.2 – 52.0*	19.3	*46.6*	1.1	*2.8*	17.6	*43.7*
Internal base + secondary branch	14	12.3 – 16.4	*30.6 – 38.9*	15.2	*36.7*	1.2	*2.3*	12.3	*30.6*
Internal spur	12	6.4 – 8.5	*15.0 – 20.7*	7.3	*17.7*	0.5	*1.5*	7.6	*18.9*
Internal branches length ratio	14	70% – 86%	–	79%	–	4%	–	70%	–
Claw 2 heights									
External primary branch	14	19.0 – 23.4	*47.2 – 56.6*	21.8	*52.6*	1.5	*3.1*	20.7	*51.4*
External base + secondary branch	14	14.5 – 17.6	*36.4 – 42.5*	16.3	*39.4*	1.0	*1.9*	14.8	*36.8*
External spur	10	5.4 – 8.0	*12.6 – 19.4*	6.8	*16.5*	0.8	*2.1*	?	*?*
External branches length ratio	14	71% – 81%	–	75%	–	3%	–	72%	–
Internal primary branch	14	18.9 – 23.0	*46.3 – 54.9*	21.1	*51.0*	1.4	*2.8*	20.0	*49.7*
Internal base + secondary branch	14	14.6 – 16.5	*35.5 – 40.2*	15.6	*37.8*	0.6	*1.5*	14.6	*36.2*
Internal spur	12	6.6 – 9.7	*16.2 – 23.5*	8.2	*19.8*	1.1	*2.4*	7.6	*18.9*
Internal branches length ratio	14	68% – 80%	–	74%	–	4%	–	73%	–
Claw 3 heights									
External primary branch	13	20.5 – 24.8	*49.3 – 58.3*	22.5	*54.2*	1.4	*3.3*	?	*?*
External base + secondary branch	14	15.0 – 18.0	*36.2 – 43.1*	16.4	*39.6*	1.1	*2.3*	15.3	*38.0*
External spur	12	5.0 – 8.0	*11.6 – 19.4*	6.5	*15.6*	0.9	*2.3*	5.8	*14.3*
External branches length ratio	13	63% – 81%	–	74%	–	4%	–	?	–
Internal primary branch	14	19.5 – 23.1	*46.0 – 56.0*	21.3	*51.4*	1.2	*2.9*	20.4	*50.5*

Table 9 (continued). Measurements (in µm) and *pt* values of selected morphological structures of senior specimens of *Milnesium quiranae* sp. nov. mounted in polyvinyl-lactophenol medium.

Character	N	Range		Mean		SD		Holotype	
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*
Internal base + secondary branch	13	14.0 – 16.5	*34.8 – 40.2*	15.3	*37.1*	0.8	*1.7*	14.0	*34.8*
Internal spur	14	7.5 – 9.8	*17.9 – 23.3*	8.5	*20.5*	0.8	*1.9*	9.4	*23.3*
Internal branches length ratio	13	66% – 77%	–	72%	–	4%	–	69%	–
Claw 4 heights									
Anterior primary branch	14	23.5 – 27.5	*56.4 – 65.6*	25.7	*61.9*	1.5	*2.7*	23.6	*58.7*
Anterior base + secondary branch	14	17.1 – 20.4	*42.4 – 49.6*	18.9	*45.5*	0.9	*2.2*	17.1	*42.5*
Anterior spur	13	7.5 – 9.8	*17.8 – 23.7*	8.6	*20.7*	0.8	*1.9*	8.2	*20.4*
Anterior branches length ratio	14	67% – 79%	–	74%	–	3%	–	72%	–
Posterior primary branch	14	26.0 – 28.9	*60.1 – 69.7*	27.3	*65.9*	1.1	*2.8*	26.0	*64.6*
Posterior base + secondary branch	14	18.0 – 21.3	*44.0 – 51.7*	19.5	*47.0*	1.1	*2.4*	18.0	*44.6*
Posterior spur	11	6.4 – 8.1	*15.4 – 20.7*	7.0	*16.9*	0.6	*1.6*	?	*?*
Posterior branches length ratio	14	68% – 76%	–	71%	–	2%	–	69%	–

different *pt* of many claw heights: external primary and secondary branch I [45.6–54.9] and [34.2–41.8] in *M. quiranae* vs [*22.8*] and [*22.8*] respectively in *M. brachyungue*; external primary and secondary branch II [*47.6–56.6*] and [*36.4–42.5*] in *M. quiranae* vs [*24.5*] and [*23.9*] respectively in *M. brachyungue*; external primary and secondary branch III [*49.3–58.3*] and [*36.2–43.1*] in *M. quiranae* vs [*27.1*] and [*23.7*] respectively in *M. brachyungue*; posterior primary and secondary branch IV [*60.1–69.7*] and [*44.0–51.7*] in *M. quiranae* vs [*33.1*] and [*24.6*] respectively in *M. brachyungue*.

6. *Milnesium burgessi* only known from the type locality in USA; by different body colour: reddish in *M. quiranae* sp. nov. vs transparent to yellow in *M. burgessi*; pseudoplates absent in the new species vs present in *M. burgessi*; statistically significant differences of *pt* of all branches I–IV in *M. quiranae* (Table 11) including anterior primary branch IV [*56.4–65.6*] in *M. quiranae* vs [*70.7–89–6*] in *M. burgessi* (statistical significance not calculated due to completely separate ranges).

7. *Milnesium eurystomum* known from Greenland (type locality), from Argentina and Chile (Maucci 1996), Mongolia (Kaczmarek & Michalczyk 2006), Arkansas, USA (Land *et al.* 2012), Alaska, USA (Johansson *et al*. 2013), Norway, United Kindong (Scotland) (Morek *et al.* 2020a), by different body colour: reddish in *M. quiranae* vs brownish in *M. eurystomum*; no evident ontogenetic shape change of the buccal tube in *M. quiranae* (which remains more or less cylindrical) vs marked ontogenetic shape change in *M. eurystomum* (the tube becomes definitely funnel-shaped); statistically significant higher buccal tube posterior/anterior width ratio, 80–97%, mean 92% in *M. quiranae* vs 53–92%, mean 75% in *M. eurystomum* ($t_{15} = 4.72$, $p < 0.001$); statistically significant higher *pt* of stylet supports insertion point [*67.5–73.6*, mean *70.5*] in *M. quiranae* vs [*60.3–69.8*, mean *65.1*] in *M. eurystomum* ($t_{15} = 4.51$, $p < 0.001$); different *pt* of external primary branch I and III [*45.6–54.9*] and [*49.3–58.3*] in *M. quiranae* vs [*30.3–43.3*] and [*31.8–48.1*] respectively in *M. eurystomum*; internal primary branch II [*46.3–54.9*] in *M. quiranae* vs [*30.5–45.6*] in *M. eurystomum* posterior primary branch IV [*60.1–69.7*] in *M. quiranae* vs [*35.4–58.9*] in *M. eurystomum*; statistically significant differences of *pt* of many other claw heights (Table 11).

Table 10 (continued on next page). Measurements (in µm) and *pt* values of selected morphological structures of intermediate specimens of *Milnesium quiranae* sp. nov. mounted in polyvinyl-lactophenol medium. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: N = number of specimens or structures measured; SD = standard deviation.

Character	N	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	10	476 – 568	*1240 – 1511*	539	*1415*	32	*86*
Peribuccal papillae length	5	7.7 – 8.3	*20.1 – 21.9*	8.0	*21.1*	0.2	*0.8*
Lateral papillae length	8	6.0 – 8.0	*15.0 – 21.1*	6.6	*17.3*	0.7	*1.9*
Buccal tube							
Length	10	37.0 – 40.0	–	38.1	–	0.8	–
Stylet support insertion point	10	25.8 – 27.0	*65.5 – 71.1*	26.2	*68.8*	0.3	*1.5*
Anterior width	10	18.4 – 21.5	*49.0 – 56.1*	20.3	*53.4*	1.0	*2.6*
Standard width	10	19.3 – 23.3	*52.2 – 60.7*	21.4	*56.1*	1.2	*2.6*
Posterior width	10	17.7 – 19.8	*44.3 – 52.1*	18.5	*48.6*	0.8	*2.5*
Standard width/length ratio	10	52% – 61%	–	56%	–	3%	–
Posterior/anterior width ratio	10	85% – 98%	–	91%	–	4%	–
Claw 1 lengths							
External primary branch	10	15.4 – 18.3	*41.6 – 48.1*	17.5	*45.9*	0.8	*1.9*
External base + secondary branch	9	12.6 – 13.6	*33.3 – 35.8*	13.2	*34.7*	0.3	*0.9*
External spur	5	4.2 – 6.3	*11.1 – 16.4*	4.9	*12.8*	0.8	*2.1*
External branches length ratio	9	72% – 82%	–	76%	–	3%	–
Internal primary branch	9	14.4 – 18.1	*38.8 – 47.6*	16.8	*44.2*	1.1	*2.5*
Internal base + secondary branch	10	12.0 – 15.1	*31.0 – 39.7*	12.7	*33.3*	0.9	*2.4*
Internal spur	8	5.3 – 6.5	*14.1 – 17.0*	6.0	*15.7*	0.4	*1.0*
Internal branches length ratio	9	66% – 87%	–	74%	–	6%	–
Claw 2 lengths							
External primary branch	10	17.0 – 19.8	*45.9 – 51.1*	18.6	*48.9*	0.9	*1.8*
External base + secondary branch	10	12.5 – 14.4	*33.2 – 37.8*	13.5	*35.4*	0.7	*1.4*
External spur	9	4.6 – 7.0	*12.2 – 18.4*	5.3	*13.9*	0.8	*2.0*
External branches length ratio	10	65% – 75%	–	72%	–	3%	–
Internal primary branch	10	16.7 – 19.0	*45.2 – 50.0*	18.2	*47.8*	0.7	*1.8*
Internal base + secondary branch	10	12.0 – 14.4	*31.9 – 37.9*	13.6	*35.7*	0.7	*1.6*
Internal spur	9	5.2 – 7.0	*13.7 – 18.6*	6.3	*16.6*	0.5	*1.5*
Internal branches length ratio	10	66% – 79%	–	75%	–	4%	–
Claw 3 lengths							
External primary branch	10	17.2 – 20.8	*46.5 – 52.6*	19.2	*50.4*	1.0	*2.0*
External base + secondary branch	10	12.5 – 14.8	*33.8 – 39.0*	13.9	*36.4*	0.8	*1.8*
External spur	8	5.0 – 7.8	*13.0 – 19.5*	5.8	*15.1*	1.0	*2.3*
External branches length ratio	10	67% – 79%	–	72%	–	4%	–
Internal primary branch	10	16.2 – 20.0	*43.9 – 52.6*	18.4	*48.4*	1.2	*2.7*
Internal base + secondary branch	9	12.5 – 14.5	*33.8 – 38.2*	13.5	*35.5*	0.6	*1.5*

Table 10 (continued). Measurements (in µm) and *pt* values of selected morphological structures of intermediate specimens of *Milnesium quiranae* sp. nov. mounted in polyvinyl-lactophenol medium.

Character	N	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Internal spur	10	6.4 –7.4	*16.7 – 19.5*	7.1	*18.7*	0.3	*0.9*
Internal branches length ratio	9	67% –79%	–	74%	–	4%	–
Claw 4 lengths							
Anterior primary branch	9	20.9 –23.1	*55.7 – 60.7*	22.3	*58.5*	0.9	*1.9*
Anterior base + secondary branch	10	13.9 –17.2	*37.2 – 45.3*	15.5	*40.7*	1.2	*2.6*
Anterior spur	8	5.6 –9.0	*15.1 – 23.5*	7.0	*18.3*	1.1	*2.7*
Anterior branches length ratio	9	62% –79%	–	70%	–	5%	–
Posterior primary branch	10	22.2 –26.0	*60.1 – 67.9*	24.6	*64.7*	1.2	*2.6*
Posterior base + secondary branch	10	14.7 –17.9	*39.7 – 47.1*	16.4	*43.0*	1.0	*2.2*
Posterior spur	5	5.0 –7.6	*13.2 – 20.2*	6.2	*16.4*	1.2	*3.2*
Posterior branches length ratio	10	64% –71%	–	66%	–	2%	–

8. *Milnesium longiungue*, only known from the type locality in India, by different body colour: reddish in *M. quiranae* sp. nov. vs white in *M. longiungue*; different buccal tube width: higher *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*33.8–59.1*] in *M. longiungue*; accessory points present in *M. quiranae* vs absent in *M. longiungue*; higher *pt* of the stylet support insertion point: [*67.5–73.6*] in *M. quiranae* vs [*59.1–66.7*] in *M. longiungue*; statistically significant differences about *pt* of external primary branch III (Table 11); lower *pt* of posterior primary branch IV [*60.1–69.7*] in *M. quiranae* vs [*81.8–92.4*] in *M. longiungue.*

9. *Milnesium pseudotardigradum*, only known from the type locality in Iceland, by different body colour: reddish in *M. quiranae* sp. nov. vs yellowish in *M. pseudotardigradum*; statistically significant higher *pt* of the peribuccal papillae [*22.6–31.6*, mean 25.8] in *M. quiranae* vs [*13.7–24.8*, mean *19.9*] in *M. pseudotardigradum* ($t_7 = 2.56$, $p < 0.05$); statistically significant higher *pt* of the lateral papillae [*18.8–27.2*, mean 21.5] in *M. quiranae* vs [*11.0–21.8*, mean *15.6*] in *M. pseudotardigradum*; $t_{15} = 3.83$, $p < 0.001$; different buccal tube width: *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*25.5–50.8*] in *M. pseudotardigradum*; claw configuration: [3-3]-[3-3] in all the specimens (young and senior specimens) in *M. quiranae* vs [3-3]-[3-3] only in hatchlings in *M. pseudotardigradum*; changes during the ontogeny of claw configuration are absent in *M. quiranae* vs with double change in *M. pseudotardigradum.*

10. *Milnesium sandrae*, only known from the type locality in Hawaii (USA), by different body colour, reddish in *M. quiranae* vs transparent in *M. sandrae*; different buccal tube width: *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*44.9–48.0*] in *M. sandrae*; *pt* of the stylet support insertion point: [*67.5–73.6*] in *M. quiranae* vs [*58.0–60.5*] in *M. sandrae*; by different *pt* of many claw heights: external primary branches I–III [*45.6–54.9*]-[*47.2–56.6*]-[*49.3–58.3*] in *M. quiranae* vs [*38.8–43.5*]-[*42.4–46.6*]-[*43.4–46.1*] respectively in *M. sandrae*; posterior primary and secondary branch IV [*60.1–69.7*] and [*44.0–51.7*] in *M. quiranae* vs [*54.0–57.1*] and [*38.0–40.2*] respectively in *M. sandrae*, and statistically significant differences of *pt* of external secondary branches I–III (Table 11).

11. *Milnesium shilohae*, only known from the type locality in Hawaii (USA), by different body colour: reddish in *M. quiranae* sp. nov. vs white or transparent in *M. shilohae*; statistically significant difference of *pt* of lateral papillae [*18.8–27.2*, mean *21.5*] in *M. quiranae* vs [*12.8–21.8*, mean *17.2*] in

Table 11 (continued on next page). Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of claw heights between senior specimens of *Milnesium quiranae* sp. nov. and the similar species.

Species / Character	***M. quiranae*** sp. nov.	***M. asiaticum***	***M. barbadosense***	***M. bohleberi***	***M. burgessi***	***M. eurystomum***	***M. longiungue***	***M. sandrae***	***M. shillohe***
Claw/LegI									
External primary branch	45.6–54.9 (49.3)		30.2–50.1 (41.0) $t_{15}=3.42$, $p< 0.01$		52.2–79.5 (62.7) $t_{15}=-4.92$, $p<0.001$				
External secondary branch	34.2–41.8 (39.5)	29.2–36.0 (32.4) $t_{15}=6.12$, $p< 0.001$	23.9–36.9 (30.7) $t_{15}=5.85$, $p< 0.001$		38.8–55.2 (45.2) $t_{15}=-3.54$, $p<0.001$	26.4–38.7 (31.7) $t_{15}=-4.85$, $p<0.001$		32.8–35.4 (34.1) $t_{15}=-8.69$, $p<0.001$	29.5–35.5 (32.7) $t_{15}=-8.01$, $p<0.001$
Internal primary branch	43.2–52.0 (46.6)				47.9–75.6 (60.0) $t_{15}=-4.83$, $p< 0.001$	28.5–44.9 (35.0) $t_{15}=-5.65$, $p<0.001$			
Internal secondary branch	30.6–38.9 (36.8)				36.7–53.7 (44.2) $t_{15}=-4.37$, $p< 0.001$				
Claw/Leg II									
External primary branch	47.2–56.6 (52.7)		29.8–59.6 (42.4) $t_{15}=2.68$, $p< 0.01$		46.1–70.2 (62.2) $t_{15}=-2.69$, $p< 0.01$	32.9–47.9 (38.4) $t_{15}=-7.37$, $p<0.001$			
External secondary branch	36.4–42.5 (39.3)		22.3–47.4 (29.6) $t_{15}=2.19$, $p< 0.05$		40.8–51.5 (45.4) $t_{15}=-5.38$, $p< 0.001$			33.3–37.8 (35.5) $t_{15}=-6.01$, $p<0.001$	
Internal primary branch	46.3–54.9 (51.2)				42.6–76.6 (59.6) $t_{15}=-2.26$, $p< 0.05$				
Internal secondary branch	35.5–40.2 (37.8)			28.4–33.5 (32.4) $t_{15}=7.68$, $p< 0.001$	35.2–52.3 (42.8) $t_{14}=-2.98$, $p< 0.01$				
Claw/Leg III									
External primary branch	49.3–58.3 (54.2)		30.0–54.9 (43.7) $t_{14}=3.71$, $p< 0.001$		49.7–76.1 (62.9) $t_{14}=-2.84$, $p< 0.01$		57.1–73.5 (65.4) $t_{14}=5.68$, $p<0.001$		

Table 11 (continued on next page). Statistically significant differences (through one-side Student *t*-tests) of overlapping *pt* ranges of claw heights between senior specimens of *Milnesium quiranae* sp. nov. and the similar species.

Species / Character	*M. quiranae* sp. nov.	*M. asiaticum*	*M. barbadosense*	*M. bohleberi*	*M. burgessi*	*M. eurystomum*	*M. longiungue*	*M. sandrae*	*M. shillohe*
External secondary branch	36.2–43.1 (39.5)	30.9–37.3 (34.1) t_{15}=5.90, p< 0.001	23.6–41.1 (30.4) t_{15}=3.70, p< 0.001		40.4–58.2 (48.2) t_{15}=–4.49, p< 0.001			34.7–36.7 (35.5) t_{15}=-5.77, p<0.001	29.7–37.1 (32.6) t_{13}=4.01, p<0.001
Internal primary branch	46.0–56.0 (51.5)				49.9–74.4 (61.7) t_{15}=–4.10, p< 0.001	30.3–48.7 (38.1) t_{15}=–5.91, p<0.001			
Internal secondary branch	34.8–40.2 (37.1)			30.7–35.8 (34.0) t_{13}=4.84, p< 0.001	36.5–51.3 (44.7) t_{13}=–3.43, p< 0.01				
Claw/LegIV									
Anterior primary branch	56.4–65.6 (62.0)					32.2–59.7 (43.7) t_{14}=4.77, p< 0.001			
Anterior secondary branch	41.4–49.6 (45.3)			35.7–41.4 (37.5) t_{15}=8.93, p< 0.001	41.1–61.6 (49.6) t_{15}=–2.06, p< 0.05	29.8–43.8 (36.7) t_{15}=–4.26, p<0.001			35.2–42.7 (39.0) t_{15}=4.88, p<0.001
Posterior primary branch	60.1–69.7 (65.7)	63.9–76.0 (69.7) t_{15}=–3.05, p< 0.01	35.5–66.3 (48.7) t_{15}=4.38, p< 0.001		66.6–96.2 (84.3) t_{15}=–4.82, p< 0.001				
Posterior secondary branch	44.0–51.7 (46.8)	36.8–46.7 (41.1) t_{15}=4.42, p< 0.001	24.3–42.5 (34.0) t_{15}=6.56, p< 0.001		44.5–60.5 (52.6) t_{15}=–3.26, p< 0.01	30.4–47.7 (37.2) t_{15}=–4.18, p< 0.001			35.9–44.3 (39.7) t_{15}=6.28, p<0.001

M. shilohae ($t_{15} = 3.73$, $p < 0.001$); different buccal tube width: lower *pt* of standard width, [*59.1–67.9*] in *M. quiranae* vs [*47.1–55.9*] in *M. shilohae*; lower *pt* of stylet support insertion point: [*67.5–73.6*] in *M. quiranae* vs [*75.5–77.5*] in *M. shilohae*; different *pt* of many claw heights: external primary branch I [*45.6–54.9*] in *M. quiranae* vs [*34.2–40.3*] in *M. shilohae*, external primary and secondary branches II [*47.2–56.6*] and [*36.4–42.5*] in *M. quiranae* vs [*37.4–44.1*] and [*28.2–35.9*] respectively in *M. shilohae*; internal primary and secondary branches II [*46.3–54.9*] and [*35.5–40.2*] in *M. quiranae* vs [*35.7–42.0*] and [*28.8–34.5*] respectively in *M. shilohae*; external primary branch III [*49.3–58.3*] in *M. quiranae* vs [*35.2–46.8*] in *M. shilahoe*; statistically significant differences of *pt* of external secondary branch III (Table 11); different *pt* anterior and posterior primary branches IV [*56.4–65.6*] and [*60.1–69.7*] in *M. quiranae* vs [*42.6–51.1*] and [*48.3–55.5*] respectively in *M. shilohae* and statistically significant differences about *pt* of external secondary branch I, external secondary branch III and anterior and posterior secondary branches IV (Table 11).

12. *Milnesium swansoni* only known from the type locality in USA; by different body colour: reddish in *M. quiranae* sp. nov. vs transparent to yellow in *M. swansoni*; higher *pt* of buccal tube standard width [*59.1–67.9*] in *M. quiranae* vs [*39.2–42.2*] in *M. swansoni*, statistically significant higher *pt* of the stylet support insertion point: [*67.5–73.6*] in *M. quiranae* vs [*66.6–68.2*] in *M. swansoni* ($t_{15} = 7.39$, $p < 0.001$).

13. *Milnesium tumanovi* only known from the type locality in Yalta (Crimea) by body colour: reddish in *M. quiranae* sp. nov. vs transparent in *M. tumanovi*; different buccal tube width: higher *pt* of standard width [*59.1–67.9*], in *M. quiranae* vs [*55.1*] in *M. tumanovi*; higher *pt* of the stylet support insertion point: [*67.5–73.6*] in *M. quiranae* vs [*52.3*] in *M. tumanovi*; different *pt* of the external primary branches I–III [*45.6–58.3*], [*47.2–56.6*] and [*49.3–58.3*] respectively in *M. quiranae* vs [*43.0*], [*43.4*] and [*32.6*] in *M. tumanovi*, external secondary branches I and III [*34.2–41.8*], [*36.2–43.1*] respectively in *M. quiranae* vs [*32.6*] and [*33.0*] respectively in *M. tumanovi*, posterior primary and secondary branch IV [*60.1–69.7*] and [*44.0–51.7*] in *M. quiranae* vs [*55.3*] and [*42.2*] respectively in *M. tumanovi.*

14. *Milnesium validum*, only known from the type locality in Antarctica, by different body colour, reddish in *M. quiranae* sp. nov. vs colourless in *M. validum*; different buccal tube width: higher *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*29.9–43.9*] in *M. validum*; higher *pt* of stylet support insertion point: [*67.5–73.6*] in *M. quiranae* vs [*62.0–65.1*] in *M. validum*; higher *pt* of several claw heights: external primary and secondary branches I [*45.6–54.9*] and [*34.2–41.8*] in *M. quiranae* vs [*36.0–38.3*] and [*25.4–28.6*] respectively in *M. validum*; external primary and secondary branches II [*47.2.-56.6*] and [*36.4–42.5*] in *M. quiranae* vs [*37.2–42.1*] and [*27.0–30.3*] respectively in *M. validum*; external primary and secondary branches III [*49.4–58.3*] and [*36.2–43.1*] in *M. quiranae* vs [*38.5–42.5*] and [*30.4*] respectively in *M. validum*, and posterior primary and secondary branches IV [*60.1–69.7*] and [*41.4–49.6*] in *M. quiranae* vs [*47.9–49.3*] and [*28.8–32.9*] respectively in *M. validum*.

15. *Milnesium zsalakoae* only known from the type locality in USA by different body colour: reddish in *M. quiranae* sp. nov. vs white or transparent in *M. zsalakoae*; different buccal tube width: *pt* of standard width [*59.1–67.9*] in *M. quiranae* vs [*36.8–41.9*] in *M. zsalakoae*; accessory points present in *M. quiranae* vs absent in *M. zsalakoae*; different *pt* of several claw heights: internal primary branches I–III [*43.2–52.0*], [*46.3–54.9*], [*46.0–56.0*] in *M. quiranae*. vs [*64.4–68.6*], [*64.7–80.4*], [*80.5–88.6*], respectively in *M. zsalakoae*; internal secondary branch I [*30.6–38.9*] in *M. quiranae* vs [*45.4–64.7*] in *M. zsalakoae* and anterior primary branch IV [*56.4–65.6*] in *M. quiranae* vs [*94.8–102.9*] in *M. zsalakoae.*

With regard to young and intermediate specimens of *M. quiranae* sp. nov., as already indicated, differences of senior specimens with the above mentioned species tend to remain valid, because the only ontogenetic changes in the new species pertain to the buccal tube width and the medioventral peribuccal papilla reduction; thus, apart from those two characters, the already provided differential diagnosis is valid also for young and intermediate specimens of *M. quiranae* (e.g., considering body colour, presence/absence of eyes, *pt* of stylet support insertion point and of claw heights). It is only worth mentioning that for *M. burgessi*, *M. longiungue*, *M. pseudotardigradum*, *M. sandrae*, *M. shilohae* and *M. tumanovi*, the buccal tube width is comparable with with young and/or intermediate specimens of *M. quiranae*, but in all cases there are the other differences that keep the species well differentiated. We here provide detailed comparison of young specimens of *M. quiranae* only with *M. minutum*, for the reasons expressed above.

Young specimens of *M. quiranae* sp. nov. differ from *M. minutum* (only known from the type locality in Italy) by different body colour: reddish in *M. quiranae* sp. nov. vs transparent in *M. minutum*; different buccal tube width: higher *pt* of buccal tube standard width [*43.2–51.5*] in *M. quiranae* vs [*38.6–42.4*] in *M. minutum*; higher *pt* of the stylet support insertion point: [*67.4–75.1*] in *M. quiranae* vs [*63.0–65.9*] in *M. minutum*; different *pt* of many claw heights: external primary and secondary branches I, [*39.8–49.8*] and [*31.2–40.6*] in *M. quiranae* vs [*39.1*] and [*28.3*] in *M. minutum*; external primary and secondary branches II [*45.0–54.5*] and [*33.5–43.3*] in *M. quiranae* vs [*42.2–44.3*] and [*29.5–31.4*] respectively in *M. minutum*; posterior secondary branches IV [*37.4–47.2*] in *M. quiranae* vs [*33.5–34.5*] in *M. minutum.*

Dichotomous key of the species of Milnesium Doyère, 1840 from South America

1. Dorsal cuticle smooth 2
– Dorsal cuticle ornamented (pseudopores-reticulum-dimples) 5

2. Claw configuration [2-2]-[2-2] .... ***M. kogui*** Londoño *et al*., 2015
– Claw configuration [3-3]-[3-3] 3

3. BT funnel-shaped in adults (posterior/anterior width ratio lower than 65%) ....
....***M. eurystomum*** Maucci, 1991 (amended by Michalczyk *et al*. 2012b; amended by Morek *et al*. 2020a)
– BT about cylindrical in adults (posterior/anterior width ratio higher than 75%) 4

4. *Pt* of BT standard width lower than 52 in adults .... ***M. brachyungue*** Binda & Pilato, 1990
– *Pt* of BT standard width higher than 52 in adults ....***M. quiranae*** sp. nov.

5. Claw configuration [3-3]-[3-3] 6
– Claw configuration different 7

6. Nine rows of pseudoplates, *pt* of SS [*63.8–66.7*]; *pt* of BT standard width [*58.1–65.6*] ....
....***M. beatae*** Roszkowska *et al*., 2015
– Without pseudoplates, *pt* of SS [*70.0–73.7*], *pt* of BT standard width [*24.2–32.3*] ....
.... ***M. argentinum*** Roszkowska *et al*., 2015

7. Claw configuration [2-2]-[2-2] ....***M. katarzynae*** Kaczmarek *et al*., 2004
– Claw configuration different 8

8. Claw configuration [2-3]-[2-2], *pt* of BT standard width [*46.1–56.9*], with 9 rows of pseudoplates ...
.... ***M. irenae*** sp. nov.
– Claw configuration [2-3]-[3-2] 9

9. Cuticular dimples arranged in 10 bands, small tubercles around all claw bases, *pt* of BT standard width [*55.2–64.0*], young specimens with claw configuration [2-2]-[2-2] .... ***M. pelufforum*** sp. nov.
– Cuticular dimples present not forming bands, *pt* of BT standard width [*33.0–38.0*], no tubercles around claw bases .... ***M. krzysztofi*** Kaczmarek & Michalczyk, 2007

Abbreviations: BT = buccal tube; SS = stylet support insertion point on the buccal tube.

Discussion

The discovery of the three new species of *Milnesium* described in the present paper provides an opportunity to comment on the species of this genus. In the past, ontogenetic changes were not known in the genus *Milnesium*, until the scientific contribution of Morek *et al.* (2016) describing *M. variefidum*, followed by Moreno-Talamantes *et al.* (2019, 2020), Surmacz *et al.* (2019), Morek *et al.* (2020a) and Sugiura *et al.* (2020). It is worth mentioning that two of the three species described in the present paper show ontogenetic changes: *M. pelufforum* sp. nov. shows changes in the dorsal cuticle characteristics, buccal tube width and claw configuration, while *M. quiranae* sp. nov. shows changes in buccal tube width and the medioventral buccal papilla reduction. All these recent discoveries lead to the suspicion that ontogenetic changes in the genus *Milnesium* may be rather common, but have not yet been reported for species already described. For example, about the ontogenetic change affecting the buccal tube width, young specimens with a more slender buccal tube and senior specimens with a wider one, were reported for *M. eurystomum*, *M. pseudotardigradum*, and here for *M. pelufforum* and *M. quiranae* We believe this could be a common situation, because for several species, the range of *pt* of the buccal tube width in the species description appears quite wide, possibly hiding the ontogenetic change under discussion. Those species are: *M. alpigenum* Ehrenberg, 1853 (*pt* of the buccal tube standard width *25.5–42.3*, specimen size 367–877 µm; Morek *et al*. 2016, 2019b), *M. barbadosense* (*pt* of the buccal tube standard width *27.2–49.7*, specimen size 283–686 µm; Meyer & Hinton 2012), *M. burgessi* Schlabach, Donaldson, Hobelman, Miller & Lowman, 2018 (*pt* of the buccal tube standard width *52.9–68.5*, specimen size 343–1030 µm; Schlabach *et al.* 2018), *M. reductum* Pilato, Binda & Lisi, 2002 (*pt* of the buccal tube standard width *20.0–39.8*, specimen size 363–730 µm; Morek *et al.* 2020b) and *M. tardigradum* (*pt* of the buccal tube standard width *18.4–51.8*, specimen size 278–789 µm; Morek *et al.* 2019a).

Even in some recent species descriptions/redescriptions that report ontogenetic change, metric characters of specimens of all sizes are reported together. This could hide important morphometric differences between young and senior specimens, e.g., for buccal tube width. Therefore, we strongly recommend that future species descriptions/redescriptions should always report separate morphometric characters for young and senior specimens.

We also suggest, for species in which ontogenetic change is verified, that the differential diagnosis treats young and senior specimens separately if possible and useful, with comparisons to similar species, in order to provide more precise, complete differential diagnosis which facilitates species distinction.

Another, more recent achievement (firstly thanks to Michalczyk *et al.* 2012a) regards the discovery of reduction of the medio-ventral peribuccal papilla. We found this in *M. irenae* sp. nov. and *M. quiranae* sp. nov. Maybe this trait might prove to be, with further investigations, less rare than it appears now. For the moment it has been reported (additionally to the two new species mentioned above) for *M. alpigenum*, *M. beasleyi*, *M. bohleberi*, *M. eurystomum*, *M. granulatum*, *M. inceptum* Morek, Suzuki, Schill, Georgiev, Yankova, Marley & Michalczyk, 2019b, *M. pacificum*, *M. tardigradum* and *M. variefidum*. In addition, we noticed that such papilla looks to be reduced also in *M. almatyense* (Morek *et al.* 2020b: fig. 2c), *M. berladnicorum* (Morek *et al*. 2016: fig. 6b) and *M. reductum* (Morek *et al.* 2020b: fig. 4b). It is also important to ascertain in the various species if ontogenetic reduction of such papilla may occur, like we found in *M. quiranae*.

About more detailed characters of the species described in the present paper, we find it interesting to discuss some features of the cuticular ornamentation of *M. pelufforum* sp. nov. and *M. irenae* sp. nov.

Milnesium pelufforum sp. nov. has ten bands of sculptured cuticle coinciding with ten rows of pseudoplates, while, until now, the maximum number of those bands/rows was nine. This because the new species has the first band and row in the first cephalic subsegment, where, until now, no band of sculpture or row of pseudoplates has been reported. This raises the need of updating the way to indicate the pseudoplate formula which was provided by Moreno-Talamantes *et al.* (2019). As a matter of fact, it is necessary from now on to deal with ten possible rows/bands, which is the maximum number possible considering there are ten subsegments on the entire body (head plus four trunk segment, each one divided into two subsegments at least regarding the cuticle). We therefore propose to always indicate all ten rows for correct comparison between species, according to Table 3.

Milnesium pelufforum sp. nov. also shows cuticular dimples provided with internal structures of variable appearance. Based on the literature, no other species of the genus has been reported having these characters until now, but it is not possible to exclude that some species described in the past might have these characters which passed unnoticed. Further investigations are required to ascertain whether presence and shape of the dimple internal structures are constant. Under PCM, we could not spot those internal structures inside all dimples, and their shape appeared variable; however, we cannot exclude that this is just due to matter of focus under the microscope and position of the structures in the preparation, so that those internal structures might be present in all dimples and always with the same morphology.

Regarding the cuticular sculpture of *M. irenae* sp. nov., with the today available data it looks one of the most complex ones within the genus. It also includes a reticulation which is different from all other 'reticular' sculptures of the other congeneric species. We mean that the latter ones actually have dimples close to one another (we ascertained this detail from the species descriptions and related photos) which gives the appearance of a reticulation. In *M. irenae*, instead, the reticulation does not look as consisting of dimples but of a delicate mesh appearing more similar to reticulations present in Parachela (e.g., several species of Isohypsibiidae and Doryphoribiidae). Lisi (2011) described the reticulation of some species of Doryphoribiidae as consisting of cuticular ridges delimiting a mesh, and this looks exactly the case for *M. irenae.* Until now, the only similar case could be the delicate reticulation of the hatchlings of *M. almatyense* (see Morek *et al.* 2020b). It is clear that deeper investigations, especially using SEM, are needed (including for the new species described in the present paper) to clarify the still pending problems.

Conclusion

Valuable data were obtained from the review of a limited number of slides of the tardigrade collection deposited at the National University of La Pampa, containing 10 870 specimens in total. This shows how important it is to investigate and describe in detail biological archives that represent the biodiversity of the natural heritage of a given place and time; such work, based on descriptive morphology of the species, can also provide contributions for deciphering both intra- and interspecific variability.

Thanks to the analysis of specimens of the genus *Milnesium* deposited in the above mentioned collection, three previously unknown species were described, so that the number of species in this genus in South America increases from 8 to 11 and in Argentina in particular from 5 to 8. The results of this research also contribute to gain knowledge about the family Milnesiidae (e.g., about morphological details and ontogenetic aspects), and provide information about faunistics of tardigrades of the southern hemisphere, as yet insufficiently known.

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Supplementary material

For all the following Excel files (1–6), the Excel template "Apochela" (ver. 1.4) from the tardigrade Register (Michalczyk & Kaczmarek 2013) was used.

Supp. file 1. Measurements of *Milnesium pelufforum* young. Complete morphometric dataset of the young specimens of *Milnesium pelufforum* sp. nov. (from which Table 1 in the paper derives) (Excel file). https://doi.org/10.5852/ejt.2022.822.1807.6929

Supp. file 2. Measurements of *Milnesium pelufforum* senior. Complete morphometric dataset of the senior specimens of *Milnesium pelufforum* sp. nov. (from which Table 2 in the paper derives) (Excel file). https://doi.org/10.5852/ejt.2022.822.1807.6931

Supp. file 3. Measurements of *Milnesium irenae*. Complete morphometric dataset of all specimens of *Milnesium irenae* sp. nov. (from which Table 6 in the paper derives) (Excel file). https://doi.org/10.5852/ejt.2022.822.1807.6933

Supp. file 4. Measurements of *Milnesium quiranae* young. Complete morphometric dataset of the young specimens of *Milnesium quiranae* sp. nov. (from which Table 8 in the paper derives) (Excel file). https://doi.org/10.5852/ejt.2022.822.1807.6935

Supp. file 5. Measurements of *Milnesium quiranae* senior. Complete morphometric dataset of the senior specimens of *Milnesium quirane* sp. nov. (from which Table 9 in the paper derives) (Excel file).
https://doi.org/10.5852/ejt.2022.822.1807.6937

Supp. file 6. Measurements of *Milnesium quiranae* intermediate. Complete morphometric dataset of the intermediate specimens of *Milnesium quiranae* sp. nov. (from which Table 10 in the paper derives) (Excel file). https://doi.org/10.5852/ejt.2022.822.1807.6939

Supp. file 7. Supplementary figure – statistics for life stages. Figure illustrating the results of the analysis of Surmacz *et al.* (2020) for detection of different life stages (through correlation between growth in body length and buccal tube length) applied to the entire population of each new species of the present paper. **A**. *Milnesium pelufforum* sp. nov.. Two evident clusters corresponding to young and senior specimens were outlined. **B**. *Milnesium quiranae* sp. nov. Three evident clusters corresponding to young, intermediate and senior specimens were outlined. **C**. *Milnesium irenae* sp. nov. No cluster was distinguishable with the available dataset (png file). https://doi.org/10.5852/ejt.2022.822.1807.6941