Roszkowska et al. Zoological Studies (2015) 54:12
DOI 10.1186/s40555-014-0082-7

Zoological Studies
a SpringerOpen Journal

RESEARCH Open Access

The genus *Milnesium* Doyère, 1840 (Tardigrada) in South America with descriptions of two new species from Argentina and discussion of the feeding behaviour in the family Milnesiidae

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Abstract

Background: The diversity and distribution of the tardigrades in South America are rather poor and selective, as is information about their feeding behaviour and diet. To date, only ca. 210 tardigrade taxa have been reported from the region of South America. In the present paper, we provide an update of the distribution of the genus *Milnesium* in South America and discuss some aspects of the feeding behaviour in the family Milnesiidae.

Results: In seven moss samples collected in the Argentinean province of Río Negro, 31 specimens, 4 exuviae and 32 eggs belonging to the genus *Milnesium* were found. Among them, four species were identified: *Milnesium argentinum* sp. nov., *Milnesium beatae* sp. nov., *Milnesium brachyungue* and *Milnesium granulatum*. By its dorsal sculpture, *M. argentinum* sp. nov. is most similar to *M. beatae* sp. nov., *Milnesium beasleyi* and *Milnesium berladnicorum*, but it differs from *M. beasleyi* and *M. berladnicorum* mainly by having a different claw configuration and from *M. beatae* and *M. beasleyi* by having stylet supports inserted in a more caudal position and by some other morphometric characters. In the width of its buccal tube and the claw configuration [3-3]-[3-3], *M. beatae* sp. nov. is most similar to *Milnesium bohleberi*, *M. brachyungue* and *Milnesium eurystomum*, but it differs from them mainly by having a sculptured dorsal cuticle and by some other morphometric characters.

Conclusions: The study discusses distribution and taxonomic problems of the *Milnesium* species known from South America. As of now, nine *Milnesium* taxa are known from this region (including two new species reported in this paper). Additionally, the study broadens our knowledge of tardigrades' feeding behaviour, provides some details about their diet and suggests that the type of prey chosen by some species belonging to the family Milnesiidae may be associated with the width of their buccal tube.

Keywords: Milnesiidae; *Milnesium argentinum* sp. nov; *Milnesium beatae* sp. nov; Nahuel Huapi National Park; Neotropical region; New records; Prey selection; Río Negro; Taxonomy; Water bears

Background

South America, with an area of almost 18 million km^2, is located mostly in the Southern Hemisphere, with a relatively small portion (ca. 10%) in the Northern Hemisphere. It includes 12 sovereign countries. Geographically, the western part of South America is dominated by the Andes while the eastern part contains both highland regions and large river basins such as the Amazon, Paraná and Orinoco. Most of the continent lies in the tropics and the entire South American territory belongs to Neotropical ecozone (Peel et al. 2007, Holt et al. 2013).

Argentina, located in southern part of the continent, shares land borders with Chile across the Andes to the west, Bolivia and Paraguay to the north, Brazil to the northeast, Uruguay and the South Atlantic Ocean to the east and the Drake Passage to the south. Argentina is the eighth largest country in the world and the second

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largest in Latin America. It is subdivided into 23 provinces and one autonomous city - Buenos Aires. The exceptionally diverse climate depends on the geographic regional division and ranges from tropical in the north to subpolar in the far continental south (Edwards 2008).

Río Negro, one of the 23 provinces of Argentina, is located at the northern edge of Patagonia. The central region of the province is dominated by a series of plateaus and isolated hills with altitude ranging from 600 to 1,000 m asl. Towards the west, the foothills of the Andes are dominated by a series of low valleys. The climate of the province is temperate at low elevations and very harsh in the highest Andean peaks (Edwards 2008).

The Phylum Tardigrada consists currently of ca. 1,200 species (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Vicente and Bertolani 2013; Degma et al. 2009-2014) that inhabit terrestrial and aquatic (freshwater and marine) environments throughout the world (Ramazzotti and Maucci 1983). Our knowledge of the diversity and distribution of South American water bears is poor and selective. To date, ca. 210 taxa (including seven *Milnesium* species) have been reported from this region (mostly from Argentina, Chile and Brazil) (McInnes 1994; Binda and Pilato 1994a,b, 1999a,b; Pilato and Binda 1994, 1996, 1998; Maucci 1996; Claps and Rossi 1997; Claxton 1998; Pilato and Patanè 1998; Dastych et al. 1998; Pilato et al. 1998, 2000, 2001, 2002, 2003, 2004; Dastych 1999a,b, 2000, Pilato 2000, 2007; Jerez Jaimes and Narváez Parra 2001; Nickel et al. 2001; Peluffo et al. 2002, 2007; Michalczyk and Kaczmarek 2003, 2004, 2005, 2006; Marley 2006; Moly de Peluffo et al. 2006; Degma et al. 2008; Kaczmarek and Michalczyk 2009; Rossi et al. 2009; Montoya et al. 2010; Lisi 2011; Guidetti et al. 2013; Meyer 2013; Kaczmarek et al. 2014a, b; Lisi et al. 2014; Melo et al. 2014).

The genus *Milnesium* (Doyère 1840) is known from many localities, from the Antarctic through tropical and temperate to Arctic regions (Michalczyk et al. 2012a,b). Since the genus was recently redescribed (Michalczyk et al. 2012a,b), many new records and species have been reported from various localities throughout the world (e.g. Kaczmarek et al. 2012a,b, 2014b; Meyer and Hinton 2012; Johansson et al. 2013; Hinton et al. 2013; Meyer et al. 2013, 2014; Shaw and Miller 2013; Trygvadóttir and Kristensen 2013; Bartels et al. 2014; Ciobanu et al. 2014a,b; Lisi et al. 2014; Zawierucha et al. 2014; Melo et al. 2014). Taking into consideration that some morphological characters were not mentioned in older records of the genus members, all the *Milnesium* records before 2012 should be verified (Michalczyk et al. 2012a,b). Until now in South America, only seven *Milnesium* species have been reported (see the 'Results' section). In this paper, two new species of this genus are described and illustrated. Additionally, two new records of *Milnesium* species for Argentina are given. The distribution and taxonomic problems of all *Milnesium* species known from South America are also discussed.

All *Milnesium* species, with their wide and relatively short buccal tube connected with a large pharynx without placoids, are considered carnivorous, but details of their diet are still very poorly known. They can feed on rotiferas, nematodes, other tardigrades or even on amoebas (e.g. Kinchin 1990; Suzuki 2003; Nelson et al. 2010; Miller and Williams 2012). In this paper, we discuss some details of the diet of *Milnesium* species (and more general the entire family Milnesiidae) in connection with the different constructions of the buccal tube.

Methods

Seven moss samples were collected from trees and rocks from various localities in Nahuel Huapi National Park (Patagonia, southern Argentina) in 2006 and 2012 by Dawid Diduszko, Łukasz Kaczmarek and Marta Prange (see the 'List of samples' section) and four species of the genus *Milnesium* were found. All samples were collected and examined for tardigrades using standard methods (Dastych 1980; Ramazzotti and Maucci 1983). After extraction, all specimens, exuviae and eggs were fixed and mounted on microscope slides in Hoyer's medium. Observations, measurements and photomicrographs were taken using phase contrast microscopy (PCM) (Olympus BX41 with digital camera ARTCAM-300Mi, Olympus Corporation, Shinjuku-ku, Japan). All measurements (determined with QuickPhoto Camera 2.3) are given in micrometres [μm].

Morphometric data were handled using the 'Apochela' ver. 1.1 template available from the Tardigrada Register (Michalczyk and Kaczmarek 2013). Structures were measured only if their orientations were suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. All measurements followed protocols in Tumanov (2006). Buccal tube width was measured at three points as suggested by Michalczyk et al. (2012a,b). The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage (Pilato 1981). The *pt* values are always provided in [square brackets and in *italics*]. Configuration of the number of claw points on the secondary branches ('claw configuration') is given according to Michalczyk et al. (2012a,b).

Species were identified using the keys in Michalczyk et al. (2012a,b) and other original descriptions/re-descriptions (Binda and Pilato 1990; Ramazzotti 1962; Kaczmarek et al. 2012a, b; Michalczyk et al. 2012a,b). Characteristics and measurements of the species used in the differential diagnosis are given according to the original descriptions (Ramazzotti 1962; Binda and Pilato 1990; Maucci 1991; Pilato et al. 2002; Kaczmarek et al. 2004, 2012a, b; Tumanov 2006; Kaczmarek and Michalczyk 2007;

Meyer and Hinton 2010, 2012; Michalczyk et al. 2012a, b; Bartels et al. 2014; Ciobanu et al. 2014b) or are based on direct examination of fixed specimens (holotype and paratypes of *M. beasleyi*, *M. berladnicorum*, *M. bohleberi*, *M. granulatum*, *M. katarzynae*, *M. krzysztofi* and specimens of *M. eurystomum* from Spitsbergen). Tardigrade taxonomy follows Marley et al. (2011). In the list of the species, we use Roman numerals to indicate the sample code (see the 'List of samples' and 'Results' sections) and Arabic numerals to designate the number of specimens and eggs (see the 'Results' section).

Raw data underlying the description of *Milnesium argentinum* sp. nov. and *Milnesium beatae* sp. nov. are deposited in the Tardigrada Register (Michalczyk and Kaczmarek 2013) under http://www.tardigrada.net/register/0015.htm and http://www.tardigrada.net/register/0016.htm, respectively.

List of samples

I. 41°20′S, 71°30′W, ca. 850 m asl: Río Negro, Nahuel Huapi National Park, Bariloche, Mirador Lago Mascardi, moss from rock, coll. Dawid Diduszko, 22 February 2012.
II. 41°12′S, 71°50′W, ca. 1,000 m asl: Río Negro, Nahuel Huapi National Park, Ventisquero Negro, car parking near small bar, *Nothofagus* forest, moss from rocks, coll. Łukasz Kaczmarek, 27 January 2006.
III. 41°12′S, 71°50′W, ca. 1,000 m asl: Río Negro, Nahuel Huapi National Park, Ventisquero Negro, car parking near small bar, *Nothofagus* forest, moss from rocks, coll. Łukasz Kaczmarek, 27 January 2006.
IV. 41°12′S, 71°50′W, ca. 1,000 m asl: Río Negro, Nahuel Huapi National Park, Ventisquero Negro, car parking near small bar, *Nothofagus* forest, moss from rocks, coll. Łukasz Kaczmarek, 27 January 2006.
V. 41°12′S, 71°50′W, ca. 1,000 m asl: Río Negro, Nahuel Huapi National Park, Ventisquero Negro, car parking near small bar, *Nothofagus* forest, moss from tree, coll. Łukasz Kaczmarek, 27 January 2006.
VI. 41°12′S, 71°28′W, ca. 1,400 m asl: Río Negro, Nahuel Huapi National Park, Bariloche, 1.5 km from Refugio Frey, *Nothofagus* forest, moss from stone, coll. Marta Prange, 26 January 2006.
VII. 41°13′S, 71°27′W, ca. 1,200 m asl: Río Negro, Nahuel Huapi National Park, on the trail to Refugio Frey, near to Van Titter stream, *Nothofagus* forest, moss from stone, coll. Marta Prange, 26 January 2006.

Results

Taxonomic account

Phylum: Tardigrada Doyère 1840.
Class: Eutardigrada Richters 1926.
Order: Apochela Schuster et al. 1980.
Family: Milnesiidae Ramazzotti 1962.
Genus: *Milnesium* Doyère 1840.

Milnesium argentinum sp. nov.

http://www.tardigrada.net/register/0015.htm.

Material examined: Eighteen females, all from Nahuel Huapi National Park, Río Negro, Argentina, mosses samples from rocks and stones, coll. Dawid Diduszko, Łukasz Kaczmarek and Marta Prange.

Type material: Holotype and three paratypes in sample VII.

Additional material: two specimens in sample I, ten specimens in sample III and two specimens in sample IV.

Diagnosis

Adults (Figure 1a) (measurements in micrometres, pt ratios and statistics in Table 1): The body rose before fixation and transparent afterwards, eyes present. Cuticle sculptured with pseudopores (0.4 to 0.7) not arranged in bands, sparsely distributed and not forming reticular design (Figure 2b). Six peribuccal papillae and six peribuccal lamellae around the mouth opening present. Two cephalic papillae positioned laterally. Peribuccal and cephalic papillae similar in length.

Buccal apparatus of the *Milnesium* type (Figure 2a). Buccal tube rather narrow and long (standard width on average 27% of its length) and funnel-shaped, wider anteriorly (posterior diameter on average 80% of the anterior diameter). Pharyngeal bulb elongated, pear-shaped and without placoids or septulum.

Claws of the *Milnesium* type, slender (Figure 3a,b). Primary branches on all legs with small but distinct accessory points detaching from the branch at its greatest curvature (Figure 3b). Secondary branches with rounded basal thickenings (Figure 3b). All secondary branches on all legs with three points (claw configuration: [3-3]-[3-3]). Single, long transverse, cuticular bars under claws I to III present (Figure 3a).

Eggs: Oval, smooth and deposited in exuvium as in all other known *Milnesium* species.

Males not observed.

Locus typicus: Argentina, 41°13′S, 71°27′W, ca. 1,200 m asl., Río Negro Province, Nahuel Huapi National Park.

Etymology: The new species is named after the country of Argentina, where the species was collected.

Type depositories: The holotype and 14 paratypes are deposited in the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University in Poznań, Umultowska 89, Poznań, Poland; two paratypes are deposited at the Natural History Museum, University of Copenhagen Universitetsparken 15, DK-2100 Copenhagen, Denmark and one paratype is deposited at collection of

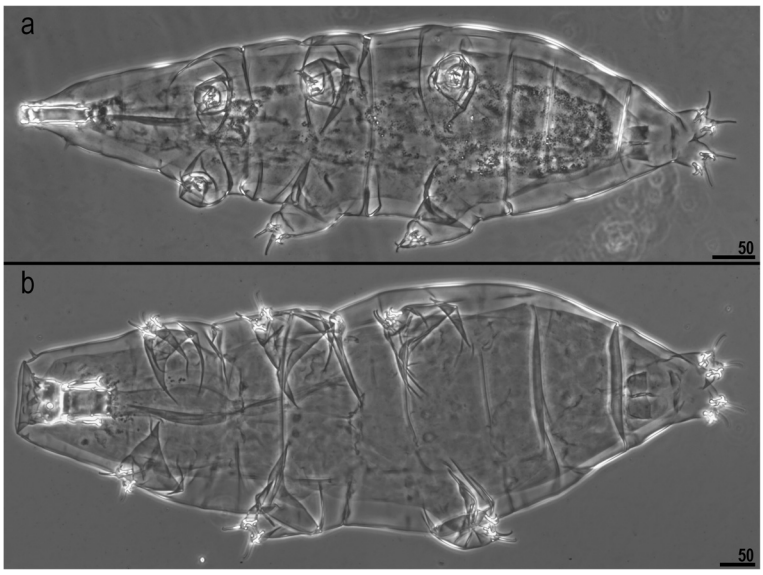

Figure 1 *Milnesium argentinum* sp. nov. and *Milnesium beatae* sp. nov. *Milnesium argentinum* sp. nov.: **(a)** habitus (ventral view) and *Milnesium beatae* sp. nov.: **(b)** habitus (ventral view) (both PCM).

Binda and Pilato, Museum of the Department of Animal Biology 'Marcello La Greca', University of Catania, Italy.

Differential diagnosis

Based on having a sculptured dorsal cuticle, *M. argentinum* sp. nov. belongs to the *granulatum* group (Michalczyk et al. 2012a,b). The new species with three points on the secondary branches of all claws (claw configuration [3-3]-[3-3]) is most similar to *M. beasleyi* Kaczmarek et al. 2012a, b, *M. beatae* sp. nov. and *M. berladnicorum* Ciobanu et al. 2014b, but it differs from the following:

1. *M. beasleyi*, known only from Turkey (Kaczmarek et al. 2012a, b) by different claw configuration ([3-3]-[3-3] in *M. argentinum* sp. nov. *vs.* [2-3]-[3-2] in *M. beasleyi*), presence of rounded basal thickenings under secondary branches of claws, stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*61.6-65.6*] in *M. beasleyi*) and slightly smaller claws I to IV (see Table 1 and Table one in Kaczmarek et al. (2012a, b) for the exact differences in dimensions of claws).
2. *M. beatae* sp. nov. by stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*63.8-66.7*] in *M. beatae* sp. nov.), smaller *pt* of width of buccal tube ([*30.3-39.8*], [*24.2-32.3*], [*23.1-33.6*] anterior, standard and posterior, respectively, in *M. argentinum* sp. nov. vs. [*70.3-78.9*], [*58.1-65.6*], [*49.4-56.5*] anterior, standard and posterior, respectively, in *M. beatae* sp. nov.) and smaller claws I to IV (see Tables 1 and 2 for the exact differences in dimensions of claws).
3. *M. berladnicorum*, known only from Romania (Ciobanu et al. 2014b) by different claw configuration ([3-3]-[3-3] in *M. argentinum* sp. nov. vs. [2-3]-[2-2] in *M. berladnicorum*) and smaller claws I to IV (see Table 1 and Table one in Ciobanu et al. (2014a, b) for the exact differences in dimensions of claws).

Besides the species mentioned above, *M. argentinum* sp. nov. is similar to other species of the genus *Milnesium* with the claw configuration [3-3]-[3-3] (*M. alabamae* Wallendorf and Miller 2009, *M. antarcticum* Tumanov 2006, *M. asiaticum* Tumanov 2006, *M. barbadosense* Meyer and Hinton 2012, *M. bohleberi* Bartels et al. 2014, *M. brachyungue* Binda and Pilato 1990, *M. eurystomum* Maucci 1991, *M. granulatum* Ramazzotti 1962, *M. longiungue* Tumanov 2006 and *M. zsalakoae* Meyer and Hinton 2010) or sculptured cuticle (*M. beasleyi* Kaczmarek et al. 2012a, b, *M. katarzynae* Kaczmarek et al. 2004, *M. krzysztofi* Kaczmarek and Michalczyk 2007 and *Milnesium reticulatum* Pilato et al. 2002).

The new species differs from the following:

4. *M. alabamae*, known only from the USA (Wallendorf and Miller 2009) by different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. argentinum* sp. nov. vs. pseudopores arranged in bands (especially in caudal region), densely distributed and forming a reticular design in *M. alabamae*), presence of accessory points on main branches of claws, presence of eyes and stylet

Table 1 Measurements and *pt* values of selected morphological structures of *Milnesium argentinum* sp. nov. mounted in Hoyer's medium

Character	N	Range						Mean		SD		Holotype	
		µm			*pt*			µm	*pt*	µm	*pt*	µm	*pt*
Body length	12	445	-	890	*921*	-	*1,290*	707	*1,093*	141	*123*	890	*1,211*
Peribuccal papillae length	10	6.7	-	12.4	*14.1*	-	*18.6*	10.3	*16.3*	1.7	*1.1*	11.6	*15.8*
Lateral papillae length	9	5.5	-	11.4	*9.4*	-	*17.6*	9.0	*14.1*	1.9	*2.3*	?	*?*
Buccal tube													
Length	12	47.6	-	74.2		-		64.3	-	8.6	-	73.5	-
Stylet support insertion point	12	35.1	-	52.3	*70.0*	-	*73.7*	46.0	*71.7*	5.8	*1.4*	52.3	*71.2*
Anterior width	12	16.0	-	29.5	*30.3*	-	*39.8*	22.3	*34.5*	4.3	*3.4*	29.1	*39.6*
Standard width	12	13.0	-	24.0	*24.2*	-	*32.3*	17.7	*27.4*	3.5	*2.8*	22.0	*29.9*
Posterior width	12	12.2	-	24.5	*23.1*	-	*33.6*	17.8	*27.6*	3.9	*3.4*	24.5	*33.3*
Standard width/length ratio	12	24%	-	32%		-		27%	-	3%	-	30%	-
Posterior/anterior width ratio	12	76%	-	87%		-		80%	-	4%	-	84%	-
Claw 1 lengths													
External primary branch	10	12.4	-	20.5	*24.3*	-	*28.6*	16.7	*26.3*	2.6	*1.5*	20.5	*27.9*
External base + secondary branch	9	10.5	-	17.8	*22.0*	-	*25.2*	14.4	*23.0*	2.4	*1.2*	17.8	*24.2*
External spur	8	3.7	-	5.8	*7.1*	-	*8.2*	4.8	*7.7*	0.8	*0.4*	?	*?*
Internal primary branch	11	12.9	-	18.7	*21.1*	-	*28.9*	15.8	*24.8*	2..1	*2.0*	17.4	*23.7*
Internal base + secondary branch	10	11.1	-	18.2	*21.1*	-	*26.1*	14.8	*23.2*	2.5	*1.5*	17.3	*23.5*
Internal spur	10	3.4	-	5.7	*7.1*	-	*8.8*	5.0	*7.8*	0.9	*0.6*	5.6	*7.6*
Claw 2 lengths													
External primary branch	10	14.3	-	20.5	*24.7*	-	*28.5*	17.8	*26.6*	2.0	*1.3*	20.5	*27.9*
External base + secondary branch	12	11.3	-	17.9	*22.1*	-	*26.3*	15.2	*23.7*	2.0	*1.3*	17.9	*24.4*
External spur	6	2.8	-	5.5	*5.9*	-	*8.5*	4.0	*6.7*	1.0	*1.0*	?	*?*
Internal primary branch	10	12.6	-	20.8	*23.1*	-	*30.5*	17.2	*26.4*	2.8	*2.5*	20.8	*28.3*
Internal base + secondary branch	11	11.6	-	16.5	*21.2*	-	*25.2*	14.8	*22.5*	1.6	*1.2*	16.1	*21.9*
Internal spur	9	3.8	-	6.3	*6.6*	-	*8.8*	5.3	*7.9*	0.9	*0.8*	6.3	*8.6*
Claw 3 lengths													
External primary branch	11	12.8	-	22.3	*23.4*	-	*30.3*	17.7	*27.3*	3.2	*2.1*	22.3	*30.3*
External base + secondary branch	10	11.9	-	17.1	*22.5*	-	*25.1*	14.9	*23.3*	2.1	*0.9*	17.1	*23.3*
External spur	7	3.3	-	5.3	*5.9*	-	*7.6*	4.5	*6.9*	0.8	*0.6*	?	*?*
Internal primary branch	11	13.9	-	21.4	*24.4*	-	*28.8*	17.5	*27.0*	2.6	*1.5*	20.7	*28.2*
Internal base + secondary branch	10	12.7	-	17.8	*20.3*	-	*25.7*	15.2	*23.1*	1.9	*1.4*	17.2	*23.4*
Internal spur	8	4.2	-	6.9	*6.8*	-	*9.9*	5.7	*8.5*	1.0	*1.1*	6.2	*8.4*
Claw 4 lengths													
Anterior primary branch	10	14.7	-	24.3	*29.9*	-	*33.8*	20.5	*31.8*	3.6	*1.3*	24.2	*32.9*
Anterior base + secondary branch	11	12.2	-	20.3	*24.4*	-	*28.8*	16.8	*26.0*	2.7	*1.4*	20.3	*27.6*
Anterior spur	11	3.8	-	7.9	*8.0*	-	*10.8*	6.3	*9.7*	1.2	*0.8*	7.3	*9.9*
Posterior primary branch	11	13.5	-	25.8	*28.4*	-	*36.4*	21.3	*33.0*	4.0	*2.7*	25.4	*34.6*
Posterior base + secondary branch	11	12.8	-	20.2	*25.0*	-	*28.3*	17.3	*26.8*	2.7	*1.0*	20.2	*27.5*
Posterior spur	8	3.3	-	6.2	*6.9*	-	*8.4*	4.9	*7.8*	1.0	*0.6*	6.2	*8.4*

Range refers to the smallest and the largest structure among all measured specimens.
The *pt* values are provided in *italics*.
N, number of specimens/structures measured; SD, standard deviation; ?, no data.

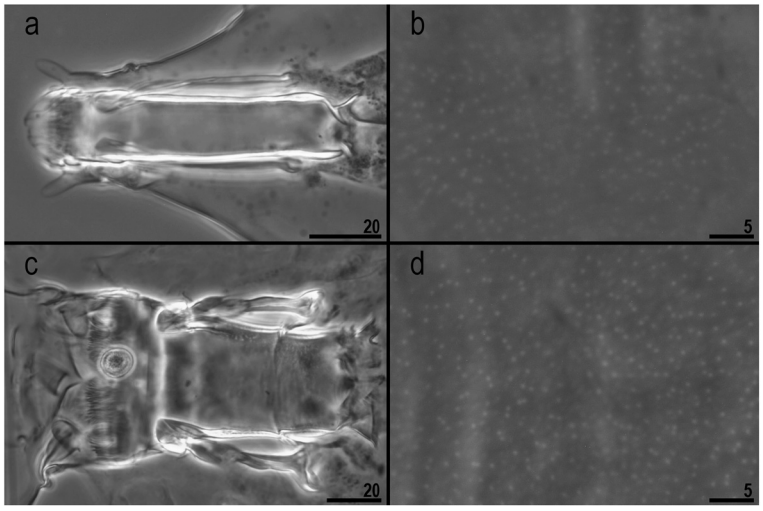

Figure 2 *Milnesium argentinum* sp. nov. and *Milnesium beatae* sp. nov. *Milnesium argentinum* sp. nov.: **(a)** buccal tube (ventral view); **(b)** dorsal cuticle with pseudopores and *Milnesium beatae* sp. nov.: **(c)** buccal tube (ventral view); **(d)** dorsal cuticle with pseudopores (all PCM).

supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*63.7*] in *M. alabamae* (in specimen 514.9 in length)).

5. *M. antarcticum*, known only from Antarctica (Tumanov 2006) by having sculptured dorsal cuticle and slightly smaller claws I to IV (see Table 1 and Table two in Tumanov (2006) for the exact differences in dimensions of claws).
6. *M. asiaticum*, known only from Kirghizia, Romania and Svalbard (Tumanov 2006; Kaczmarek et al. 2012a, b; Ciobanu et al. 2014a, b) by having sculptured dorsal cuticle and stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*63.9-66.9*] in *M. asiaticum*).
7. *M. barbadosense*, known only from Barbados (Meyer and Hinton 2012) by having sculptured dorsal

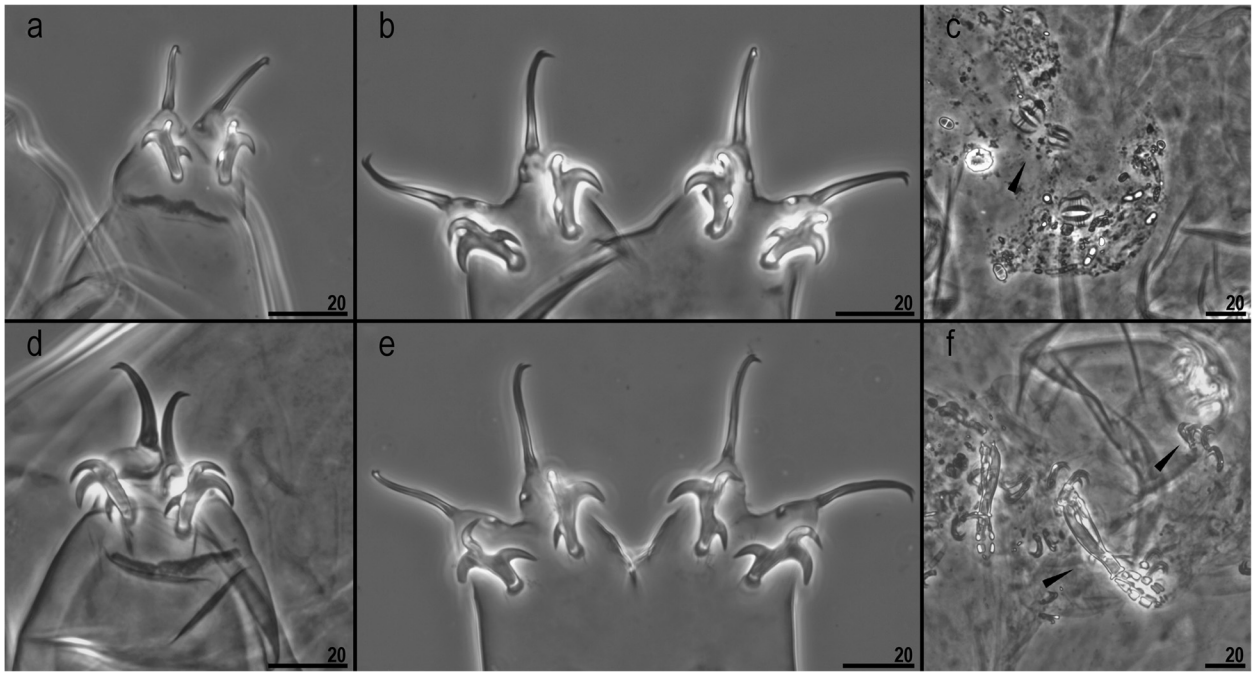

Figure 3 *Milnesium argentinum* sp. nov. and *Milnesium beatae* sp. nov. *Milnesium argentinum* sp. nov.: **(a)** claws II; **(b)** claws IV; **(c)** rotifer mastaxes in the gut (black arrowhead) and *Milnesium beatae* sp. nov.: **(d)** claws I; **(e)** claws IV; **(f)** tardigrade buccal apparatuses and claws in the gut (black arrowheads) (all PCM).

Table 2 Measurements and *pt* values of selected morphological structures of *Milnesium beatae* sp. nov. mounted in Hoyer's medium

Character	N	Range						Mean		SD		Holotype	
		μm			*pt*			μm	*pt*	μm	*pt*	μm	*pt*
Body length	7	662	-	1,011	*1,259*	-	*1,524*	864	*1,401*	144	*97*	979	*1,494*
Peribuccal papillae length	7	9.8	-	13.2	*18.0*	-	*23.6*	12.0	*19.7*	1.1	*1.9*	13.2	*20.2*
Lateral papillae length	6	10.0	-	14.8	*19.0*	-	*21.1*	12.8	*20.2*	1.6	*0.9*	13.2	*20.2*
Buccal tube													
Length	7	51.6	-	70.2		-		61.4	-	6.8	-	65.5	-
Stylet support insertion point	7	33.8	-	45.0	*63.8*	-	*66.7*	40.0	*65.1*	4.3	*1.1*	43.3	*66.1*
Anterior width	7	37.0	-	53.5	*70.3*	-	*78.9*	45.7	*74.3*	6.6	*3.7*	51.7	*78.9*
Standard width	7	32.0	-	42.5	*58.1*	-	*65.6*	38.0	*61.9*	4.2	*2.6*	39.2	*59.8*
Posterior width	7	25.7	-	39.0	*49.4*	-	*56.5*	33.1	*53.6*	5.2	*2.8*	37.0	*56.5*
Standard width/length ratio	7	58%	-	66%		-		62%	-	3%	-	60%	-
Posterior/anterior width ratio	7	69%	-	76%		-		72%	-	3%	-	72%	-
Claw 1 lengths													
External primary branch	7	17.9	-	26.4	*33.6*	-	*41.4*	22.0	*35.8*	3.1	*2.7*	22.0	*33.6*
External base + secondary branch	6	13.6	-	22.1	*26.4*	-	*34.6*	19.0	*30.9*	3.8	*3.0*	20.8	*31.8*
External spur	4	5.0	-	6.5	*8.0*	-	*10.3*	5.6	*9.2*	0.7	*1.0*	5.8	*8.9*
Internal primary branch	6	16.4	-	25.4	*31.2*	-	*39.8*	22.0	*34.9*	3.2	*3.0*	23.6	*36.0*
Internal base + secondary branch	6	13.9	-	22.3	*26.9*	-	*33.4*	19.1	*30.9*	3.8	*3.0*	21.5	*32.8*
Internal spur	5	4.4	-	7.4	*8.5*	-	*10.5*	5.8	*9.5*	1.2	*0.8*	?	*?*
Claw 2 lengths													
External primary branch	7	19.1	-	27.3	*36.9*	-	*42.4*	24.5	*39.8*	3.6	*2.3*	27.3	*41.7*
External base + secondary branch	6	14.8	-	22.9	*28.7*	-	*34.1*	19.4	*31.6*	3.4	*2.1*	21.1	*32.2*
External spur	5	5.5	-	7.9	*8.0*	-	*12.1*	6.3	*10.5*	1.1	*1.5*	7.9	*12.1*
Internal primary branch	6	18.6	-	25.4	*35.4*	-	*37.4*	22.2	*36.3*	2.9	*0.7*	24.5	*37.4*
Internal base + secondary branch	7	14.4	-	21.8	*27.9*	-	*33.3*	19.1	*31.0*	3.1	*2.2*	21.8	*33.3*
Internal spur	6	5.2	-	7.7	*10.1*	-	*12.3*	6.5	*10.6*	1.1	*0.9*	6.8	*10.4*
Claw 3 lengths													
External primary branch	7	18.4	-	26.7	*35.7*	-	*41.4*	23.8	*38.7*	3.5	*2.6*	26.7	*40.8*
External base + secondary branch	6	14.8	-	22.9	*28.7*	-	*36.5*	19.8	*32.5*	3.6	*3.0*	?	*?*
External spur	5	4.7	-	6.9	*8.9*	-	*10.5*	5.9	*9.7*	1.1	*0.7*	?	*?*
Internal primary branch	5	17.6	-	26.4	*34.1*	-	*41.2*	22.9	*37.8*	4.4	*3.2*	?	*?*
Internal base + secondary branch	6	15.5	-	23.1	*29.8*	-	*36.8*	20.0	*32.6*	3.4	*2.7*	21.7	*33.1*
Internal spur	5	5.8	-	8.9	*10.5*	-	*14.2*	7.3	*11.9*	1.1	*1.4*	6.9	*10.5*
Claw 4 lengths													
Anterior primary branch	6	23.5	-	31.5	*43.1*	-	*49.4*	28.7	*45.6*	2.9	*2.5*	28.2	*43.1*
Anterior base + secondary branch	6	17.7	-	25.5	*33.4*	-	*40.0*	22.9	*36.4*	2.8	*3.2*	21.9	*33.4*
Anterior spur	5	6.4	-	10.0	*12.2*	-	*14.2*	8.2	*13.0*	1.3	*0.8*	?	*?*
Posterior primary branch	6	24.0	-	32.6	*45.3*	-	*51.5*	30.0	*47.5*	3.4	*2.5*	32.6	*49.8*
Posterior base + secondary branch	6	18.2	-	26.1	*34.6*	-	*40.8*	24.0	*37.9*	2.9	*2.2*	24.1	*36..8*
Posterior spur	5	6.2	-	8.3	*11.2*	-	*11.8*	7.2	*11.5*	0.8	*0.3*	?	*?*

Range refers to the smallest and the largest structure among all measured specimens.
The *pt* values are provided in *italics*.
N, number of specimens/structures measured; SD, standard deviation; ?, no data.

cuticle, presence of eyes and slightly smaller claws I to IV (see Table 1 and Table four in Meyer and Hinton (2012) for the exact differences in dimensions of claws).

8. *M. bohleberi*, known only from the USA (Bartels et al. 2014) by having sculptured dorsal cuticle, lower *pt* of papillae length ([*14.1-18.6*], [*9.4-17.6*] peribuccal and lateral, respectively, in *M. argentinum* sp. nov. vs. [*27.2-32.3*], [*17.7-21.2*] peribuccal and lateral, respectively, in *M. bohleberi*), smaller *pt* of width of buccal tube ([*30.3-39.8*], [*24.2-32.3*], [*23.1-33.6*] anterior, standard and posterior, respectively in *M. argentinum* sp. nov. vs. [*63.4-74.7*], [*54.5-64.0*], [*52.4-62.0*] anterior, standard and posterior, respectively in *M. bohleberi*) and smaller *pt* of claws I to IV (see Table 1 and Table one in Bartels et al. (2014) for the exact differences in dimensions of claws).
9. *M. brachyungue*, known only from Argentina (present study) and Chile (Binda and Pilato 1990) by having sculptured dorsal cuticle and stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*69.4*] in *M. brachyungue* (in specimen 729.0 in length)).
10. *M. eurystomum*, known from Argentina, Chile, Greenland, Mongolia (probably, see comments below), Spain, Svalbard and USA (Maucci 1991, 1996; Kaczmarek and Michalczyk 2006; Guil 2008; Kaczmarek et al. 2012a, b; Land et al. 2012; Johansson et al. 2013) by having sculptured dorsal cuticle, stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*60.0-60.3*] in *M. eurystomum*) and smaller *pt* of buccal tube width ([*30.3-39.8*], [*24.2-32.3*], [*23.1-33.6*] anterior, standard and posterior, respectively, in *M. argentinum* sp. nov. vs. [*72.1-75.8*], [*61.8-64.8*], [*43.7-57.9*] anterior, standard and posterior, respectively, in *M. eurystomum*).
11. *M. granulatum*, known from Argentina (present study), Chile, Colombia, Italy, Romania and the USA (Ramazzotti 1962; Ciobanu et al. 2014a, b; Bartels et al. 2014; Melo et al. (2014)) by different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming reticular design in *M. argentinum* sp. nov. vs. pseudopores densely distributed and forming a reticular design in *M. granulatum*), stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*66.5*] in *M. granulatum* (in specimen 338.3 in length)), smaller *pt* of width of buccal tube ([*30.3-39.8*], [*24.2-32.3*], [*23.1-33.6*] anterior, standard and posterior, respectively, in *M. argentinum* sp. nov. vs. [*46,3*], [*39.0*], [*40.1*] anterior, standard and posterior, respectively, in *M. granulatum* (in specimen 338.3 in length)) and slightly smaller claws I to IV (see Table 1 and Table two in Michalczyk et al. (2012a,b) for the exact differences in dimensions of claws).
12. *M. katarzynae*, known from China, Colombia, Costa Rica and Taiwan (Kaczmarek et al. 2004, 2014a; Yin and Li 2011; Melo et al. (2014)) by different claw configuration ([3-3]-[3-3] in *M. argentinum* sp. nov. vs. [2-2]-[2-2] in *M. katarzynae*), different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. argentinum* sp. nov. vs. pseudopores densely distributed and forming a reticular design in *M. katarzynae*), presence of eyes and smaller summed *pt* of external primary branches of claws I to IV([*23.4-33.8*] in *M. argentinum* sp. nov. vs. [*40.0-43.8*] in *M. katarzynae*).
13. *M. krzysztofi*, known from Colombia, Costa Rica and Peru (Kaczmarek and Michalczyk 2007; Kaczmarek et al. 2014a,b; Lisi et al. 2014; Melo et al. (2014)) by different claw configuration ([3-3]-[3-3] in *M. argentinum* sp. nov. vs. [2-3]-[3-2] in *M. krzysztofi*), different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. argentinum* sp. nov. vs. pseudopores densely distributed and forming a reticular design in *M. krzysztofi*), presence of eyes, stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*63.3-67.3*] in *M. krzysztofi*), slightly lower *pt* of buccal tube standard width ([*24.2-32.3*] in *M. argentinum* sp. nov. vs. [*33.1-38.4*] in *M. krzysztofi*) and slightly smaller claws I to IV (see Table 1 and Table one in Kaczmarek and Michalczyk (2007) for the exact differences in dimensions of claws).
14. *M. longiungue*, known only from India (Tumanov 2006) by having sculptured dorsal cuticle, presence of accessory points on main branches of claws, stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*59.1-66.7*] in *M. longiungue*), lower *pt* of standard buccal tube width ([*24.2-32.3*] in *M. argentinum* sp. nov. vs. [*33.8-59.1*] in *M. longiungue*) and slightly smaller claws I to IV (see Table 1 and Table six in Tumanov (2006) for the exact differences in dimensions of claws).
15. *M. reticulatum*, known only from Seychelles (Pilato et al. 2002) by different claw configuration ([3-3]-[3-3] in *M. argentinum* sp. nov. vs. [2-3]-[3-2] in *M. reticulatum*), different dorsal

sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. argentinum* sp. nov. vs. pseudopores arranged in nine sculptured bands, forming a reticular design in *M. reticulatum*), absence of cuticular gibbosities, having six peribuccal lamellae (four in *M. reticulatum*), stylet supports inserted in more caudal position ([*70.0-73.7*] in *M. argentinum* sp. nov. vs. [*68.5-69.8*] in *M. reticulatum*) and slightly smaller claws I to IV (see Table 1 and Table two in Pilato et al. (2002) for the exact differences in dimensions of claws).

16. *M. zsalakoae*, known only from Arizona and New Mexico, U.S.A. (Meyer and Hinton 2010) by having sculptured dorsal cuticle, presence of accessory points on main branches of claws, lower *pt* of buccal tube standard width ([*24.2-32.3*] in *M. argentinum* sp. nov. vs. [*36.8-41.9*] in *M. zsalakoae*) and smaller summed *pt* of internal claws and spurs I to IV (internal primary branch [*21.1-36.4*], internal base + secondary branch [*20.3-28.3*], internal spur [*6.6-9.9*] in *M. argentinum* sp. nov. vs. internal primary branch [*64.4-102.9*], internal base + secondary branch [*32.9-56.8*], internal spur [*11.1-23.2*] in *M. zsalakoae*).

Milnesium beatae sp. nov.

http://www.tardigrada.net/register/0016.htm.

Material examined: Seven females, all from Nahuel Huapi National Park, Río Negro, Argentina, moss sample from rocks, coll. Łukasz Kaczmarek.

Type material: Holotype and six paratypes in the sample III.

Diagnosis

Adults (Figure 1b) (measurements in micrometres, pt ratios and statistics in Table 2): The body rose before fixation and transparent afterwards, eyes present. Cuticle sculptured with pseudopores (0.3 to 0.6) not arranged in bands, sparsely distributed and not forming reticular design (Figure 2d). Six peribuccal papillae and six peribuccal lamellae around the mouth opening present. Two cephalic papillae positioned laterally. Peribuccal and cephalic papillae similar in length.

Buccal apparatus of the *Milnesium* type (Figure 2c). Buccal tube wide and short (standard width on average 62% of its length) and funnel-shaped, wider anteriorly (posterior diameter on average 72% of the anterior diameter). Pharyngeal bulb elongated, pear-shaped and without placoids or septulum.

Claws of the *Milnesium* type, slender (Figure 3d,e). Primary branches on all legs with small, but distinct accessory points detaching from the branch at its greatest curvature (Figure 3d,e). Secondary branches with rounded basal thickenings (Figure 3d,e). All secondary branches on all legs with three points (claw configuration: [3-3]-[3-3]). Single, long transverse, cuticular bars under claws I-III present (Figure 3d).

Eggs: Oval, smooth and deposited in exuvium as in all other known *Milnesium* species.

Males not observed.

Locus typicus: Argentina, 41°12′S, 71°50′W, ca. 1,000 m asl., Río Negro, Nahuel Huapi National Park.

Etymology: This species is named after first author's secondary school Biology teacher - Mrs. Beata Ostasiewicz.

Type depositories: The holotype and all paratypes are deposited in the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University in Poznań, Umultowska 89, Poznań, Poland.

Differential diagnosis

By having a sculptured dorsal cuticle, *M. beatae* sp. nov. belongs to the *granulatum* group within the genus (Michalczyk et al. 2012a,b). This new species with three points on the secondary branches of all claws [3-3]-[3-3] and very short and wide buccal tube is most similar to *M. bohleberi*, *M. brachyungue* Binda and Pilato 1990 and *M. eurystomum*, but it differs from the following:

1. *M. bohleberi* by having sculptured dorsal cuticle and lower *pt* of peribuccal papillae length ([*18.0-23.6*] in *M. beatae* sp. nov. vs. [*27.2-32.3*] in *M. bohleberi*).
2. *M. brachyungue* by having sculptured dorsal cuticle, stylet supports inserted in more cephalic position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*69.4*] in *M. brachyungue* (in specimen 729.0 in length)) and lower *pt* of claws I to IV (see Table 2 and Table one in Binda and Pilato (1990) for the exact differences in dimensions of claws).
3. *M. eurystomum* by having sculptured dorsal cuticle, stylet supports inserted in more caudal position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*60.0-60.3*] in *M. eurystomum*) and slightly different *pt* of claws I to IV (see Table 2 and Table two in Michalczyk et al. (2012a) for the exact differences in dimensions of claws).

Besides the abovementioned the most similar species, *M. beatae* sp. nov. is similar to other species of the genus *Milnesium* with the claw configuration [3-3]-[3-3] (*M. alabamae*, *M. antarcticum*, *M. asiaticum*, *M. barbadosense*, *M. eurystomum*, *M. granulatum*, *M. longiungue* and *M. zsalakoae*) or with a sculptured cuticle (*M. beasleyi*, *M. katarzynae*, *M. krzysztofi* and *M. reticulatum*).

This new species differs from the following:

4. *M. alabamae* by different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. beatae* sp. nov. vs. pseudopores arranged in bands (especially in caudal region), densely distributed and forming a reticular design in *M. alabamae*), presence of accessory points on main branches of claws, presence of eyes and lower *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*29.5-44.0*] in *M. alabamae*).
5. *M. antarcticum* by having sculptured dorsal cuticle, higher *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*35.4-43.9*] in *M. antarcticum*) and stylet supports inserted in more cephalic position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*70.0-73.7*] in *M. antarcticum*).
6. *M. argentinum* sp. nov. by stylet supports inserted in more cephalic position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*70.0-73.7*] in *M. argentinum* sp. nov.), lower *pt* of width of buccal tube ([*70.3-78.9*], [*58.1-65.6*], [*49.4-56.5*] anterior, standard and posterior, respectively, in *M. beatae* sp. nov. vs. [*30.3-39.8*], [*24.2-32.3*], [*23.1-33.6*] anterior, standard and posterior, respectively, in *M. argentinum* sp. nov. and longer claws I to IV (see Tables 1 and 2 for the exact differences in dimensions of claws).
7. *M. asiaticum* by having sculptured dorsal cuticle, lower *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*30.0-41.6*] in *M. asiaticum*) and slightly different *pt* of claws I to IV (see Table 2 and Table four in Tumanov (2006) for the exact differences in dimensions of claws).
8. *M. barbadosense* by having sculptured dorsal cuticle, presence of eyes and lower *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*27.2-49.7*] in *M. barbadosense*).
9. *M. beasleyi* by different claw configuration ([3-3]-[3-3] in *M. beatae* sp. nov. vs. [2-3]-[3-2] in *M. beasleyi*), presence of rounded basal thickenings under secondary branches of claws, higher *pt* of width of buccal tube ([*70.3-78.9*], [*58.1-65.6*], [*49.4-56.5*] anterior, standard and posterior, respectively, in *M. beatae* sp. nov. vs. [*35.3-41.8*], [*31.2-39.8*], [*29.6-33.2*] anterior, standard and posterior, respectively, in *M. beaslei*,]) and slightly smaller claws I to IV (see Table 2 and Table one in Kaczmarek et al. (2012a, b) for the exact differences in dimensions of claws).
10. *M. berladnicorum* by different claw configuration ([3-3]-[3-3] in *M. beatae* sp. nov vs. [2-3]-[2-2] in *M. berladnicorum*), higher *pt* of width of buccal tube ([*70.3-78.9*], [*58.1-65.6*], [*49.4-56.5*] anterior, standard and posterior, respectively, in *M. beatae* sp. nov. vs. [*35.0-47.1*], [*30.6-38.9*], [*27.7-36.0*] anterior, standard and posterior, respectively, in *M. berladnicorum*]) and smaller claws I to IV (see Table 2 and Table one in Ciobanu et al. (2014b) for exact differences in dimensions of claws).
11. *M. granulatum* by different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. beatae* sp. nov. vs. pseudopores densely distributed and forming a reticular design in *M. granulatum*), higher *pt* of width of buccal tube ([*70.3-78.9*], [*58.1-65.6*], [*49.4-56.5*] anterior, standard and posterior, respectively, in *M. beatae* sp. nov. vs. [*46,3*], [*39.0*], [*40.1*] anterior, standard and posterior, respectively, in *M. granulatum* (in specimen 338.3 in length)) and slightly larger claws I to IV (see Table 2 and Table two in Kaczmarek et al. (2012a, b) for the exact differences in dimensions of claws).
12. *M. katarzynae* by a different claw configuration ([3-3]-[3-3] in *M. beatae* sp. nov. vs. [2-2]-[2-2] in *M. katarzynae*), different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. beatae* sp. nov. vs. pseudopores densely distributed and forming a reticular design in *M. katarzynae*), presence of eyes, higher *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*21.7-26.6*] in *M. katarzynae* and stylet supports inserted in more cephalic position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*73.3-78.3*] in *M. katarzynae*).
13. *M. krzysztofi* by different claw configuration ([3-3]-[3-3] in *M. beatae* sp. nov. vs. [2-3]-[3-2] in *M. krzysztofi*), different dorsal sculpture (pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. beatae* sp. nov. vs. pseudopores densely distributed and forming a reticular design in *M. krzysztofi*), presence of eyes, higher *pt* of buccal tube standard width ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*33.1-38.4*] in *M. krzysztofi*) and smaller claws I to IV (see Table 2 and Table one in Kaczmarek and Michalczyk (2007) for the exact differences in dimensions of claws).
14. *M. longiungue* by having sculptured dorsal cuticle, presence of accessory points on the main branches of claws, slightly higher *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*33.8-59.1*] in *M. longiungue*) and smaller claws I to IV (see Table 2 and Table six in Tumanov (2006) for the exact differences in dimensions of claws).
15. *M. reticulatum* by different claw configuration ([3-3]-[3-3] in *M. beatae* sp. nov. vs. [2-3]-[3-2] in *M. reticulatum*), different dorsal sculpture

(pseudopores not arranged in bands, sparsely distributed and not forming a reticular design in *M. beatae* sp. nov. vs. pseudopores arranged in nine sculptured bands, forming a reticular design in *M. reticulatum*), absence of cuticular gibbosities, having six peribuccal lamellae (four in *M. reticulatum*), stylet supports inserted in more cephalic position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*68.5-69.8*] in *M. reticulatum*), higher *pt* of standard width of buccal tube ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*30.4-37.4*] in *M. reticulatum*) and different measurements of claws I to IV (see Table 2 and Table two in Pilato et al. (2002) for the exact differences in dimensions of claws).

16. *M. zsalakoae* by having sculptured dorsal cuticle, presence of accessory points on main branches of claws, higher *pt* of buccal tube standard width ([*58.1-65.6*] in *M. beatae* sp. nov. vs. [*36.8-41.9*] in *M. zsalakoae*), stylet supports inserted in more cephalic position ([*63.8-66.7*] in *M. beatae* sp. nov. vs. [*68.2-71.1*] in *M. zsalakoae*) and higher *pt* of internal claws I to IV (see Table 2 and the description of *M. zsalakoae* in Meyer and Hinton (2010) for the exact differences in dimensions of claws).

Milnesium brachyungue Binda and Pilato, 1990

Terra typica: Chile (South America).

Localities and number of specimens in present studies: Five females in sample VI.

Remarks: Until now this species was considered as an endemic of Chile (Binda and Pilato 1990) (Table 3). This is a second report of the species and a new record for Argentina. *Milnesium brachyungue* is relatively similar to *M. tardigradum* and it is possible that the specimens of *M. tardigradum* sensu stricto reported from South America (especially from southern regions of Chile or Argentina) in the past belong rather to *M. brachyungue*, but this hypothesis needs to be confirmed. We strongly suggest that all specimens reported as *M. tardigradum* s. s. from this region should be re-examined and determined based on the modern taxonomic characters (see also Michalczyk et al. 2012a,b).

Milnesium eurystomum Maucci, 1991

Terra typica: Denmark (Arctic, Greenland).

Localities and number of specimens in present studies: None.

Remarks: Species reported from Argentina, Chile, Mongolia (probably), Spain, Svalbard, USA and *locus typicus* on Greenland (Maucci 1991, 1996; Kaczmarek and Michalczyk 2006; Guil 2008; Kaczmarek et al. 2012a, b; Land et al. 2012; Johansson et al. 2013) (Table 3). This species is characterized by a very wide and relatively short buccal tube, claw configuration [3-3]-[3-3] and smooth cuticle. Recently, a similar species (with a short and wide buccal tube), *M. bohleberi*, was described from North America (USA, North Carolina and Tennessee) (Bartels et al. 2014). In this situation, we suggest that the examples, especially from South and North America, should be re-examined (see also Michalczyk et al. 2012a,b). In Mongolia this species was reported as *M.* cf. *eurystomum* Maucci 1991 (Kaczmarek and Michalczyk 2006) and its presence in this region needs confirmation.

Minesium granulatum Ramazzotti, 1962

Terra typica: Chile (South America).

Localities and number of specimens in present studies: Two females in sample II.

Remarks: Species reported from Argentina, Chile, Colombia, Italy, Romania and USA (Ramazzotti 1962; Michalczyk et al. 2012a,b; Ciobanu et al. 2014a; Melo et al. 2014) (Table 3). As mentioned by Bartels et al. (2014), such wide and discontinuous geographic distribution can suggest a complex of cryptic species, but testing this hypothesis requires molecular data which are not yet available. We suggest that at least the examples

Table 3 Distribution of the species of genus *Milnesium* in South America

Species	Country									
	Argentina	Bolivia	Brazil	Chile	Colombia	Ecuador	Paraguay	Peru	Uruguay	Venezuela
M. argentinum	X									
M. beatae	X									
M. brachyungue	X			X						
M. eurystomum	X			X						
M. granulatum	X			X	X					
M. katarzynae					X					
M. krzysztofi					X			X		
M. t. tardigradum	X	X	X	X	X	X	X	X	X	X
M. t. trispinosa			X	X						

from Europe should be carefully re-examined (see also Michalczyk et al. 2012a,b).

Milnesium katarzynae Kaczmarek et al., 2004

Terra typica: China (Asia).

Localities and number of specimens in present studies: None.

Remarks: Until now, this species was known only from China (*locus typicus*) (Kaczmarek et al. 2004), Costa Rica (Kaczmarek et al. 2014a), Colombia (Melo et al. 2014) and Taiwan (Yin and Li 2011) (Table 3). Such distribution may suggest a pantropical distribution of this species.

Milnesium krzysztofi Kaczmarek and Michalczyk 2007

Terra typica: Costa Rica (Central America).

Localities and number of specimens in present studies: None.

Remarks: Until now, this species was known only from Costa Rica (*locus typicus*) (Kaczmarek and Michalczyk 2007), Colombia (Lisi et al. 2014; Melo et al. 2014) and Peru (Kaczmarek et al. 2014a,b) (Table 3). Such distribution may suggest a neotropical distribution of this species.

Milnesium tardigradum tardigradum Doyère 1840

Terra typica: Chile (South America).

Localities and number of specimens in present studies: None.

Remarks: This species has been reported from many localities in South America (McInnes 1994, see also the 'Discussion' section and Table 3). For over a century, *Milnesium* was considered to be a monotypic, highly cosmopolitan genus. However, currently there are more than 20 species within the genus and it is very likely that the zoogeographic range of *M. tardigradum* s. s. is limited to the Palaearctic (Michalczyk et al. 2012a,b). This means that all South American reports for *M. tardigradum* that occurred prior to the re-description of *M. tardigradum* s. s. (Michalczyk et al. 2012a,b) should be considered dubious and need a re-examination based on the modern taxonomy of the genus *Milnesium*. In other words, currently, there is no evidence that *M. tardigradum* s. s. inhabits South America; thus, its records may represent a number of different *Milnesium* taxa (Michalczyk et al. 2012a,b, see also remarks on other *Milnesium* species, especially on *M. brachyungue*).

Milnesium tardigradum trispinosa Rahm 1932 [T]

Terra typica: Chile (South America).

Localities and number of specimens in present studies: None.

Remarks: A subspecies reported from single localities in Chile and Brazil (Table 3). This unique subspecies is characterized by the presence of short spines on dorsal cuticle. It should be probably promoted to the species level, but the re-description based on type material (or based on specimens collected in the type locality) is necessary (Michalczyk et al. 2012a,b).

Discussion

Distribution

Milnesium species have been recorded from many localities throughout the world (Michalczyk et al. 2012a,b) and one of them, *M. swolenskyi* Bertolani and Grimaldi 2000, is also known from Cretaceous amber collected in the USA (Bertolani and Grimaldi 2000). For a long time, *M. tardigradum* Doyère 1840 was the only recognised species in the genus and it has been considered as an extremely cosmopolitan taxon (e.g. Ramazzotti and Maucci 1983). In the past, even morphologically very different specimens were still considered as only different forms or subspecies of the type *M. tardigradum* (e.g. Rahm 1932; Ramazzotti 1962; Sudzuki 1964; Horning et al. 1978; Nelson and Horning 1979; Dastych 1984). Interestingly, even that Nederström 1919 described a new *Milnesium* species, *Milnesium quadrifidum*, this new taxon was later recognised only as a specific form of *M. tardigradum* (Szymańska 1994 according to Marcus 1936) and finally excluded from the list of tardigrade taxa at all. It was not until 1990 that Binda and Pilato described a new *Milnesium* species, *M. brachyungue*, from Tierra del Fuego. Later, new species were described only occasionally mainly due to the lack of a clear diagnosis of the nominal species *M. tardigradum* s. s. (Maucci 1991; Bertolani and Grimaldi 2000; Pilato et al. 2002; Yang 2003; Kaczmarek et al. 2004). In 2006, Tumanov published the first partial revision of the genus *Milnesium* and described five new species. Since this time, many new species have been described and now 25 taxa (including the two new species described in this paper) are known in the genus (Degma et al. 2009-2014). The majority of the newly described species are known only from their type localities and Michalczyk et al. (2012a,b) hypothesised that *M. tardigradum* s. s. is not a cosmopolitan species, but previous reports from various localities should be re-examined in light of new taxonomic characters because they probably belong to different species (see also Meyer 2013). Based on the present data, most of the species from the genus *Milnesium* have truly restricted geographic ranges, as was also shown in other tardigrade genera (e.g. Pilato and Binda 2001).

As of now, nine *Milnesium* taxa are known from South America (including two new species reported in this paper) (Table 3). These are *M. argentinum* sp. nov. and *M. beatae* sp. nov. (currently restricted to Argentina), *M. brachyungue* (known from Argentina and Chile), *M. eurystomum* (known from Argentina, Chile, Greenland, Mongolia (probably), Spain, Svalbard and USA), *M.*

granulatum (known from Argentina, Chile, Colombia, Italy, Romania and USA), *M. katarzynae* (known from China, Colombia, Costa Rica and Taiwan), *M. krzysztofi* (known from Colombia, Costa Rica and Peru), *M. tardigradum* sensu lato (known from Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela) and *M. t. trispinosa* (known from Chile and Brazil) (Richters (1911a); Murray and Wailes (1913); Heinis (1914); Rahm (1931, 1932); de Barros (1943); (du Bois-Reymond 1944); Ramazzotti (1957, 1962, 1964); Iharos (1963); Mihelčič (1967, 1972) Rossi and Claps (1980, 1989, 1991); Claps and Rossi (1981, 1984, 1988, 1997); Maucci (1988); Binda and Pilato (1990), Maucci (1991, 1996); Séméria (1993); McInnes (1994); Jerez Jaimes and Narváez Parra (2001); Nickel et al. (2001); Pilato et al.(2003); Kaczmarek et al. (2004, 2012a,b); Kaczmarek and Michalczyk (2006, 2007); Moly de Peluffo et al.(2006); Peluffo et al.(2007); Guil (2008); Rossi et al.(2009); Kaczmarek et al. (2012a,b), Land *et al.* (2012), Michalczyk *et al.* (2012a,b), Johansson *et al.* (2013); Ciobanu et al. (2014a); Lisi et al. (2014); Melo et al. (2014)).

As shown above, only *M. tardigradum* s. l. has a wide distribution in South America, however the presence of this species in this region is doubtful and needs confirmation. The present study seems to confirm this hypothesis, because although four *Milnesium* species were found in the samples studied, none of them was recognised as the nominal *M. tardigradum*. This also confirms the hypothesis proposed by Michalczyk et al. (2012a,b) that *M. tardigradum* have rather a narrower geographic range (restricted to Holarctic or Palearctic), although more extensive studies (molecular) are necessary in other regions of South America.

Similar to *M. tardigradum* s.s., other species of the genus *Milnesium* also have restricted geographic ranges (e.g. *M. alabamae*, *M. almatyense* Tumanov 2006, *M. antarcticum*, *M. barbadosense*, *M. beasleyi*, *M. jacobi* Meyer and Hinton 2010, *M. brachyungue*, *M. dujiangensis* Yang 2003, *M. lagniappe* Meyer et al. 2013, *M. longiungue*, *M. reductum* Tumanov 2006, *M. reticulatum*, *M. tetralamellatum* Pilato and Binda 1991 and *M. zsalakoae*), which seems to support the hypothesis that none of the *Milnesium* species is cosmopolitan (Bartels et al. 2014). However, as was also shown by Bartels et al. (2014), some of the *Milnesium* species have rather wide and discontinuous geographic ranges (*M. asiaticum*, *M. eurystomum*, *M. granulatum*, *M. katarzynae* and *M. krzysztofi*), which can suggest the presence of cryptic species complexes or a specific distribution of some of the taxa (e.g. pantropical for *M. katarzynae* or neotropical for *M. krzysztofi*). In this situation, only detailed morphological and molecular studies could shed light on this issue.

Remarks on feeding behaviour

Very little is known about food preferences in tardigrades, although, many different types of food sources have been reported, i.e. plant cell fluids, algae, bacteria, protozoa and small invertebrates like nematodes, rotifers and other tardigrades (for the review, see Schill et al. 2011). Previous studies have focused on the following: a) the rate of consumption, e.g. in the carnivorous *Paramacrobiotus richtersi* (Murray, 1911), b) predator-prey interactions (*P. richtersi* vs. nematodes), c) population dynamics and food availability in the herbivorous tardigrade *Apodibius confusus* Dastych, 1983, d) tardigrade food preferences in the herbivorous *Echiniscus granulatus* (Doyère, 1840), *Macrobiotus sapiens* Binda and Pilato, 1984, *M. persimilis* Binda and Pilato, 1972, *Richtersius coronifer* (Richters, 1903) and the carnivorous *P. richtersi*, e) life histories of some species and f) different aspects of tardigrade histology (e.g. Suzuki 2003; Hohberg and Traunspurger 2005, 2009; Poprawa 2005, 2011; Hohberg 2006; Horikawa et al. 2008; Hohberg et al. 2011; Lemloh et al. 2011; Schill et al. 2011; Schill 2013), give us some incomplete information on tardigrade feeding behaviour.

The species in the genus *Milnesium* are relatively large (occasionally more than 2 mm but most often between 0.5 to 1.0 mm) and inhabit mainly limno-terrestrial habitats (Guil 2008). Their buccal tube is wide, relatively short, connected with a large pharynx without placoids and associated with large buccal lamellae on a wide mouth ring, which is in agreement with the definition of the 'carnivore type' of buccal apparatus proposed by Guidetti et al. (2012). All *Milnesium* species are considered carnivorous and feed on other small invertebrates like rotifers, nematodes, tardigrades and sometimes also on amoebas (e.g. Kinchin 1990; Suzuki 2003; Nelson et al. 2010; Miller and Williams 2012).

In contrast to other predatory tardigrades from genera such as *Bertolanius* Özdikmen, 2008, *Macrobiotus* C.A.S. Schultze, 1834 or *Paramacrobiotus* Guidetti et al., 2009, *Milnesium* species suck the entire prey into the gut rather than only single cells or body fluids (McInnes et al. 2001). However, also species from the genus *Bertolanius* (i.e. *Bertolanius weglarskae* (Dastych 1972)) can suck in entire prey (see Figure 1a in Guidetti and Bertolani 2001). McInnes et al. (2001) analysed the diet of sub-Antarctic specimens of *M.* cf. *tardigradum*. They found in the guts of *M.* cf. *tardigradum* not only remnants but also entire specimens of three different species of Rotifera (Bdelloidea: *Adineta* sp., *Philodina* spp. and Monogononta: *Dicranophorus* sp.), unidentified species of Nematoda and two species of Tardigrada (*Diphascon* sp. and *Calohypsibius* cf. *ornatus*). Miller and Williams (2012) reported a Greenland tardigrade *M. eurystomum* feeding on shelled (testate amoebae) (similar to *Euglypha*).

Additionally, the same authors suggested that *M. eurystomum* has the ability to properly orient their prey, so that the amoeba can be extracted by the sucking action and later the empty shell can be expelled. However, specific differences in the feeding behaviour in connection with the structure of buccal apparatus in different *Milnesium* species have never been discussed.

In the present research, a total of 25 adult specimens of two new *Milnesium* species: *M. argentinum* (18 specimens) and *M. beatae* (7 specimens) were studied. Both species were found in the same region of Nahuel Huapi National Park (Río Negro Province, Argentina) in a few moss samples. Five specimens (two specimens of *M. argentinum* sp. nov. and three specimens of *M. beatae* sp. nov.) were found with the remnants of food (rotifer mastaxes and tardigrade buccal apparatuses and claws) in their guts. Both species were found in the moss samples (sometimes also together in the same piece of moss) that also contained rotifers, nematodes and other tardigrades (*Diphascon chilenense* Plate, 1888, *Echiniscus bigranulatus* Richters 1907, *Hebesuncus mollispinus* Pilato et al. 2012, *Macrobiotus* cf. *andinus*, *M. harmsworthi* group, *M. kazmierskii* Kaczmarek and Michalczyk 2009, *M. patagonicus* Maucci 1988, *M. szeptyckii* Kaczmarek and Michalczyk 2009, *Mopsechiniscus granulosus* Mihelčič 1967). Both new *Milnesium* species were able to choose from the same type of prey, because the prey were similar in every sample.

The main morphological differences between *M. argentinum* sp. nov. and *M. beatae* sp. nov. are visible in the structure of buccal tube. *Milnesium argentinum* sp. nov. has a relatively long and narrow buccal tube while *M. beatae* sp. nov. has a relatively short and wide buccal tube (in the specimens of similar lengths, see Tables 1 and 2). Based on gut contents, both species seem also to have a different diet: the two specimens of *M. argentinum* sp. nov. fed on undefined species of rotifers (Figure 3c), while the three specimens of *M. beatae* sp. nov. fed on other tardigrades (exclusively *M. szeptyckii*) (Figure 3f). In general, specimens of *M. szeptyckii* are larger (360 to 650 μm) and more bulky (a wider buccal tube is probably necessary to swallow them) than typical rotifers which are shorter (100 to 500 μm) and more slender (a narrower buccal tube is sufficient to swallow them). Although the numbers are small, these data suggest that the buccal tube width may affect the type of prey consumed. However, it should be also noted that McInnes et al. (2001) showed that both tardigrades and rotifers were found in the gut of the same specimen of *M.* cf. *tardigradum*. But, according the photo (Figure 1a in McInnes et al. 2001), the *Milnesium* species studied by McInnes et al. (2001) had rather long and narrow buccal tube (similarly to *M. argentinum* sp. nov.) and fed on rather small and slender prey (rotifers, nematodes and small and slender tardigrades from the genera *Diphascon* Plate, 1888 and *Calohypsibius* Thulin 1928), which is in agreement with the observations made in the present research.

Although neither McInnes et al. (2001) observations nor the present research provided a definitive answer whether the buccal tube width has a crucial role in the choice of different types of prey, these studies are the basis for further, more comprehensive studies.

Such studies are necessary also to understand the evolution of buccal apparatuses in other genera of the family Milnesiidae (*Bergtrollus* Dastych 2011, *Limmenius* Horning, Schuster and Grigarick, 1978 and *Milnesioides* Claxton 1999). However, we know almost nothing about feeding behaviour and prey choice of the members of these monophyletic genera. Only Claxton (1999) mentioned some specimens of *M. exsertum* that had remnants of rotifers in their guts. This is a very interesting observation, because this species has a very long and narrow buccal tube, similarly to the 'rotifer-feeding' *M. argentinum* sp. nov.

Based on the available data, it can be initially hypothesised that the species from the family Milnesiidae with long and narrow buccal tubes (e.g. *M. argentinum* sp. nov., Antarctic *M.* cf. *tardigradum* or *M. exsertum*) choose smaller and more slender prey (rotifers or small slender tardigrades from genera *Diphascon* or *Calohypsibius*) in contrast to the species with relatively short and wide buccal tubes (e.g. *M. beatae* sp. nov. or *M. eurystomum*) which choose more robust and larger or armoured prey (large and robust tardigrades from the genus *Macrobiotus* or testate amoebae). Certainly, such conclusions based only on the 'post-mortem' studied specimens give us only very limited knowledge on the food that had been eaten just before death (preservation) of the animal. Such information needs confirmation in further, more detailed experimental studies with molecular methods (e.g. Schill et al. 2011) or based directly on the observations in cultured animals (e.g. Suzuki 2003).

Conclusions

Milnesium argentinum and *M. beatae* are new taxa for science. As of now, nine *Milnesium* taxa are known from South America (including the two new species and two newly recorded taxa for Argentina, reported in this paper). The presence of *M. tardigradum* s. s. in South America needs confirmation and for now it should be considered as dubious. It is probable that width of buccal tube may limit a prey size and play an important role in the feeding behaviour in the family Milnesiidae. Studies on the feeding behaviour in tardigrades are in initial phase and definitive conclusions are not possible at this moment.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MR examined and analysed the material, identified and described the species, made the measurements, figures and tables and drafted the manuscript. MO made the measurements and tables. ŁK collected part of the material, invented the research conception, analysed the examined material, identified and described the species, corrected tables and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements
The authors want to thank Prof. Diane Nelson of East Tennessee State University for very valuable comments to the manuscript and help in improving of the English. This work was partially supported by the Polish Ministry of Science and Higher Education via the 'Iuventus Plus' programme (grant: IP2010 015570, Invertebrate biodiversity in temporary ponds of Costa Rica - verification of the 'Great American Biotic Interchange' hypothesis) and by the Prometeo Project of the Secretariat for Higher Education, Science, Technology and Innovation of the Republic of Ecuador. Studies have been conducted in the framework of activities of BARg (Biodiversity and Astrobiology Research group).

Received: 15 August 2014 Accepted: 10 December 2014
Published online: 14 January 2015

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