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Tardigrades from northwestern Patagonia (Neuquén Province, Argentina) with the description of three new species

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Abstract

Fifteen moss and lichen samples collected in localities of the Neuquén province, Argentina, contained tardigrades and their eggs. Eighteen species were found, three being new to science: *Macrobiotus neuquensis* **sp. nov.**, *Macrobiotus tehuelchensis* **sp. nov.**, and *Minibiotus claxtonae* **sp. nov.** *Macrobiotus neuquensis* **sp. nov.** can be differentiated from the other species of the *harmsworthi* group by the presence of a cuticular bar and a refractile zone in the first three pairs of legs. Also the characteristics of the eggs such as the shape and distribution of processes allow a distinction from other species. *Macrobiotus tehuelchensis* **sp. nov.** differs from other similar species by the measurements of some structures of the buccal apparatus and the characteristics of the eggs (the shape of the processes). *Minibiotus claxtonae* **sp. nov.** is characterized by the presence of three macroplacoids, a microplacoid, and cuticular pores arranged in transverse bands. The new species of *Minibiotus* is distinguished from other species of the genus by the distribution and shape of the pores (round and star shaped), presence of granulation on the legs, and some characteristics of the eggs (*e.g.*, the absence of a membrane and the shape of the processes).

Key words: *Macrobiotus neuquensis* **sp. nov.**, *Macrobiotus tehuelchensis* **sp. nov.**, *Minibiotus claxtonae* **sp. nov.**, Tardigrada, *Nothofagus* forests, Argentina

Introduction

The tardigrade fauna of the Neotropical region has been poorly characterized (Pilato *et al.*, 2001). The Andinopatagónica region, with forests of *Nothofagus*, has been the most widely studied since the 1960s (Iharos, 1963; Mihelcic, 1967; Claps & Rossi, 1981; Maucci, 1988; Rossi & Claps, 1989; Pilato, 1990; Pilato & Binda, 1996; Pilato & Patané, 1998; Pilato *et al*., 1998; Binda & Pilato, 1999[a], 1999[b]; Dastych, 2000) except for the sector that represents the northern limit of the region and includes the Neuquén province. Claps & Rossi (1981), however, noted the presence of *Pseudechiniscus novaezeelandiae* (Richters), *E. viridis* Murray, *Hypsibius convergens* (Urbanowicz), *Macrobiotus hibiscus* De Barros, *M. hufelandi* Schultze, *M. richtersi* Murray, *M. subintermedius* (Ramazzotti) in the Quetrihue Peninsula, a region which constitutes a boundary between the Neuquén and Río Negro Provinces.

The aim of this paper is to report additional tardigrade records for Patagonia with new findings both for the Neuquén Province and for Argentina in general. Three new species are also described and illustrated.

Materials and methods

The following 15 samples included four tree-lichen samples, seven soil-moss samples, one soil-lichen sample, and one sample of moss on wood:

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Sample 5: Hua Hum, Junín de los Andes, at *ca*. 700 m asl (40°07'S, 71°39' W), from mosses on soil and rock (II-03), Claps & Rossi coll.

Samples 6 and 7: Rucachoroi Lake, Aluminé, at *ca*. 900 m asl (39°12' S, 70°55' W), from moss on soil and lichen on a tree, respectively (XII-2004), J. Girini coll.

Samples 8 and 9: Villa Pehuenia, Moquehue Lake, Aluminé, at *ca*. 1300 m asl (38°53'S, 71°13'W), from moss on soil and lichen on a tree, respectively (XII-2004), J. Girini coll.

Sample 10: Ñorquinco pond, Aluminé, at *ca*. 1,000 m asl, from moss and lichen on soil (I-2005), J. Girini coll.

Samples 11 and 12: Quillén Lake, Aluminé, from lichen on tree and moss on soil, respectively, (I-2005), J. Girini coll.

Sample 13: Rucachoroi Lake, Aluminé, at *ca*. 900 m asl, from lichen on a tree, (XII-2004), J. Girini coll.

Samples 14 and 15: Abra Ancha, Aluminé, from lichen and moss on soil, respectively (XII-2004), J. Girini coll.

Sample 16: Rucachoroi Lake, Aluminé, from moss on wood (XII-2004), J. Girini coll.

Sample 19: Epulauquen Lake, at *ca*. 1500 m asl (36°50'S, 71°00'W), from mosses on soil and rock (I-2006), L. Solari coll.

Samples 17 and 18 were negative for tardigrade fauna.

The samples were placed in paper bags and left to dry naturally; in the laboratory, they were first soaked in tap water overnight, next thoroughly washed with water, and then filtered through a 1 mm sieve. The filtered material was finally transferred to an Erlenmeyer flask. The samples were shaken vigorously, washed a second time to release eggs and additional remaining tardigrades, and finally soaked in water overnight before examination under a dissecting microscope and mounting of the tardigrades in polyvinyl lactophenol and Faure mounting medium. Structures were measured only if their orientation was suitable. Body length was measured from the mouth to the posterior end of the body excluding the hind legs. The buccal tube length and the level of the stylet support insertion point were measured according to Pilato (1981). The buccal tube width was defined as the external diameter at the level of the stylet support insertion point. The lengths of the claw branches were measured from the base of the claw to the top of each branch, excluding accessory points. The *pt* value is defined as the ratio of the length of a given structure to the length of the buccal tube expressed as a percent (Pilato, 1981). In addition to the traditional nomenclature, the terminology proposed by Michalczyk & Kaczmarek (2003) for describing the oral armature within the genus *Macrobiotus* Schultze was employed. Species were identified on the basis of the key to the world fauna of Tardigrada (Ramazzotti & Maucci, 1983) and the findings from several original papers.

Photomicrographs were taken with a Nomarski differential-interference-contrast microscope (DIC) and/or under phase-contrast optics. All drawings were made by means of a camera lucida attached to a phase-contrast microscope.

Results

Eighteen species were recognized in the collected samples (Table 1). The maximum species richness (8) and the minimum (1) were recorded in the Aluminé samples.

***Antechiniscus lateromamillatus* (Ramazzotti, 1964)**

This identification constitutes the first record of this species in Argentina, it being previously documented only in Chile and New Zealand (McInnes, 1994).

TABLE 1. Species found in the samples collected in Neuquén province.

Species / samples	5	6	7	8	9	10	11	12	13	14	15	16	19
Acutuncus antarcticus		*											
Antechiniscus lateromamillatus							*						
Echiniscus bigranulatus	*		*			*	*	*					
Echiniscus merokensis										*	*		
Echiniscus markezi										*			
Mopsechiniscus granulosus							*						
Milnesium tardigradum	*			*			*		*		*		
Diphascon tenue		*			*								
Hypsibius convergens											*		
Hypsibius microps				*	*		*						
Hypsibius pallidus										*			*
Isohypsibius sculptus	*	*											
Macrobiotus neuquensis **sp. nov**.	*	*	*								*		*
Macrobiotus patagonicus	*			*		*						*	*
Macrobiotus tehuelchensis **sp. nov**.						*							
Minibiotus claxtonae **sp. nov.**							*						
Minibiotus subintermedius										*			
Ramazzottius baumanni	*	*					*						

***Echiniscus bigranulatus* Richters, 1908**

This species exhibits a restricted distribution, consisting of South and Central America according to Michalczyk & Kaczmarek (2006), with it being widely distributed in Argentina (in the provinces of Buenos Aires, Misiones, Corrientes, Entre Ríos, Tucumán, Salta, Río Negro, Santa Cruz, and Tierra del Fuego) (Claps *et al*., 2008). Previously in a review of tardigrade distribution, McInnes (1994) cited its presence in Europe, Africa, Indonesia, and New Zealand.

***Echiniscus markezi* Mihelcic, 1971**

This species has only been recorded in Río Negro by Mihelcic (1971–72).

***Echiniscus merokensis* Richters, 1904**

Our finding disagrees with Kristensen's (1987) suggestion about the restricted preference of this species to a soil habitat. This species is considered cosmopolitan (McInnes, 1994; Fontoura *et al*., 2008). In Argentina, *E. merokensis* was previously recorded in Santa Cruz and Tierra del Fuego (Claps *et al.*, 2008).

***Mopsechiniscus granulosus* Mihelcic, 1967**

This species has been previously recorded only in the Río Negro province (McInnes, 1994).

***Milnesium tardigradum* Doyère, 1840**

The collected specimens were identified by means of the key given by Tumanov (2006) and comparison with a new species of Kaczmarek and Michalczyk (2007) for the recognition of all known species. According to Tumanov (2006), *M. tardigradum* must be considered widely distributed but not cosmopolitan.

***Acutuncus antarcticus* (Richters 1904)**

This species has been recorded in Antarctica (Dastych, 1991); the records from Europe, Asia, and North America were considered dubious by this author. In Argentina, it has been found previously in Tierra del

Fuego (Claps *et al.*, 2008). All specimens examined exhibited the characteristics given in Dastych's redescription..

***Hypsibius convergens* (Urbanowicz 1925)**

This species is considered to be cosmopolitan, or at least widely distributed (McInnes, 1994), and has been previously recorded in the majority of the provinces of Argentina (Claps *et al.*, 2008).

***Hypsibius microps* Thulin 1928**

This species is considered to be widely distributed and as such has been recorded in America, Europe, and Asia (McInnes, 1994). In Argentina, it was identified in the Buenos Aires, Entre Ríos, and Santa Cruz Provinces (Claps *et al.*, 2008).

***Hypsibius pallidus* Thulin, 1911**

This species is widely distributed (Europe; Africa; Asia; North, Central, and South America (McInnes, 1994)). This record is the first documentation for Argentina.

***Isohypsibius sculptus* (Ramazzotti, 1962)**

This species has been previously recorded in Chile and Mexico (McInnes, 1994). In Argentina, the first record came from Río Negro Province (Rossi & Claps, 1989).

***Diphascon tenue* Thulin, 1928**

This species has been recorded in Europe (McInnes, 1994). In Argentina, the records are restricted to Río Negro Province and to the south Atlantic islands (Claps *et al.*, 2008).

***Macrobiotus patagonicus* Maucci, 1988**

This species has been restricted to the southern sector of Argentina and Chile (McInnes, 1994).

***Minibiotus subintermedius* (Ramazzotti, 1962)**

In Argentina, the first record was for Chubut Province (Claps *et al.*, 2008).

***Ramazzottius baumanni* (Ramazzotti 1962)**

This species has been previously recorded in New Zealand, North, Central and South America (McInnes, 1994). In Argentina, it has been cited in the majority of provinces (Claps *et al.*, 2008).

***Macrobiotus neuquensis* sp. nov.**

Figs. 1–2

Material examined: The holotype and the paratypes are from moss samples on soil and rock collected in Hua Hum, Junín de los Andes, at *ca*. 700 m asl (40°07'S 71°39' W), and from mosses on soil and lichen on a tree near Rucachoroi Lake, Aluminé, at *ca*. 900 m asl (39°12' S, 70°55' W), collected in December, 2004.

Type repository: The holotype (slide N° Nq. 6.6) is preserved at the Museum of La Plata (La Plata University). The paratypes are preserved in the collection of G. Rossi and M. C. Claps.

Specific diagnosis: *Macrobiotus* with an oral-cavity armature of the *harmsworthi* type, three macroplacoids, and one microplacoid; with eye spots, cuticle smooth, legs without granulation, claws of the *hufelandi* type, lunules smooth on all legs, the presence of a cuticular bar on the first three pairs of legs, and a refractile zone. These structures are less visible on the fourth pair of legs. The eggs have conical processes with reticular sculpture, all of them in contact with each other. The chorion is invisible.

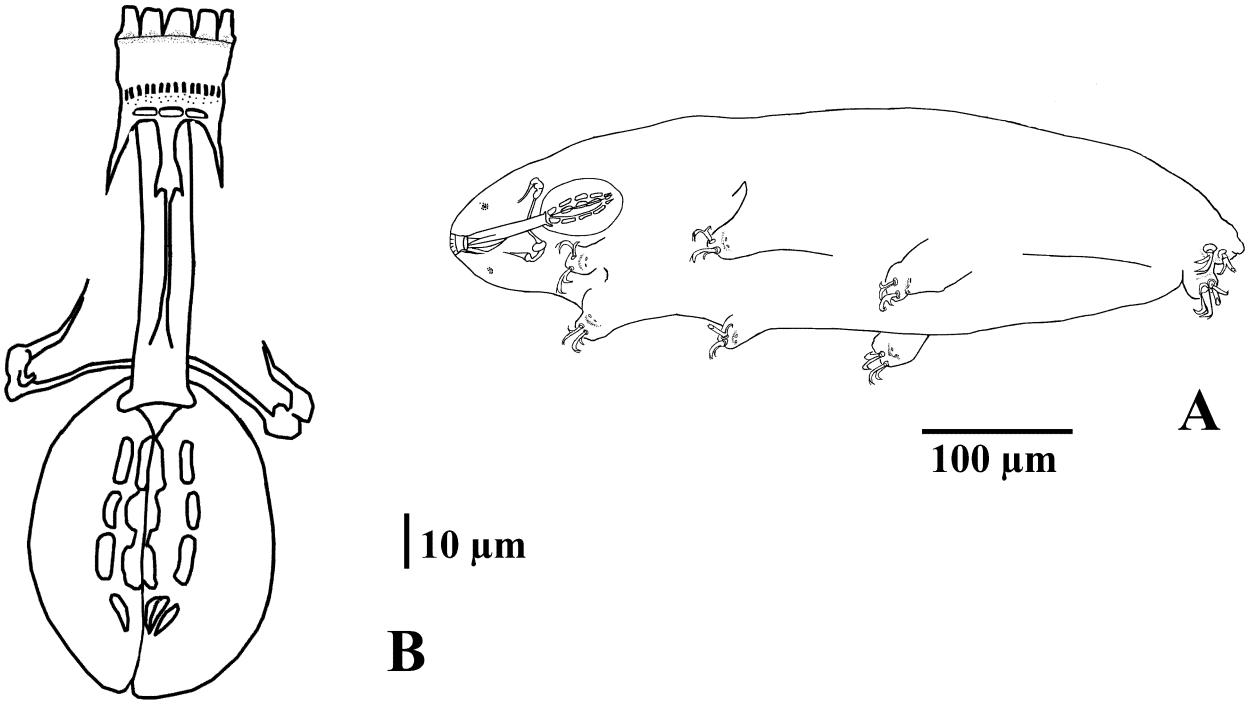

FIGURE 1. *Macrobiotus neuquensis* **sp. nov.** A, habitus ; B, buccopharyngeal apparatus.

Description of the holotype: The body length is 503.4 µm. The body (Fig. 1A) is transparent (white) with eyes in anterior position (Fig. 2A). The cuticle is smooth, without pores or pearls. The mouth is anterior and surrounded by a ring of 10 peribuccal lamellae. The oral-cavity armature is of the *harmsworthi* type. The teeth of the anterior band—or first band according to the Michalczyk and Kaczmarek (2003) terminology—are smaller, within the anterior border, and are in contact with the lamellae. The posterior band of teeth in the shape of a crown (second band) consists of conical teeth with granules in the posterior portion. The transverse ridges (third band) are small, while the laterals have a smooth margin. The buccal tube is 52 µm long and 10 µm wide (*pt* = 19.2) (Figs. 1B, 2A). Stylet supports are inserted on the buccal tube at 38 µm (*pt* = 73). The pharyngeal bulb is oval with distinct, triangular apophyses, three macroplacoids, and a microplacoid. The first macroplacoid is wider in the middle section, its length being 9.3 µm (*pt* = 17.8); the second, more pyramidal, is 8.2 µm long (*pt* = 15.7); the third is 10.2 µm long (*pt* = 19.6) and with a structurally different constriction in its terminal part. The microplacoid is 6 µm long (*pt* = 11.5) and separate from the third macroplacoid. The macroplacoid row is 28 µm long (*pt* = 53.8; Figs. 1B, 2A). The claws are of the *hufelandi* type, having primary branches with distinct accessory points (Fig. 2B). The lunules are smooth on all legs (Fig. 2C). Between the lunules and the refractile zone of the first three pairs of legs there is a cuticular bar (Fig. 2B), only visible under phase contrast or by DIC and not detected on the fourth pair of legs. On the first pair of legs, the primary branch has a basal portion and measures 12 µm (*pt* = 23.0), while the secondary branch is 10.5 µm long (*pt* = 20.1). On the second pair of legs, the primary and secondary branches are 12 (*pt* = 23.0) and 10 µm (*pt* = 19.2), respectively. On the third and fourth pair of legs, these branches measure 11 (*pt* = 21.1) and 8 µm (*pt* = 15.3) and 11 (*pt* = 21.12) and 9 µm (*pt* = 17.3), respectively.

The eggs, laid freely, are spherical and contain conical processes having a truncated apex (Fig. 2D). The processes, 9–12 around the circumference, have a 10–25 µm diameter at their base (Fig. 2F). The surface of the processes contains dense and uniform reticular sculpture with isodiametric meshes (Fig. 2E). The bases of the processes are in contact, and the union with the shell is by either connecting cords or a continuation of the sculpture itself, for this reason the shell is not visible (Fig. 2F). The processes are 7–15 µm height. We found one egg with an embryo (Table 2), thus allowing us to confirm that the eggs are without a doubt from *M. neuquensis* **sp. nov.** (Fig. 2D).

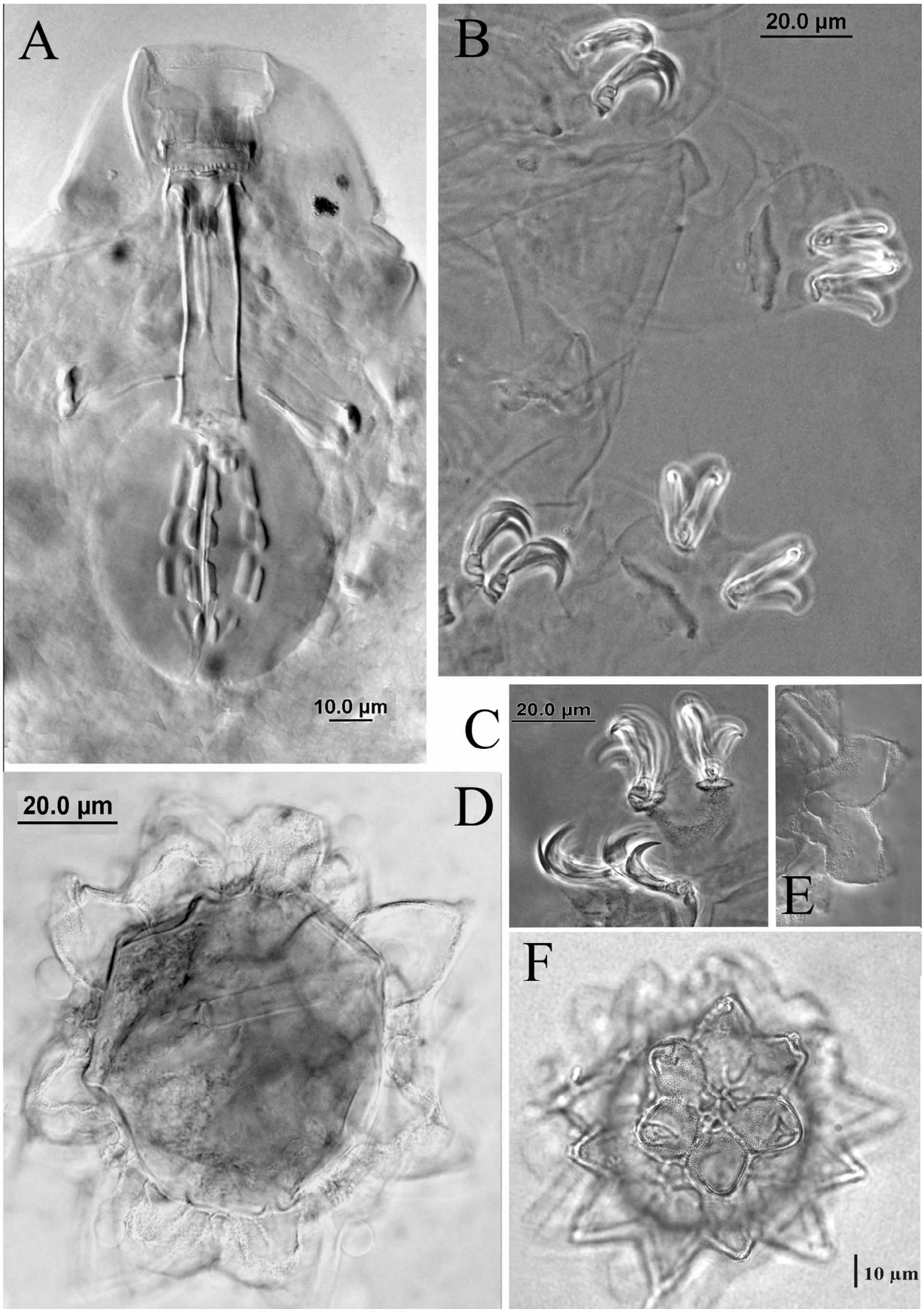

FIGURE 2. *Macrobiotus neuquensis* **sp. nov.** A, buccopharyngeal apparatus ; B, detail of claws showing cuticular bars; C, detail of claws showing lunules and accessory points; D, egg with embryo; E, detail of egg processes; F, detail of egg sculpture, showing the bases of the processes in contact.

TABLE 2. Measurements [in µm] and *pt* values of selected morphological structures of the holotype and mean values of other measured specimens (n = 23) of *Macrobiotus neuquensis* **sp. nov**. (minimum and maximum refers to the smallest and the largest structure found among all measured specimens; SD, standard deviation).

	Holotype	Mean	S. D.	Minimum	Maximum	Embryonaded egg
Body length	503.4	437.5	92.2	285.0	570.4	
Buccal tube length	52.0	50.2	10.6	37.1	69.4	32.0
Buccal tube width	10.0	9.5	1.7	7	12.4	4.8
pt	19.2	19.3	3.2	15.1	26.7	15.5
Stylet supports insertion	38.0	38.5	8.0	28.7	52.0	25.0
pt	73.0	76.8	2.1	73.1	79.5	78.1
Entire placoid row	34.0	32.2	8.2	22.3	44.8	15.5
pt	65.3	64.0	4.1	57.6	70.2	50.0
Macroplacoid row	28.0	25.1	6.8	16.8	34.7	12.2
pt	53.8	49.7	3.8	44.7	56.0	39.4
Macroplacoid 1	9.3	8.0	1.9	4.9	10.4	3.3
pt	17.8	15.9	2.0	13.2	19.1	10.6
Macroplacoid 2	8.2	6.5	1.6	4.9	9.1	3.0
pt	15.7	13.1	1.7	10.4	15.8	9.7
Macroplacoid 3	10.2	8.5	1.8	5.9	11.1	3.3
pt	19.6	17.2	1.8	15.0	20.4	10.6
Microplacoid	6.0	4.9	1.5	2.7	7.4	1.8
pt	11.5	9.8	1.8	7.0	11.5	5.8
Claw 1 primary branch	12.0	13.	4.4	8.5	19.8	
pt	23	27.8	3.2	21.2	29.8	
Claw 1 secondary branch	10.5	10.9	3.5	6.0	17.3	
pt	20.1	20.0	3.1	16.2	24.9	
Claw 2 primary branch	12.0	13.7	5.0	8.0	22.3	
pt	23.0	25.0	4.1	21.2	32.1	
Claw 2 secondary branch	10.0	10.9	3.6	6.5	17.3	
pt	19.2	20.1	2.8	17.3	24.9	
Claw 3 primary branch	11.0	13.6	4.8	8.0	19.8	
pt	21.1	24.4	3.6	20.5	29.8	
Claw 3 secondary branch	8.0	11.2	3.8	7.0	17.3	
pt	15.3	20.6	3.1	15.4	24.9	
Claw 4 primary branch	11.0	15.1	5.0	9.0	24.8	
pt	21.1	32.0	13.2	21.2	67.1	
Claw 4 secondary branch	9.0	11.6	3.8	7.0	17.3	
pt	17.3	21.1	3.1	17.3	26.1	
Pharyngeal bulb length	65.2	65.7	4.3	62.0	69.4	
Pharyngeal bulb width	52.8	53.1	1.3	52.0	54.5	

Etymology: The name *neuquensis* refers to the province that includes the *locus typicus* of this new species.

Differential diagnosis: *Macrobiotus neuquensis* **sp. nov.** belongs to the *harmsworthi* group, whose

species exhibit the following characteristics in common: three macroplacoids in the form of an arch, a microplacoid in the pharyngeal bulb, plus a cuticle without pores. The presence of the cuticular bar and a refractile zone in the legs along with the eggs having conical processes in contact with one another are the principal characteristics differentiating *M. neuquensis* **sp. nov.** from the other species of the group.

This new species is similar to *Macrobiotus chieregoi* Maucci & Durante 1980, but differs from the latter by having eyes and a wider buccal tube. In addition, the processes of the eggs of *M. neuquensis* **sp. nov.** are shorter than those of *M. chieregoi*. *Macrobiotus neuquensis* **sp. nov.** differs from *Macrobiotus krynauwi* Dastych & Harris 1995 in having a smooth cuticle without pearls, a longer and wider buccal tube, bigger macroplacoids, and smaller claws. *Macrobiotus neuquensis* **sp. nov.** also differs from *Macrobiotus blocki* Dastych 1984 and *Macrobiotus ovostriatus* Pilato & Patané 1998 with respect to the characteristics of the eggs (Table 3). *Macrobiotus neuquensis* **sp. nov.** is furthermore unlike *Macrobiotus reinhardti* Michalczyk & Kaczmarek 2003 in having a shorter third macroplacoid, a wider buccal tube, and longer processes on the eggs, among other morphological features.

Remarks: Measurements and *pt* values of selected morphological structures for all specimens are presented in Table 2.

TABLE 3. Egg characteristics of *Macrobiotus neuquensis* **sp. nov.** compared with those mentioned for similar species.

	M. neuquensis **sp. nov.**	*M. coronatus*	*M. harmsworthi*	*M. simulans*	*M. blocki*	*M. ovostriatus*	*M. krynauwi*
Diameter without processes	87–94	42–55	69–84	43–82	70–90	64–70	98–115
Diameter with processes	100–117	55–71	88–104	95–99	90–130	92–97	130–145
Processes: number	9–10	13–14	14–22	11–13	15–24	12	16–21
pattern		spaced	spaced	spaced	close	spaced	close
surface	dotted	dotted		dotted	dotted		dotted
height	7–15	9.6	15	11	10–25	19	9–14
basal diameter	10–25	10.4	15–22	11–16	8–14	14	9–15
base	sculptured	serrated	smooth	serrated	smooth	with thickenings	smooth
tip	short	median	median	short	long	long	long
Shell		dotted		dotted	smooth	dotted	

***Macrobiotus tehuelchensis* sp. nov.**
Figs. 3 and 4

Material examined: The holotype and paratypes are from moss and lichen samples from soil collected near Ñorquinco Pond, Aluminé, *ca.* 1000 m asl, in January, 2005.

Type repository: The holotype (slide N° Nq. 10.6) is preserved at the Museum of La Plata (La Plata University). The paratypes are preserved in the collection of G. Rossi and M.C. Claps.

Specific diagnosis: *Macrobiotus* with oral-cavity armature of the *echinogenitus* type and claws of the *hufelandi* type, three macroplacoids, and a microplacoid; without eye spots; and with small smooth lunules on all legs**.** The eggs have conical processes with a slender, translucent, and flexible distal portion. The egg shell is not visible.

Description of the holotype: The colorless body has a length of 494 µm, eye spots are absent, and the cuticle is smooth (Fig. 3A). The mouth is terminal and the buccal tube rigid with ventral lamina (Fig. 4A). The buccal armature corresponds to the *echinogenitus* type (Pilato, 1972), resembling the mouth of *Macrobiotus pallari* Maucci 1954 with 10 large peribucal lamellae, an anterior band of numerous small, fine teeth all around the wall. The posterior band of teeth is formed by 3–4 rows of larger teeth than those present in the anterior band but smaller than those of the posterior band. The median ventral ridge is rectangular in shape and nearly the same size as the dorsal ridge. The ventrolateral and dorsal ridges always have smooth margins (Fig. 3B). The buccal tube is 67 µm long and 12.4 µm wide (*pt* = 18.5); the pharyngeal bulb has apophyses, three macroplacoids, and a well developed microplacoid (Figs. 3C, 4A). The insertion of the stylets is at 47.1 µm (*pt* = 70.4). The lengths of the macroplacoids are 9.9 µm (*pt* = 14.8), 8.7 µm (*pt* = 13.0), 8.7 µm (*pt* = 13.0); that of the microplacoid, which is separate from the third macroplacoid, is 5.0 µm (*pt* = 7.4). The lengths of the entire placoid and macroplacoid rows are 37.2 µm (*pt* = 55.6) and 32.2 µm (*pt* = 48.1), respectively. The claws are of the *hufelandi* type with thin accessory points on the main branches, and small lunules with smooth margins are also present in the fourth pair of legs (Figs. 4B, 4C).

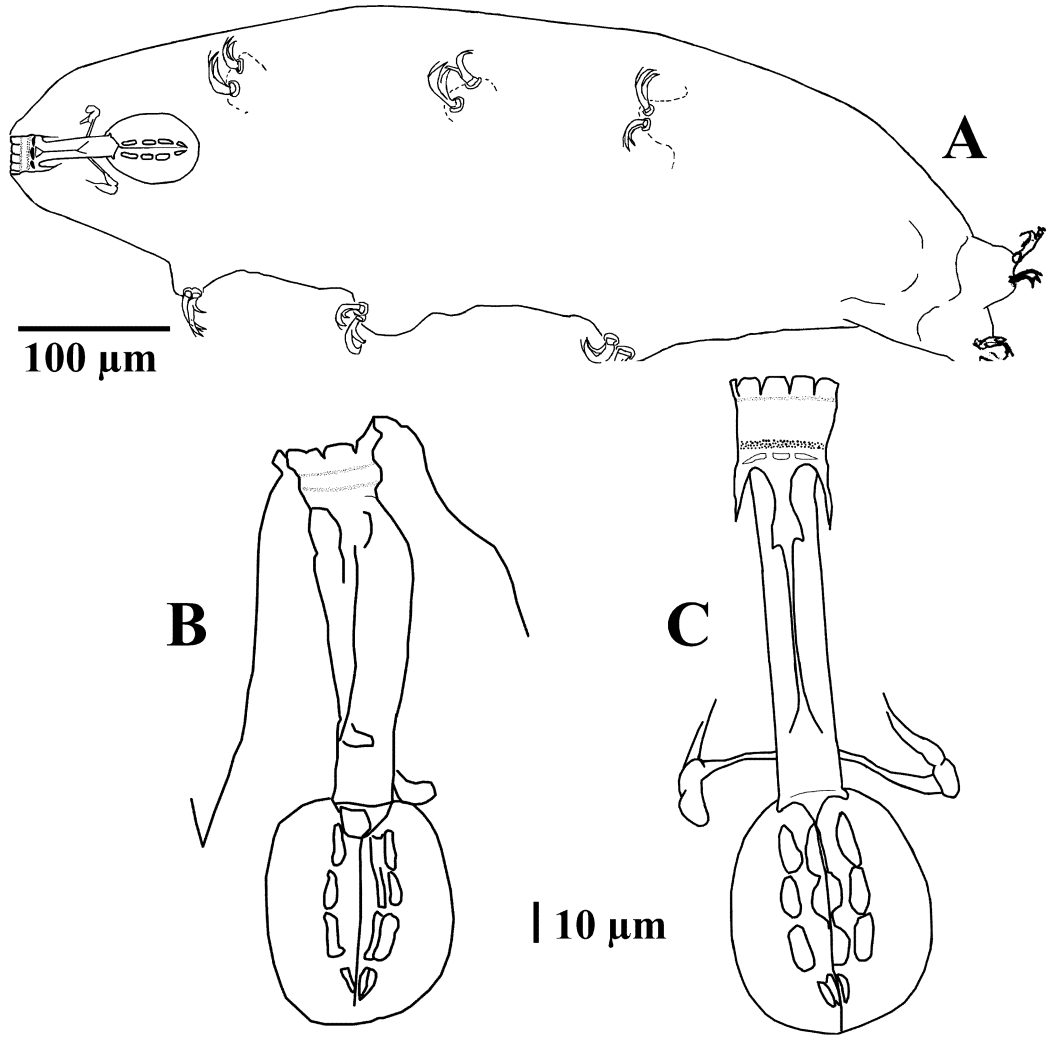

FIGURE 3. *Macrobiotus tehuelchensis* **sp. nov.** A, habitus ; B, buccopharyngeal apparatus in lateral view; C, buccopharyngeal apparatus in ventral view.

The eggs, laid freely, are a translucent white-gray and are spherical with conical processes (nine on each egg circumference). The mean diameters excluding and including processes are 77 and 150 µm, respectively. The processes have a slender, translucent and flexible distal portion with parallel linear markings (Fig. 4D).

The maximum length of each complete process is nearly 50 µm, the cone 10–15 um, and the translucent distal portion 30–40 µm. The bases are reticulated, irregular, and with a diameter of 24–26 µm. The shell is apparently the continuation of the striation of the processes (Figs. 4D, E).

We found one egg with an embryo, thus allowing us to confirm that the eggs are definitely from *M. tehuelchensis* (Fig. 4F).

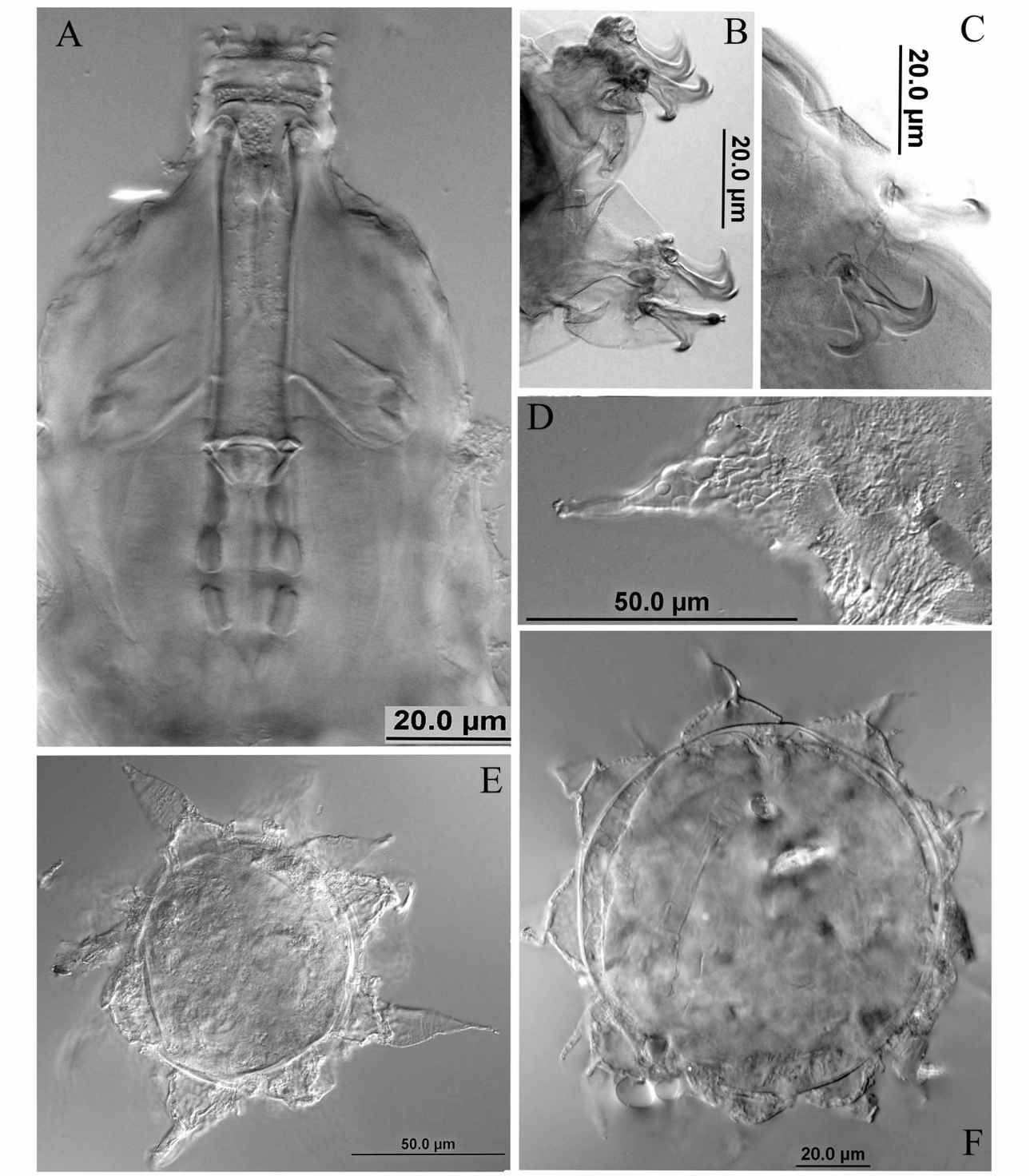

FIGURE 4. *Macrobiotus tehuelchensis* **sp. nov.** A, buccopharyngeal apparatus. B, claws of the fourth pair of legs; C, detail of a claw, showing the lunule; D, detail of the egg processes; E, egg; F, egg with embryo.

TABLE 4. Measurements [in µm] and *pt* values of selected morphological structures of the holotype and mean values of other measured specimens (n = 7) of *Macrobiotus tehuelchensis* **sp. nov.** (minimum and maximum refers to the smallest and the largest structure found among all measured specimens; SD, standard deviation).

	Holotype	Mean	S. D.	Minimum	Maximum
Body length	494.0	484.7	111.8	316.0	666.0
Buccal tube length	67.0	66.3	7.9	57.0	79.4
Buccal tube width	12.4	11.7	2.1	9.9	14.9
pt	18.5	17.5	1.4	14.8	18.8
Stylet supports insertion	47.1	47.8	4.7	42.2	54.6
pt	70.4	72.4	2.1	68.8	74.1
Entire placoid row	37.2	34.1	6.8	27.3	42.2
pt	55.6	53.5	4.6	47.8	58.6
Macroplacoid row	32.2	33.9	1.4	32.2	34.7
pt	48.1	46.7	2.6	43.8	48.3
Macroplacoid 1	9.9	9.7	2.0	7.4	12.4
pt	14.8	14.6	1.6	13.0	17.2
Macroplacoid 2	8.7	8.1	1.0	7.4	9.9
pt	13.0	12.3	0.8	11.1	13.0
Macroplacoid 3	8.7	8.6	1.5	7.4	11.2
pt	13.0	12.9	0.9	11.5	14.1
Microplacoid	5.0	5.8	1.3	5.0	7.4
pt	7.4	8.5	1.2	7.4	10.3
Claw 1 primary branch	14.9	16.1	1.8	14.9	17.4
pt	22.2	23.2	1.4	22.2	24.1
Claw 1 secondary branch	12.4	12.4	0.0	12.4	12.4
pt	18.5	17.9	0.9	17.2	18.5
Claw 2 primary branch	14.9	16.1	1.8	14.9	17.4
pt	22.2	23.2	1.4	22.2	24.1
Claw 2 secondary branch	12.4	13.6	1.8	12.4	14.9
pt	18.5	19.6	1.5	18.5	20.7
Claw 3 primary branch	17.4	17.4	0.0	17.4	17.4
pt	25.9	25.0	1.3	24.1	25.9
Claw 3 secondary branch	14.9	14.9	0.0	14.9	14.9
pt	22.2	21.5	1.1	20.7	22.2
Claw 4 primary branch	17.4	16.9	2.9	12.4	19.8
pt	25.9	26.3	2.9	21.7	30.8
Claw 4 secondary branch	14.9	13.6	2.6	9.9	17.4
pt	22.2	21.2	2.4	17.4	24.1

Etymology: The species is named after the extinct aboriginal inhabitants of the Patagonia (the Tehuelches).

Differential diagnosis: Depending on the measurements of the macroplacoids, this species may be identified as a different species with respect to *Macrobiotus richtersi* Murray, 1911 or to *Macrobiotus liviae* Ramazzotti, 1962. The differences from these species are the points of insertion of the stylets, the *pt* of the

buccal tube width, and the size of the micoplacoid. The main difference resides in the structure of egg processes, which are more numerous and truncate in *M. richtersi* and without the distal portion in *M. liviae*. The new species is similar to *Macrobiotus mauccii* Pilato, 1974, but differs from that species by having a wider buccal tube, longer macroplacoids, and a smaller microplacoid. Moreover, *M. tehuelchensis* **sp. nov.** has much longer claws than those mentioned for *M. mauccii,* and the eggs also show clear differences from those described for that species. *Macrobiotus tehuelchensis* **sp. nov.** differs from *Macrobiotus snaresensis* Horning Schuster & Grigarick, 1978 by the length and width of the buccal tube, the size of the macroplacoids, the separate microplacoid, and the claws, as well as the morphologic characteristics of the eggs.

Remarks: The paratypes of this species have the same qualitative characteristics as the holotype. The dimensions of some structures of the specimens are indicated in Table 4.

***Minibiotus claxtonae* sp. nov.**
Fig. 5

Material examined: Holotype and paratypes from lichen samples on trees, collected near Quillen Lake, Aluminé during January of 2005.

Type repository: The holotype (slide N° Nq 11.11) is preserved at the Museum of La Plata (La Plata University). The paratypes are preserved in the collection of G. Rossi and M. C. Claps

Specific diagnosis: The cuticle is largely smooth, but with 10 transverse bands of roughness. Star-shaped pores are present on the cuticle surface and on all pairs of legs. Eye spots are also present. The buccal tube contains two curvatures. The insertion of the stylet support is near the middle of the buccal tube and is nearest to the pharyngeal bulb; the pharynx exhibits apophyses, three square macroplacoids, and a small microplacoid. On all legs there is granulation along with small, smooth lunules. The eggs contain cup-shaped processes and are not covered by a membrane.

Description of the holotype: The body length is 208 µm with posterior eyes present (Fig 5A). The cuticle is smooth, with 10 transverse bands and pores having two shapes: small and round (nearly 1 µm in diameter) or conspicuously star-shaped. These stellate pores are aligned in longitudinal rows and separated, and the round rings are intermixed (Fig. 5E). Each leg contains stars and granulation that appear denser at the end of the legs on all four pairs (Fig. 5C). The mouth is anteroventral with a buccal tube 23 µm long and 2 µm wide (*pt* = 8.7), having two bends (anterior and posterior). The pharyngeal bulb is with noticeable apophyses, three near-square macroplacoids, and a small microplacoid. The stylets are inserted at 16 µm (*pt* = 69.6). The macroplacoid lengths are very similar: the first, 2.2 µm (*pt* = 9.6); the second; 2 µm (*pt* = 8.7); and the third, 2.3 µm (*pt* = 10.0). The small microplacoid is 1 µm long (*pt* = 4.3). The macroplacoid row is 9 µm long (*pt* = 39.1) (Fig. 5B). The claws are short and robust, with smooth lunules and the primary branches of internal and external claws have distinct accessory points (Fig. 5D).

The eggs have a non-reticulated shell. The egg diameter, with and without processes, fluctuates between 82–86 µm and 68–70 µm, respectively. There are *ca*. 22 processes around the egg's circumference and nearly 62 are found within a given hemisphere. The processes are in the form of an egg cup. Each process is not covered by a separate membrane, is without ornamentation at the base, and has a length of 7 µm and a width at the base of *ca.* 5 µm (Fig. 5F).

Etymology: This species is named after Dr. Sandra Claxton of the Macquarie University, Australia in recognition of her contribution to our knowledge of the genus *Minibiotus* in particular and to our understanding of the tardigrades in general.

Differential diagnosis: *Minibiotus claxtonae* **sp. nov.** differs from *Minibiotus hufelandioides* (Murray, 1910) by its smaller macroplacoids, the first macoplacoid not being obscured by the apophysis, and the absence of denticles in the lunules. In the egg, a ring of pores at the base of the processes is absent. *Minibiotus claxtonae* **sp. nov.** differs from *Minibiotus aquatilis* Claxton, 1998 mainly by characteristics of the egg. The *pt* values of different structures are very similar but the absence of denticles in the lunules is evident.

Remarks: The measurements and *pt* values of selected morphological structures for all specimens are given in Table 5

TABLE 5. Measurements [in µm] and *pt* values of selected morphological structures of the holotype and mean values of other measured specimens (n = 23) of *Minibotus claxtonae* **sp. nov.** (minimum and maximum refers to the smallest and the largest structure found among all measured specimens; SD, standard deviation).

	Holotype	Mean	S. D.	Minimum	Maximum
Body length	208.3	238.0	40.0	187.5	315.0
Buccal tube length	23.0	23.0	2.4	18.0	28.0
Buccal tube width	2.0	2.0	0.3	1.0	2.4
pt	8.7	8.5	1.0	5.6	10.0
Stylet supports insertion	16.0	15.0	2.5	10.0	19.0
pt	69.6	62.1	10.3	33.3	78.7
Entire placoid row	10.0	9.0	1.6	4.0	11.0
pt	39.1	35.3	6.4	21.3	47.8
Macroplacoid row	9.0	8.0	1.1	6.0	10.0
pt	38.3	33.2	4.1	29.2	43.5
Macroplacoid 1	2.2	2.0	0.5	1.0	3.0
pt	9.6	8.7	1.4	5.6	11.3
Macroplacoid 2	2.0	2.0	0.3	1.0	2.5
pt	8.7	8.3	1.1	5.6	10.0
Macroplacoid 3	2.3	2.0	0.4	1.0	3.0
pt	10.0	9.0	1.6	5.6	12.2
Microplacoid	1.0	1.0	0.0	0.9	1.0
pt	4.3	4.0	0.7	2.3	4.8
Claw 1 primary branch	6.0	6.0	1.0	5.0	8.0
pt	22.6	27.2	3.7	21.7	34.0
Claw 1 secondary branch	4.3	5.0	0.8	4.0	6.2
pt	18.7	20.7	2.7	17.5	25.5
Claw 2 primary branch	6.0	6.0	1.4	3.2	7.3
pt	26.1	29.7	3.2	23.9	35.0
Claw 2 secondary branch		5.0	1.1	3.2	7.0
pt	26.1	22.2	3.7	16.0	26.9
Claw 3 primary branch	7.0	7.0	1.3	5.0	9.0
pt	30.4	29.2	3.5	23.0	34.0
Claw 3 secondary branch	5.5	5.0	1.2	3.0	6.5
pt	23.9	21.4	4.1	15.0	26.1
Claw 4 primary branch	7.5	8.0	1.6	5.0	10.2
pt	32.6	28.4	7.6	16.7	42.5
Claw 4 secondary branch		5.0	1.4	3.0	8.0
pt	21.7	22.9	5.0	15.0	34.0
Pharyngeal bulb length	19.0	21.0	4.4	16.0	29.0
Pharyngeal bulb width	16.0	17.0	2.8	13.0	21.0

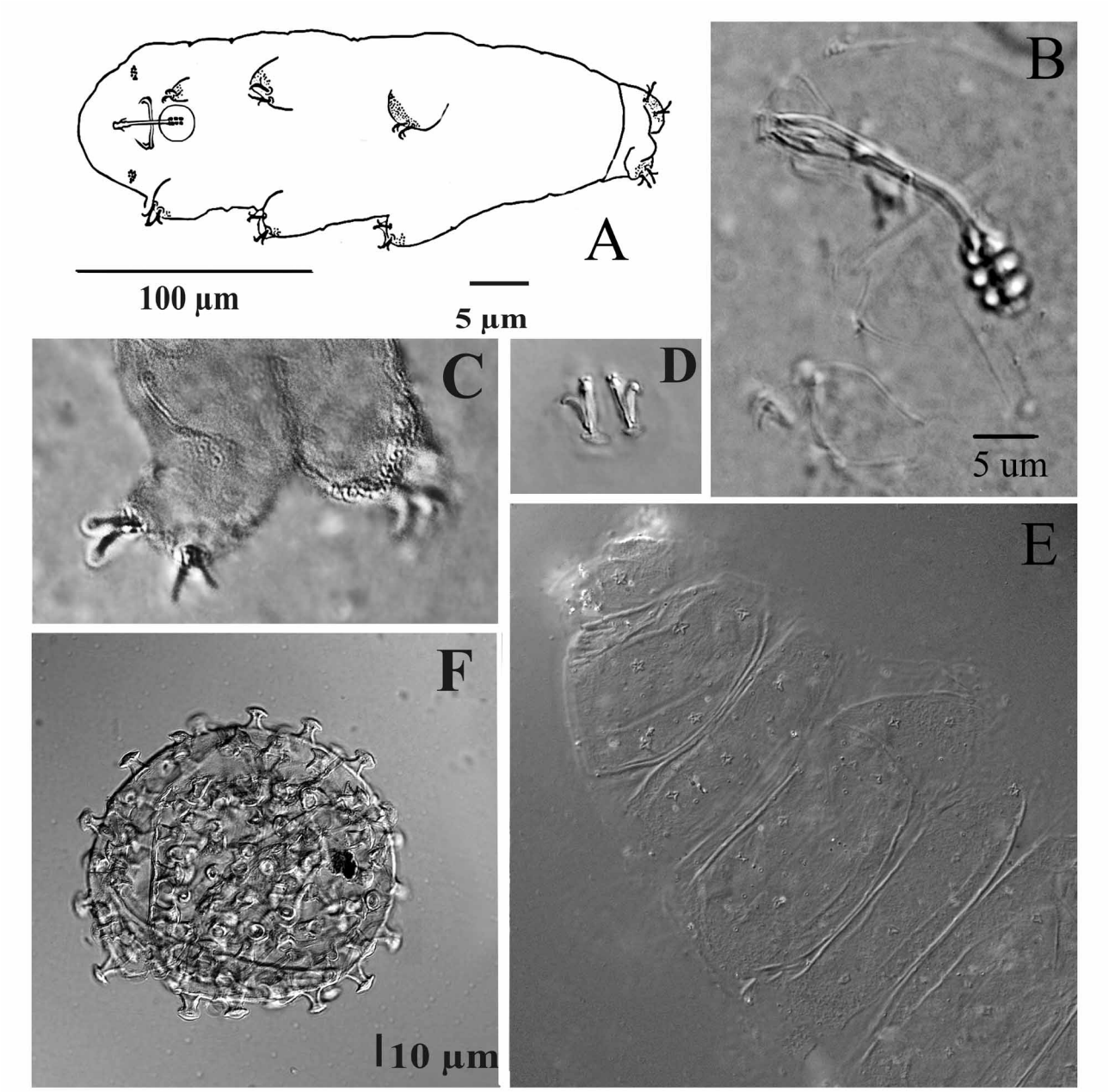

FIGURE 5. *Minibiotus claxtonae* **sp. nov.** A, habitus; B, buccopharyngeal apparatus in lateral view; C, claws of the fourth pair of legs, showing the granulation; D, detail of a claw, showing the lunules; E, cuticle with the round and star-shaped pores; F, egg.

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