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MINIBIOTUS ACADIANUS (EUTARDIGRADA: MACROBIOTIDAE), A NEW SPECIES OF TARDIGRADA FROM SOUTHERN LOUISIANA, U.S.A.

Harry A. Meyer^{1,2} and Megan N. Domingue¹

ABSTRACT.—Over 200 species of freshwater and terrestrial water bears (phylum Tardigrada) are known to occur in North America. Of these, 16 species have been collected in Louisiana. Foliose and fruticose lichens collected on 18 April 2010 in Acadia Parish, Louisiana, were stored in paper envelopes and later soaked in tap water overnight. Tardigrade specimens and eggs were extracted and mounted in polyvinyl lactophenol. The samples contained a new species of tardigrade. *Minibiotus acadianus* sp. n. has a buccal tube with single anterior curvature, 2 macroplacoids and 1 microplacoid. Small gibbosities are present on the fourth pair of legs, in 3 caudal rows, and in a single row at the level of the third pair of legs. *Minibiotus acadianus* sp. n. differs from the most similar tardigrade species, *Minibiotus fallax* (found in Australia and Florida), in having a shorter and wider buccal tube, a different pattern of gibbosities, and short, peg-shaped processes in addition to long, filamentous processes on the eggs. Reexamination of specimens identified as *M. fallax* in an earlier paper on the Tardigrada of Louisiana indicates that they are, in fact, *M. acadianus* sp. n., suggesting that the new species is widely distributed in Louisiana.

RESUMEN.—Se conocen más de 200 especies de osos de agua (phylum Tardigrada) terrestres y de agua dulce que ocurren en Norteamérica. De éstas, 16 se han colectado en Louisiana. Se colectaron líquenes foliosos y fruticosos en la parroquia de Acadia, Louisiana, el 18 de abril de 2010, los cuales fueron guardados en sobres de papel y después remojados en agua de la llave durante toda la noche. Se extrajeron especímenes y huevos de tardígrados y se fijaron en polivinilo lactofenol. Las muestras contenían una nueva especie de tardígrado. *Minibiotus acadianus* sp. n. tiene un tubo bucal con una única curvatura anterior, dos macroplacoides y un microplacode. Pequeñas gibosidades están presentes en el cuarto par de patas, en tres hileras caudales y en una sola hilera al nivel del tercer par de patas. *Minibiotus acadianus* sp. n. difiere de la especie más próxima, *Minibiotus fallax*, una especie que se encontró en Australia y Florida, porque tiene un tubo bucal más corto y ancho, un patrón distinto de gibosidades y apófisis cortas en forma de estaquilla en los huevos, además de apófisis largas y filamentosas. Una reexaminación de los especímenes identificados como *M. fallax* en una publicación previa sobre los tardígrados de Louisiana indica que son más bien *M. acadianus* sp. n., lo cual sugiere que esta nueva especie tiene una distribución extensa en Louisiana.

Tardigrades (phylum Tardigrada), commonly known as water bears, are microscopic animals found in marine, freshwater, and terrestrial habitats. Terrestrial species occur in mosses, lichens, liverworts, and leaf litter, and are renowned for their ability to enter a cryptobiotic state (anhydrobiosis) in response to desiccation. Terrestrial tardigrades include both herbivorous and carnivorous species that feed on nematodes, rotifers, and other tardigrades (Lehmann et al. 2007). Over 200 species of freshwater and terrestrial tardigrade are known to occur in North America (Meyer and Hinton 2007). Of these, 16 are known to occur in Louisiana (Meyer 2001, Hinton and Meyer 2007).

We describe a new species belonging to the genus *Minibiotus* collected from lichens in Acadia Parish, Louisiana, southeastern United States (in Louisiana, a parish is a unit of local

administration corresponding to a county in other American states). Only one species of water bear, *Milnesium tardigradum*, has been found previously in Acadia Parish (Hinton and Meyer 2007).

METHODS

The second author collected lichen samples in Crowley, Acadia Parish, Louisiana, on 18 April 2010; sampling was not quantitative. The foliose lichen *Parmotrema perforatum* (Jacq.) A. Massal. and the fruticose lichen *Ramalina stenospora* Müll. Arg. were collected from trees and fallen branches. Samples were stored in sealed paper envelopes or paper bags.

In the laboratory, samples were placed in tap water and soaked overnight to rehydrate anhydrobiotic water bears. After sieving (42- μ m

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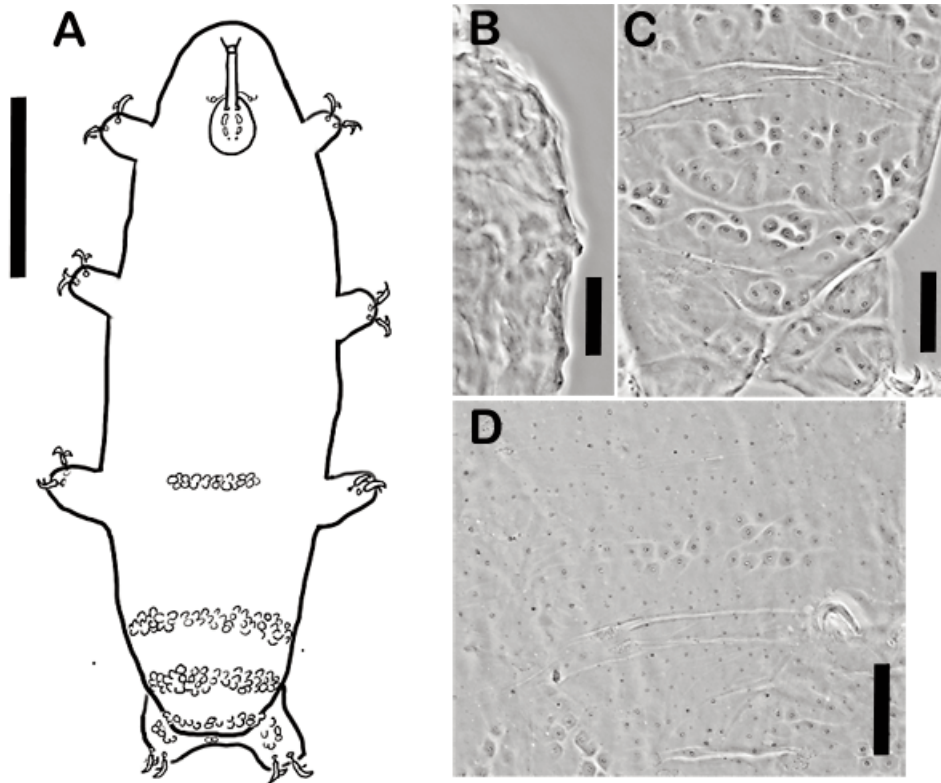


Fig. 1. *Minibiotus acadianus* sp. n.: A, habitus; B, posterior gibbosities; lateral view of dorsum near leg II; C, posterior and leg IV gibbosities, dorsal view; D, gibbosities at level of leg III. Scale bars: A, 100 μ m; B–D, 20 μ m.

mesh diameter), the samples were examined with a dissecting microscope for the presence of tardigrade specimens and eggs. Specimens and eggs were mounted on slides in polyvinyl lactophenol and examined under oil immersion using a phase-contrast microscope. Anatomical terminology follows Guidetti and Bertolani (2005). Specimens were measured using imaging software (NIS-Elements D 2.30, SPI), and all morphological measurements are given in micrometers (μ m). The *pt* index, expressed as a percentage, is the ratio of the length of a given structure to that of the buccal tube (Pilato 1981). In type descriptions, *pt* indices are put in bracketed italics. Buccal tube length was measured from the anterior margin of the stylet sheaths to the base of the tube, while the stylet insertion point was measured from the anterior margin of the stylet sheaths to the point of insertion on the buccal tube. Buccal tube width was measured as the external diameter at the level of stylet support insertion. Lengths of primary and

secondary branches of claws were measured from base to apex, including accessory points.

TAXONOMIC ACCOUNT

Minibiotus acadianus sp. n. (Figs. 1–3, Tables 1–2)

DIAGNOSIS.—A *Minibiotus* species with 2 macroplacoids and 1 microplacoid; gibbosities on the fourth pair of legs, in 3 caudal bands, and in a band at the level of the third pair of legs; eggs with short, peg-shaped processes and sometimes long, filamentous processes.

MATERIAL EXAMINED.—One holotype, 8 paratypes, and 9 eggs (including 1 embryonate egg): foliose and fruticose lichen from trees and fallen branches in a wooded lot behind Woodlawn Cemetery on South Avenue F, Crowley, Acadia Parish, Louisiana, U.S.A. (30°11'30.23"N, 92°22'23.43"W; elevation 20 m asl).

DEPOSIT OF TYPES.—The holotype (SMLA 9560) and paratypes (SMLA 9550, 9552, 9555,

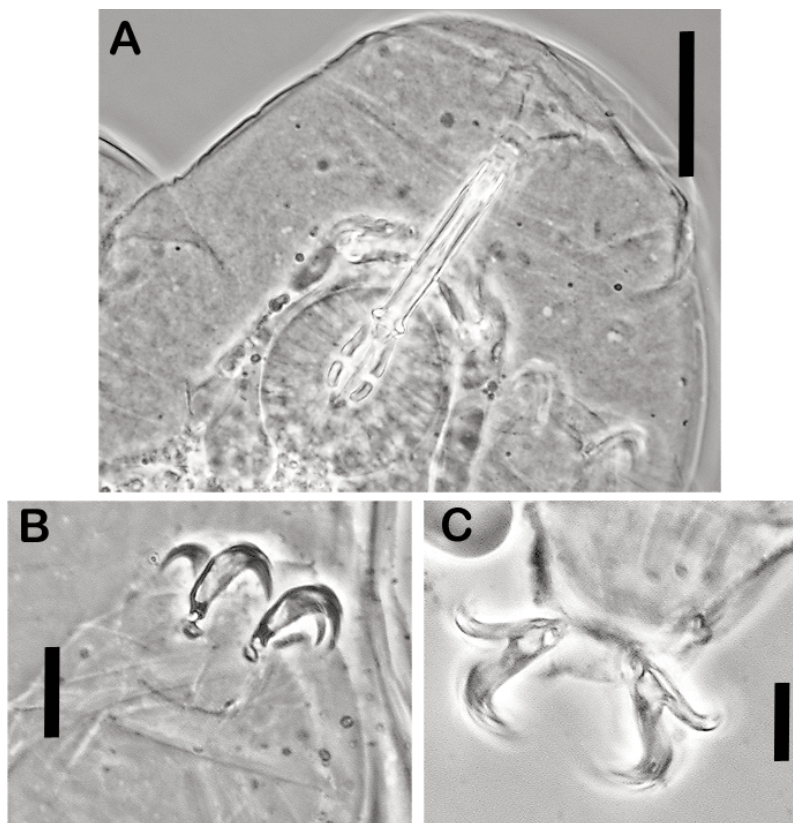


Fig. 2. *Minibiotus acadianus* sp. n.: A, buccopharyngeal apparatus; B, claw of leg II; C, claw of leg IV. Scale bars: A, 20 μ m; B–C, 10 μ m.

TABLE 1. Morphometric data and *pt* values of selected characters of 9 specimens of *Minibiotus acadianus* sp. n. from Acadia Parish, Louisiana, USA. Characters are lengths (μ m) unless otherwise indicated. Range refers to the smallest and largest structure found among all measured specimens. Abbreviations: *N* = number of specimens measured, SD = standard deviation, n.a. = not applicable, *pt* = *pt* index.

Character	<i>N</i>	Range		Mean		SD	
		Length	<i>pt</i>	Length	<i>pt</i>	Length	<i>pt</i>
Body	9	285.0–445.4	n.a.	365.3	n.a.	56.7	n.a.
Buccal tube	9	26.0–28.2	n.a.	28.2	n.a.	1.4	n.a.
Stylet support insertion point	9	17.8–20.4	66.0–71.1	19.3	68.6	0.8	1.9
Buccal tube external diameter	9	2.6–4.2	10.4–13.6	3.6	12.6	0.4	1.0
First macroplacoid	9	5.3–6.8	19.3–22.4	5.9	21.1	0.5	1.4
Second macroplacoid	9	3.4–4.4	12.5–15.7	4.0	14.1	0.3	1.0
Macroplacoid row	9	9.4–11.7	35.4–39.4	10.5	37.3	0.8	1.4
Microplacoid	9	1.0–2.1	3.8–7.4	1.6	5.5	0.4	1.2
Placoid row	9	11.0–14.0	41.7–49.3	12.5	44.5	1.1	2.8
Primary claw branch, Leg I	4	9.7–11.5	36.2–42.0	10.7	39.7	0.8	2.6
Secondary claw branch, Leg I	4	8.1–9.6	30.2–33.8	9.0	32.3	0.6	1.7
Primary claw branch, Leg II	6	10.9–12.0	39.2–43.5	11.5	41.2	0.4	1.7
Secondary claw branch, Leg II	6	9.2–10.4	30.4–35.6	9.6	33.6	0.4	1.9
Primary claw branch, Leg III	4	10.8–12.1	36.9–43.1	11.5	40.7	0.6	2.8
Secondary claw branch, Leg III	4	8.3–10.1	26.9–35.6	9.2	32.6	0.7	3.9
Primary claw branch, Leg IV	5	11.0–13.2	42.3–47.8	12.7	45.6	0.7	2.2
Secondary claw branch, Leg IV	5	9.2–10.7	34.6–37.7	9.9	36.4	0.7	1.2

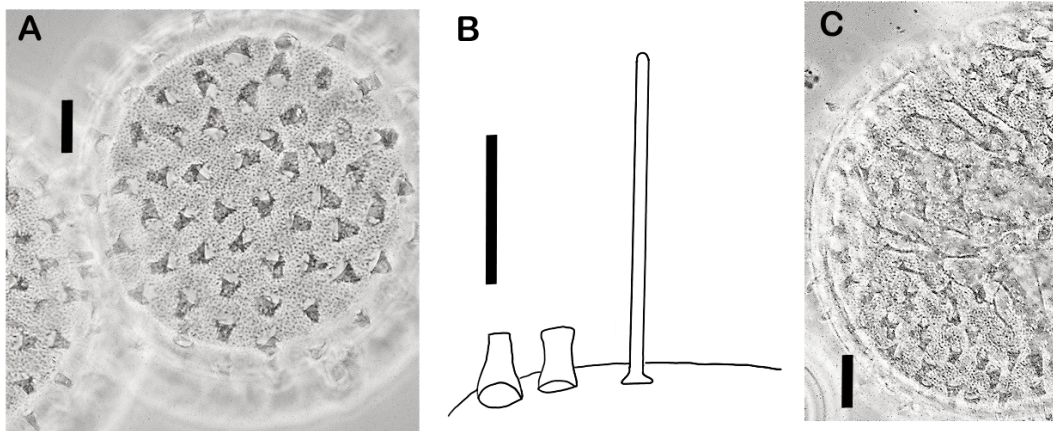


Fig. 3. *Minibiotus acadianus* sp. n.: A, egg with peg-shaped processes; B, egg processes; C, egg with mixed peg-shaped and filamentous processes. Scale bars: 10 μ m.

TABLE 2. Morphometric data of selected structures of 8 eggs of *Minibiotus acadianus* sp. n. from Acadia Parish, Louisiana, USA. Diameter, height, and distance in micrometers (μ m). Range refers to the smallest and largest structure found among all measured specimens.

Character	Range
Diameter without processes	38.0–72.8
Diameter with processes	43.6–79.9
Height of peg-shaped processes	2.0–4.4
Diameter of peg-shaped process bases	2.2–4.3
Length of filamentous processes	5.1–17.1
Diameter of filamentous process bases	1.5–2.5
Distance between processes	1.7–4.7
Number of processes around circumference	28–38
Approx. number of processes in hemisphere	75–110

9557–9561) are deposited in the W.A.K. Seale Museum, Department of Biology and Health Sciences, McNeese State University, Lake Charles, Louisiana 70609, U.S.A.

DESCRIPTION.—Holotype: body length 414.7, white or transparent (Fig. 1A). Eyes absent. Cuticle with numerous small circular pores (approximately 1.0 wide). Gibbosities present in 3 caudal rows of variable width and on fourth pair of legs (Figs. 1B, 1C). Additional band of gibbosities at level of third pair of legs (Fig. 1D). Most gibbosities polygonal or elliptical, 3.1–4.8 wide, with well-developed central pore. Some gibbosities fused into larger formations, maximum measured 9.1 long by 4.1 wide.

Mouth anteroventral. Oral cavity teeth absent or not visible with light microscopy. Buccal tube rigid, with single anterior curvature, 30.9 long, 4.2 [13.6] wide (Fig. 2A). Buccal tube wall

thickened below stylet support insertion point. Stylet supports inserted on buccal tube at 20.4 [66.0]. Ventral support inserted at 15.6 [50.5]. Apophyses in pharynx triangular, close to first macroplacoid. First macroplacoid rod shaped, with central constriction, 6.8 [22.2] long; second rod shaped, with subterminal constriction, 4.4 [14.2] long. Macroplacoid row 11.7 [37.9] long. Microplacoid shaped like an elongated tear drop, 1.8 [5.8] long. Entire placoid row 13.3 [43.0] long. Pharyngeal bulb round, 32.9 [106.5] wide, 34.1 [110.4] long.

Granulation present on all legs. Claws of *hufelandi* type short and stout, with well-developed accessory points on primary branches (Figs. 2B, 2C). Claw lengths: leg II primary branch (pb) 12 [43.5], secondary branch (sb) 9.4 [30.4]; leg III pb 11.4 [36.9], sb 8.3, 26.9; leg IV pb 13.7 [42.7], sb 10.7 [34.6]. Claw lunules small, smooth edged. Lunule widths: leg II 2.5 [8.1], leg III 2.1 [6.8], leg IV 2.7 [8.7].

Morphometric data and summary statistics for the holotype and 8 paratypes are given in Table 1.

Eggs white or transparent, laid freely. Surface punctate (Fig. 3A). Two types of processes: short, peg-shaped processes (Fig. 3A, 3B) and thin, blunt-ended filamentous processes (Fig. 3B, 3C). Peg-shaped processes always present, with uniform distribution (Fig. 3A). Filamentous processes sometimes absent, sometimes sparse, sometimes abundant (Fig. 3C). Measurements of some structures from 8 eggs are given in Table 2. The presence of embryonation (buccal tube and placoids) in one egg makes the assignment

of these eggs to *Minibiotus acadianus* sp. n. definitive.

DIFFERENTIAL DIAGNOSIS.—Guidetti et al. (2007) listed a suite of 10 characters shared by most, though not all, species of *Minibiotus*. These characters are (1) anteroventral mouth, (2) oral cavity teeth absent or strongly reduced, (3) rigid, narrow buccal tube (width $pt \leq 12$), (4) buccal tube thickened below the point of insertion of stylet supports, (5) stylet supports inserted at a considerable distance from the posterior end of the buccal tube ($pt \leq 73$), (6) short ventral lamina ($pt \leq 73$), (7) short macroplacoid row length ($pt \leq 42$), (8) double curvature of the buccal tube, (9) first macroplacoid located very close to the pharyngeal apophyses, and (10) 3 almost rounded macroplacoids. *Minibiotus acadianus* sp. n. exhibits characters 1, 2, 4, 5, 6, 7, and 9. However, it has 2 macroplacoids, and its buccal tube is somewhat wider (mean $pt = 12.6$) and has only one curvature.

Most species of *Minibiotus* have 3 macroplacoids, a character which differentiates them from *Minibiotus acadianus* sp. n. Only *Minibiotus scopulus* Claxton, 1998; *M. africanus* Binda and Pilato, 1995; *M. diphascoides* (Iharos, 1969); and *M. fallax* Pilato, Claxton, and Binda, 1989, have 2 macroplacoids. The presence of posterior gibbosities differentiates *Minibiotus acadianus* sp. n. from *M. scopulus*, *M. africanus*, and *M. diphascoides*.

Minibiotus acadianus sp. n. most closely resembles *Minibiotus fallax*; in the most recently published key to *Minibiotus* (Claxton 1998), the new species keys out to the space occupied by *M. fallax*. Both species have posterior bands of gibbosities, cuticles with numerous small pores, and eggs with punctate shells and long, thin processes (Pilato et al. 1989). The 2 species can be distinguished by several independent characters:

1. *Minibiotus acadianus* sp. n. has a buccal tube that is shorter (in specimens of comparable size, 28.2 in the new species and 30.6 in *M. fallax*) and wider (in specimens of comparable size, 3.6 [pt 12.6] in the new species and 2.9 [pt 9.2] in *M. fallax*). The stylet support insertion point is more posterior in the new species (pt 66.0–71.1 in *M. acadianus* sp. n. and pt 62.8–66.6 in *M. fallax*).
2. Gibbosities extend forward as far as the second pair of legs in *M. fallax* but only as far as the third in the new species. Gibbosities are present on the fourth pair of legs in *M. acadianus* sp. n. but not in *M. fallax*. The largest gibbosity measured in *M. fallax* is 15.8 long by 10.0 wide; the largest gibbosity in *M. acadianus* sp. n. is 9.1 by 4.3.

3. Eyes and bands of pigment are present in *M. fallax* but not in the new species.
4. The numerous short, peg-shaped processes in the eggs of *M. acadianus* are not present in *M. fallax*.

REMARKS.—Tardigrades with narrow buccal tubes, including species in the genus *Minibiotus*, are known to feed on plant material (Lehmann et al. 2007). Feeding has not been observed in *Minibiotus acadianus* sp. n., but the narrowness of its buccal tube suggests that it is probably an herbivore.

ETYMOLOGY.—The specific name *acadianus* is a masculine adjective commemorating both Acadia Parish and Acadiana, the region of southern Louisiana known for its large Cajun population.

DISCUSSION

Meyer (2008) found *Minibiotus fallax* specimens and eggs in Florida, including the far western counties. The species has not been reported from Mississippi or Alabama (Hinton and Meyer 2007, 2009). Hinton and Meyer (2007) reported the presence of *Minibiotus fallax*, but no eggs, in cryptogams from East Feliciana, Iberia, Iberville, Ouachita, Richland, St. Bernard, Vermillion, and Vernon parishes in Louisiana. Reexamination of the Louisiana specimens indicates that they are *Minibiotus acadianus* sp. n., suggesting that the new species is distributed widely in the state.

Hinton and Meyer (2007) hypothesized that 4 species (*Echiniscus cavagnaroi* Schuster and Grigarick, 1966, *Echiniscus kofordi* Schuster and Grigarick, 1966, *Minibiotus fallax*, and an undescribed *Macrobotus* cf. *hufelandi*) constitute a distinctive regional tardigrade fauna within North America. We now add *Minibiotus acadianus* sp. n. to this list. We also hypothesize that the border between the distributions of *Minibiotus acadianus* sp. n. and *Minibiotus fallax* occurs somewhere from eastern Louisiana to Alabama.

ACKNOWLEDGMENTS

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