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The Tardigrada of southern Africa, with the description of *Minibiotus harrylewisi*, a new species from KwaZulu-Natal, South Africa (Eutardigrada: Macrobiotidae)

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ABSTRACT

Nine species of tardigrades (Phylum Tardigrada) were found in moss and lichen samples in KwaZulu-Natal, South Africa, with two species being also present in Lesotho. *Macrobiotus richtersi*, *Minibiotus intermedius*, *Hypsibius convergens*, *Ramazzottius theroni*, *Milnesium tardigradum*, and *Echiniscus duboisi* were previously known to be part of the southern African fauna. *Macrobiotus iharosi* has not been reported before from this region. One species, *Macrobiotus* cf. *echinogenitus*, could be identified only to species complex because eggs were not found. One South African species, *Minibiotus harrylewisi* sp. n., is new to science and is described and illustrated. It differs from other *Minibiotus* species in its adult cuticle and its egg ornamentation. It most closely resembles *M. furcatus*, from which it differs in having smooth lunules on leg IV, smaller eyes, smaller and more rounded posterior cuticular pores, and eggs with a smooth shell and much longer, non-bifurcate processes. These new records bring to 61 the number of tardigrade species reported from southern African mosses, lichens and soil.

KEY WORDS: Tardigrada, Lesotho, South Africa, KwaZulu-Natal, new species, new records, check list.

INTRODUCTION

The first investigations of tardigrades (Phylum Tardigrada) in southern Africa were made in the early decades of the twentieth century, when Murray (1907, 1913) found 16 species in South Africa. Since then, studies of tardigrades in southern Africa (herein defined as encompassing modern Angola, Zambia, Malawi, Namibia, Zimbabwe, Botswana, Swaziland, Mozambique, Lesotho, and South Africa) have been sporadic. Currently, 14 papers report the presence in southern Africa of 16 genera and 60 species, including those identified only to genus or species complex (Murray 1907, 1913; Marcus 1936; da Cunha & do Nascimento Ribeiro 1964; Dastych 1980, 1992, 1993; Binda 1984; Pilato *et al*. 1991; Van Rompu *et al*. 1995; Jørgensen 2001; Middleton 2003; Kaczmarek & Michalczyk 2004; Kaczmarek *et al*. 2006).

Tardigrades have not been reported from Zambia and Swaziland. Most southern African tardigrades have been collected from cryptogams (Table 1). Soil has only rarely been sampled, and freshwater only once.

In this paper we report tardigrades collected from cryptogams in Lesotho and KwaZulu-Natal, South Africa.

MATERIAL AND METHODS

All samples were collected in midwinter on 9–11 July, 2008. Foliose lichens were collected from a stone fence in Sani Pass (SP), Lesotho and from *Acacia* trees at Hluhluwe-Imfolozi Game Park (HGP) and Tala Private Game Reserve (TPGR) in KwaZulu-Natal Province, South Africa. In KwaZulu-Natal ground moss was collected in Underberg. Eight samples were stored in paper envelopes until laboratory processing. Moss and lichen samples were soaked overnight in tap water and examined using a

TABLE 1

Distribution of tardigrade species collected in southern Africa.

Abbreviations. Nations: AO – Angola, BW – Botswana, LS – Lesotho, MW – Malawi, MZ – Mozambique, NA – Namibia, SA – South Africa, ZW – Zimbabwe. South African provinces: CC – Cape Colony (where the original reference does not permit assigning to modern provinces), GT – Gauteng, KN – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NW – North West, WC – Western Cape. Biogeographical regions: Af – Afrtotropical, An – Antarctic and subantarctic, Au – Australian, I – Indomalayan, Na – Nearctic, Nt – Neotropical, NZ – New Zealand, O – Oceania, P – Palearctic. Substrates: M – moss, L – lichen, C – unspecified cryptogam, S – soil, A – aquatic sediment. References: B – Binda (1984), D^1 – Dastych (1980), D^2 – Dastych (1992), D^3 – Dastych (1993), DN – da Cunha & do Nascimento Ribeiro (1964), J – Jørgensen (2001), KB – Kaczmarek *et al*. (2006), KM – Kaczmarek & Michalczyk (2004), Ma – Marcus (1936), Mi – Middleton (2003), Mu^1 – Murray (1907), Mu^2 – Murray (1913), P – Pilato *et al*. (1991), V – Van Rompu *et al*. (1995).

Species	Substrate	Location and Reference	Biogeography
Bryodelphax parvulus Thulin, 1928	L	AO(DN)	Na, Nt, P, Af, I
Echiniscus sp.	C	LP(Mi), NW(Mi), ZW(Mi)	
Echiniscus africanus Murray, 1907	M	AO(DN), CC(Mu^1), LP(Mu^2)	Af, I
Echiniscus cf. *africanus*	C	LS(Mi)	
Echiniscus angolensis da Cunha & do Nascimiento Ribeiro, 1964	L	AO(DN), BW(Mi)	Nt, Af
Echiniscus arctomys Ehrenberg, 1853	M	CC(Mu^1)	Na, Nt, P, Af, I, O, Au, NZ, An
Echiniscus bigranulatus Richters, 1907	C	BW(Mi)	Nt, P, Af, I, NZ
Echiniscus crassispinosus Murray, 1907	M	CC(Mu^1)	Nt, P, Af
Echiniscus duboisi Richters, 1902	M	CC(Mu^2)	Nt, Af, I, Au
Echiniscus kerguelensis Richters, 1904		NA(Ma)	Na, Nt, P, Af, Au, NZ, An
Echiniscus limae da Cunha & do Nascimento Ribeiro, 1964	L	AO(DN)	Af
Echiniscus longispinosus Murray, 1907	M	CC(Mu^1), WC(B,P)	Af
Echiniscus merokensis Richters, 1904	M	AO(DN)	Na, Nt, P, Af
Echiniscus perarmatus Murray, 1907	M	CC(Mu^1)	Na, Nt, Af, O
Echiniscus pusae Marcus, 1928		CC(Ma)	Af, I
Pseudechiniscus bispinosus (Murray, 1907)	M	CC(Mu^1)	Nt, Af
Pseudechiniscus jiroveci Bartoš, 1963	M	WC(B,P)	P, Af
Pseudechiniscus suillus (Ehrenberg, 1853)	M	AO(DN), LP(Mu^2)	Na, Nt, P, Af, I, O, Au, NZ, An
Doryphoribius citrinus (Maucci, 1972)	M	WC(B)	P, Af, I
Hypsibius sp.	C	LS(Mi)	
Hypsibius convergens (Urbanowicz, 1925)	M	GT(KM)	Na, Nt, P, Af, I, O, Au, NZ, An
Hypsibius dujardini (Doyère, 1840)	M	WC(B)	Na, Nt, P, Af, I, O, NZ, An
Hypsibius maculatus (Iharos, 1969)	M	GT/MP(KM)	P, Af
Hypsibius scabropygus Cuénot, 1929		NA(Ma)	Na, P, Af
Isohypsibius deconincki Pilato, 1971	S	KN(B)	P, Af
Isohypsibius kristenseni Pilato, Catanzaro & Binda, 1989	S	MZ(P)	P, Af
Isohypsibius malawensis Jørgensen, 2001	A	MW(J)	Af
Isohypsibius nodosus (Murray, 1907)	M	CC(Mu^1)	Na, Nt, P, Af, I, O, NZ
Isohypsibius pseudoundulatus (da Cunha & do Nascimento Ribeiro, 1964)	M	AO(DN)	Af
Isohypsibius sattleri (Richters, 1902)	M	AO(DN), WC(B)	Na, Nt, P, Af, I, Au, NZ, An
Isohypsibius schaudinni (Richters, 1909)	S	ZW(V)	Na, Nt, P, Af, I
Ramazzottius sp.	C	LS(Mi)	
Ramazzottius oberhaeuseri (Doyère, 1840)	L	AO(DN)	Nt, Nt, P, Af, I, O, NZ, An

TABLE 1 *(continued)*

Species	Substrate	Location and Reference	Biogeography
Ramazzottius szeptycki (Dastych, 1980)	M	LP(D^1)	Af
Ramazzottius theroni Dastych, 1983	L	WC(D^3)	Af
Diphascon (*Adropion*) *scoticum* Murray, 1905	M	CC(Mu^2)	Na, Nt, P, Af, I, O, Au, NZ, An
Diphascon (*Diphascon*) *zaniewi* Kaczmarek & Michalczyk, 2004	M	NW(KM)	Af
Paradiphascon manningi Dastych, 1992	M, S	WC(D^2)	Af
Astatumen trinacriae (Arcidiacono, 1962)	M, S	WC(B), ZW(V)	Na, P, Af, I
Haplomacrobiotus seductor Pilato & Beasley, 1987	M	GT/MP(KB)	Na, Af
Calcarobiotus filmeri Dastych, 1993	S	MP(D^3)	Af
Calcarobiotus occulti Dastych, 1993	M, S	KN(D^3)	Af
Macrobiotus areolatus Murray, 1907	M	MZ(B)	Na, Nt, P, Af, I, O, Au, NZ
Macrobiotus drakensbergi Dastych, 1993	M	LP(D^3)	Af
Macrobiotus echinogenitus Richters, 1904	M	CC(Mu^1)	Na, Nt, P, Af, I, Au, NZ, An
Macrobiotus furciger Murray, 1906	M	WC(B)	Na, Nt, P, Af, I, NZ, An
Macrobiotus hufelandi C.A.S. Schultze, 1834	M, L	AO(DN), CC(Mu^1), LP(Mu^2)	Na, Nt, P, Af, I, O, Au, NZ, An
Macrobiotus cf. *hufelandi*	C	BW(Mi), LS(Mi)	
Macrobiotus nuragicus Pilato & Sperlinga, 1975	S	KN(B)	P, Af, I
Macrobiotus occidentalis Murray, 1910	M	AO(DN)	Na, Nt, P, Af, O, Au, NZ
Macrobiotus richtersi Murray, 1911	M, S	AO(DN), GT(Mu^2), WC(B), ZW(V)	Na, Nt, P, Af, I, O, Au, NZ
Macrobiotus cf. *richtersi*	M	LS(Mi), GT/MP(KM), WC(B)	
Macrobiotus sapiens Binda & Pilato, 1984	L	NA(P)	P, Af, I
Minibiotus sp.	C	LS(Mi)	
Minibiotus crassidens (Murray, 1907)	M, L	CC(Mu^1)	P, Af, O, Au
Minibiotus furcatus (Ehrenberg, 1859)	L	AO(DN)	Na, Nt, P, Af, I
Minibiotus harrylewisi **sp. n.**	L	KN(this paper)	Af
Minibiotus hufelandioides (Murray, 1910)	M	LP(Mu^2)	P, Af, O, Au
Minibiotus intermedius (Plate, 1888)	M, L	AO(DN), BW(Mi), NA(Ma), GT/MP(KM), WC(B,P)	Na, Nt, P, Af, I, O, Au, NZ, An
Milnesium tardigradum Doyère, 1840	M, L	AO(DN), BW(Mi), LS(Mi), NA(Ma), CC(Mu^1), LP(Mi), NW(Mi), ZW(Mi)	Na, Nt, P, Af, I, O, Au, NZ, An
Apodibius nuntius Binda, 1984	M, S	MZ(B), ZW(V)	Af

stereoscopic microscope. Specimens were mounted on slides in polyvinyl lactophenol and examined with phase contrast microscopy.

Tardigrades were identified using keys and descriptions in Nelson & McInnes (2002) and Ramazzotti & Maucci (1983), and by reference to the current literature. Anatomical terminology, global species lists, and current taxonomy and nomenclature are given according to Guidetti & Bertolani (2005) and Degma & Guidetti (2007). Comments on tardigrade zoogeography are based primarily on McInnes (1994).

All morphological measurements are given in micrometres. The *pt* index is the ratio of the length of a given structure to that of the buccal tube expressed as a percentage

(Pilato 1981). Buccal tube length and the point of stylet support insertion were measured from the anterior margin of the stylet sheaths to the base of the tube. Buccal tube widths were measured as the external diameter of the buccal tube at the level of the stylet support insertion. The lengths of the primary and secondary branches of the claws were measured from the base to the apex, including accessory points.

The material has been deposited in the following collections: SMLA – W.A.K. Seale Museum, Department of Biology and Health Sciences, McNeese State University, Lake Charles, Louisiana, USA; NMSA – Natal Museum, Pietermaritzburg, South Africa.

TAXONOMY

Class Heterotardigrada Marcus, 1927
Order Echiniscoidea Richters, 1926
Family Echiniscidae Thulin, 1928
Genus *Echiniscus* Schulze, 1840

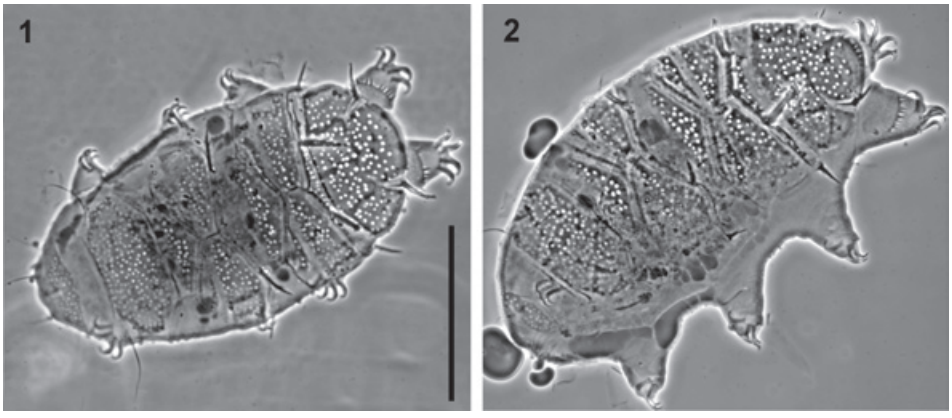
Twelve of the 158 species of *Echiniscus* have been reported in southern Africa (Table 1).

Echiniscus duboisi Richters, 1902

Figs 1–7, Table 2

Description: Descriptions of this species indicate considerable variation in the shape, length, and number of lateral and dorsal spines (Ramazzotti & Maucci 1983). In Richter's (1902) description and illustration, lateral spines *B* through *E* are serrated, and have the same length as the smooth dorsal spines C^d and D^d. Specimens from Cape Colony (Murray 1913), Brazil (de Barros 1942), and New Guinea (Iharos 1967) lack dorsal and lateral spines at *B*. Illustrations of *E. duboisi* vary considerably in their depiction of its habitus, cuticle, and spines, and only de Barros (1942) and Iharos (1967) provide limited morphometric data.

Table 2 presents morphometric data for twelve specimens of *Echiniscus duboisi*. Cuticular ornamentation consists of widely-spaced depressions ranging in diameter from

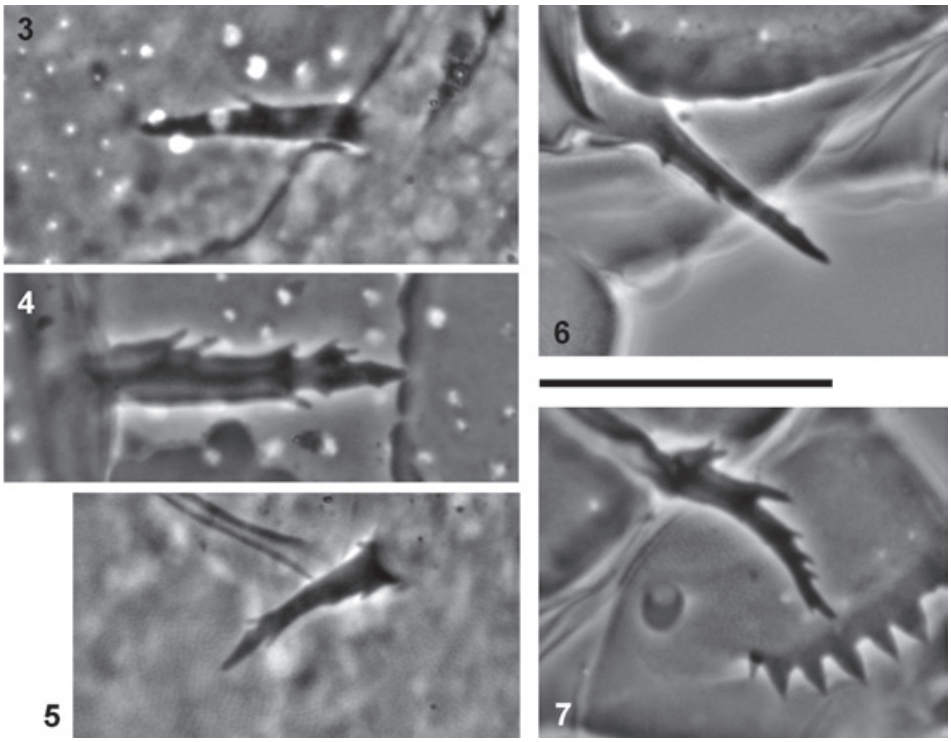


Figs 1, 2. Habitus of *Echiniscus duboisi*, KwaZulu-Natal, South Africa: (1) dorsal, (2) lateral. Scale bar = 100 µm.

TABLE 2

Morphometric data of selected structures on 12 specimens of *Echiniscus duboisi* from KwaZulu-Natal, South Africa. Ranges and means are lengths in µm. Range refers to the smallest and largest structure found among all measured specimens (a zero indicates the structure was not present on some specimens). Abbreviations: *N* – number of specimens measured, SD – standard deviation.

Character	*N*	Range	Mean	SD
Body	12	176.6–292.9	219.4	40.8
Internal cirrus	8	6.5–15.5	11.2	2.7
Cephalic papilla	9	3.9–8.4	6.0	9.5
External cirrus	9	9.1–23.7	16.7	4.4
Cirrus *A*	12	19.6–33.5	28.8	4.0
Clava	11	3.3–7.2	5.2	1.3
Lateral spine *C*	9	0–16.4	10.9	3.0
Lateral spine *D*	5	0–16.7	13.7	2.8
Lateral spine *E*	12	4.7–17.6	12.8	3.2
Dorsal spine C^d	11	0–15.9	11.7	4.2
Dorsal spine D^d	12	12.2–25.1	20.3	4.1
Leg I spine	8	1.3–2.5	2.0	0.4
Leg IV papilla	11	2.2–4.1	3.2	0.6
No. of teeth on dentate collar, Leg IV	12	6–11	8.6	1.3
Dentate teeth, Leg IV	4	1.7–4.6	3.4	1.0



Figs 3–7. *Echiniscus duboisi*, KwaZulu-Natal, South Africa: (3) dorsal spine C^d, (4) dorsal spine D^d, (5) lateral spine *C*, (6) lateral spine *D*, (7) lateral spine *E*. Scale bar = 20 µm.

1.0–3.1 (Figs 1, 2). Cirri and spines are shorter, and the number of teeth on the dentate collars fewer, in South African than in Brazilian specimens. Three specimens lack lateral spine *C*, seven lateral spine *D*, and one dorsal spine D^d. Dorsal spines, especially D^d, are robust and serrated, with wide variation in number and size of teeth (Figs 3, 4). Lateral spines *C* and *D* are either serrated or smooth; *E* is always serrated (Figs 5–7).

Material examined: SOUTH AFRICA: *KwaZulu-Natal*: 24 specimens, TPGR, from foliose lichens on *Acacia* trees, 17 deposited at SMLA (accession numbers 9340–9346, 9349) and 7 at NMSA (accession number 9347).

Distribution: *E. duboisi* has not been found in the Nearctic or Palearctic realms, but has been widely reported elsewhere, including South Africa (Table 1).

Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Family Hypsibiidae Pilato, 1969
Genus *Hypsibius* Thulin, 1928

Four of the 41 species of *Hypsibius* are known from southern Africa (Table 1).

Hypsibius convergens (Urbanowicz, 1925)

Material examined: SOUTH AFRICA: *KwaZulu-Natal*: 36 specimens, HGP, in foliose lichens from *Acacia* trees (SMLA accession numbers 9361, 9362, 9364). *Milnesium tardigradum*, *Minibiotus intermedius*, and *Macrobiotus richtersi* were present in the same samples.

Distribution: *H. convergens* is a cosmopolitan species, previously found in Gauteng Province, South Africa (Table 1).

Genus *Ramazzottius* Binda & Pilato, 1986

Three of the 24 species of *Ramazzottius* are known from southern Africa (Table 1).

Ramazzottius theroni Dastych, 1993

Material examined: SOUTH AFRICA: *KwaZulu-Natal*: 31 specimens and 1 egg, HGP, in foliose lichens on *Acacia* trees (SMLA accession numbers 9340, 9341, 9353, 9355, 9357; NMSA accession number 9347). *Echiniscus duboisi*, *Macrobiotus iharosi*, *Minibiotus harrylewisi* sp. n., *Min. intermedius*, and *Milnesium tardigradum* were present in the same samples.

Distribution: Hitherto this species was known only from its type locality in Western Cape Province, South Africa.

Family Macrobiotidae Thulin, 1928
Genus *Macrobiotus* Schulze, 1834

Species of *Macrobiotus* cannot be identified without their eggs. Older literature often did not employ the rigorous standards of identification used today (Pilato & Binda 2001; Guidetti & Bertolani 2005). Up to now, 10 of the 161 species in this genus have been reported in southern Africa (Table 1).

Macrobiotus cf. *echinogenitus* Richters, 1904

Material examined: SOUTH AFRICA: *KwaZulu-Natal*: 1 specimen, Underberg, in moss (SMLA accession number 9359).

Distribution: Murray (1907) reported the presence of *M. echinogenitus*, a cosmopolitan species, in Cape Colony, South Africa. He did not record where in Cape Colony the specimens were collected but presumably they were from the modern provinces of Eastern Cape or Western Cape.

Remarks: The specimen keys to *Macrobiotus echinogenitus* in Ramazzotti & Maucci (1983). Its buccal and claw structure are consistent with *M. echinogenitus*, but in the absence of eggs, further identification is not possible.

Macrobiotus iharosi Pilato, Binda & Catanzaro, 1991

Material examined: SOUTH AFRICA: *KwaZulu-Natal*: 36 specimens and 8 eggs, TPGR, in foliose lichens on *Acacia* trees (SMLA accession numbers 9340, 9346, 9356, 9357; NMSA accession number 9347). *Ramazzottius theroni*, *Milnesium tardigradum*, *Minibiotus harrylewisi* sp. n., *Min. intermedius*, and *Echiniscus duboisi* were found in the same samples.

Distribution: The known distribution of *M. iharosi* has hitherto been restricted to Tanzania and the Democratic Republic of Congo (Pilato *et al*. 1991, 2003).

Macrobiotus richtersi Murray, 1911

Material examined: SOUTH AFRICA: *KwaZulu-Natal*: 5 specimens and 1 egg, TPGR, in foliose lichen on an *Acacia* tree (SMLA accession numbers 9363, 9364). *Hypsibius convergens* and *Minibiotus intermedius* were present in the same sample.

Distribution: This cosmopolitan species has previously been found in Angola, South Africa, and Zimbabwe (Table 1).

Genus *Minibiotus* Schuster, Nelson, Grigarick & Christenberry, 1980

Minibiotus currently consists of 42 species, and four of them are known to occur in southern Africa (Table 1). *Minibiotus africanus* occurs elsewhere in sub-Saharan Africa (Binda *et al*. 2001).

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*=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*=73); (6) short ventral lamina (*pt*=73); (7) short macroplacoid row length (*pt*=42); (8) double curvature of the buccal tube; (9) first macroplacoid located very close to the pharyngeal apophyses; and (10) three almost rounded macroplacoids.

Minibiotus harrylewisi sp. n.

Figs 8–17; Tables 3, 4

Etymology: The specific epithet honors the first author's father, Harry Lewis Meyer, in recognition of his unstinting support of his son's academic career.

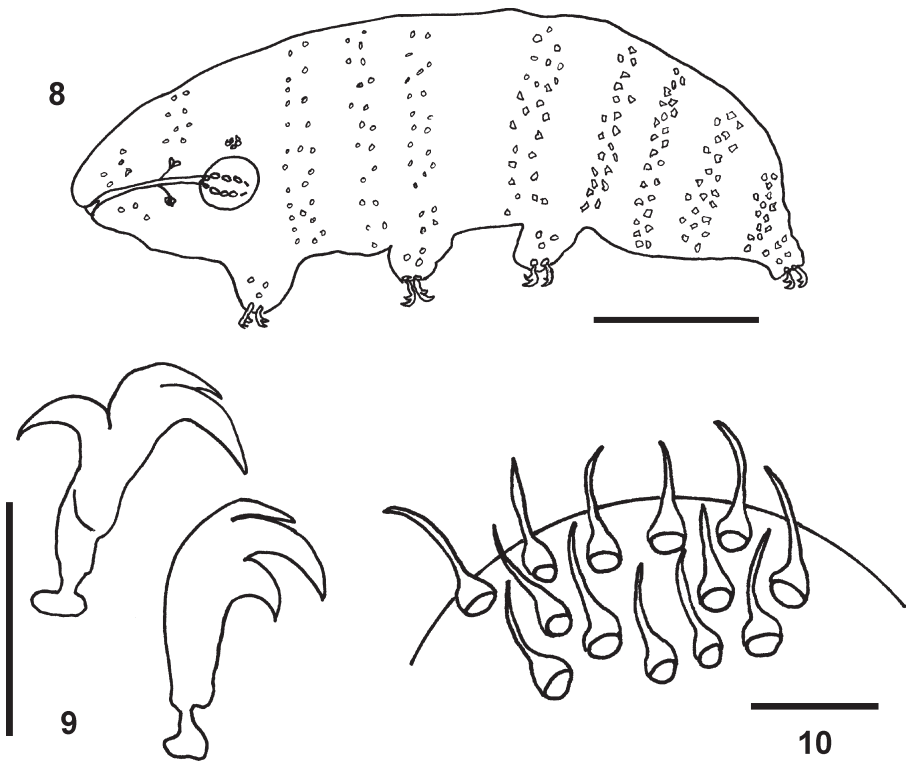
Description (adult measurements are given from holotype unless otherwise indicated; *pt* index in brackets and italics):

Body length 336.0. Body white or transparent. Eyespots present in posterior position. Cuticle without sculpture or granulation (including legs), with 10 bands of circular, oval, trefoil, or quadrifoil pores (no stellate pores); bands 2–6 pores wide (Figs 8, 11–

13). Circular and oval pores more common on anterior region of body; trefoil and quadrifoil more common on posterior body and also on legs. Pores significantly larger on posterior half of body (Fig. 11) than on anterior half (Fig. 12) (1.3, n=32 on anterior end; 1.8, n=27 on posterior end; $p<0.0001$; combined data from 10 specimens), and on legs (Fig. 13) than on rest of body (1.9, n=37 on legs; 1.5, n=60 on body; $p<0.001$; combined data from 10 specimens).

Peribuccal papillae absent. Oral cavity armature absent or not visible with light microscopy. Mouth anteroventral. Buccal tube rigid, 27.9 long and 2.6 [*9.3*] wide (Fig. 14), with double curvature (Fig. 14). Stylet support inserted on buccal tube at 17.7 [*63.4*]. Short ventral lamina, 17.9 [*63.*4] long. Pharyngeal apophyses triangular and close to first macroplacoid. First macroplacoid round and 2.7 [*9.*7] long; second pear-shaped, 2.0 [*7.2*] long; third also pear-shaped, 2.5 [*9.0*] long. Macroplacoid row 9.8 [*35.1*] long. Indistinct, rod-shaped microplacoid 1.0 [*3.6*] long. Placoid row 11.7 [*39.5*] long. Pharyngeal bulb round, 35.5×36.0.

Claws short and slender. Primary branches of all claws with distinct accessory points. All claws with smooth-edged (not dentate or crenate) lunules but well developed only on leg IV. Leg I: primary branch with basal claw 7.5 [*26.9*] long, secondary branch 5.6 [*20.1*] long. Leg II: pb with bc 7.8 [*28.0*] long, sb 4.7 [*16.8*] long. Leg III: pb with bc

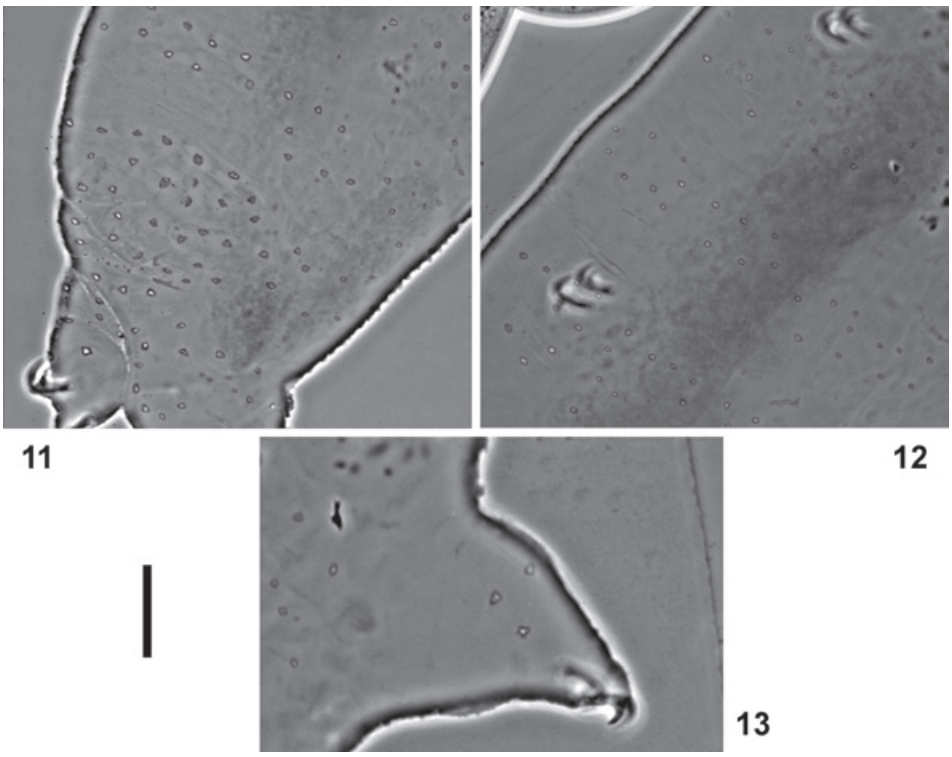


Figs 8–10. *Minibiotus harrylewisi* sp. n., KwaZulu-Natal, South Africa: (8) habitus, (9) claw of leg III, (10) egg. Scale bar in Fig. 8 = 100 µm, in Figs 9, 10 = 10 µm.

TABLE 3

Morphometric data and *pt* values of selected structures of 10 specimens of *Minibiotus harrylewisi* sp. n. All lengths in µm. Range refers to the smallest and largest structure found among all measured specimens (a zero indicates the structure was not present on some specimens). Abbreviations: *N* – number of specimens measured, SD – standard deviation, n.a. – not applicable, *pt* – *pt* index.

		Range		Mean		SD	
Character	*N*	Length	*pt*	Length	*pt*	Length	*pt*
Body	10	200.3–386.6	n.a.	304.2	n.a.	65.7	n.a.
Buccal tube	10	19.7–32.9	n.a.	28.1	n.a.	4.3	n.a.
Stylet support insertion	10	12.1–21.8	61.4–67.6	18.1	64.1	3.1	3.8
Buccal tube width	10	1.9–3.4	6.7–11.2	2.6	9.3	0.5	1.3
First macroplacoid	10	1.3–3.7	5.2–12.4	2.2	8.0	0.7	2.1
Second macroplacoid	10	1.2–2.6	6.0–8.6	2.0	7.2	0.4	0.9
Third macroplacoid	10	1.2–2.9	4.7–10.0	2.2	7.7	0.6	1.8
Macroplacoid row	10	5.6–10.9	26.3–36.6	9.1	32.3	1.8	3.7
Microplacoid	7	0–2.6	0–7.9	1.4	4.5	0.7	2.2
Placoid row	7	10.4–34.7	34.6–46.0	11.8	39.4	1.2	1.0
Primary claw branch, Leg I	10	5.5–9.7	23.1–32.6	7.7	27.6	2.9	6.4
Secondary claw branch, Leg I	10	4.0–7.0	16.4–23.1	5.6	19.9	1.0	1.9
Primary claw branch, Leg II	10	6.3–10.5	27.4–35.2	8.3	29.7	2.6	8.5
Secondary claw branch, Leg II	10	3.6–7.9	16.8–27.1	5.8	20.5	1.5	3.6
Primary claw branch, Leg III	10	6.5–9.8	27.9–34.3	8.8	31.1	1.2	2.4
Secondary claw branch, Leg III	10	3.4–8.0	16.0–26.8	6.4	22.4	2.4	1.6
Primary claw branch, Leg IV	10	6.7–13.6	26.9–45.6	9.9	35.2	2.1	4.7
Secondary claw branch, Leg IV	10	4.5–9.9	19.3–33.2	6.9	2.0	24.4	4.5



Figs 11–13. Pores of *Minibiotus harrylewisi* sp. n., KwaZulu-Natal, South Africa: (11) posterior pores, (12) anterior pores, (13) pores of leg I. Scale bar = 20 µm.

TABLE 4

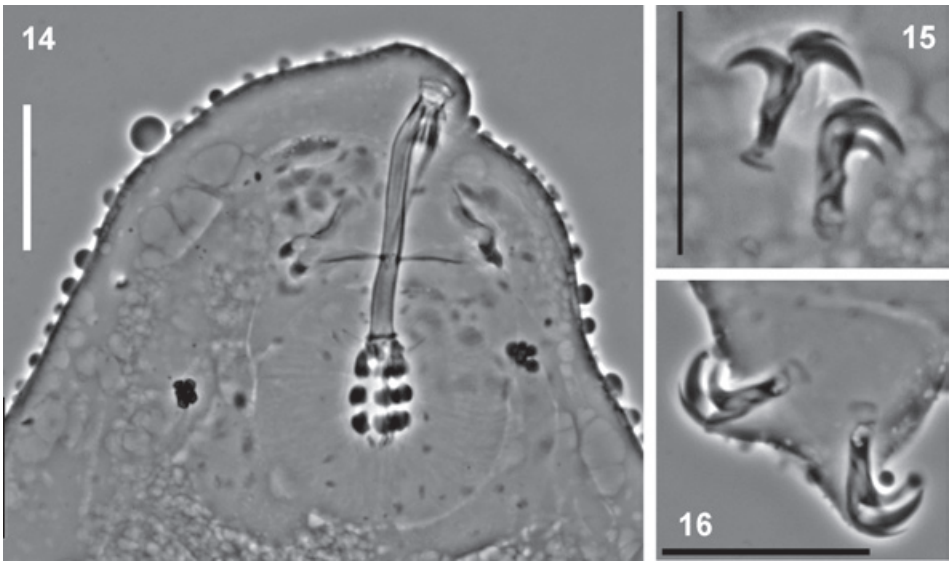
Morphometric data of selected structures on seven eggs of *Minibiotus harrylewisi* sp. n. Ranges and means are in µm. Range refers to the smallest and largest structure found among all measured specimens. Abbreviations: *N* – number of specimens measured, SD – standard deviation.

Character	*N*	Range	Mean	SD
Diameter without processes	7	66.1–80.0	70.7	5.4
Diameter with processes	7	78.2–101.9	88.5	7.2
Height of process	9	7.6–12.8	10.2	1.4
Width of process base	9	3.2–5.6	4.3	0.7
Distance between processes	9	1.3–4.1	2.8	4.0
Number of processes around circumference	7	32.0–41.0	36.6	3.2
Number of processes in optical section	5	94.0–166.0	118.8	32.6

8.6 [*28.7*] long, sb 7.3 [*26.2*] long (Fig. 15). Leg IV pb with bc 9.3 [*33.0*] in length, sb 6.6 [*23.7*] (Fig. 16).

Eggs white/colourless, laid freely, with smooth surface (Fig. 17), with 32–41 long, thin, non-membranous processes around circumference. Processes densely packed, in the form of elongated, tapering cones with bulbous base. Size variable (Table 4).

Comparison: The presence of three macroplacoids in *M. harrylewisi* sp. n. differentiates it from species of *Minibiotus* with two. About half of the species in the genus have sculptured, granulated, or smooth cuticles; *M. harrylewisi* sp. n. differs from these in having a cuticle without sculpture or granulation, but having numerous pores. Among species with pores, the eggs of several have short processes covered with membranes, unlike the long, thin, non-membranous processes of *M. harrylewisi* sp. n. Among species with pores and conical egg processes, the new species lacks the large leg pore or large stellate pores characteristic of *M. constellatus* Michalczyk & Kaczmarek, 2003, *M. siderius* Pilato, Binda & Lisi, 2003, *M. gumersindoi* Guil & Guidetti, 2005, or *M. eichhorni*



Figs 14–16. *Minibiotus harrylewisi* sp. n., KwaZulu-Natal, South Africa: (14) bucco-pharyngeal apparatus, (15, 16) claws of leg III and leg IV. Scale bars = 20 µm.

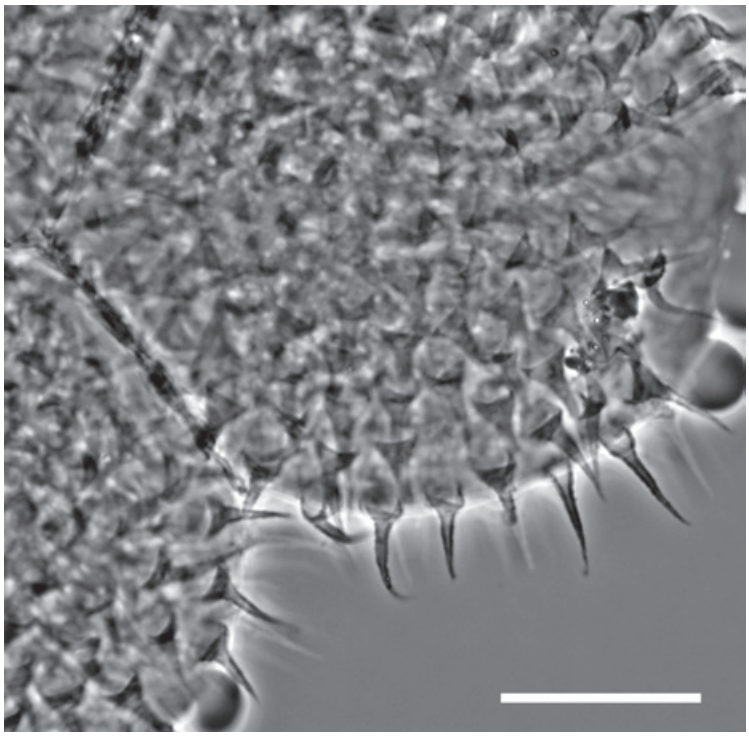


Fig. 17. Egg of *Minibiotus harrylewisi* sp. n., KwaZulu-Natal, South Africa. Scale bar = 20 µm.

Michalczyk & Kaczmarek, 2004. Unlike *M. vinciguerrae* Binda & Pilato, 1992, the pores of *M. harrylewisi* sp. n. are arranged in distinct bands. The new species differs from *M. pustulatus* Ramazzotti, 1959 in having much smaller caudal pores (mean of caudal pores 1.8 in the new species and 6.7 in *M. pustulatus*) and longer egg processes, and from *M. bisoctus* Horning, Schuster & Grigarick, 1978 in possessing eyes, smaller pores (1.3–1.9 in the new species and 3 in *M. bisoctus*), and lack of leg granulation. Unlike *M. ethelae*, the caudal cuticle of *M. harrylewisi* sp. n. is not thickened. The new species most closely resembles *M. furcatus*. The lunules of Leg IV in *M. harrylewisi* sp. n. are not dentate. The pores of the new species, especially those on the anterior body, are smaller and more rounded. The egg processes in *M. harrylewisi* sp. n. are twice as long as in *M. furcatus*, are never bifurcated, and the egg shell is never punctate.

Variation: Microplacoid often absent on smallest specimens. Division between the two most anterior and between the two most posterior bands of pores not distinct in some specimens.

Holotype: SOUTH AFRICA: *KwaZulu-Natal*: TPGR, 11.vii.1988, foliose lichen on *Acacia* trees, H.A. Meyer, deposited in W.A.K. Seale Museum, Department of Biology and Health Sciences, McNeese State University, Lake Charles, Louisiana, USA (accession number 9349).

Paratypes and other material examined: 6 paratypes and 7 eggs, same data as holotype (accession numbers 9340, 9343, 9346, 9349); 2 additional eggs, 109 additional specimens, same collection data as holotype; 1 paratype and 1 egg, same collection data as holotype, deposited in the Natal Museum, Pietermaritzburg, South Africa (accession numbers 9347, 9348); 1 cluster of three connected eggs found; 1 embryonated egg found with buccal apparatus and placoids.

Distribution: The new species is know from the type locality only. *Echiniscus duboisi*, *Ramazzottius theroni*, *Macrobiotus iharosi*, *Minibiotus intermedius*, and *Milnesium tardigradum* were present in the same lichens.

Minibiotus intermedius (Plate, 1888)

Material examined: SOUTH AFRICA & LESOTHO: 19 specimens, SP, HGP and TPGR, foliose lichens (SMLA accession numbers 9352, 9358, 9362, 9365). Also present in the same samples were *Hypsibius convergens*, *Ramazzottius theroni*, *Macrobiotus iharosi*, *M. richtersi*, *Minibiotus harrylewisi* sp. n., *Milnesium tardigradum*, and *Echiniscus duboisi*.

Distribution: A cosmopolitan species, *Minibiotus intermedius* has previously been found in Angola, Botswana, Namibia, and South Africa (Table 1).

Order Apochela Schuster, Nelson, Grigarick & Christenberry, 1980
Family Milnesiidae Ramazzotti, 1962
Genus *Milnesium* Doyère, 1840

Thirteen recent species of *Milnesium* are known to science. Of these, only one has been found in southern Africa (Table 1).

Milnesium tardigradum Doyère, 1840

Material examined: SOUTH AFRICA & LESOTHO: 15 specimens, SP, HGP and TPGR, in foliose lichens (SMLA accession numbers 9340, 9342, 9343, 9349, 9352, 9356–9358, 9361, 9362). Present in the same samples were *Hypsibius convergens*, *Ramazzottius theroni*, *Macrobiotus iharosi*, *Minibiotus harrylewisi* sp. n., *Minibiotus intermedius*, and *Echiniscus duboisi*.

Distribution: *Milnesium tardigradum* is a cosmopolitan species and has previously been found in Angola, Botswana, Lesotho, Namibia, South Africa, and Botswana (Table 1).

DISCUSSION AND CONCLUSIONS

In samples from KwaZulu-Natal and Lesotho nine tardigrade species were found. Two species were present in Lesotho and all nine were present in KwaZulu-Natal. Six species (*M. richtersi*, *M. intermedius*, *H. convergens*, *R. theroni*, *M.tardigradum*, and *E. duboisi*) were previously known from southern Africa, but *M. iharosi* represents a new record for the region. One species, *Macrobiotus* cf. *echinogenitus*, could not be identified beyond species complex, because no eggs were found. One South African species, *M. harrylewisi* sp. n., is new to science.

Pilato and Binda (2001) categorized 6.8 % of all non-marine tardigrade species as cosmopolitan in distribution, i.e., reported in five or more biogeographical regions. In southern Africa 21 (40.4 %) of the fully identified species are cosmopolitan (Table 1). In comparison, only 17.6 % of Nearctic tardigrade species are cosmopolitan (Meyer & Hinton 2007). While the proportion of southern African tardigrade fauna that is cosmopolitan is relatively high, the endemic proportion is correspondingly low. The known distribution of 11 species (21.2 %) is limited to southern Africa (Table 1), compared to 30.0 % endemic to North America (Meyer & Hinton 2007). On a global scale, 68.4 % of all terrestrial tardigrade species are found in only one biogeographic realm (Pilato & Binda 2001).

This study should be considered a preliminary survey of tardigrades in KwaZulu-Natal, South Africa. No doubt additional species remain to be found. Tardigrade

distributions are extremely patchy at fine spatial scales (Meyer 2006), and only the rigorous sampling programs employed by All Taxa Biological Inventories (e.g. Bartels & Nelson 2006) can estimate their true diversity.

Guil and Cabrero-Sañudo (2007) noted that almost none of the authors of papers on tardigrades from the Afrotropical and Indomalayan realms are from those areas themselves. Among the authors of papers about southern African tardigrades, all except Middleton (2003) have been from Europe or North America. Our understanding of the distribution and abundance of tardigrades in southern Africa will benefit when researchers from the region play a greater role in their study.

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