

# First records of water bears (Phylum Tardigrada) from Swaziland

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Academic editor: N. Akkari | Received 21 December 2017 | Accepted 21 March 2018 | Published 13 April 2018

<http://zoobank.org/57C2DACF-6941-4C9D-B991-C9823115B2CE>

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**Citation:** Meyer HA, Tsaliki M, Hinton JG (2018) First records of water bears (Phylum Tardigrada) from Swaziland. African Invertebrates 59(1): 47–53. <https://doi.org/10.3897/AfrInvertebr.59.23191>

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## Abstract

There are no published records of water bears (Phylum Tardigrada) from Swaziland. Two samples of foliose lichen collected in 2010 contained nine tardigrade specimens and one egg belonging to five genera and seven species: *Echiniscus* cf. *quadrispinosus*, *Milnesium* sp., *Milnesium* cf. *bohleberi*, *Hypsibius* cf. *convergens*, *Ramazottius* sp., *Macrobiotus* cf. *pallarii* and *Minibiotus harrylewisi*. *Milnesium* sp. resembles *Milnesium lagniappe*, a species from southeastern USA, in its cuticle, and possibly in the number of peribuccal lamellae. Specimens from Lesotho and South Africa previously identified as *Milnesium tardigradum* are in fact *Milnesium* cf. *bohleberi*. The habitus of *Ramazottius* sp. is consistent with *R. theroni*, a southern African species, but due to the condition of the specimen the presence of cuticular sculpture cannot be definitively ruled out. *Macrobiotus* cf. *pallarii* differs from *M. pallarii sensu stricto* in some structural details of the egg processes. This is the first record of *Minibiotus harrylewisi* outside of its type location in KwaZulu-Natal, South Africa.

## Keywords

tardigrades, Lesotho, KwaZulu-Natal, *Echiniscus quadrispinosus*, *Minibiotus harrylewisi*, *Milnesium*

## Introduction

Water bears (Phylum Tardigrada) are minute animals closely related to arthropods (Bertolani et al. 2014). Terrestrial tardigrades are common in cryptogams, leaf litter and soil. The tardigrade fauna of Africa remains poorly known in comparison to Europe or the Americas; many African countries have few or no published records (Middleton 2003, McInnes et al. 2017).

The tardigrades of southern Africa were reviewed by Middleton (2003) and Meyer and Hinton (2009). The latter paper noted that the presence of *Milnesium tardigradum* Doyère, 1840 has been widely reported from southern Africa. However, records of *Milnesium tardigradum* outside Europe should be regarded as tentative unless confirmed (Michalczyk et al. 2012a, 2012b; McInnes et al. 2017). While *M. tardigradum* has two or three points on the secondary branches of its claws (claw formula [2-3]-[3-2]), many species have three on all claws ([3-3]-[3-3]). We reexamined specimens from Lesotho and KwaZulu-Natal, South Africa identified by Meyer and Hinton (2009) as *M. tardigradum*. Their claw formula is [3-3]-[3-3]. The overall habitus and morphometric dimensions of these animals are consistent with *Milnesium bohleberi* Bartels, Nelson, Kaczmarek and Michalczyk, 2014, a North American species, although their claws are less robust. We therefore amend the earlier identification to *Milnesium* cf. *bohleberi*. Murray (1907), in reporting the presence of *Milnesium tardigradum* in western South Africa, also noted that it had three points on all secondary claws, so it cannot have been *M. tardigradum sensu stricto*.

In this paper we present the first tardigrade records from the Kingdom of Swaziland.

## Material and methods

Two samples of foliose lichen were collected on 28 May 2010 by a McNeese State University undergraduate from two trees in the Ezulwini Valley, Hhohho Region, Kingdom of Swaziland. We soaked specimens overnight in water, inspected them with a dissecting microscope, and mounted them in polyvinyl lactophenol. Specimens were measured using imaging software (NIS-Elements D 2.30, SPI). We measured body lengths from the mouth to the posterior end, excluding legs IV. The *pt* index is the length of a structure divided by the length of the buccal tube expressed as a percentage (Pilato 1981). Specimens were identified using published keys and by comparing them to literature descriptions and slides in our collection.

The specimens are deposited in the W.A.K. Seale Museum, Department of Biology, McNeese State University, Lake Charles, Louisiana, USA.

## Results and discussion

The samples contained nine tardigrade specimens and one egg belonging to five genera and seven species.

**Echiniscidae Thulin, 1928*****Echiniscus* C.A.S. Schutze, 1840*****Echiniscus* cf. *quadrispinosus* Richters, 1902**

One specimen. Tree 1, Slide SMLA 9800. This specimen belongs to the *spinulosus-quadrispinosus* complex of species in the genus *Echiniscus*, and most closely resembles *Echiniscus quadrispinosus*. This is the first report of this species complex from southern Africa.

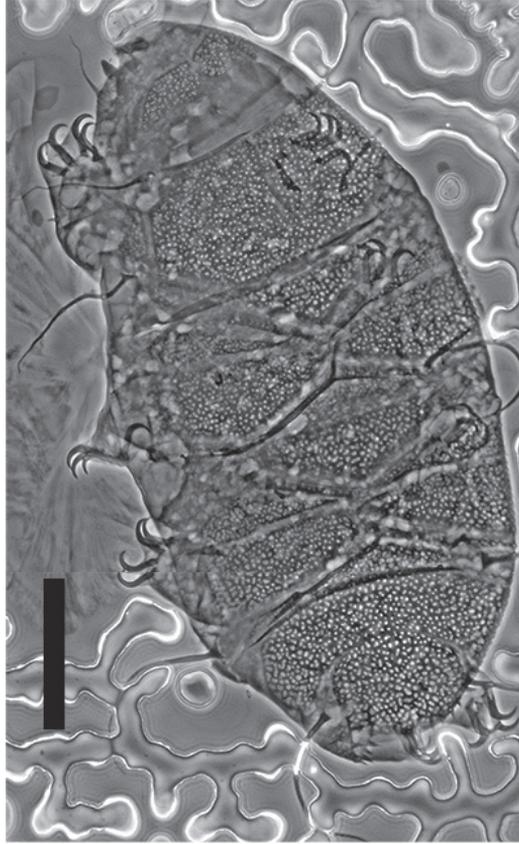
Description: Sex undetermined. Colour red when alive. Plate sculpture comprised of pores of variable size (Figure 1). Ventral cuticle with patches of fine granulation. Pedal plates well developed. Internal claws with spurs (Figure 1). Body 282 µm, scapular plate 52.2 µm, cirrus *internus* 21.8 µm, cephalic papilla 6.5 µm, cirrus *externus* 23.8 µm, clava 6.1 µm, cirrus *A* 59.2 µm, filament *B* 61.1 µm, filament *C* 87.5 µm, spine *C<sup>d</sup>* 21.7 µm, filament *D* 75.4 µm, spine *D<sup>l</sup>* 6.7 µm, spine *D* 15.9 µm, filament *E* 71.8 µm, spine on leg I 3.6 µm, claw I branch 15.2 µm, claw I spur 3.9 µm, claw II branch 14.9 µm, claw II spur 2.8 µm, claw III branch 14.2 µm, claw III spur 3.52 µm, claw IV branch 17.0 µm, claw IV spur 3.4 µm. Ten large teeth (maximum length 5.7 µm) on dentate collar. Leg IV papilla not visible. Appendages C, *C<sup>l</sup>* and *D<sup>l</sup>* absent on right side; *D<sup>d</sup>* absent on left.

**Milnesiidae Ramazzotti, 1962*****Milnesium* Doyère, 1840*****Milnesium* sp.**

One female, length 666 µm. Tree 2, SMLA 9801. This specimen is similar to *Milnesium lagniappe* Meyer, Hinton & Dupré, 2013, a species found in southeastern USA, in having [2-3]-[3-2] claw configuration and a cuticle which lacks gibbosities but has bands of dorsal sculpture consisting of a reticular design with irregular polygons. The African specimen has six peribuccal lamellae; Meyer et al. (2013) reported four for *M. lagniappe*. However, Morek et al. (2016) state that light microscopy of peribuccal lamellae is often misleading, and small lamellae can only be detected by scanning electron microscopy. The absence of gibbosities, and possibly the number of lamellae, also differentiate the African animal from *Milnesium reticulatum* Pilato, Binda and Lisi, 2002, a species known only from the Seychelles.

***Milnesium* cf. *bohleberi* Bartels, Nelson, Kaczmarek & Michalczyk, 2014**

One female, length 674 µm. Tree 1, SMLA 9803. The habitus and morphometric dimensions of this specimen are similar to *Milnesium bohleberi*. Buccal tube length and width are within the range of *M. bohleberi* of comparable size. However, its primary



**Figure 1.** *Echiniscus* cf. *quadrispinosus* from Swaziland. Scale bars: 50  $\mu$ m.

and secondary claws are not so robust as those of *M. bohleberi*, and are proportionately longer (e.g., claw IV anterior primary branch *pt* is 45.4–52.3 in *M. bohleberi* and 73.4 in Swaziland; claw IV anterior secondary branch *pt* is 35.7–41.4 in *M. bohleberi* and 45.9 in Swaziland). It is probably the same undescribed species, as reported by Meyer and Hinton (2009), found in KwaZulu-Natal, South Africa, which borders Swaziland.

### **Hypsibiidae Pilato, 1969**

#### ***Hypsibius* Ehrenberg, 1848**

#### ***Hypsibius* cf. *convergens* (Urbanowicz, 1925)**

One specimen, length 244  $\mu$ m. Tree 2, SMLA 9802. The habitus of this specimen is consistent with species in the *Hypsibius convergens* species complex.

**Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008**  
***Ramazzottius* Binda & Pilato, 1986**

***Ramazzottius* sp.**

One specimen, length 200  $\mu\text{m}$ . Tree 1, SMLA 9804. Two species in this genus have been found in South Africa, *Ramazzottius szeptycki* (Dastych, 1980) and *R. theroni* Dastych, 1993. Both species have sculptured dorsal cuticles. The Swaziland specimen has no visible sculpture; however, the mounting of the specimen is not ideal, and the presence of sculpture cannot be ruled out. Otherwise, the habitus of the specimen is consistent with *R. theroni*.

**Macrobiotidae Thulin, 1928**

***Macrobiotus* C.A.S. Schultze, 1834**

***Macrobiotus* cf. *pallarii* Maucci, 1954**

Two specimens, lengths 288  $\mu\text{m}$  and 457  $\mu\text{m}$ . Tree 2, SMLA 9802. The buccopharyngeal apparatus and claws of these animals are consistent with *Macrobiotus pallarii* Maucci, 1954, a species with a wide distribution in Europe and North America. A single unembryonated egg present in the same sample is similar to the egg of *M. pallarii* in having cone-shaped, reticulated processes surrounded by a single row of areolae; however in the African egg the processes are rounded rather than tapering, and the number of areolae surrounding them is ten to twelve rather than eight or nine.

***Minibiotus* R.O. Schuster, 1980 (in Schuster et al. 1980)**

***Minibiotus harrylewisi* Meyer & Hinton, 2009**

Two specimens, lengths 297  $\mu\text{m}$  and 298  $\mu\text{m}$ . Tree 2, SMLA 9803, 9804. The habitus and morphometric dimensions of these specimens conform in all respects with the original description and type material from KwaZulu-Natal, South Africa. This is the first record of the species outside the type location.

**Acknowledgements**

We thank the student who collected the samples. Unfortunately, her name has been lost and she graduated without leaving contact information. Piotr Gąsiorek provided valuable advice on the *Echiniscus* specimen.

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