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Juliana G. Hinton McNeese State University, jhinton@mcneese.edu

Harry A. Meyer

Brad Peet

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# A Contribution to the Tardigrade Fauna of Georgia, USA

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# A CONTRIBUTION TO THE TARDIGRADE FAUNA OF GEORGIA, USA

Juliana G. Hinton\*, Harry A. Meyer, and Brad Peet Department of Biology, McNeese State University Lake Charles, Louisiana, 70609 \*Corresponding author, jhinton@mcneese.edu

#### ABSTRACT

Tardigrada (water bears) is a phylum of microscopic animals commonly found in mosses, lichens, leaf litter, and freshwater. There are no published records of marine tardigrades from Georgia. Twelve species have been reported from four counties in the state of Georgia, USA. Eighteen species of water bear were present in lichen, moss, and leaf litter samples from eight additional counties in northern and central Georgia. Ten species – *Pseudechiniscus suillus, Milnesium bohleberi, Hypsibius convergens, Astatumen trinacriae, Macrobiotus anemone, Macrobiotus* cf. echinogenitus, Macrobiotus cf. islandicus, *Macrobiotus spectabilis, Paramacrobiotus* cf. areolatus, and *Paramacrobiotus tonollii* – are new to the fauna of Georgia.

*Keywords:* Tardigrada, bryofauna, water bear, biodiversity of Georgia

#### **INTRODUCTION**

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 herbivores and carnivorous species that feed on nematodes, rotifers, and other tardigrades.

Four papers have been published on the terrestrial tardigrades of Georgia (Bernard 1977; Christenberry 1979; Christenberry and Mason 1979; Hinton and Meyer 2009). These papers recorded a total of twelve species from Fayette, Hall, Liberty, and Putnam Counties. There are no published records of freshwater tardigrades from Georgia (Meyer 2013) or marine tardigrades from Georgia's coastal waters (Kaczmarek, Bartels, et al. 2015; Miller and Perry 2016).

Hinton and Meyer (2009) found *Minibiotus jonesorum* in Fayette County, Georgia, but misidentified the specimen as *Minibiotus furcatus* Ehrenberg, 1859. The tardigrades identified by Hinton and Meyer (2009) as *Milnesium tardigradum* Doyère, 1840 are now corrected to *Milnesium bohleberi*.

In this paper we report the tardigrades in moss, lichen, and leaf litter samples collected from 2008 to 2013 in eight counties in northern and central Georgia.

#### **MATERIALS AND METHODS**

Moss and lichen samples were collected and stored in paper envelopes. In the laboratory the cryptogams were soaked overnight in water and inspected for tardigrades with a dissecting microscope (Nikon SMZ-U Zoom 1:10). Specimens and eggs were mounted in polyvinyl lactophenol and examined under 100x oil immersion using phase microscopy (Nikon Eclipse 50i).

Tardigrade specimens were identified using keys in Ramazzotti and Maucci (1983), Nelson and McInnes (2002), and Pilato and Binda (2010), and by reference to original descriptions. Tardigrade taxonomy and nomenclature follow Guidetti and Bertolani (2005), Degma and Guidetti (2007), and Degma et al. (2009-2015). Comments on species distribution are based on McInnes (1994), Meyer (2013), Kaczmarek et al. (2014), and Kaczmarek, Michalczyk, et al. (2015). Species or species groups are considered cosmopolitan if they meet the criterion of Pilato and Binda (2001), namely that they have been reported from five or more ecozones. A Garmin nüvi GPS receiver was used for coordinates.

# **Collection sites**

Site 1: Red Top Mountain State Park and Lodge, Barstow County, Georgia, USA; 6 July 2008; Campsite 40: 34°08′28″N, 84°25′51″W, elevation 285 m; White Tail Trail: 34°08′15″N, 84°39′47″W, elevation 268 m; legit H.A. Meyer and K.R. Jones.

Sample 1a: Campsite 40; moss on a rock; slide SMLA 14060.

Sample 1b: Campsite 40; moss on a rock; slide SMLA 14061.

Sample 1c: Campsite 40; squamulose lichen on a rock; slides SMLA 14062-14063.

Sample 1d: Campsite 40; squamulose lichen on a rock; slides SMLA 14064-14065.

Sample 1e: Campsite 40; leaf litter; slides SMLA 14066-14067.

Sample 1f: Campsite 40; leaf litter; slide SMLA 14068.

Sample 1g: Campsite 40; moss and lichen; slide SMLA 14069.

Sample 1h: Campsite 40; moss and lichen; slides SMLA 14070-14075.

Sample 1i: White Tail Trail; lichen on a fallen branch; slide SMLA 14076.

Sample 1j: White Tail Trail; leaf litter; slides SMLA 14077-14079.

Sample 1k: White Tail Trail; leaf litter; slide SMLA 14080.

Sample 11: White Tail Trail; moss on a tree; slide SMLA 14081.

Sample 1m. White Tail Trail; foliose lichen on a fallen branch; slides SMLA 14082-14086.

Site 2: Mercer University, Macon, Bibb County, Georgia, USA; collected 11 June 2013; 32°49′34″N, 83°39′09″W, elevation 138 m, moss mat in pine needles on the ground; slide SMLA 14087; legit H.A. Meyer.

Site 3: Sandy Creek Nature Center, Clarke County, Georgia, USA; collected 13 June 2013; ADA Interpretive Center: 33°59'11"N, 83°58'98"W, elevation 202 m; Piedmont Prairie Trail: 33°59'18"N, 83°22'57"W, elevation 211 m; legit H.A. Meyer.

Sample 3a: ADA Interpretive Center; lichen on a log; slide SMLA 14088. Sample 3b: Piedmont Prairie Trail: lichen and moss on a log; slides SMLA 14089-14090.

Sample 3c: Piedmont Prairie Trail: moss on a log; slide SMLA 14091. Sample 3d: Piedmont Prairie Trail: moss on a log; slide SMLA 14092.

- Site 4: University of Georgia Visitor Center, Athens, Clarke County, Georgia, USA; collected 13 June 2013; 33°56′08″N, 83°22′13″W, elevation 205 m; moss on a tree; slides SMLA 14093-14094; legit H.A. Meyer.
- Site 5: 774 Rocky Branch Lane, Evans, Columbia County, Georgia, USA; collected 27 April 2012; 33°33'26"N, 82°08'53"W, elevation 91 m; lichen on a tree; SMLA 14095; legit S. Phillips.

Site 6: Kennesaw Mountain National Historical Park, Cobb County, Georgia, USA; collected 15 July 2008 and 4 January 2000; 33°58'35"N, 84°34'45"W, elevation 550 m; legit D.H. Jones and H.A. Meyer. Sample 6a: moss on a rock; slide SMLA 14096. Sample 6b: moss on a rock; slides SMLA 14097-14099. Sample 6c: moss on a rock; slides SMLA 15027-15028. Sample 6d: foliose lichen on a rock; slides SMLA 15029-15030. Sample 6e: moss on a rock; slide SMLA 15031. Sample 6f: foliose lichen on a rock; slides SMLA 15032-15033. Sample 6g: foliose lichen on a rock; slides SMLA 15034-15036. Sample 6g: foliose lichen on a rock; slides SMLA 12024. Sample 6i: moss on a rock; slides SMLA 12024. Sample 6i: moss on a rock; slides SMLA 12025.

- Site 7: Stone Mountain Park, DeKalb County, Georgia, USA; collected 18 July 2008; 33°48′40″N, 84°08′48″W; elevation 280 m; foliose lichen on a tree; slide SMLA 12028; legit D.H. Jones and S.H. Jones.
- Site 8: Sweetwater Creek State Park, Douglas County, Georgia, USA; collected 12 June 2013; 33°45′27″N, 84°38′22″W; elevation 295 m; legit H.A. Meyer. Sample 8a: moss on a log; slide SMLA 15055. Sample 8b: moss on a log; slide SMLA 15056.
- Site 9: Fairfax Museum and Heritage Center, Black Rock Mountain State Park, Rabun County, Georgia, USA; collected 14 June 2013; 34°54′24″N, 83°23′57″W; elevation 792 m; legit H.A. Meyer. Sample 9a: moss on a rock; slides 15037-15042 Sample 9b: foliose lichen on a rock; slides 15042-15043.

# RESULTS

Five hundred nineteen tardigrade specimens and 16 eggs were collected, representing nine genera and 18 species. Some species could only be identified to species group. Ten species or species groups were new records for Georgia.

# TAXONOMIC ACCOUNT

# Echiniscus virginicus Riggin, 1962

Samples 1i, 3b; 6g, f; 9a. 26 specimens. *Echiniscus virginicus* has been reported from Central America, Hawaii, the West Indies, Venezuela, and eastern USA. Riggin (1962) described the presence of spines B, C, C<sup>d</sup>, D, D<sup>d</sup>, and E in the holotype of *E. virginicus*, but Christenberry and Mason (1979) reported that B was absent in specimens they considered subadults (113-165  $\mu$ m long). In specimens from Kennesaw Mountain, Georgia (147-245  $\mu$ m long) we found considerable variation in the presence or absence of dorsal spines; spines B, C, D, and E were present in all specimens, but

C<sup>d</sup> was present in only 38%, and D<sup>d</sup> in 85%. Christenberry and Mason (1979) provided scanning electron micrographs and detailed line drawings of *E. virginicus*, but no phase contrast micrographs. Figure 1 shows the cuticular sculpture and claw configurations of an *E. virginicus* from Black Rock Mountain State Park.



*Figure 1. Echiniscus virginicus* specimen from Georgia, dorsolateral perspective, illustrating cuticular sculpture, spines, and claws. Phase contrast microscopy. The scale bar indicates 20  $\mu$ m. A. Anterior. B. Medial. C. Posterior. D. Claws of legs II, III, and IV.

#### Pseudechiniscus suillus (Ehrenberg, 1853)

Samples 1d, h; 3b, g; 6b, i; 9a. 83 specimens. *Pseudechiniscus suillus* is considered a cosmopolitan species. This is the first report of this species from Georgia.

# *Milnesium bohleberi* Bartels, Nelson, Kaczmarek, and Michalczyk, 2014

Samples 1a, c, d, h, i, m; 3a, b; 4, 5; 6b, 6d, 6f-j, 7, 8a, 9a. 96 specimens and one exuvium with seven eggs. The habitus, range of morphometric values, and a\* values (morphometric values corrected to account for allometric effects as recommended by Bartels et al. 2011) are consistent with the description in Bartels et al. (2014) and the key in Morek et al. (2016). This is the first report of this species from Georgia.

# Diphascon pingue Marcus, 1936

Samples 1i, m; 3g. 10 specimens. These specimens keyed out to *Diphascon pingue* in Fontoura and Pilato (2007)'s key to the species of the *D. pingue* group. *Diphascon pingue* is considered a cosmopolitan species.

# Hypsibius convergens Urbanowicz, 1925

Samples 5, 9a. Three specimens. *Hypsibius convergens* is considered a cosmopolitan species. This is the first report of this species from Georgia.

#### Astatumen trinacriae Arcidiacono, 1962

Samples 3c, 4. Two specimens. *Astatumen trinacriae* is considered a cosmopolitan species. This is the first report of this species from Georgia.

# Macrobiotus anemone Meyer, Domingue, and Hinton, 2014

Sample 5. Five specimens and one egg. *Macrobiotus anemone* has been reported from Florida and Louisiana. This is the first report of this species from Georgia.

# Macrobiotus cf. echinogenitus Richters, 1903

Samples 1g-i, l, m; 6b-d, f, g, i, j. 91 specimens. The absence of eggs precluded further identification. This is the first report of this cosmopolitan species group from Georgia.

# Macrobiotus harmsworthi Murray, 1907

Sample 9a. One specimen and one egg. *Macrobiotus harmsworthi* is considered a cosmopolitan species.

# Macrobiotus cf. harmsworthi

Samples 1e, f, j, k; 2, 3b. 32 specimens. The absence of eggs precluded further identification.

### *Macrobiotus hibiscus* de Barros, 1942

Site 9a, b. 16 specimens and one egg.

# Macrobiotus cf. hufelandi C.A.S. Schultze, 1834

Samples 3b, 4. Seven specimens. The absence of eggs precluded further identification.

# Macrobiotus cf. islandicus Richters, 1904

Samples 1a, b; 4; 6a. 11 specimens. The absence of eggs precluded further identification. This the first report of this species group from Georgia.

## Macrobiotus spectabilis Thulin, 1928

Sample 9a. Four specimens. This the first report of this species from Georgia.

# Minibiotus intermedius Plate, 1888

Samples 1c, g, m; 6a-c, e, f. 48 specimens and three eggs. *Minibiotus intermedius* is considered a cosmopolitan species.

#### Minibiotus cf. intermedius Plate, 1888

Samples 3b-d; 5, 9a. 16 specimens. The absence of eggs precluded further identification.

#### *Minibiotus jonesorum* Meyer, Lyon, Nelson, and Hinton, 2010 Sample 3a, 6g, 8a. 11 specimens.

## Paramacrobiotus cf. areolatus Murray, 1907

Samples 6i, 9b. Eight specimens. The absence of eggs precluded further identification. This is the first report of this cosmopolitan species group from Georgia.

# Paramacrobiotus richtersi Murray, 1911

Samples 6a, h. 15 specimens and one egg. *Paramacrobiotus richtersi* is considered a cosmopolitan species.

#### Paramacrobiotus tonollii Ramazzotti, 1956

Samples 3c, 4, 8b. 34 specimens and two eggs. This is the first report of this species from Georgia.

#### DISCUSSION

With the results of this study 22 species of terrestrial tardigrade are now recorded as occurring in Georgia, USA. All tardigrade collecting in Georgia has focused on moss, lichen, and leaf litter – the soil, freshwater, and marine tardigrade fauna of the state remain unknown. Much remains to be learned about the tardigrades of Georgia. For example, based on data from Louisiana and Florida, Hinton and Meyer (2007) and Meyer and Domingue (2011) hypothesized that five species – *Echiniscus cavagnaroi* Schuster and Grigarick, 1966, *Echiniscus kofordi* Schuster and Grigarick, 1966, *Macrobiotus acadianus* (Meyer and Domingue 2011), *Macrobiotus anemone*, and *Minibiotus fallax* Pilato, Claxton, and Binda, 1989 – constitute a distinctive regional tardigrade fauna within the southeastern USA. *Macrobiotus anemone* has now been found in Georgia, but the other four species have not.

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