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Author(s): Juliana G. Hinton, Harry A. Meyer, Nola D. McDaniel, Cynthia B. Bergeron, Shailaja J. Keely and Amie Matte

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Lone Star Bears: The Tardigrada of Big Thicket National Preserve, Texas

Juliana G. Hinton^{1,*}, Harry A. Meyer¹, Nola D. McDaniel³, Cynthia B. Bergeron⁴,
Shailaja J. Keely², and Amie Matte¹

Abstract - We completed an all taxa biological inventory (ATBI) for water bears (Phylum Tardigrada) in the Big Thicket National Preserve (BTNP) of southeastern Texas begun in 2006. Our inventory is based on methods used in a tardigrade ATBI of the Great Smoky Mountains National Park (GSMNP). We sampled lichens, mosses, liverworts, leaf litter, soil, and aquatic vegetation. Tardigrades were present in 47% of terrestrial and 3% of freshwater samples. We identified 631 tardigrade specimens and 63 eggs, representing 13 genera and 37 species. Tardigrade species richness in BTNP is approximately half that in GSMNP. Lower diversity in BTNP is partly explained by the absence of the altitudinal variation characteristic of GSMNP. Freshwater tardigrades were especially poorly represented at BTNP. Streams and lakes in BTNP are eutrophic and muddy, and may be poor habitats for tardigrades. This study confirms previous findings that the high tardigrade diversity found in mountainous areas may not be typical of other landscapes, and that in North America, tardigrades are less numerous and diverse in southern coastal plains and flatlands than in northern forests and mountains.

Introduction

Tardigrades (Phylum Tardigrada), commonly known as water bears, are microscopic animals found in marine, freshwater, and terrestrial habitats. Terrestrial species occur in cryptogams (mosses, lichens, and liverworts), soil, and leaf litter, and are renowned for their ability to enter a cryptobiotic state (anhydrobiosis) in response to desiccation. Over 960 tardigrade species of marine, freshwater, and terrestrial habitats are known worldwide (Degma et al. 2011). The presence of more than 200 freshwater and terrestrial species has been reported in North America (Meyer and Hinton 2007).

The tardigrade fauna of Texas has received only sporadic attention. Chitwood (1951) and Mehlen (1969) published the only records of marine tardigrades from Texas waters. Nineteen freshwater and terrestrial species have been reported from Texas (Beasley 1968, 1988, 2001; Caskey 1971; Mehlen 1969; Meyer and Hinton 2010). Caskey (1971) provided the only record of tardigrades within the counties of the Big Thicket (Hardin, Jasper, Jefferson, Liberty, and Tyler), in which he found 6 species.

Most papers on Tardigrada of Texas were written before the development of rigorous standards of specific diagnosis. Some species regarded in older literature

¹Department of Biology and Health Sciences, McNeese State University, Lake Charles, LA 70609. ²Department of Mathematics, Computer Science, and Statistics, McNeese State University, Lake Charles, LA 70609. ³710 Todd Jude Road, Lake Charles, LA 70607. ⁴Corresponding author - jhinton@mcneese.edu.

as cosmopolitan have proven to belong to complexes of morphologically similar species. Such records must be considered tentative unless confirmed (see Nelson and Bartels 2007 for a review of this problem). Michalczyk et al. (2012) determined that all records of *Milnesium tardigradum* Doyère outside its type locality in Europe must be considered *Milnesium* sp. or *Milnesium* cf. *tardigradum* until reappraised. Three studies (Beasley 1968, Caskey 1971, Mehlen 1969) have reported the presence of *M. tardigradum* in Texas. Using the criteria set forth in Michalczyk et al. (2012), we conclude that these records must now be corrected to *Milnesium tardigradum* sensu lato in Beasley (1968) and Mehlen (1969) and to *Milnesium* cf. *tardigradum* in Caskey (1971).

The majority of studies of limnoterrestrial tardigrades (i.e., those inhabiting freshwater, cryptogams, soil, and leaf litter) have had a narrow geographical focus, concentrating on the diversity and ecology of a single site or at most a relatively small area. Some investigators have surveyed the tardigrade fauna of larger regions, but only a few have used intensive sampling to compile a multihabitat inventory of a region (e.g., Dastych 1987 in Poland). Tardigrade distributions are extremely patchy at fine spatial scales (Meyer 2006). Rigorous sampling programs, such as those conducted by the ATBI in Great Smoky Mountains National Park (GSMNP) (Bartels and Nelson 2006, 2007; Nelson and Bartels 2007) can estimate their true diversity.

In this paper, we report results of a multihabitat inventory of the freshwater and terrestrial tardigrades of Big Thicket National Preserve (BTNP), TX.

Methods

Study site

The geological history of the Big Thicket in southeast Texas, with its surface formations, drainage patterns, and soil types, has produced a unique collection of diverse plant communities (Watson 2006). Our sampling was conducted within BTNP, an area set aside by the Federal government for preservation. It consists of 14 geographically separated units comprising about 38,931 ha. Elevation ranges from 0 to 8 m. Eight major natural communities are recognized: *Pinus palustris* Mill. (Longleaf Pine) uplands, pine savannah-wetlands, *Fagus* sp. (beech)-*Magnolia* sp. (magnolia) flats, *Pinus taeda* L. (Loblolly Pine) flats, and *Sabal* sp. (palmetto)-hardwood flats, *Taxodium distichum* (L.) Rich. (cypress)-*Nyssa* sp. (tupelo) swamps, an acid bog/baygall, and an arid sandyland (Watson 2006). Plant types found in these communities range from hydrophilic to xerophilic species.

Collection and analysis

We thoroughly sampled nine units of BTNP: Beaumont, Beech Creek, Big Sandy Creek, Canyonlands, Hickory Creek Savannah, Turkey Creek, Lance Rosier, Menard Creek, and Upper Neches River Corridor Unit. Sampling was not quantitative. We collected cryptogams, leaf litter, and soil, and stored the samples in paper envelopes or bags. We preserved aquatic vegetation in 70% ethanol.

Prior to examination, we placed samples in tap water and soaked them overnight. We examined them using a dissecting microscope (Nikon SMZ-U Zoom 1:10) with phase contrast microscopy (Nikon Eclipse 50i). We mounted any tardigrades observed on slides in polyvinyl lactophenol.

We identified Tardigrades using keys and descriptions in Nelson and McInnes (2002), Pilato and Binda (2010), and Ramazzotti and Maucci (1983), and by referring to the primary literature. We obtained anatomical terminology, global species lists, and current taxonomic nomenclature from Degma et al. (2011), Degma and Guidetti (2007), and Guidetti and Bertolani (2005).

We used the statistical program EstimateS Version 8.2.0 to estimate species richness (Colwell 2006), using the seven estimators included in EstimateS (Chao 1, Chao 2, ACE, ICE, Jackknife 1, Jackknife 2, and Bootstrap). No single estimator is accurate for all tardigrade habitats (Bartels and Nelson 2007). To eliminate biases in estimated species richness caused by the patchy distribution of tardigrades, we used 100 randomizations and set patchiness at zero.

Results

Samples collected in BTNP contained 631 identifiable tardigrade specimens and 63 eggs, representing 13 genera and 37 species. Table 1 lists the tardigrades collected, the substrates with which they were associated, whether they are new records for BTNP or Texas, and whether they were also found in GSMNP. Eighty-four (47%) terrestrial samples and 70 (97%) aquatic samples did not contain tardigrade specimens.

Ten species are new to the fauna of Texas, and 25 are new to the fauna of BTNP. *Pseudechiniscus juanitae* De Barros and *Pseudobiotus longiunguis* Iharos are new records for North America. Thirteen species present in BTNP were also found in GSMNP.

Voucher specimens of all species are deposited in the W.A.K. Seale Museum, Department of Biology and Health Sciences, McNeese State University, Lake Charles, LA (slides SMLA 10,000-10,102).

Mean estimates of tardigrade species richness in BTNP predicted by EstimateS ranged from 41 to 65 (Chao 1: 65, Chao 2: 55, Jack 1: 49, Jack 2: 58, Bootstrap: 41, ACE: 54, and ICE: 50). Confidence intervals were large (95% CI: Chaos 1: 41–180, Chaos 2: 40–106).

Discussion

Our results suggest that freshwater and terrestrial tardigrade biodiversity in BTNP appears to be considerably lower than in GSMNP. Bartels and Nelson (2007) collected 73 species and estimated the species richness to be 96 at GSMNP. Both the number of species collected and the estimated species richness in BTNP are roughly half that in GSMNP. The number of tardigrade species found in a cryptogam sample usually ranges from 1 to 6, although more than 10 are sometimes found (Ramazzotti and Maucci 1983). In BTNP, the number of species per sample ranged from 1 to 4. This low range of species per sample is characteristic of tardigrade collections in

Gulf Coast areas (Hinton and Meyer 2007, 2009; Hinton et al. 2010; Meyer 2001, 2008). The majority of our terrestrial tardigrade samples contained no specimens, a result similar to that of Kaczmarek et al. (2011) who noted that only 48% of their cryptogam samples from Costa Rica were positive for tardigrade specimens or eggs. Neither the GSMNP nor the BTNP surveys employed quantitative sampling, but it is worth noting that the number of tardigrade specimens collected in GSMNP was an order of magnitude greater than in BTNP (Bartels and Nelson 2007).

Table 1. List of tardigrade species collected in Big Thicket, TX, showing the substrates with which they were associated, whether they were new records for BTNP or Texas, and whether they have been found in GSMNP. Abbreviations: C = Cryptogam (unspecified lichen, liverwort, or moss), Fw = Freshwater, Lc = Lichen, Ll = Leaf litter or soil, Lv = Liverwort, M = Moss, N = Bird nest.

Species	Substrate	New to		Present in
		Texas	BTNP	
<i>Echiniscus mauccii</i> Ramazzotti, 1956	C	Yes	Yes	Yes
<i>Echiniscus tamus</i> Mehlen, 1969	C	No	Yes	No
<i>Echiniscus virginicus</i> Riggin, 1962	Lc	Yes	Yes	Yes
<i>Echiniscus wendti</i> Richters, 1903	Lc	No	Yes	No
<i>Pseudechiniscus brevimontanus</i> Kendall-Fite and Nelson, 1996	Lc, M	Yes	Yes	Yes
<i>Pseudechiniscus juanitae</i> de Barros, 1939	M	Yes	Yes	No
<i>Pseudechiniscus suillus</i> (Ehrenberg, 1853)	M	Yes	Yes	Yes
<i>Milnesium jacobii</i> Meyer and Hinton, 2010	M	No	Yes	No
<i>Milnesium</i> cf. <i>tardigradum</i> Doyère, 1840	Ll	No	No	No
<i>Isohypsibius saltursus</i> Schuster, Toftner and Grigarick, 1978	Lc	Yes	Yes	No
<i>Isohypsibius schaudinni</i> (Richters, 1909)	M	No	Yes	No
<i>Pseudobiotus kathmanae</i> Nelson, Marley and Bertolani, 1999	M	No	Yes	No
<i>Pseudobiotus longiunguis</i> (Iharos, 1968)	C	Yes	Yes	No
<i>Ramazzottius baumanni</i> (Ramazzotti, 1962)	M	Yes	Yes	Yes
<i>Ramazzottius</i> sp.	Ll	No	No	Yes
<i>Diphascon</i> (<i>Diphascon</i>) <i>alpinum</i> Murray, 1906	M	No	Yes	No
<i>Diphascon</i> (<i>Diphascon</i>) <i>chilenense</i> Plate, 1888	N	No	No	No
<i>Diphascon</i> (<i>Diphascon</i>) <i>pingue</i> (Marcus, 1936)	Ll	No	Yes	Yes
<i>Diphascon</i> (<i>Adropion</i>) <i>scoticum</i> Murray, 1095	C	No	Yes	Yes
<i>Itaquascon umbellinae</i> de Barros, 1939	Ll	No	Yes	No
<i>Macrobiotus coronatus</i> de Barros, 1942	Ll	No	No	No
<i>Macrobiotus echinogenitus</i> Richters, 1904	C	No	No	No
<i>Macrobiotus</i> cf. <i>harmsworthi</i>	Ll, M	No	No	Yes
<i>Macrobiotus</i> cf. <i>hufelandi</i>	Ll	No	No	Yes
<i>Macrobiotus islandicus</i> Richters, 1904	Ll, M	No	Yes	Yes
<i>Macrobiotus</i> cf. <i>liviae</i> Ramazzotti, 1962	Lv	No	No	No
<i>Macrobiotus occidentalis</i> Murray, 1910	Ll	No	No	No
<i>Macrobiotus</i> cf. <i>spectabilis</i> Thulin, 1928	M	No	Yes	No
<i>Minibiotus acadianus</i> Meyer and Domingue, 2011	M	No	Yes	No
<i>Minibiotus intermedius</i> (Plate, 1888)	Lc	No	No	Yes
<i>Paramacrobiotus areolatus</i> (Murray, 1907)	Ll, M	No	No	No
<i>Paramacrobiotus richtersi</i> (Murray, 1911)	Lc, Lv, M	No	Yes	No
<i>Paramacrobiotus tonollii</i> (Ramazzotti, 1956)	M, Lv	No	Yes	Yes
<i>Paramacrobiotus areolatus/tonollii</i>	Lc, Lv, M	No	No	No
<i>Murrayon pullari</i> (Murray, 1907)	C	No	Yes	No
<i>Murrayon</i> sp.	Fw, M	Yes	Yes	No
<i>Murrayon</i> sp. n.	Ll	Yes	Yes	No

The low tardigrade diversity that we observed in BTNP is consistent with other studies conducted in Gulf Coast states (Caskey 1971, Hinton and Meyer 2007, Hinton et al. 2010, Meyer 2008). Freshwater tardigrades are especially poorly represented in BTNP. The vast majority of aquatic samples had no specimens. Streams and rivers in BTNP are relatively slow moving, eutrophic, and laden with sediment, and may be poor habitat for aquatic tardigrades (McFatter et al. 2007). Altitudinal variation in BTNP is much less than in mountainous GSMNP. The absence of altitudinal variation in BTNP partly explains its lower tardigrade diversity; other studies have shown that the number of tardigrade species increases with altitude (Dastyh 1987, Rodriguez-Roda 1951).

Many studies reporting high tardigrade diversity in well-sampled areas (e.g., Dastyh 1987, in Poland; Bartels and Nelson 2007, in GSMNP; Kaczmarek et al. 2011, in Costa Rica) have been conducted in mountainous areas. The present study emphasizes that such results may not be typical of other landscapes. In North America, tardigrades appear to be less numerous and diverse in southern coastal plains and flatlands than in northern forests and mountains.

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