Marine tardigrades from South Carolina, USA

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Until now, there have been only three studies of marine tardigrades from South Carolina, USA. The Fall 2015 invertebrate zoology class from Warren Wilson College collected four sediment samples from Huntington Beach State Park: shallow beach sand, deep beach sand, shallow salt marsh sediment, and deep salt marsh sediment. No tardigrades were found in the salt marsh, but two species were found in the beach samples. *Batillipes pennaki* Marcus, 1946 was found in shallow and deep beach sand, and three specimens of a potentially new species of *Stygarctus* Schulz, 1951 were found in the deep beach sand at groundwater level. Subsequent collections to find more specimens of *Stygarctus* have been unsuccessful so far, but a third species record, *Orzeliscus belopus* du Bois-Reymond Marcus, 1952, *sensu lato* was verified. Three eutardigrade species were also found in the beach sand, but we presume these were "accidentals" from terrestrial environments. A summary of known marine tardigrades from South Carolina is presented.

Introduction

Only one record exists in peer-reviewed literature with species-level identifications for marine tardigrades from South Carolina, USA (Fleeger 1978, three species). Coull (1985) reported marine tardigrades in SC, but these were identified only to phylum. A master’s thesis (Gaugler 2002) listed 11 intertidal species from beach sand at Huntington Beach State Park (HBSP), Georgetown County, SC, but these results were never published, descriptions were inadequate, and all specimens have been misplaced. Thus, all records from Gaugler’s thesis need verification before they can be considered valid. In the fall of 2015, the invertebrate zoology class from Warren Wilson College collected beach sand at Huntington Beach State Park and found two tardigrade species. One is a possible new species in the genus *Stygarctus*. Subsequent collections were made at HBSP in an unsuccessful attempt to uncover more specimens of *Stygarctus*. Here we summarize previous records from SC, and we report the results of our collections, thus validating some records from Gaugler (2002).

Material and Methods

On 13 Sep 2015, sediment samples were collected at two sites at Huntington Beach State Park: beach sand samples were collected near the boardwalk at the north end of the park (33° 30.862′ N, 79° 2.916′ W), and salt marsh samples were collected at Oaks Creek (33° 31.406′ N, 79° 3.731′ W). At the beach, sand was collected in the mid-intertidal zone. One sample was taken from the top 2–4 cm of surface sand. Another sample was taken at the groundwater level about 0.5–1 m below the surface. The deeper beach sample contained more shell fragments and was coarser than the shallow sample. Intertidal samples at the salt marsh included a very shallow surface sample consisting of only about the top 1 cm. This was light brown in color with finer sand and some silt compared to the beach sand. Immediately below this layer was a black, anoxic mix of fine sand and mud. We collected a sample of this black sediment, approximately 2–10 cm below the surface. Sediment analysis was not conducted.

For all four samples (shallow beach, deep beach, shallow marsh, deep marsh), 15 individual students collected small amounts of sediment by hand, trowel or shovel. Individual student collections were separated by at least 1 m from each other. These were combined into a single 5-gallon bucket until a total of four liters of sediment was collected for each sample. The freshwater shock method was used (Kristensen 1983), and after the sediment was agitated the supernatant was decanted through a 64 μm sieve and backwashed with saltwater. This was repeated for a second washing. Samples were fixed with 4% buffered formalin. Samples were sorted under a stereomicroscope, animals were mounted with polyvinyl alcohol, and cover slips were sealed with fingernail polish. Animals were examined with phase contrast (PhC) and differential interference contrast (DIC) with an Olympus BX60 microscope equipped with a Jenoptics C14+ digital camera. Measurements were completed with i-Solution v. 8.1 morphometric software. Drawings were made with a Wacom Cintiq 13HD Touch pen display.

Two additional collecting trips were made to HBSP. In January of 2016 four liter sand samples were taken in the mid-tide zone and processed as above. On 21 Jul 2016 seven samples were collected totalling about 70 liters of sand. These were spread between high tide, mid-tide, and upper low tide zones. These samples were processed as above, except no preservative was used and fresh samples were processed within two days of collecting. Additionally, the July samples were subjected to five freshwater washings.

Identifications were based on keys in Ramazzotti & Maucci (1983), de Zio Grimaldi et al. (1987), Pollock (1971), and Rho et al. (1999). Original species descriptions were also consulted.

Voucher specimens from these collections are in the collection of Paul J. Bartels at Warren Wilson College.

Results

All marine tardigrade species reported for South Carolina are listed in Table 1, including those found in this study.

In our fall 2015 collections, the two beach samples contained large numbers of nematodes and copepods, as well as other meiofauna. The shallow beach sample contained 24 *Batillipes pennaki* and the deep beach sample contained 13 *B. pennaki* and three specimens of a possible new *Stygarctus* (two males, one female). The shallow marsh sample contained a smaller number of nematodes and copepods, many turbellarians, but no tardigrades. The deep marsh sample was nearly devoid of meiofauna.

No tardigrades were found in the January 2016 samples.

In the July 2016 samples, huge numbers of various meiofauna were found with nematodes, copepods and turbellarians being most abundant. *Batillipes pennaki* was the most common tardigrade found in these samples, and it occurred from high to low tide samples. In the one high tide sample there were hundreds of *B. pennaki*. Three specimens of *Orzeliscus belopus sensu lato* were found in mid to low tide samples. We also found five eutardigrades which we presume are “accidentals” from terrestrial
habitats. That is, these are not likely to be commonly found in marine habitats. There were three specimens of *Minibiotus pustulatus* (Ramazzotti, 1959), which is a new record for South Carolina (Meyer 2013), one specimen of *Minibiotus intermedius* (Plate, 1888), and one specimen of a very unusual *Macrobiotus* C.A.S. Schultze, 1834 with bands of brown or maroon pigment. No eggs were found precluding further identification. These eutardigrades were in the mid and low tide samples. Unfortunately, we found no additional specimens of *Stygarcus*. The marine tardigrades found in our collections are discussed below.

**Taxonomic accounts**

Phylum: Tardigrada Doyère, 1840  
Class: Heterotardigrada Marcus, 1927  
Order: Arthrotardigrada Marcus, 1927  
Family: Batillipedidae Ramazzotti, 1962  
Genus: *Batillipes* Ramazzotti, 1962  
*Batillipes pennaki* Marcus, 1946

![Figure 1](image1.png)

Figure 1. Photographs (DIC) of *Batillipes pennaki* from Huntington Beach State Park, South Carolina, USA.  
A = habitus, scale bar = 100 μm.  
B = tail-like caudal appendage and femur (arrow) of leg IV, scale bar = 10 μm.  
C = constriction of clava (arrow), scale bar = 10 μm.

The specimens collected from HBSP were compared with *B. pennaki* from Massachussetts from the collection of Dr. Leland Pollock and specimens previously collected in North Carolina by the lead author (unpublished).

There were no obvious differences in the specimens from South Carolina, North Carolina, and Massachusetts, and they all perfectly matched the descriptions of *B. pennaki* in Marcus (1946) and the keys of Pollock (1971) and Rho et al. (1999). The identifying characters are the oddly shaped femur of leg IV (Fig. 1B), the single sharply pointed caudal appendage arising from a pedestal (Fig. 1B), and the constriction in the clava (Fig. 1C).

Our specimens match the description of *Orzeliscus belopus* in McKirdy et al. 1976. This includes some differences from the original description by du Bois-Reymond Marcus (1952), including the degree of enlargement at the base of the primary clavae, and the “blister-like” lateral swellings between legs III and IV (Fig. 2B). Our specimens clearly differ from *Orzeliscus septentrionalis* Schulz, 1953 by the presence of the sensory papillae on leg IV (Fig. 2A). McKirdy et al. (1976), however, discuss problems with the original descriptions of both species in this genus, and Kaczmarek et al. (2015) concluded that the *Orzeliscus* species are in need of redescription. Thus, we identify our specimens as *Orzeliscus belopus sensu lato*.

![Figure 2](image2.png)

Figure 2. Photographs of *Orzeliscus belopus sensu lato* from Huntington Beach State Park, South Carolina. DIC, scale bars = 50 μm.  
A = Mid-body focal plane, arrows indicate leg IV sensory papillae.  
B = Ventral focal plane, arrows indicate “blister-like” lateral swellings between legs III and IV.
Family: Stygarctidae Schulz, 1951
Subfamily: Stygarctinae Schulz, 1951
Genus: Stygarctus Schulz, 1951
Stygarctus cf. bradypus Schulz, 1951

Two males and one female were found in our samples. Several specimens identified as Stygarctus bradypus Schulz, 1951 from Edisto Beach, SC from the Pollock collection were also examined. There were a total of three slides with five animals in the Pollock collection. Only one of these individuals was a recognizable female, and this individual was compared to our female specimen. Our specimens match the original description of Stygarctus bradypus in almost all characters. However, the seminal receptacles of our female specimen (Fig. 4 A,B) are strikingly different from S. bradypus (Fig. 4 C) and all other members of the genus, as follows. The paired seminal receptacles have openings through the cuticle 1 μm lateral to and 1 μm posterior to the gonopore. The seminal receptacle ducts form multiple loose loops terminating in an enlarged seminal vesicle projecting well caudally. The right vesicle is clearly observable, but the left vesicle is not visible. In addition to the differences in the seminal receptacles, the presumptive new species also appears to differ from S. bradypus by less prominent lateral lobes on the caudal dorsal plate. Based on Figs. 1 & 2 in Schulz (1951) and Fig. 9E in de Zio Grimaldi et al. (2003), these lateral lobes are quite elongate in S. bradypus.

In the single female specimen identified as S. bradypus from Edisto Beach, SC in the Pollock collection, the gonopore, anus, and connecting cuticular thickening closely match our specimen from Huntington Beach. The seminal receptacles are poorly preserved in the Pollock specimen, but loose loops can be seen on one side. Pollock’s SC specimen, then, is much like our SC specimen, and is probably the same species.

Discussion

Only one published report lists tardigrade species from South Carolina (Fleeger 1978). Fleeger sampled subtidal sand and intertidal mud and listed three taxa identified to species (Table 1). Echiniscoides sigismundi now has several recognized subspecies and is a cryptic species complex (Kristensen & Hallas 1980, Faurby et al. 2012). No subspecies was given in Fleeger (1978), and this record should be given as E. sigismundi sensu lato. Gaugler’s thesis (2002) on beach tardigrades in SC listed 11 previously known species, six additional unidentified Batillipes (Table 1), and one specimen of Paradoxipus Kristensen & Higgins, 1989 that was not identified to species. Since none of these were published in peer-reviewed literature, since the descriptions were very brief, and since all slides from this collection have been misplaced, each of his records is in need of verification.

Our collections yielded three species of marine tardigrades from beach intertidal sand. Batillipes pennaki can now be considered a confirmed species from SC. Previous U.S. Atlantic Coast records of this species include the states of Massachusetts (Marcus 1946, Pollock 1970), New Hampshire (Pollock 1970), New York (Martinez 1975), and Florida (McKirdy 1975), but no additional records in North America have been reported in the past 30 years. Batillipes pennaki is widely distributed (see Kaczmarek et al. 2015 for records of all marine tardigrades and an interactive map).
Table 1. Marine tardigrade reports from South Carolina, USA. Bold font indicates marine species that have been published in peer reviewed literature, which accordingly can be considered valid records. HBSP=Huntington Beach State Park. * = terrestrial eutardigrades that are probably not true marine species. Gaugler thesis information used with permission of the author.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Habitat</th>
<th>Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Batillipes bullacaudatus</em></td>
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<td>intertidal sand</td>
<td>Gaugler 2002</td>
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<td><em>Batillipes carnonensis</em></td>
<td>North Inlet, Georgetown County</td>
<td>subtidal sand</td>
<td>Fleeger 1978</td>
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<td><em>Batillipes cf. dicrocercus</em></td>
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<td>Gaugler 2002</td>
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<tr>
<td><em>Batillipes pennakii</em></td>
<td>HBSP, Georgetown County</td>
<td>intertidal sand</td>
<td>Gaugler 2002, this study</td>
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<tr>
<td><em>Batillipes roscoffensis</em></td>
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<td>Gaugler 2002</td>
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<td><em>Batillipes sp. A</em></td>
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<td>intertidal sand</td>
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<td><em>Batillipes sp. C</em></td>
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<td>intertidal sand</td>
<td>Gaugler 2002</td>
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<td>Gaugler 2002</td>
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<tr>
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<td>intertidal sand</td>
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<td><em>Echiniscoides sigismundi sensu lato</em></td>
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<tr>
<td><em>Halechiniscus remanei</em></td>
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<td><em>Paradoxipus sp.</em></td>
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<td><em>Stygarctus bradypus</em></td>
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<td>intertidal sand</td>
<td>Gaugler 2002</td>
</tr>
<tr>
<td><em>Stygarctus cf. bradypus</em></td>
<td>HBSP, Georgetown County</td>
<td>intertidal sand</td>
<td>this study</td>
</tr>
</tbody>
</table>
and Miller & Perry 2016 for a summary of marine tardigrades in the Americas. It lives in both intertidal and subtidal sand (de Zio Grimaldi and D’Addabbo 2001). Bollitellus pennakii is relatively well studied, and a good review of the literature for this species can be found in Zawierucha et al. (2013).

Oreizicus belopus sensu lato is extremely broadly distributed in a wide diversity of sediment types and depths, but this distribution is dubious due to the poor original description (Kaczmarek et al. 2015). A revision of this genus is needed.

The Stygarctus specimens found at HBSP have a unique seminal receptacle morphology and appear to be a new species. J.G. Hansen (pers. comm.) believes that S. bradypus is a cryptic species complex with morphological differences largely confined to the anatomy of the seminal receptacles. Fujimoto (2014) recently described a new species, Stygarctus avatorii in the S. bradypus complex, based primarily on the morphology of seminal receptacles. Hansen et al. (2012) described other marine tardigrades based on seminal receptacles. We believe that describing a new species in our case is premature, however, until additional female specimens are found. We will continue our search.

The HBSP Stygarctus specimens raise questions about previous records of S. bradypus from the U.S. East Coast, the Western Atlantic, and the Caribbean. Stygarctus bradypus has been reported from Massachusetts (Uhlig 1968, McGinty & Higgins 1968), Virginia (McGinty & Higgins 1968), North Carolina (Lindgren 1971, McGinty & Higgins 1968), the Bimini Islands in the Bahamas (Renaud-Debyser 1959), and Jamaica (McGinty & Higgins 1968), as well as in South Carolina in Gaugler’s thesis (2003), but the female reproductive anatomy was not described in any of these publications. Thus, it is possible that these previous records of S. bradypus from the Western Atlantic might be the same species as our HBSP Stygarctus. We propose that all previous records from this region should be listed as S. bradypus sensu lato in future publications.

**Acknowledgements**

Support for field collections for this work was provided by the Department of Biology of Warren Wilson College for the invertebrate zoology class field trip of 2015. Students in this class were Lilvia Bradbury, Olivia Delaune, Michael Giambalvo, Isabel Harger, Maya Heubner, Miron Honer, Evan Lamb, Meredith Langford, Christina Myslinski, Adaline Pann, Sean Reckert, Ellina Smith, Hope Smith, Oscar Thompson, Alisha Weaver, and Christopher Weber. We thank Hope Smith for returning to Huntington Beach State Park on a second ill-fated attempt to collect Stygarctus, and Margo Nottoli for assistance with the July 2016 collections. We also thank Dr. Leland Pollock for the generous donation of his entire marine tardigrade slide collection which is now housed at Warren Wilson College in the personal collection of the lead author. East Tennessee State University provided partial support for Dr. Diane Nelson.

**Notes and References**

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http://dx.doi.org/10.1111/j.1439-0469.1983.tb00285.x


Journal of the South Carolina Academy of Science, [2017], 15(1) | 47

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