TARDIGRADES: AN IMAGING APPROACH,

A RECORD OF OCCURRENCE, AND A BIODIVERSITY INVENTORY

By

STEVEN LOUIS SCHULZE

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Dr. John Dighton

And approved by

_____

Dr. John Dighton

_____

Dr. William Saidel

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Dr. Emma Perry

_____

Dr. Jennifer Oberle

Camden, New Jersey

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THESIS ABSTRACT

Tardigrades: An Imaging Approach,

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by STEVEN LOUIS SCHULZE

Thesis Director:

Dr. John Dighton

Three unrelated studies that address several aspects of the biology of tardigrades—morphology, records of occurrence, and local biodiversity—are herein described.

Chapter 1 is a collaborative effort and meant to provide supplementary scanning electron micrographs for a forthcoming description of a genus of tardigrade. Three micrographs illustrate the structures that will be used to distinguish this genus from its confamilials. An *In toto* lateral view presents the external structures relative to one another. A second micrograph shows a dentate collar at the distal end of each of the fourth pair of legs, a posterior sensory organ (cirrus E), basal spurs at the base of two of four claws on each leg, and a ventral plate. The third micrograph illustrates an appendage on the second leg (p2) of the animal and a lateral appendage (C′) at the posterior sinistral margin of the first paired plate (II). This image also reveals patterning on the plate margin and the leg. A fourth image presents the tip of a feeding stylet, which is normally retracted into the body.

Chapter 2 compares unknown marine specimens collected in New Jersey to *Neoechiniscoides pollocki* Møbjerg *et al.*, 2019 (=*Echiniscoides pollocki* Hallas &

Kristensen, 1982). We provisionally consider the specimens *Neoechiniscoides* cf. *pollocki*, due to their warty cuticle, cephalic sensory appendages (internal and external cirri) that terminate in star-like arrays of projections, 8,8,8,7 claw configuration, and the absence of tertiary clavae.

Chapter 3 is an inventory of tardigrades on Plummers Island, Maryland that adds the phylum Tardigrada to the list of known taxa from the island. The genera *Astatumen* and *Diphascon* are new records for the state of Maryland, while the genera *Milnesium*, *Macrobiotus*, and *Minibiotus* were also recovered. Tardigrades were found in higher abundance in the eastern sites than the western sites, and eggs were only recovered from two sites.

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DEDICATION

For my grandfather, Louis Norato, a true gentleman and the kindest person I've ever known.

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Strange is this little creature, because the whole organization of his body is extraordinary and strange and because his external appearance, at the first sight, has the closest similarity to a little bear. This also led me to give him the name little water bear.

–Johann A. E. Goeze, 1773

Translated from the German
by Hartmut Greven (2015)

General Introduction

The phylum Tardigrada (It: *tardi*, 'slow'; *grado*, 'walker') is a group of about 1,300 described species of microscopic invertebrates known as tardigrades (Degma *et al.* 2019; Spallanzani 1776). Most are no longer than 500 µm, but some, such as the predatory *Milnesium tardigradum* Doyère, 1840, may exceed one millimeter (Kinchin 1994; Møbjerg *et al.* 2018). Their bodies exhibit bilateral symmetry, are dorsoventrally flattened, have distinct anterior and posterior ends, have a mouth, and have eight lobopodous limbs that, with the exception of five genera, terminate in claws (Dastych 1983; May 1948; Pilato 1969a, 1969b; Pilato & Beasley 1987). These animals may occur in marine, freshwater, or terrestrial environments—from the oceanic abyssal plain to the Himalayas (Dey & Mondal 2018; Romano *et al.* 2011), from Arctic glaciers to Antarctic lakes (Cathey *et al.* 1981; De Smet & Van Rompu 1994), and from subtropical deserts to tropical rainforests (Gąsiorek & Vončina 2019; Pilato *et al.* 1991).

In the first published description of a tardigrade, Goeze (1773) reported collecting his "little water bears" from duckweed in stagnant water from his hometown. Eichhorn (1775) similarly collected tardigrades from bodies of water near his home in Poland, while Corti (1774) and Spallanzani (1776) reported them from sediment collected in rain gutters. In each instance, the authors described their outward appearance and curious gait, both resembling a bear's or caterpillar's, and recognized, significantly, their ability to survive desiccation. It is this combination of peculiarity, resilience, and omnipresence that has led to dedicated studies of the biology of tardigrades.

Since their discovery, tardigrades have been included among arachnids, insects, or crustaceans (Müller 1785; Plate 1889; Schultze 1834; von Siebold 1848; see also Greven 2018), mostly owing to a misdiagnosis of articulation in their limbs. They were later included in the defunct phylum Aschelminthes, based on similarities with nematodes in the structure of their feeding apparatuses (Greven 2018; Jorgensen *et al.* 2018; see also Kinchin 1994). Each grouping, though now antiquated, maintained a relationship of tardigrades with arthropods or nematodes. Current consensus, supported by molecular analyses and life history traits, places Tardigrada within the clade Ecdysozoa Aguinaldo *et al.*, 1997, whose taxa undergo ecdysis (molting) and have lost locomotory cilia (Nielson 1995). Ecdysozoans are the cycloneuralians (Loricifera, Kinorhyncha, Priapulida, Nematomorpha, and Nematoda) and panarthropods (Tardigrada, Arthropoda, Onychophora). The panarthropods are distinguished from the cycloneuralians by their segmented bodies and limbs with terminal hooks (Jørgensen *et al.* 2018; Nielsen 2012). Molecular data and comparisons of morphology among the panarthropods have inferred or supported tardigrades as a sister group to either onychophorans, arthropods, or an arthropod-onychophoran clade (Budd 2001; Jørgensen *et al.* 2018; Nielsen 2012).

The phylum Tardigrada is divided into four classes: Heterotardigrada Marcus, 1927; Eutardigrada Marcus, 1927; the recently erected Apotardigrada Guil *et al.*, 2019 comb. Schuster *et al.*, 1980; and the dubious monospecific Mesotardigrada Rahm, 1937, whose type specimen was lost and type locale destroyed in an earthquake (Guil *et al.* 2019). At a glance, heterotardigrades can be distinguished from eutardigrades and apotardigrades by their numerous and prominent cephalic appendages, cuticular sculpturing, a distinct anus and gonopore, and unbranched claws; apotardigrades and eutardigrades have a

cloaca, branched claws, reduced or absent appendages, and slightly sculptured or unsculptured cuticles (Jørgensen *et al.* 2018). Additionally, Apotardigrades have double claws whose primary and secondary branches are completely separated and have a pharyngeal bulb without cuticular elements known as placoids (Guil *et al.* 2019).

Heterotardigrada is further divided into the orders Arthrotardigrada Marcus, 1927 and Echiniscoidea Richters, 1926. The arthrotardigrades have an external cephalic sensory appendage (median cirrus) that is unique to the order, while echiniscoideans lack a median cirrus and have reduced lateral, leg, and sensory appendages. Eutardigrada includes four recently erected orders that are primarily distinguished by the morphology of their claws and their feeding apparatuses (Guil *et al.* 2019). Despite this morphological diversity, tardigrade taxa do exhibit common anatomical and physiological features.

Tardigrades—marine, freshwater, and even terrestrial—are collectively aquatic; they require a film of water to be metabolically and reproductively active. Terrestrial species are commonly found in the periodically inundated interstitial spaces between leaves of moss or lobes of lichen, while marine (intertidal, benthic, pelagic, and abyssal) and freshwater species are frequently or continuously submerged in water (Ramazzotti & Maucci 1983). The oxygenated water diffuses across the soft, tri-layered tardigrade cuticle and into the body's haemocoel. This body cavity circulates extracellular fluids, gas, and nutrients among the tardigrade's internal organs and also contains what are thought to be storage cells that supplement the animal with protein and fat during lean times (Hyra *et al.* 2016b). Their primary source of nutrition, however, is quite varied and may include plants, fungi, bacteria, or other microinvertebrates (Møbjerg *et al.* 2018).

Feeding preference is poorly understood, and the aforementioned diets are rarely assigned to any one group. Known predatory tardigrades have, however, been observed 'foraging'. Claws and locomotory muscles and a ventral nervous system allow the animal to crawl toward and potentially differentiate prey items, but the underlying strategies must be investigated further (Hohberg & Traunspurger 2009). When a tardigrade does feed, two stylets extend from the mouth and pierce a cell wall or membrane. The cellular contents are ingested through suction and pass through the gut where they are digested (Møbjerg *et al.* 2018). Waste is egested through an anus or cloaca, which serves as an excretory orifice and, like the gonopore in the heterotardigrades, a reproductive organ. If favorable environmental conditions persist, tardigrades will reproduce—with or without fertilization—and a female will lay eggs (Altiero *et al.* 2018). Those eggs will eventually hatch, and the juveniles will grow into adults through a series of instars. If unfavorable conditions arise, tardigrades will enter a dormant stage known as cryptobiosis.

Cryptobiosis, or 'latent life', is a state of dormancy through which an organism reduces its metabolism to an imperceptible level in response to adverse environmental conditions (Keilin 1959). Desiccation, oxygen depletion, freezing, or changes in ambient salinity will trigger this physiological response, for which tardigrades are perhaps most famous (e.g., Kinchin 1994). Cryptobiosis was first reported by van Leeuwenhoek at the turn of the 18th century, and recent tardigrade research has focused on the molecular mechanisms that drive the process and their applications (Boothby *et al.* 2017; van Leeuwenhoek 1702). Understanding desiccation tolerance may allow human tissue to be preserved without the need for refrigeration (Shankar *et al.* 2019). Radiation-tolerant

tardigrades, which omics studies suggest can maintain DNA integrity when irradiated, may have genes that can be transfected to cells of cancer patients (Jönsson 2019). Rightly so, promising medical applications have pushed molecular biology to the forefront of tardigrade research, but in some cases at the risk of superseding useful morphological taxonomy and observational data. For example, some species in the Macrobiotidae: *Macrobiotus hufelandi* group are only distinguished by their DNA and are otherwise morphologically identical. The three studies that will be described here are meant to address understudied aspects of tardigradology and to highlight the importance of natural history and organismal biology.

Chapter 1.

Imaging Tardigrades: A Scanning Electron Microscopic Approach

Introduction

Scanning electron microscopy (SEM) is a useful, multidisciplinary imaging tool. It has found applications in both the physical and life sciences to visualize surface features of an object at a high magnification and with better resolution than can be achieved with light microscopy (e.g., Moropoulou *et al.* 2019; Szöke-Nagy *et al.* 2018; Thompson *et al.* 2018). This is accomplished by using electrons, rather than photons, to interact with a sample, thereby overcoming the diffraction limit on resolution imposed by an optical system (Abbe 1873; Haguenau *et al.* 2003; Thomson 1897). Resolution is the minimum distance between two objects that allows them to be seen as separate entities. This value is directly proportional to the wavelength of an energy source (Abbe 1873; de Broglie 1925; Haguenau *et al.* 2003). Electrons have a shorter wavelength than protons, and thus theoretically permit a smaller diffraction-limited separation to resolve objects, which results in better resolution. For an SEM to produce an image, a focused beam of electrons scans an object in a line-by-line raster pattern to produce signals about the object's surface. These signals arise from electrons of the object's atoms being 'kicked' out of their orbital by the high energy electrons of the beam. The high-energy signals, known as backscattered electrons (BSE), or beam electrons rebounding from an interaction with an atom's nucleus, have almost as much energy as the incident beam and require a different detection system than the low-energy signals. These low-energy signals, called secondary electrons (SE), are more easily attracted to a positively biased

electron detector. This detector uses a photomultiplier to convert an electron signal to an electronic one that is viewed on a television screen (McMullan 1995; Postek *et al.* 1980; Stintzing 1929a, b; Synge 1928).

The concept of scanning an object with an electron beam to produce images was theorized by Hugo Stintzing in 1927 and demonstrated by Max Knoll in 1935 (Knoll 1935; Stintzing 1929a, b). Knoll's demonstration employed an apparatus similar to an SEM, but which lacked condenser lenses (Knoll 1935; McMullan 1995). He initially used the apparatus to study the metallic granule targets of television camera tubes and later expanded to other metallic samples (Knoll 1941). By 1938, Manfred von Ardenne had constructed the first SEM (Knoll 1935; von Ardenne 1938a, b) just as Vladimir Zworykin, at RCA laboratories in Camden, NJ, began development of his own machine. Charles Oatley began work in 1948 on an SEM at Cambridge University that led to the first commercially available SEM. It was marketed in 1965 by the Cambridge Instrument Company and marked the initiation of its widespread use, including in the field of biology (Oatley *et al.* 1965; Stewart & Snelling 1964).

Ladislaus Marton (1934) was the first to image a biological specimen (*Drosera intermedia*) with an electron microscope of any kind (Van Dyck 1996), while Smith and Oatley (1955) was the earliest example this author could find of published SEMs of biological specimens (an amoeba and mealworm grub). Baccetti and Rosati (1971) were the first to use SEM to study tardigrades—specifically, their integument—and successive publications in the same decade expanded the gallery of tardigrade SEMs, including those of eggs (e.g., Crowe & Cooper 1971; Grigarick *et al.* 1973; Nelson 1975).

Nowadays, it would be difficult to find a species description without supplementary images from SEM (e.g., Bai *et al.* 2020; Guidetti *et al.* 2019; Perry *et al.* 2018).

In tardigradology, SEM is not a diagnostic tool; rather, it corroborates what is observed with light microscopy and reveals details that would otherwise go unseen. External structures are especially useful in distinguishing members of the ornamented class Heterotardigrada. In collaboration with Lowman *et al.* (in prep), scanning electron micrographs are contributed to better illustrate characteristics used to describe a new genus of heterotardigrade.

Methods

Tardigrades (Heterotardigrada, Echiniscoidea, Echiniscidae) recovered from lichen collected in the Malaysian rainforest were shipped from Kansas City, Missouri to the Rutgers Pinelands Field Station, New Lisbon, New Jersey in a vial of 70% ethanol courtesy of Dr. William R. Miller. At the field station, the contents of the vial were poured into a Petri dish and examined with a Nikon SMZ 1000 stereoscope using reflected fiber optic illumination (Dolan-Jenner Fiber-Lite® series 180). Specimens were transferred with an Irwin Loop from the dish to a 30-µm microporous specimen capsule (Electron Microscopy Sciences) that was immersed to just below its rim in 70% ethanol (Miller 1997; Mitchell & Miller 2008; Schram & Davidson 2012). Animals were dehydrated using a standard ethanol series (Newell 1947; Perry *et al.* 2018) and critical-point dried (Denton Vacuum DCP-1) according to local protocol at Rutgers University, Camden, NJ. Viewed with a stereomicroscope, individual specimens were removed from the capsule electrostatically with a pig's eyelash taped to a small wooden dowel (Emma

Perry pers. comm.) and positioned on double-sided tape on a stub. They were sputter-coated (Denton Vacuum Desk II) with 10 nm of Au/Pd at Rutgers University, Camden, NJ, then imaged with a Hitachi SU5000 FE-SEM at The College of New Jersey, Ewing, NJ. The use of detector bias and choice of accelerating voltage were informed by trial and error to produce the most illustrative images.

Results

Pictured are scanning electron micrographs of two specimens belonging to the undescribed genus (Heterotardigrada, Echiniscoidea, Echiniscidae). Figures 1.1, 1.3, and 1.4 are photographs of the same animal. Figure 1.1 is an *in toto* lateral view, showing lateral appendages, dorsal and ventral plates, and claws. Figure 1.2 is a detailed view of the terminal ventral plate with few pores. Also visible are the anus, gonopore, fourth pair of legs, basal spurs on the two interior claws on each leg, and the dentate collar at the posterior margin of the legs. The dextral cirrus E has broken off. Bacteria (bacilli and cocci) appear on the ventral plate and legs. Figure 1.3 is a detailed lateral view of leg appendage II and lateral appendage C. Cocci are visible under the leg appendage. Figure 1.4 is a detailed view of a grooved, piercing stylet protruding from the mouth. Bacteria did not obstruct views of any structures in Figures 1.2 or 1.3.

Discussion

A full description of the genus is beyond the scope of this work, which is simply meant to provide supplementary SEM images in collaboration with Lowman, Miller, and

others for a forthcoming description of the newly discovered tardigrade (Heterotardigrada, Echiniscoidea, Echiniscidae) (Lowman *et al.* in press).

Figures 1.1–1.3 are of publishable quality and will be used in the description of the genus. The stylet in Figure 1.4 will not be used to describe the genus, but such a structure is normally retracted into the body and is seldom photographed with SEM. The pores on ventral plate E in Figure 1.2 are incidental; ventral sculpturing will not be used to describe this new genus. Since the gonopore of the specimen is sufficiently deformed to render identification of sex impossible, a different animal is required for imaging this structure. In Figure 1.3, patterning is evident on leg II and on the margins of paired plates II and III. The light spots in this patterning might represent distinct concentrations of elements with high atomic numbers. Since the detector bias (SE) was turned off (BSE) for this micrograph, primarily high-energy backscattered electrons were detected. The intensity of each point in the image is highly correlated with atomic number, and the resultant strong emission translates to lighter areas on the specimen surface (Postek *et al.* 1980). The patterning may also be explained by the incident beam penetrating the surface of the animal to reveal underlying epicuticular pillars (Greven 1980).

An *in toto* image of the dorsal surface and detailed images of cirri A and E; leg appendages I, II, and IV; lateral appendage D; and a primary clava will be ideal supplements to the description of the genus. Ultrasonic cleaning can be employed before critical-point drying to remove residues such as bacteria (Kristensen pers. comm.). In an SEM, features such as mechanical rotation of the stage, autostigmation, and a broader suite of scan speeds are assets to the tardigradologist and could further improve the quality of the images.

Advancements such as serial block-face scanning electron microscopy (SBSEM) (Denk & Horstmann 2004; Hyra *et al.* 2016a; see also Møbjerg *et al.* 2018) and X-ray nano-computed tomography (nanoCT) (Gross *et al.* 2019) would allow 3D visualization of the internal anatomy of tardigrades, and both are used in conjunction with SEM. Sputter-coating with graphene has also been shown to overcome charging associated with heavy metal coating and therefore improve resolution (Park *et al.* 2016). The advantages of SEM over light microscopy—greater magnification, resolution, and depth of field—have led to its widespread adoption as a research instrument that enhances the field of tardigradology, particularly studies of morphology.

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Chapter 2.

A New Record of Marine Tardigrade from the Northwest Atlantic Ocean

Introduction

Marine tardigrades are found in all oceanic ecological zones, from the intertidal to the abyssal. They are smaller than their limnoterrestrial counterparts, and their habitats are often difficult to access. Consequently, few records of occurrence exist of marine tardigrades from the Cold Temperate Northwest Atlantic Marine Province, which extends from Onslow County, North Carolina, USA, northward to the northern reach of the Gulf of St. Lawrence, Canada (Miller & Perry 2016; Spalding *et al.* 2007). Records of occurrence exist for nine U.S. states within the marine province (Fig. 2.1).

Perry and Miller (2015) described *Echiniscoides wyethi* from Allen Island, Maine, while Faurby *et al.* (2011) reported *Echiniscoides* sp. from Wells, Maine. Pollock (1970c) reported *Batillipes pennaki* from Hampton Harbor, New Hampshire, and Hummon (1994) reported *Batillipes tubernatis* from Duxbury Harbor and Saquish Neck, both in Massachusetts. Eight different species were reported from the Woods Hole area in Massachusetts: *Batillipes mirus* from Wood Neck Beach (Pollock 1970a, 1975b); *Stygarctus bradypus* from MBL Beach (Uhlig 1968); *Angursa bicuspis*, *Batillipes bullacaudatus*, *Batillipes dicrocercus*, *Batillipes mirus*, *Batillipes pennaki*, *Echiniscoides sigismundi*, and *Stygarctus granulatus* from Crane's Beach (Marcus 1946; Pollock 1970a, 1970c, 1979); *Echiniscoides sigismundi* from the "Fisheries Jetty" (Pollock 1975a); and *Stygarctus bradypus* from Nobska Beach (McGinty & Higgins 1968). In Rhode Island, Hallas and Kristensen (1982) described *Echiniscoides pollocki* (=*Neoechiniscoides*

pollocki Møbjerg *et al.*, 2019) and *Echiniscoides higginsi* (=*Isoechiniscoides higginsi* Møbjerg *et al.*, 2016) from both an area north of Jamestown Bridge, Narragansett Bay and a "brackish bay near Hamilton, Rhode Island", which this author presumes is Bissel Cove, at the mouth of the Annaquatucket River. Bartsch (1982) did not report tardigrades from her three sites at the brackish bay, but did communicate to Kristensen that the tardigrades in question were indeed collected there (Reinhardt Kristensen pers. comm.). Kristensen and Hallas (1980) also reported *Echiniscoides sigismundi groenlandicus* from an unspecified location near the town of Narragansett, Rhode Island. Continuing south, Martinez (1975) reported *Batillipes mirus* and *Batillipes pennaki* from Rockaway Point, New York, while Hummon (1994) reported *Tanarctus heterodactylus* from The Shears, a sand bar in the Delaware Bay near Cape Henlopen, Delaware. McGinty and Higgins (1968) described *Batillipes bullacaudatus* and reported *Batillipes mirus*, *Halechiniscus remanei*, and *Stygarctus bradypus* from Sandy Point, Virginia, where Indian Field Creek meets the York River. Lindgren (1971) reported *Batillipes bullacaudatus*, *Batillipes mirus*, *Stygarctus bradypus*, *Stygarctus granulatus*, and *Tanarctus arborspinosus* from Bogue Beach, North Carolina, "approximately 250 m west of the Iron Steamer Pier". Finally, in the first record of marine tardigrades from the Americas, Hay (1917) described *Batillipes caudatus* from Shackleford Bank, North Carolina.

The following records are noted but not treated in this paper: The type locality of *Angursa bicuspis*, *Tanarctus dendriticus*, *Tanarctus gracilis*, and *Tanarctus heterodactylus*, off the coast of North Carolina, lies outside the 200-m isobath that Spalding *et al.* (2007) defined as the outer boundary of coastal realms, provinces, and

ecoregions, and so is not considered here (Coull *et al.* 1977; Pollock 1979; Renaud-Mornant 1980). McGinty and Higgins (1968) noted that *Echiniscoides sigismundi* is known from North Carolina and Massachusetts, but this is uncited and the locations unspecified. Pollock (1976) further mentioned two unpublished records of *Echiniscoides*—one from Seawall Beach, Acadia National Park, Maine and the other from Rye Harbor, Rye, New Hampshire. Higgins (1972) mentioned *Echiniscus sigismundi* (=*Echiniscoides sigismundi* Plate 1889) from an unspecified location in North Carolina. It should also be noted that Pollock (1970a) suspected that *E. sigismundi* occurred accidentally on Crane's Beach, as it is usually found among algae on barnacles.

Notwithstanding this smattering of records, intertidal species of the family Echiniscoididae Kristensen & Hallas, 1980 remain of particular interest. They may clarify the transition of tardigrades from marine to terrestrial and freshwater environments. Molecular and morphological studies suggest that they evolved from the exclusively marine Arthrotardigrada Marcus, 1927. Jørgensen *et al.* (2011) inferred an intermediate phylogenetic position of *Echiniscoides sigismundi* based on combined 18S, 28S, COI, and morphological data; *E. sigismundi* was found to be basal to the terrestrial family Echiniscidae but rooted by arthrotardigrades. Kristensen (1981) noted that the setae of arthrotardigrades are well-developed, with a defined cuticular portion and cuticle-forming epithelial cells. The cuticular portion of the setae is reduced in the intertidal echiniscoideans, and the cells and cuticular portion are lost completely in the terrestrial eutardigrades.

The class Heterotardigrada Marcus, 1927 is comprised of two orders: the Arthrotardigrada Marcus, 1927 and Echiniscoidea Richters, 1926. Echiniscoidea is

distinguished from the arthrotardigrades by the absence of a median cirrus, reduction of cephalic and lateral sensory organs, and reduction or absence of leg sensory organs. Within the Echiniscoidea are five families, among which is Echiniscoididae Kristensen & Hallas, 1980 (Degma *et al.* 2019). Echiniscoididae is distinguished by supernumerary claws (6–13) in adults; an unplated cuticle; papillar primary clavae and fourth leg appendages; the absence of a median cirrus, of stylet supports, and of a seminal receptacle; and an anal system with a terminal lobe and two lateral lobes (Møbjerg *et al.* 2019). Echiniscoididae is further divided into three genera: *Echiniscoides* Plate, 1889; *Isoechiniscoides* Møbjerg *et al.*, 2016; and *Neoechiniscoides* Møbjerg *et al.*, 2019. Size and shape of sensory organs, claw configuration, body size, and dorsal sculpturing are used to distinguish taxa of these genera. *Neoechiniscoides* is distinguished from its confamilials by its winged anal system (Møbjerg *et al.* 2019). Here, we report *Neoechiniscoides* cf. *pollocki*, recovered from barnacles at Barnegat Lighthouse, New Jersey, USA (Fig. 1 inset).

Methods

Barnegat Lighthouse was visited five times between November 2017 and March 2018, coincident with low tidal stages as recorded by the United States Coast Guard Barnegat Inlet monitoring station. Barnacles (*Semibalanus balanoides*) (Pollock 1998) were scraped from rocks with a putty knife and submerged in Poland Spring® or Wawa® spring water to osmotically shock any tardigrades into releasing their grasp on the barnacles. Barnacle plates were separated and broken into more easily examined pieces with

forceps. Specimens were either permanently mounted on glass slides or prepared for scanning electron microscopy (SEM).

Specimens selected for SEM were prepared according to the protocol described in Chapter 1. At the University of Maine, Orono, Maine, specimens were critical-point dried (Tousimis® Samdri® pvt-3) and sputter-coated with a 35 nm-thick layer of Au/Pd (Cressington® 108 Auto/SF). Specimens were imaged with an AMRAY 1820 SEM at the University of Maine. The specimens were later recoated with an additional 10 nm of Au/Pd (Denton Vacuum Desk II) and imaged with a Zeiss LEO 1450 EP SEM at Rutgers University, Camden, New Jersey.

Specimens selected for permanent mounting were collected with an Irwin Loop (Schram & Davidson 2012) according to Miller (1997) and viewed with a Nikon SMZ 1000 stereoscope using reflected fiber optic illumination (Dolan-Jenner Fiber-Lite® series 180). They were then transferred to a drop of Hoyer's mounting medium (Hempstead Halide) on a glass slide and covered with a glass coverslip. Four small dots were applied with a permanent marker to the top surface of the coverslip to mark the location of the tardigrade. The Hoyer's medium was allowed to dry for one week, after which the edges of the coverslips were sealed with fingernail polish; pigmented fingernail polish is preferred to clear fingernail polish, as it is more viscous and renders incomplete coverage more evident. Specimens were viewed with a Nikon Eclipse E200 compound microscope and photographed with phase contrast microscopy using an OMAX A35140U digital camera and ToupView computer software (ToupTek Photonics). Morphometric measurements of taxonomically informative traits were taken using ToupView (Møberg *et al*. 2016, 2019; Perry *et al.* 2018). Where curvature of a structure

was inherent (e.g., cirri A and E), a measurement line was drawn to approximate the curvature. Body widths were not measured if the specimen was mounted on its side. Identification was made according to Hallas and Kristensen (1982), Møbjerg *et al.* (2019), and Perry and Miller (2015).

Results

Figure 2.2 illustrates the diagnostic characters of our specimens. Table 2.1 presents morphometrics from our specimens and the type specimens of *N. pollocki*. Character names and abbreviations follow Kristensen & Hallas (1980).

Our adult specimens whose full complement of claws could be resolved (n = 21) exhibited an 8,8,8,7 claw pattern, while juveniles (n = 4) exhibited a 5,5,5,4 pattern (Table 2.1). Body lengths of our adult specimens averaged 243.10 µm, which falls within the range expected for medium-sized *Echiniscoides* and *Neoechiniscoides* taxa (Kristensen & Hallas 1980). Appendages appeared on all legs, but were most often found on legs III and IV; those on legs I, II, and IV were dome-shaped, while that on leg III was an elongate spike (Fig. 2.2E–H). Black eyespots were present as were internal and external cirri, cirri A and E, and primary clavae. Cephalic papillae (secondary clavae) were lens-shaped (Fig. 2.2B). The dorsal sculpturing consisted of irregularly arranged, polygonal granulations without a substructure of smaller points (Fig. 2.2C). Stylet bases occurred at the anterior part of the pharyngeal bulb. Cirri A and E were proximally annulated (Fig. 2.2C & D). Internal and external cirri terminated in a star-like array of projections (Fig. 2.2B). The anus was ovoid, with two lateral lobes and a smaller

terminal lobe (Fig. 2.2A). Female gonopores were encircled by a six-lobed floret, while male gonopores were trilobed (Fig. 2.2A inset).

Discussion

We provisionally consider these specimens to be *Neoechiniscoides* cf. *pollocki* Møbjerg *et al.*, 2019 (=*Echiniscoides* cf. *pollocki* Hallas & Kristensen, 1982). Only *N. pollocki* and *N. horningi* Møbjerg *et al.*, 2019 (=*Echiniscoides horningi* Miller & Kristensen, 1999) have both a warty cuticle and an 8,8,8,7 claw configuration within the genus. *N. horningi* has a pair of tertiary clavae, however, which is lacking in our specimens and in *N. pollocki* (Hallas & Kristensen 1982; Miller & Kristensen 1999; Møbjerg *et al.* 2019). The internal and external cirri of our specimens terminate in short, star-like arrays of projections, which are similar in appearance to the "terminal tufts of setae" of *Echiniscoides bruni* D'Addabbo Gallo *et al.*, 1992. Such projections also appear on *E. rugostellatus* Perry *et al.*, 2018, but those are longer, and both *E. bruni* and *E. rugostellatus* have different claw configurations than our specimens (D'Addabbo Gallo *et al.* 1992; Perry *et al.* 2018).

N. pollocki is described as having stylet bases that rest on the posterior portion of the pharyngeal bulb, while those of our specimens rest on the anterior portion (Hallas & Kristensen 1982). This characteristic is not presently used to differentiate taxa, however, and will be provisionally considered incidental. Møbjerg *et al.* (2019) distinguish *Neoechiniscoides* from the other genera of Echiniscoididae by the presence of lateral wings in the anal system. These lateral wings were not apparent in our specimens, although they are often difficult to discern. According to Møbjerg *et al.* (2019), a ventral

cuticular plate “in front of the female gonopore” was observed the two other species in the genus, except for *N. pollocki*. DNA analysis of our specimens would provide an integrative description and contribute to the molecular phylogeny of the still understudied marine tardigrades.

Acknowledgements

Thanks to Emma Perry and her family for graciously hosting me and Emma for her help in all things tardigrade-related and otherwise. Thanks to Kelly Edwards of the University of Maine and Dr. William Saidel for their expertise with SEM. Thanks to Dr. Reinhardt Kristensen for his enthusiastic assistance.

Chapter 3

A Preliminary Inventory of Tardigrades on Plummers Island, Maryland

Introduction

Biodiversity inventories are crucial to promoting environmental stewardship (NPS 2010). The presence or absence as well as abundance of certain taxa may indicate the health of an ecological system. Lichens and mosses, for example, are known to accumulate heavy metal in their tissues that can be correlated with air or water pollution (Özyiğitoğlu 2020; Radziemska *et al.* 2019). Moss- and lichen-dwelling animals such as nematodes, rotifers, and tardigrades can inform future strategies of conservation or remediation if their abundance or community composition changes in response to stressors, particularly pollution (e.g., Gerlach *et al.* 2013; Leetham *et al.* 1982). Long-term changes cannot be documented, however, without basic ecological data. Tardigrades are among the taxa whose local diversity is not well established and whose habitat preferences and trophic role are poorly understood. Their geographic distribution as a phylum is cosmopolitan, but the mid-Atlantic region has scarcely been surveyed (Kaczmarek *et al.* 2016; Meyer 2013). Plummers Island, Maryland, within that region, is an area with enough habitat diversity to support a rich community and high abundance of tardigrades (Nelson & Bartels 2007; Simmons *et al.* 2016).

Plummers Island is a five-hectare island about 15 km northwest of the District of Columbia (Fig. 1B). It lies at the northern bank of the Potomac River and is separated from mainland Maryland by a narrow culvert. The island rises in two knolls over 90 and 110 feet above sea level along gradual gradients in the East and North and steep gradients

in the South and West (Fig. 1C). Areas of lowest elevation are frequently inundated by floodwater, while the highest areas may flood centennially (Fleming 2015). Alluvial deposits at river level primarily support herbaceous vegetation. Bedrock outcrops and colluvial boulders occur at middle to high elevations and support assemblages of cryptogams, which—particularly bryophytes and lichens—are commonly inhabited by tardigrades (Ramazzotti & Maucci 1983; Wells 2004). Rainwater may also collect in depressions and fissures in the rocks to form ephemeral pools with subaqueous sediment and detritus that have been known to harbor freshwater tardigrade taxa (Ramazzotti & Maucci 1983). The remaining terrain of the island is sparsely or densely wooded with broad-leaved trees whose bark may also support cryptogams that, along with leaf litter and underlying soil, may harbor tardigrades. Low-lying riparian areas with immature, silty soil sustain sycamore, birch, and red maple. At middle elevations, red oaks begin to appear, alongside basswood and tulip-poplar in mature, sandy soil. Upland forests include hickories and oaks that are underlain by clayey hardpan (Fleming 2015; Wells 2004). The island's forested landscape and geomorphological profile support representative fauna of which a catalogue is extensive but incomplete.

The natural history of Plummers Island is well documented. Biological collecting on the island predates the turn of the twentieth century, and since its adoption in 1901 by the Washington Biologists' Field Club as the organization's base of operations has come to be known as "the most thoroughly studied island in North America" (Perry 2007). Birds, mammals, reptiles, and amphibians have all been inventoried (Johnston & Winings 1987; Manville 1968) in an ongoing series of publications dedicated to the biodiversity of the island. Seven invertebrate phyla (Annelida, Arthropoda, Bryozoa, Cnidaria, Mollusca,

Nematoda, and Platyhelminthes) are also recorded from the island, but not Tardigrada, even though this phylum has been documented in surrounding mid-Atlantic states and Maryland (Brown 2008; Kaczmarek 2016; Meyer 2013).

The mid-Atlantic region includes Delaware, Maryland, New Jersey, New York, Pennsylvania, Virginia, and West Virginia, as well as the District of Columbia (DC). Published records of non-marine tardigrades can be found for each of these locations except for Delaware, but only cumulatively do they include specimens collected from all known terrestrial and freshwater tardigrade habitats represented in the Mid-Atlantic region (Meyer 2013). Aquatic tardigrades were recovered from groundwater and moss in New York (Kim-Koutsis and Miller 2019; Strayer *et al.* 1994), while liverworts yielded specimens of tardigrades in West Virginia (Tarter & Nelson 1994). In New Jersey (Miller *et al.* in press; Shaw & Miller 2013), Maryland (Curtin 1957), and DC (Curtin 1948; Marcus 1928), leaf litter, soil, moss, and tree bark contained tardigrades, as did commonly collected lichen and moss from Virginia (Riggin 1962) and Pennsylvania (Mitchell *et al.* 2009). Hutchinson and Streu (1960) reported a tardigrade from New Jersey, but identification beyond phylum was not attempted and the specimen was not curated (George Hamilton and Herb Streu pers. comm.). Just south of the mid-Atlantic region, however, a more comprehensive, multi-habitat inventory of tardigrades in Great Smoky Mountains National Park was undertaken, and 59 species were added to the list of known taxa in the park (Bartels & Nelson 2006; Nelson & Bartels 2007). With this Plummers Island inventory, we add the phylum Tardigrada to the list of known taxa from the island and at least two new species to that of Maryland.

Methods

Plummers Island was visited July 12–13, 2018, concurrent with a low river stage (less than four feet) as recorded by the USGS water gauge near the Washington, D.C. Little Falls pump station. The low water levels allowed for wading, rather than boating, across a culvert to the island (Ralph Eckerlin pers. comm.). Thirty-five terrestrial and 15 aquatic sample sites were chosen to ensure thorough sampling of the breadth of the island and its range of elevations (Fig. 1C). Soil, leaf litter, moss, lichen, and aquatic samples were collected. Soil was removed with a one-inch diameter corer, three inches into the soil, while leaf litter was collected in a single handful (about 125 cm^3) per site. Moss or lichen was separated from its substrate (trees, boulders, bedrock outcrops, or cabin roof) with a pocket knife in about 100-cm^2 sections where possible. Terrestrial samples were stored in paper bags. A suspension of subaqueous detritus (about 100 mL) was siphoned with a turkey baster from the shallows of Rocky Run Culvert, the Potomac River, and an ephemeral pool in a rock fissure on the island. Subaqueous samples were transferred to lidded plastic cups, which were placed in a cooler and later refrigerated at the Rutgers Pinelands Field Station, New Lisbon, New Jersey.

Samples of moss from only five of the 50 total sites in the present study and one from a pilot study were analyzed for species composition and abundance of tardigrades due to time constraints; the remaining 45 samples will be analyzed in a later study (Fig. 3.1C; Table 3.2). The mosses were chosen based on their mat-forming habit and sediment-deficient substrates of metadiamictitic bedrock outcrops or boulders (Fleming 2015; Glime 2017; Mägdefrau 1982); the author has most easily found tardigrades in samples with these attributes (pers. obs.). Mosses from sites 3 and 18 and the pilot study all have

similar, branching stems in two rows; that from site 5 is julaceous—its stalks have densely overlapping leaves; that from site 16 has numerous leaves that radiate from its stalks; and the moss from site 23 has long, thin leaves that tend to the same side of a given stalk (Sargent & Lucas 2012). Tentative identifications of the mosses processed are given in Table 3.2. Each sample was allowed to air-dry for one week, then was separated into three, 0.25-g subsamples to account for tardigrades' patchy distribution within microhabitats (Degma *et al.* 2011). One, 0.25-g subsample was processed in the pilot study. Subsamples were submerged in Poland Spring® or Wawa® spring water in individual glass dishes for 24 hours to relax tardigrades. Tardigrades were retrieved, mounted, and photographed according to the light microscopy protocol described in Chapter 1. One subsample each from the five sites in the present study and one individual from the pilot study were photographed. Tardigrades were identified according to Pilato and Binda (2010). Elevations were recorded with a Garmin Foretrex 401 global positioning system and compared to Drake and Froelich (1997). Moss was tentatively identified according to Sargent and Lucas (2012).

Collection was conducted under National Park Service Scientific Research and Collecting Permit number CHOH-2018-SCI-0016. Slides will be deposited in the Smithsonian Institution, National Museum of Natural History, Department of Invertebrate Zoology, National Park Service collection.

Results

Tardigrades were found at the five sites analyzed in the present study and one site analyzed in the pilot study (Table 1). Two classes (Apotardigrada Guil *et al.*, 2019 comb.

Schuster *et al.*, 1982; and Eutardigrada Marcus, 1927) and three families (Hypsibiidae Pilato, 1969; Macrobiotidae Thulin, 1928; and Milnesiidae Ramazzotti, 1962) are represented. Five specimens of the genus *Milnesium* Doyère, 1840 and three from *Macrobiotus* Schultze, 1834 were found at Site 3, while three specimens of *Minibiotus* Schuster, 1980 were recovered from Site 5. A single representative each of *Astatumen* Pilato, 1997 and *Diphascon* Plate, 1889 were recovered from Site 5 and the pilot site, respectively, and are new records for the state of Maryland. Specimens belonging to Macrobiotidae were recovered from all five sites in the present study. Sites 3, 18, and 23 included specimens belonging to Milnesiidae, while Site 5 and the pilot site included specimens belonging to Hypsibiidae. The greatest abundance of tardigrades was found at Sites 18, 16, and 23, while Sites 3 and 5 yielded the fewest. Eggs were recovered only from sites 16 and 18 and are of the Macrobiotidae type.

Discussion

Tardigrade distribution has been shown to be influenced by dispersal mechanisms (Mogle *et al.* 2018). The presence on Plummers Island of *Diphascon* and the seldom reported *Astatumen* may be attributed to avian ectozoochorous (extrabodily) or aeolian (wind-mediated) dispersal. Nests and feathers of Neotropical migratory birds sampled in Kansas, Nebraska, and Massachusetts have yielded tardigrades, including *Diphascon* (Mogle *et al.* 2018). Johnston and Winings (1987) recorded nine Neotropical migrants on Plummers Island whose migratory range includes Bolivia, Costa Rica, and Ecuador—three countries with records of *Astatumen* (Schulenberg 2020). *Astatumen* and *Diphascon* have also been recorded west of Plummers Island, in Ohio, where desiccated

tardigrades may be carried to the island by prevailing winds (Meininger 1985; Meyer *et al.* 2011). Insects may also be vectors for dispersal of tardigrades as suggested by Pape (1986), who recovered tardigrades from bumblebee (*Bombus*) nests in Greenland. *Bombus* was recorded by Norden (2008) in her survey of bumblebees on Plummers Island. In addition to aerial dispersal, water is capable of carrying and depositing tardigrades (Miller *et al.* 2020). Curtin (1957) recovered *Milnesium, Macrobiotus,* and *Minibiotus* from Frederick County, Maryland within the Monocacy Watershed, which drains into the Potomac River above Plummers Island (ICPRB 2014).

Pollution may influence abundance of tardigrades, as Vargha *et al.* (2002) observed in Hungary. Higher concentrations of atmospheric heavy metals—particularly cadmium—that accumulated in moss significantly decreased abundances of tardigrades. Similarly, Mitchell *et al.* (2009) noted that tardigrade abundances were positively correlated with distance from roads within a Pennsylvania college campus, while Hohl *et al.* (2001) found fewer tardigrades downwind of a coal-burning power plant than upwind in a study in Missouri. In our study, airborne pollutants may explain the low abundance of tardigrades from sites 3 and 5, which are closer to the heavily trafficked American Legion Bridge than the sites with highest abundances—16, 18, and 23 (Hogan & Northam 2019). This may also explain the absence of eggs in Sites 3 and 5.

The morphology of the mosses analyzed could also explain the presence and abundance of tardigrades and eggs (Nelson *et al.* 2018). Site 5 had the lowest abundance of tardigrades, possibly owing to the julaceous habit of *Plagiobryum*; it has very tightly packed leaves along its stalk that may make it difficult for interstitial fauna to maneuver (pers. obs.). The presence of eggs in only sites 16 and 18 may be attributed their distance

from the American Legion Bridge, but their absence in site 23 could be attributed to the 'wind-swept' morphotype of *Dicranum*, which may preclude eggs from being retained on its leaves. Generally, Macrobiotidae was by far the most frequently encountered family across the five sites analyzed, which is not unexpected as it is the most speciose family of tardigrades in the phylum (Degma *et al.* 2019) and has been found in six Mid-Atlantic states and DC (Kaczmarek *et al.* 2016; Meyer 2013).

Sampling effort was limited by time and accessibility. The terrain of the island is precipitous certain areas, and its understory growth prohibitively thick in others. Several attempts to sample southwestern escarpments were abandoned, as were densely foliated areas in the Northwest. A several-day group foray would afford access to these unsampled areas. Priority will also be given to resampling aquatic environments and sampling birds' and bees' nests. Species-level identification of both tardigrades and cryptogams is necessary for statistical analyses and to draw informed conclusions about habitat preference, distribution, and abundance of tardigrades on Plummers Island.

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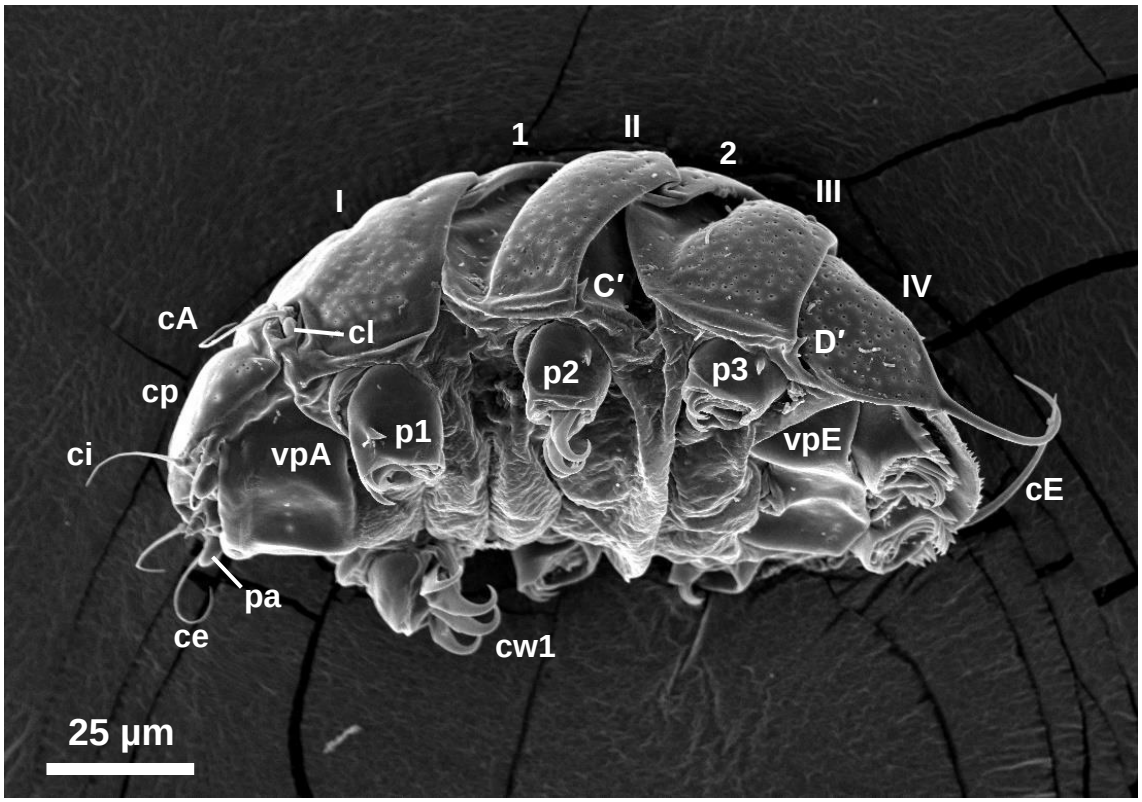

Figure 1.1. *In toto* lateral view of a heterotardigrade. The animal is facing left. 1 = first median plate, 2 = second median plate, C′ = lateral appendage C, cA = cirrus A, cE = cirrus E, ce = external cirrus, ci = internal cirrus, cl = primary clava, cp = cephalic plate, cw1 = claws of leg I, D′ = lateral appendage D, I = scapular plate, II = first paired plate, III = second paired plate, IV = terminal plate, p = leg appendage, pa = cephalic papilla, vpA = ventral plate A, vpE = ventral plate E. Detector bias on (SE). 700×. 7kV

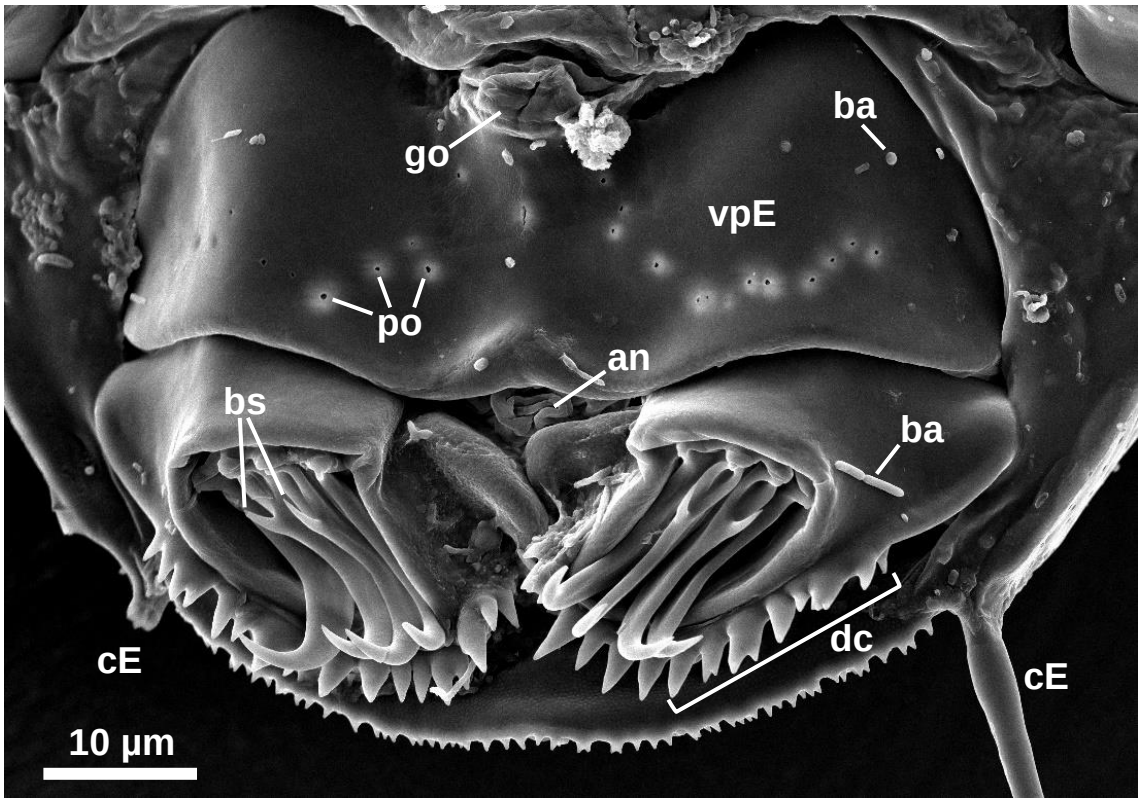

Figure 1.2. Ventral posterior view of a heterotardigrade. an = anus, ba = bacteria, bs = basal spurs, cE = cirrus E, dc = dentate collar, go = gonopore, po = pores, vpE = ventral plate E. Note that the dextral cE has broken off. Detector bias on (SE). 1,800× 7kV.

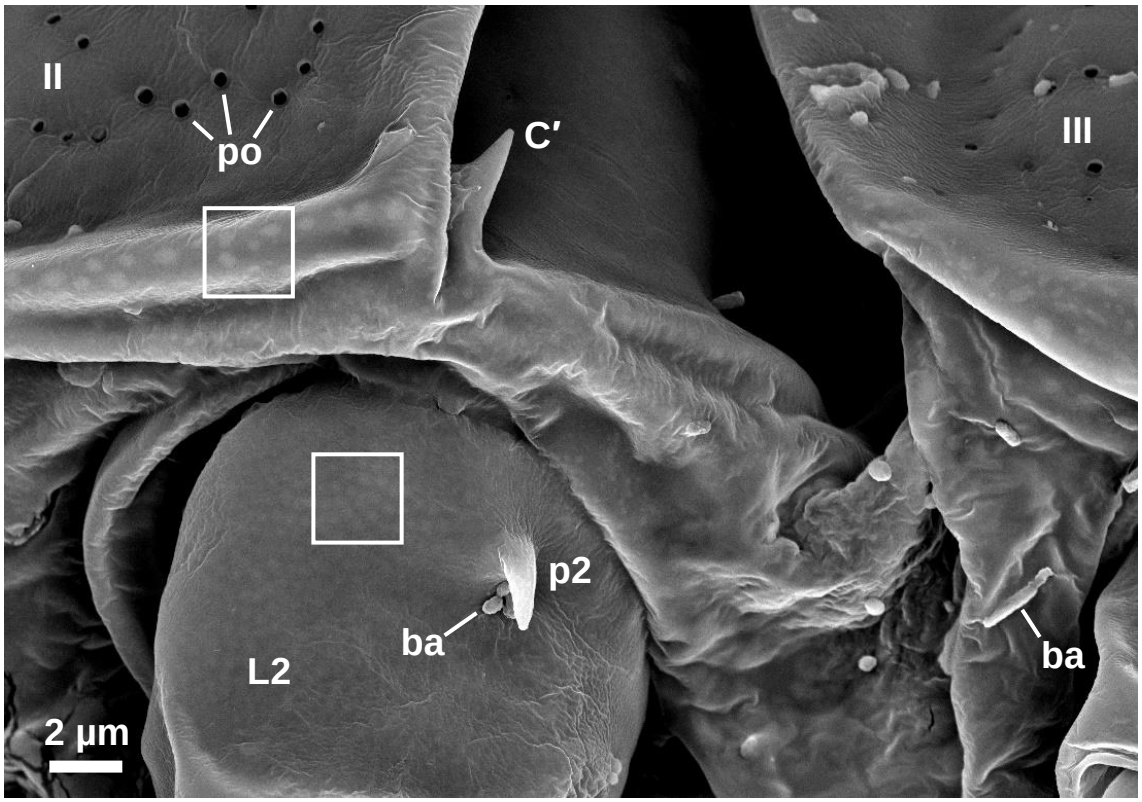

Figure 1.3. Lateral view of leg II (L2) of a heterotardigrade, with first and second paired plates (II and III) partially visible. Patterning (□) is evident on the plate margins and on the leg. ba = bacteria, C′ = lateral appendage C, p2 = leg appendage II, po = pores. Detector bias off (BSE). 4,000×. 10kV.

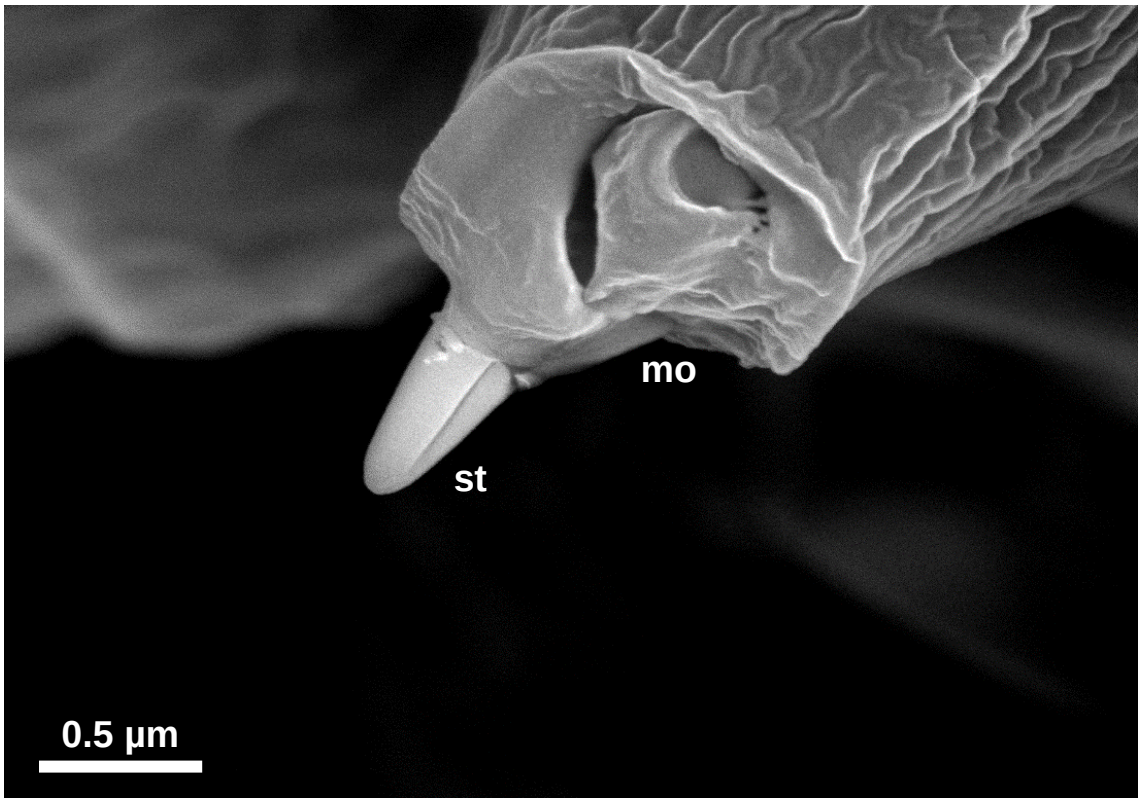

Figure 1.4. Lateral view of the tip of one of a pair of piercing stylets (st) protruding from the mouth (mo) of a heterotardigrade. Detector bias off (BSE). 35,000×. 10kV.

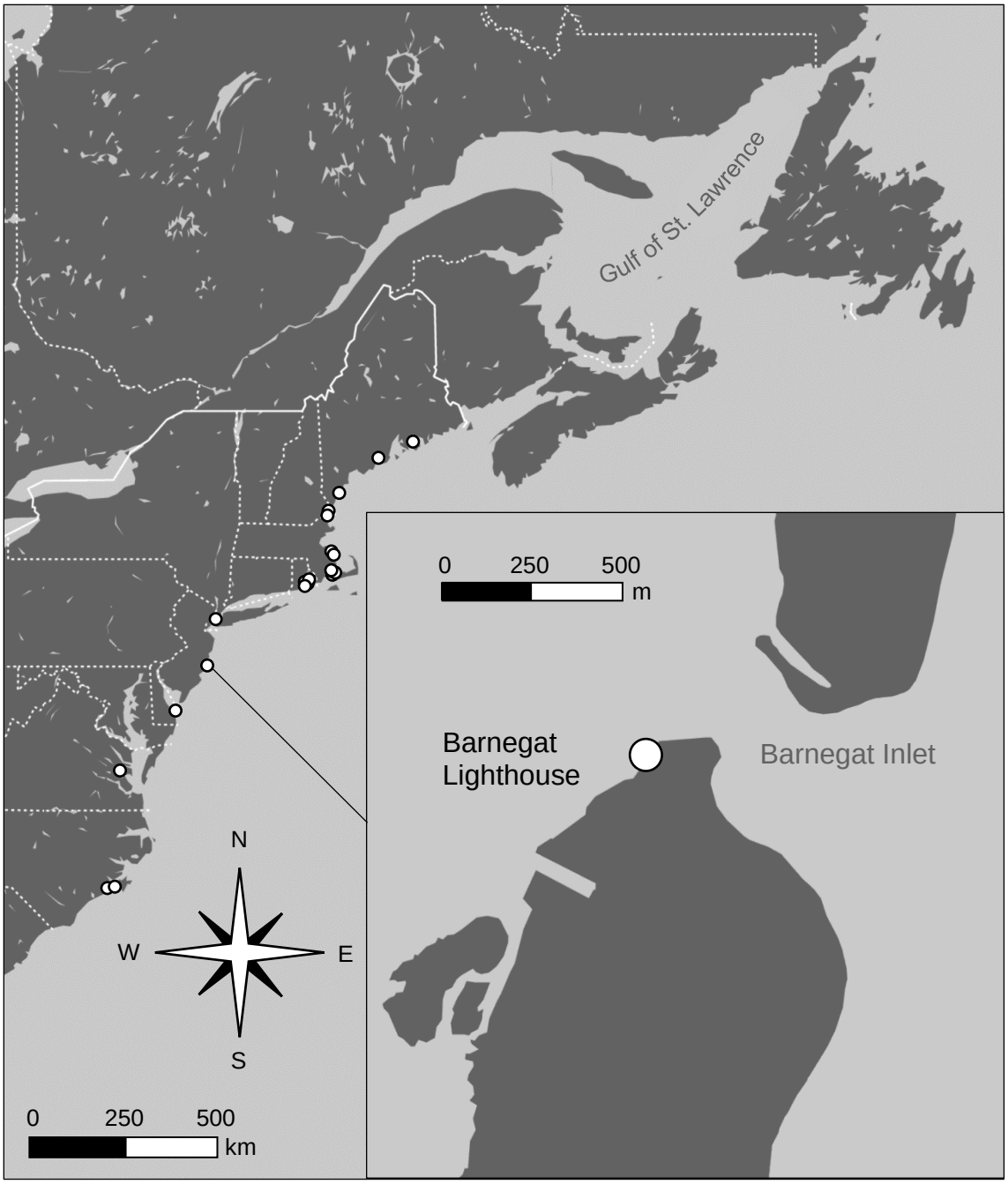

Figure 2.1. The distribution of marine tardigrades within the Cold Temperate Northwest Atlantic Marine Province. Dotted white lines represent state or province borders, solid white line an international border. White dots represent collection localities. Inset: The collection locality of the present study.

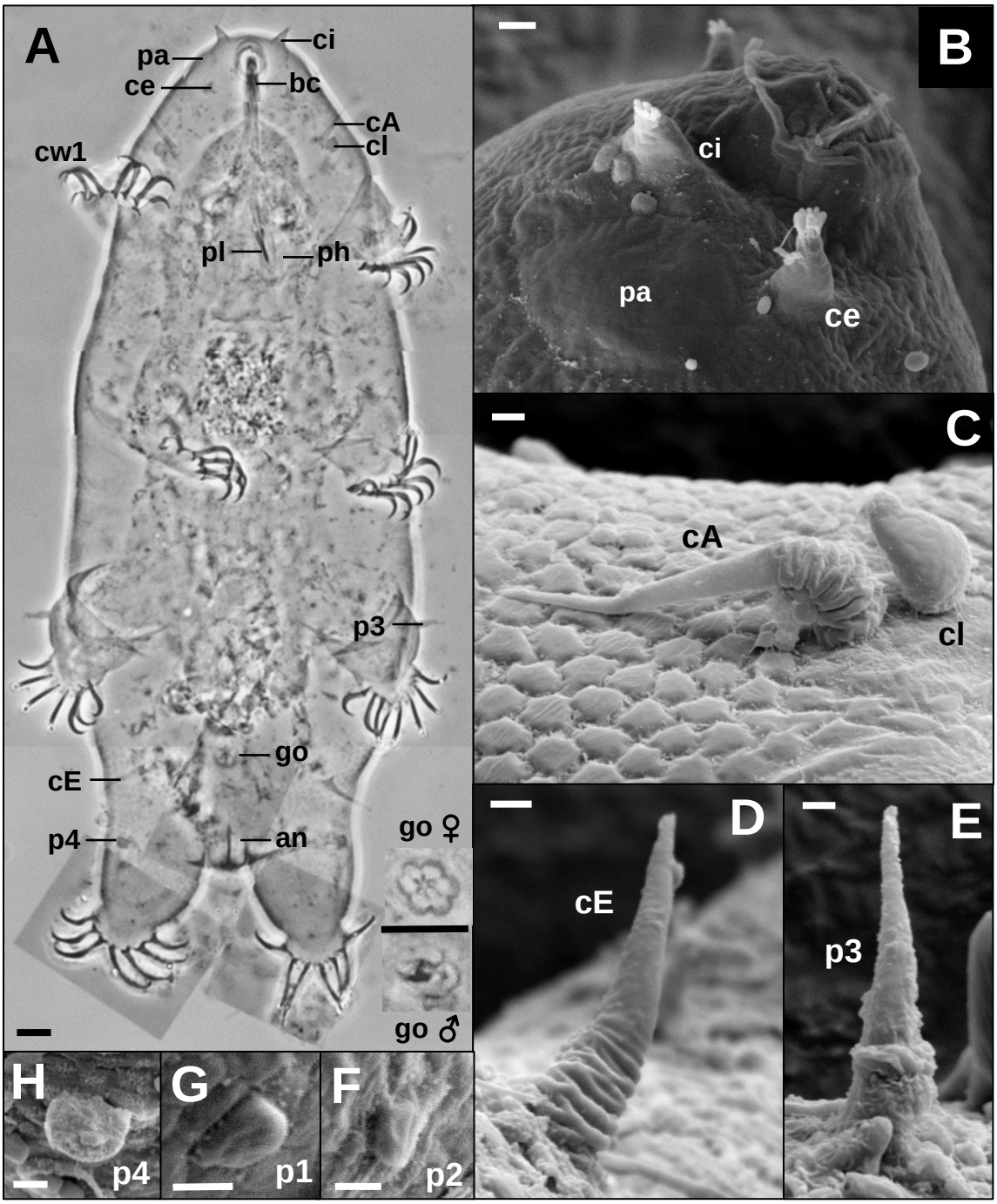

Figure 2.2. *Neoechiniscoides* cf. *pollocki*. (See Table 2.1 for abbreviations defined.) A: *in toto* dorsal view with anus (an), gonopore (go), and claws (cw) visible. Inset: female (♀) and male (♂) gonopores. B: lateral view of head, showing mouth, cephalic papilla, and ce and ci terminating in star-like arrays of projections. C: laterodorsal view of cuticle, cl, and cA, with its proximal cuticular annulations. D: cE. E–H, p3, p2, p1, and p4. A and inset, phase contrast (PC); B–H, SEM. Scale bars: A and inset, 10 µm; B–H, 1 µm.

Character (abbreviation)	Present Study					Hallas & Kristensen 1982	
	Adults			Juveniles		Holotype female	Paratype male
	Avg.	n	SD	Avg.	n		
body length (L)	243.10	26	32.43	117.23	2	281	216
body width (W)	90.31	7	18.87	45.11	1	119	76
buccal canal (bc)	44.11	14	3.55	24.01	2	48	43
bc external width	2.72	15	0.58	1.45	1	----------	----------
bc internal width	1.40	15	0.52	--------	--	----------	----------
placoids (pl)	13.39	17	1.92	6.37	2	19	11
pharyngeal bulb (ph)	20.67	9	2.50	--------	--	23	18
ph width	21.50	12	4.26	--------	--	----------	----------
stylet sheath	26.09	16	3.49	--------	--	----------	----------
leg I appendage (p1)	3.78	1	0	1.72	1	----------	----------
leg II appendage (p2)	2.82	2	0.21	2.40	1	present	present
leg III appendage (p3)	9.38	16	1.42	6.65	2	12	10
leg IV appendage (p4)	3.88	18	0.95	2.00	2	5.4	6.5
internal cirrus (ci)	7.32	24	0.94	4.62	1	7.6	5.4
external cirrus (ce)	4.86	25	1.00	3.50	1	7.6	5.4
cirrus A (cA)	13.85	28	2.32	6.38	2	17	15
cirrus E (cE)	14.85	20	3.19	8.74	2	18	16
clava (cl)	4.44	28	0.67	2.69	2	5.4	5.9
cephalic papilla (pa)	12.00	21	1.44	10.05	1	11	10
stylets	55.06	1	0	--------	--	----------	----------
number of claws leg I	7.81	21	0.40	5.25	4	8	8
number of claws leg II	7.90	21	0.30	4.50	4	8	8
number of claws leg III	7.71	21	0.46	5.33	3	8	8
number of claws leg IV	7.00	21	0	4	4	7	7

Table 2.1. Morphometrics of selected characters of *Neoechiniscoides* cf. *pollocki* (present study) and *N. pollocki* Hallas & Kristensen, 1982. All measurements are lengths in µm unless otherwise noted. Specimens from present study photographed with SEM are excluded. Strikethroughs (---) indicate no available data.

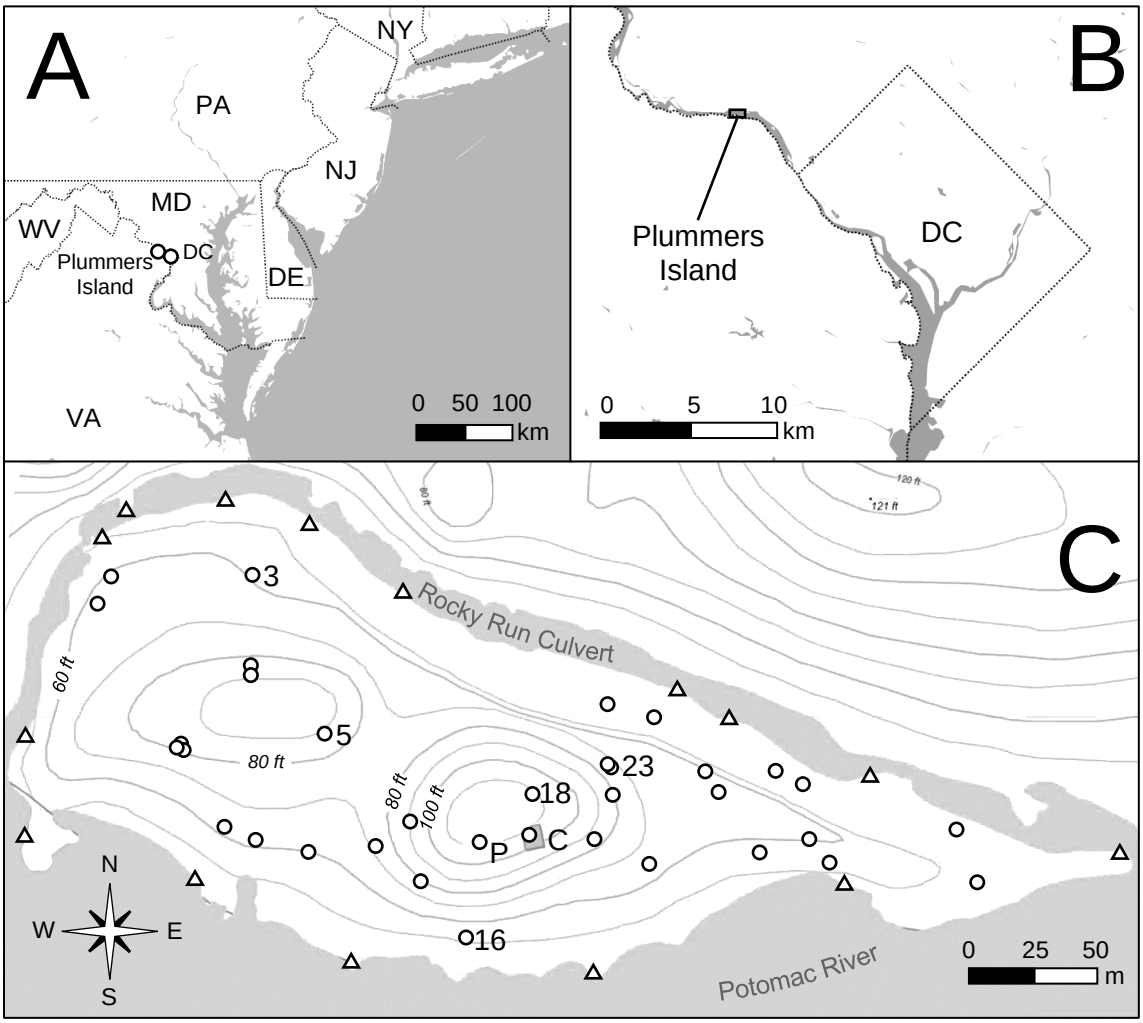

Figure 3.1. The location of Plummers Island within (A) the Mid-Atlantic region, (B) Maryland, and (C) the Potomac River. **C** is modified from Drake & Froelich (1997). Terrestrial sampling sites are represented by circles (○) and aquatic sites by triangles (Δ) in **C**. Enumerated circles indicate those sites whose samples have been completely processed. Note that isoclines are measured in feet. **A**: DC = District of Columbia, DE = Delaware, MD = Maryland, NJ = New Jersey, NY = New York, PA = Pennsylvania, VA = Virginia, WV = West Virginia. **C**: P = pilot study, C = cabin.

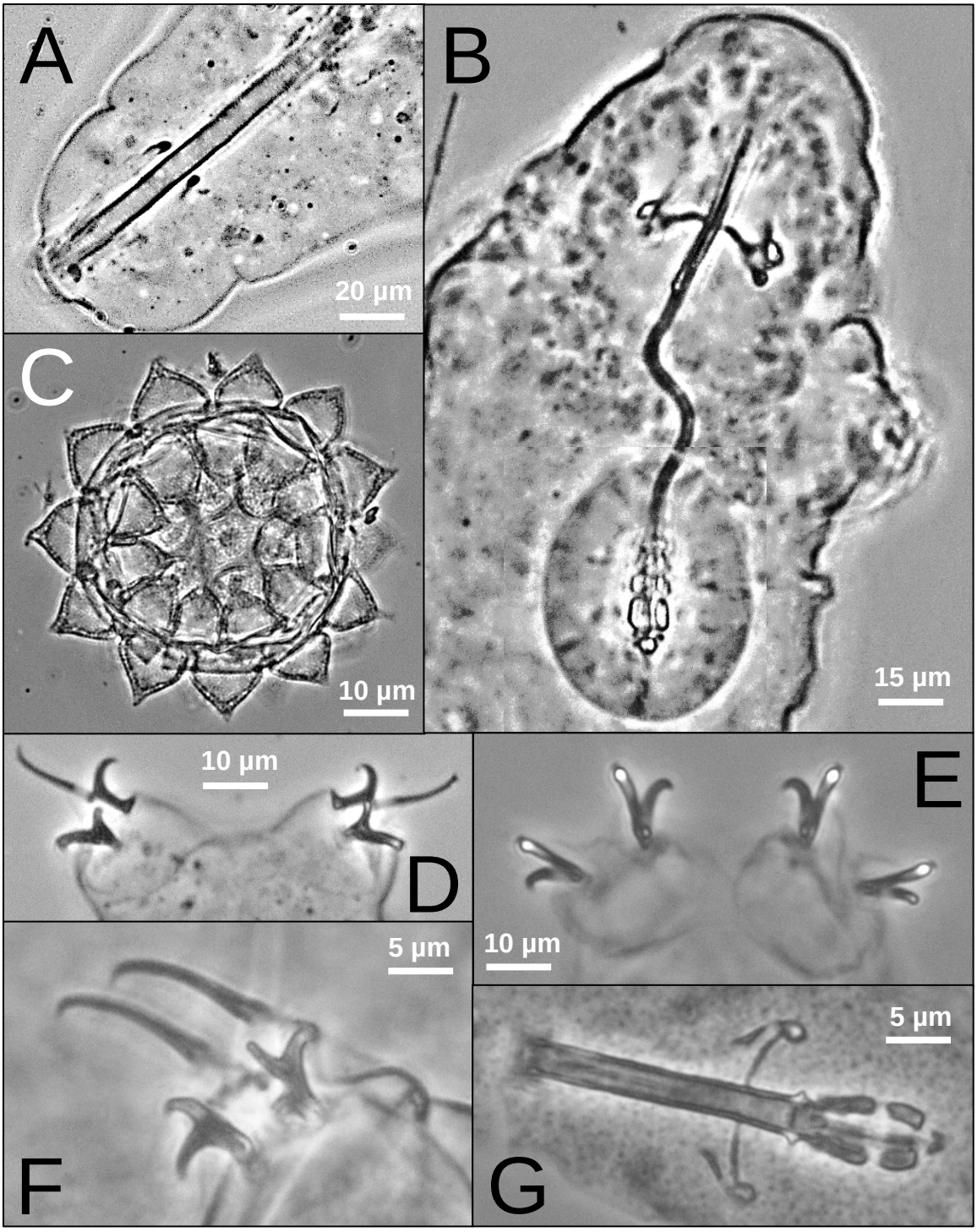

Figure 3.2. Some specimens recovered from Plummers Island. A, buccal tube of *Astatumen*; B, bucco-pharyngeal apparatus of *Diphascon*; C, Macrobiotid egg; D, Claws IV of *Astatumen*; E, Macrobiotid claws IV; F, Claws I of *Milnesium*; and G, bucco-pharyngeal apparatus of Macrobiotidae.

Tardigrades	Site					Totals	Pilot
	3	5	16	18	23		
Apotardigrada							
Milnesiidae	2			1	2	**5**	
Milnesium	(2)					**(2)**	
Eutardigrada							
Hypsibiidae		1				**1**	
Astatumen		(1)				**(1)**	
Diphascon							1*
Macrobiotidae	11	4	34	25	22	**96**	
Macrobiotus	(5)					**(5)**	
Minibiotus		(3)				**(3)**	
eggs			10*	18*		**32***	
unknown				1		**1**	
Totals	**13**	**5**	**34**	**27**	**24**	**103**	**N/A**

Table 3.1. Occurrence and abundance of tardigrades across five study sites and one pilot site. Genus abundances are included in family abundances. *Egg totals and the single occurrence of *Diphascon* are not included in total abundances.

	Site 3	Site 5	Site 16	Site 18	Site 23	Pilot
Moss	Thuidiaceae	*Plagiobryum*	*Hedwigia*	Thuidiaceae	*Dicranum*	Thuidiaceae
Forest type	Floodplain Terrace	Central Appalachian Rich Red Oak	Central Appalachian Rich Red Oak	Potomac R. Bedrock Terrace Hardpan	Potomac R. Bedrock Terrace Hardpan	Potomac R. Bedrock Terrace Hardpan
Elev.	59	90	50	122	77	121
Abun.	13	5	34	27	24	N/A
Taxa	*Milnesium Macrobiotus*	*Astatumen Minibiotus*	Macrobiotidae	Milnesiidae Macrobiotidae Unknown	Milnesiidae Macrobiotidae	*Diphascon*
Eggs	0	0	10	18	0	N/A

Table 3.2. Characterization of sites analyzed in the present study and pilot study. All mosses were collected on metadiamictite (Fleming 2015). Forest types were characterized according to Simmons *et al.* (2016). Elevations are in feet and were compared to Drake and Froelich (1997). Mosses were tentatively identified according to Sargent & Lucas (2012).

APPENDIX

Adult Character Ratios		
	Present Study	**Hallas & Kristensen 1982**
L/W	2.69	2.53
bc/ph	2.13	1.99
ph/pl	1.54	1.55
bc/pl	3.29	3.01
p2/p3	0.30	----------
p3/p4	2.42	1.96
ci/ce	1.51	1.14
ce/cE	0.33	0.34
cE/cA	1.07	1.12
cA/cl	3.12	3.28
cl/pa	0.37	0.45

Adult character ratios of *Neoechiniscoides* cf. *pollocki* (present study) and *N. pollocki* Hallas & Kristensen, 1982. (See Table 2.1 for abbreviations defined.) Strikethroughs (---) indicate no available data.

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Stintzing H. (1929b) Einrichtung zum automatischen Nachweis, Messung und Zählung von Einzelteilchen beliebiger Art, Form und Grösse nach Patent 485155, insbesondere zur Erzeugung sehr enger Strahlenbündel. German patent No. 485156.

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Steven Schulze, M.S.

Acting Manager, Rutgers Pinelands Field Station
501 Four Mile Road, New Lisbon, NJ 08064
609-304-2606 • steveschulze@gmail.com

EDUCATION

Rutgers University, Camden, NJ 2020
M.S. Biology

The College of New Jersey, Ewing, NJ 2009
B.S. Biology

TEACHING (Rutgers University unless otherwise noted)

Rutgers Pinelands Field Station Acting Manager August 2019–June 2020

Graduate Assistant August 2019–June 2020

Teaching Assistant Fall 2018–Spring 2019
Ecology of Soil Organisms Laboratory

Guest Lecturer

National Hispanic Environmental Council	December 8, 2019
Invertebrate Zoology	November 5, 2019
Microbiology	April 12, 2019
General Ecology	September 18, 2018
Principles and Practices in Quantitative Biology	October 24, 2017
Advanced Soil Ecology	October 3, 2017
Invertebrate Zoology (University of Chicago)	November 2014

Students Mentored

Josh Bushta (unaffiliated)	Kela McEntee (Johns Hopkins University)
Allison Fink (Brookdale Community College)	Elise McKean (Rutgers University)
Frank Kelly (Rutgers University)	Evan Rosenheim (Rutgers University)
Tionia Logan (Rutgers University)	Becca VanArnam (Univeristy of Miami)
Stephen Gamba (Pinelands Regional High School)	

PUBLICATIONS

Lowman M. D., Cotton H., Azizah S., **Schulze S. L.**, Miller W. R. Tardigrades of Malaysia: *Genus species* (Heterotardigrada, Echiniscoidea, Echiniscidae) a new species from Penang, Malaysia. In preparation.

Miller W. R., McCowan P. J., Perry E. S., **Schulze S. L.**, Shannon R. K., Henry C. B. S. (2020) Tardigrades of North America: New records of occurrence for three

species of green tardigrades (Heterotardigrada, Echiniscoidea, Echiniscidae, *Viridiscus*). *Transactions of the Kansas Academy of Science* 123:235–241.

Schulze S. The Inimitable Tardigrade. *On Track... New Jersey Association of Wildlife Rehabilitators Newsletter* Submitted for publication.

POSTER PRESENTATIONS (presenter underlined)

Steven Schulze and Emma Perry. "The First Record of Marine Tardigrades from New Jersey." 54th European Marine Biology Symposium, University College Dublin, Dublin, Ireland. August 25–29, 2019.

Steven Schulze and Emma Perry. "The First Record of Marine Tardigrades from New Jersey." Graduate Research and Creative Works Symposium, Rutgers University, Camden, NJ. April 16, 2019.

Steven Schulze and Emma Perry. "The First Record of Marine Tardigrades from New Jersey." Mid-Atlantic Ecological Society of America Meeting, Bowie State University, Bowie, Maryland. April 5–7, 2019.

Steven Schulze and Emma Perry. "The First Record of Marine Tardigrades from New Jersey." 14th International Symposium on Tardigrada, University of Copenhagen, Copenhagen, Denmark. July 30–August 3, 2018.

ORAL PRESENTATIONS (presenter underlined; *COVID-19 Cancellation)

Steven Schulze. "Tardigrades." The Academy of Natural Sciences of Drexel University Seminar Series, Philadelphia, PA. June 26, 2020. Invited speaker.

Steven Schulze. "A Preliminary Inventory of Tardigrades on Plummers Island, Maryland." Northeast Natural History Conference, Stamford, CT. April 18, 2020. Invited speaker.*****

Steven Schulze. "The Phylum Tardigrada." Rutgers University Biology Department Seminar Series, Camden, NJ. November 21, 2017.

Juan Larraín. "The Liverwort Tree of Life Extends its Branches: Working Towards a Model Systematic Treatment of a Hyper-diverse Lineage." The Field Museum of Natural History A. Watson Armour Seminar Series, Chicago, IL. December 10, 2014. (Acknowledgements: **Steve Schulze**)

HONORS & AWARDS

Delaware Valley Paleontological Society Paul Bond Scholarship ($1,500)	2020
Ralph Good Award ($550)	2020
Dean's Graduate Student Research Grant ($500)	2019

Dean's Graduate Student Travel Grant ($500)	2019
Dean's Graduate Student Travel Grant ($500)	2018
Washington Biologists' Field Club Research Award ($2,190)	2018
Edward J. Bloustein Distinguished Scholar Award (full academic scholarship)	2001

EMPLOYMENT

Rutgers University Pinelands Field Station, New Lisbon, NJ	Fall 2018–present
Willard Brothers Woodcutters, Trenton, NJ	May 2015–June 2019
American Science & Surplus, Chicago, IL	June 2014–April 2015
The Field Museum of Natural History, Chicago, IL	October 2013–December 2014
Chicago Botanic Garden, Glencoe, IL	May–August 2011
Earthwatch Tropic Ventures Sustainable Forestry Initiative, Patillas, PR	Mar.–June 2010
Integrity Propagation LLC, Chatsworth, NJ	April–May 2009
NJ Dept. of Agriculture, Division of Plant Industry, Trenton, NJ	Summer 2007–2009
Rutgers University Pinelands Field Station, New Lisbon, NJ	Summer 2005–2006

PUBLIC OUTREACH/CITIZENRY INVOLVEMENT (*COVID-19 Cancellation)

Panelist, vBIO (Virtual Biology Day) May 2, 2020
Rutgers University, Camden, NJ

Scientific Demonstrator

LEAP Academy Maker's Day, Camden, NJ*	March 21, 2020
Grounds for Sculpture docents, New Lisbon, NJ	December 18, 2019
Rutgers University Earth Day Celebration, Camden, NJ	April 16, 2019
Budlong Elementary School, Chicago, IL	April 21, 2015
American Science & Surplus, Chicago, IL	February 27, 2015
The Field Museum of Chicago, Chicago, IL	March 6–7, 2014

Facilitator, Carbon Neutrality and Climate Action Town Hall February 18, 2020
Rutgers University, Camden, NJ

Woodturning Instructor August 2015–November 2016
Private Homes, Allentown, Ewing, & Princeton, NJ