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ON WATER BEARS

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ABSTRACT

Tardigrades, or "water bears" are microscopic invertebrates that require water in their environment and are found in freshwater, marine and terrestrial habitats. The morphology and phylogeny of this "little known phylum" is described as are ways the naturalist might collect water bears. Examples of species distributions in different locations in the southeastern USA are given.

Key Words: tardigrade, taxonomy, phylogeny, ecology, collecting

RESUMEN

Los tardigrados, u "osos de agua" son invertebrados microscópicos que requieren agua en su ambiente y son encontrados en ambientes marinos de agua fresca y ambientes terrestres. Se describe la morfologia y la filogenia de este "filo poco conocido" y también las maneras que los naturalistas pueden recolectar los osos de agua. Se proveen ejemplares de la distribución de especies en lugares diferentes en el sureste de los Estados Unidos.

WATER BEARS: AT LAST, IN A PERSONAL REAL-LIFE INTRODUCTION

A line drawing in an already-classic entomology text book (Fig. 0) and a comment from a professor that "you are unlikely to ever see one" was my introduction to the idea of "tardigrades". Forty years later when visiting Jacksonville State University in northeastern Alabama a sign on a lab door said "Beware of the Bears", and was my invitation to actually see a living water bear. Frank Romano, the bear-room caretaker and proprietor—and now the Head of the Biology Department—said that bears were as near as the large tree on the lawn and that he could show me a living specimen in ten minutes!—not an idle boast, for in minutes and out of accumulated muck from the crotch of the tree he produced actual living water bears! For those who have never heard of tardigrades, nor the legend of their enigmatic and "by default" position in the Animal Kingdom, an "all about them and how to find them" encyclopedia entry may not mean much, but for some of us it is an invitation to a twilight zone where mythology becomes reality. (JEL)

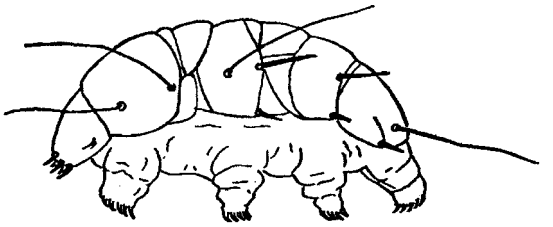

Fig. 0. The tardigrade line drawing in Herbert Ross's 1948 entomology text (p. 47), with the attribution "After U.S.D.A., B.E.P.A".

Tardigrades are microscopic invertebrates that belong to the Phylum Tardigrada (proposed by Ramazzotti in 1962). Active tardigrades require water in their environments and can be found in three main habitats; marine water, fresh water and terrestrial habitats (Kinchin 1994; Nelson 1991; Ramazzotti & Maucci, 1983). First described by Goeze in 1773. Commonly recognized as "Water Bears" (Wassar Bär) by observers, tardigrades are best classified as one of the "lesser-known phyla" (Nelson 1991). Tardigrades, the current name in use since the 18th century (adopted by Spallanzini in 1776) is also a descriptive name based on the animals lumbering gait (*tardi*-slow, *grade*-walker).

Tardigrades are generally considered cosmopolitan in their distribution and are commonly found in a variety of marine, freshwater, and terrestrial habitats: sand, algae, rooted aquatic vegetation, soil, leaf litter, mosses, lichens, and liverworts. These bilaterally symmetrical micrometazoans are generally flattened on their ventral side and convex on their dorsal side and average 250-500 µm in length as adults (see Dewel et al. 1993 for detailed morphology). Their body is composed of 5 somewhat indistinct body segments including a cephalic segment and four trunk segments each supporting a pair of legs that terminate in either claws and/or digits. The first 3 pairs of legs are directed ventrolaterally and are the primary means of locomotion, while the 4th pair is directed posteriorly and is used primarily for grasping the substrate (Fig. 1). Tardigrades feed by piercing the

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cells of bacteria, algae, plants (mosses, liverworts, and lichens) or animals (protozoans, rotifers, nematodes, larvae, and other small invertebrates) with hardened stylets and sucking out their contents using their muscular pharynx (Fig. 2). In some cases, the whole organism is ingested. Detritus may also be a major nutrient source of some species. Regardless of their specific habitat (marine, freshwater, or terrestrial), all tardigrades are aquatic, since they require a film of water surrounding the body to be active. Some, those that are limno-terrestrial, can undergo cryptobiosis when environmental conditions become unfavorable (e.g., loss of the film of water) creating an environmentally resistant state. Thus, this latent state has a significant impact on the ecological role of limnoterrestrial tardigrades.

Despite their overall abundance and presumed cosmopolitan distribution (McInnes 1994), tardigrades have been relatively neglected by invertebrate zoologists. Because of the difficulty in collecting and culturing these organisms and their apparent lack of economic importance to humans, our knowledge of tardigrades has remained in a relatively nascent state since their discovery over 200 years ago (Nelson 1991).

Tardigrade Taxonomy

Marcus (1929) established the major taxa within the phylum Tardigrada splitting the group in two, forming the classes: Heterotardigrada (armoured tardigrades) and Eutardigrada (naked tardigrades). "Naked" and "armoured" refer to the cuticular dorsal plates found in terrestrial heterotardigrades, that are absent in eutardigrades. Morphological and anatomical differences are the only characters used to identify organisms to species. Within the heterotardigrades (armoured) the main features are cephalic appendages, cuticular extensions, claws and the pattern of dorsal cuticular plates (Fig. 3). Within the eutardigrade (naked) the more important morphological characteristics are the claws, the buccopharyngeal apparatus; and the cuticle structure (smooth, granulated or bearing tubercles) (Fig. 4).

Tardigrade taxonomy stems from a number of papers but primarily from Thulin (1928) who revised the systematics of the taxon, Marcus (1929) who wrote a chapter on tardigrades in "Classes and orders in the animal realm" (Vol. 5) and a book entitled "The animal realm" (1936), Ramazzotti (1962, 1972) who published monographs on the phylum tardigrada, and Ramazzotti and Maucci (1983) who collaborated to produce the monograph entitled "The phylum tardigrada" (English translation by Beasley 1993).

Marcus named the classes Eutardigrada (meaning 'true' tardigrades) and Heterotardigrada (meaning 'other' tardigrades). The genus *Macrobiotus* was described in 1834 (a eutardigrade) and the genus *Echiniscus* was described in 1840 (a heterotardigrade). A third class, Mesotardigrada (meso = middle), was established by Rahm 1937 for *Thermozodium esakii* discovered in a hot spring near Nagasaki, Japan. Neither type material nor type locality have survived and no other mesotardigrade have been discovered – a consensus from the last symposium on tardigrades (Eighth International Symposium on Tardigrada 2000) was that this should be removed from the classification.

Tardigrade Phylogeny

Tardigrades have been closely aligned with arthropods and were described as primitive arthropods by Plate 1889 (from Kinchin 1994). The morphological characters that align them with arthropods are: vermiform animals with a cuticle, lobopodia (poorly articulated limbs) that terminate in claws, terminal mouths, caudal end (segment) terminating into the last pair of legs.

Ecology of Tardigrades

Active tardigrades require water in their environment and as noted can be found in three main habitats: marine, freshwater, and terrestrial habitats (Ramazzotti & Maucci 1983; Nelson 1991). Bryophytes, which hold water within the interstices of their cushions (mats), and leaf axils, provide ideal sites for terrestrial tardigrades. Species living in these wet terrestrial habitats are classified as limnoterrestrial, a useful term to distinguish the moss inhabiting species from the marine and freshwater species (Kinchin 1994). Ramazzotti and Maucci (1983) identified three common conditions that make the terrestrial habitat, such as mosses, suitable for tardigrades: (1) a structure that allows sufficient oxygen diffusion, (2) the ability to undergo alternate periods of wetting and drying, mainly through solar radiation and wind, and (3) one that contains sufficient food. In reference to moist-dry mosses, Kinchin (1994) stated that they share a drought tolerant pattern of adaptation to dehydration with animal groups, including tardigrades, named poikilohydry. This adaptation is advantageous to both the bryophyte and the bryofauna. Both the moss and the tardigrade can survive adverse conditions in a dormant state called a tun (Fig. 5) (anhydrobiosis for tardigrades).

Although Bertolani (1983) found that some tardigrade species were related to specific coastal dune mosses, other authors did not find enough evidence to support a direct correlation between particular moss species and particular tardigrade species (Nelson 1975; Kathman & Cross 1991). Hunter (1977) found no relationship between epiphyte species and species of tardigrades nor did Kathman and Cross (1991).

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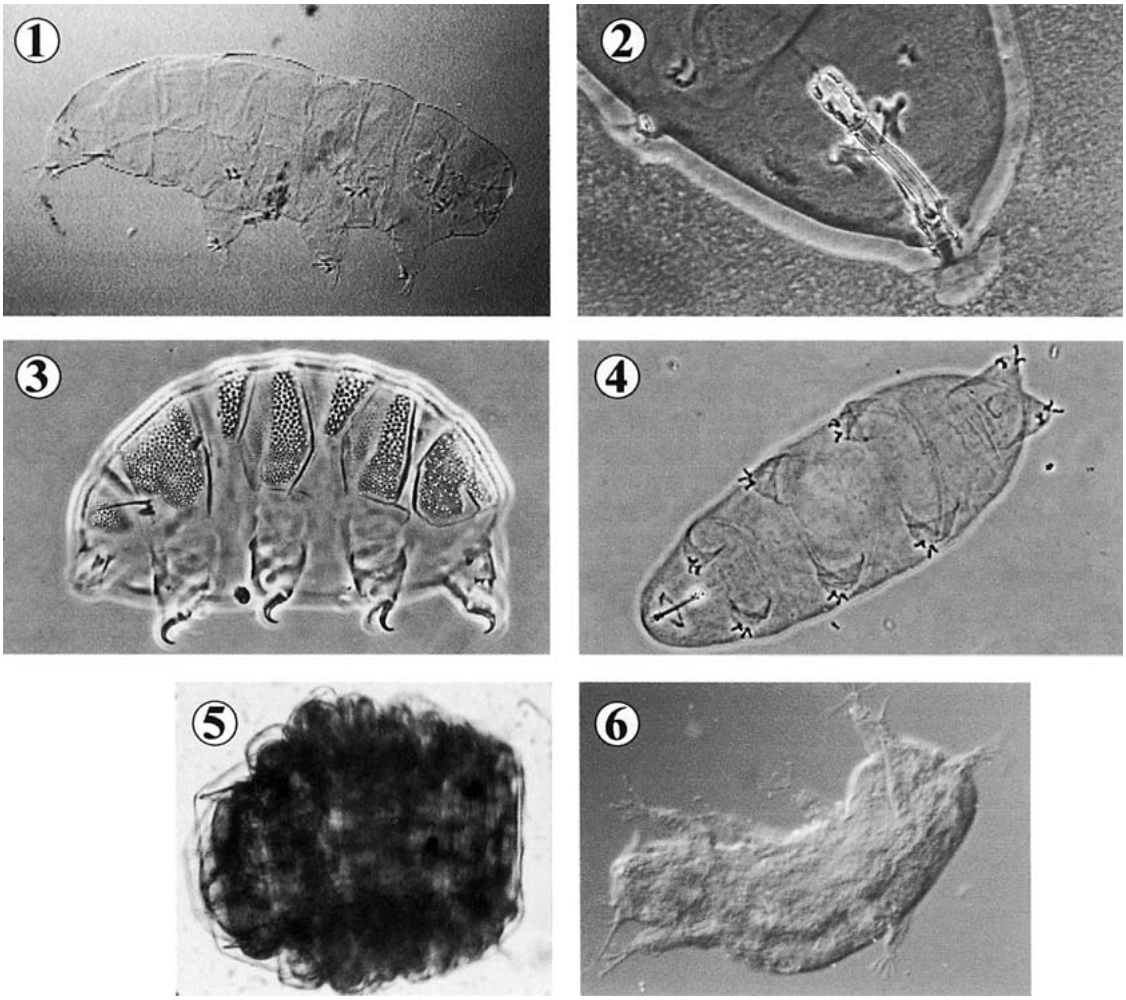

Figs. 1-6. 1) Whole mount of a Eutardigrade showing the indistinct segmentation and 4 pairs of legs. 2) Buccopharyngeal apparatus of a Eutardigrade showing the muscular pharynx, pharyngeal tube, stylet supports, and mouth. 3) Whole mount of a limnoterrestrial Heterotardigrade (*Echiniscus* sp.). 4) Whole mount of a limnoterrestrial Eutardigrade (*Hypsibius* sp.). 5) Tun formation in a Eutardigrade (*Milnesium tardigradum*). 6) Whole mount of a marine Heterotardigrade (*Batillipes* sp.).

In an effort to better understand when and where which tardigrades are abundant or rare, three ecological surveys (2 terrestrial and 1 marine) were conducted in Alabama, one on Dugger Mountain (Nichols et al. 2001), Alabama's second highest peak, one along Choccolocco Creek (Romano et al. 2001) within the riparian zone, and one on Dauphin Island. Five trees (*Quercus alba*) with cryptogams, three on north-facing slopes and two on south-facing slopes, were sampled seasonally at three sites (headwaters, midwaters, mouthwaters) along an unnamed tributary of the South Fork of Terrapin Creek. Trees were chosen based on their location outside the riparian zone at the peak, midpoint, or base of the north-facing and south-facing slopes along the creek. Seasonal and altitudinal variations in the distribution of the populations on the north- and south-facing slopes were determined. Significant seasonal and altitudinal differences were found in tardigrade abundance from samples collected at specific sites and between north- and south-facing slopes. Pooled data showed no differences in the overall abundance or number of species at each altitude. However, significant seasonal differences in both abundance and number of species were seen in pooled samples. Six sites along Choccolocco Creek were selected and 3 trees with mosses within each were surveyed for an 18 month period. No significant difference was found between tardigrade occurrence (total number of individuals) and season, moss genera, or tree species. However, there was a significant relationship be-

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tween the number of tardigrades and site, indicating the need for additional replicate samples. A marine meiofauna survey of subtidal regions of Dauphin Island, AL in the northeast region of the Gulf of Mexico was initiated 1999. Samples were taken at mile intervals from the Mobile Bay side (north) and the Gulf of Mexico side (south). A sample consisted of 500 cc's of sand collected from the subtidal zone. Meiofauna were counted and tardigrades extracted from samples. A total of 20,846 meiofaunal organisms have been observed from 11 samples. Nematodes account for 69.1%, harpactocopepods account for 13.5%, and tardigrades account for 11.1% of the collection. Miscellaneous organisms make up the remainder of the collections (5.8%) containing organisms such as foraminiferans, bivalves, cnidarians, polycheates, and kinorhynchs. The genus *Batillipes* dominated the tardigrade collection (Fig. 6).

Tardigrade Collecting

The best source of tardigrades is within moss growing on the bark of live trees or leaf litter. Moss on rocks is okay but contains a lot of dirt, making the animals even more difficult to find. Moss on soil is even worse, although you will find tardigrades in about 50% of the samples. Moss on rotten logs has very few, if any tardigrades, and you might skip that habitat. Lichens on trees and rocks are sometimes fruitful.

Following the procedure of Nelson (1975) soak the moss sample in a stoppered funnel in tap water (a bucket for leaf litter) for at least 3 hours (3-24 hours). You can leave the samples overnight in water. Realize that you are trying to induce anoxybiosis so that the tardigrades release their hold of the moss plants and are more easily removed. Remove the moss and squeeze the remaining water out into a clean beaker or jar. Some samples require vigorous shaking and squeezing to remove a sufficient quantity of tardigrades. Let the water and collected materials in the jar settle and then decant the top water. Pour the bottom layer of water and debris into a collecting jar. If too much debris, especially dirt, has been collected, the material may be sieved through a nested series. Tardigrades, and eggs, will be trapped on a #325 (45 µm) screen. Be sure to collect material from 2-3 different sized sieves, since larger tardigrades may be trapped by these. Each piece of leaf litter should be rinsed and the water in the bucket poured into a nested sieve series and collected as above.

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