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# Current Status of the Tardigrada: Evolution and Ecology[1]

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**Synopsis. The Tardigrada are bilaterally symmetrical micrometazoans with four pairs of lobopod legs terminating in claws or sucking disks. They occupy a diversity of niches in marine, freshwater, and terrestrial environments throughout the world. Some have a cosmopolitan distribution, while others are endemic. About 900 species have been described thus far, but many more species are expected as additional habitats are investigated. Most are less than 1 mm in body length and are opaque or translucent, exhibiting colors such as brown, green, orange, yellow, red, or pink in the cuticle and/or gut. Marine species are more variable in body shape and overall appearance and generally exhibit low population density with high species diversity. Reproductive modes include sexual reproduction and parthenogenesis, but much remains to be known about development. Tardigrades have a hemocoel-type of fluid-filled body cavity, a complete digestive tract, and a lobed dorsal brain with a ventral nerve cord with fused ganglia. Recent molecular analyses and additional morphological studies of the nervous system have confirmed the phylogenetic position of tardigrades as a sister group of the arthropods. The ability of tardigrades to undergo cryptobiosis has long intrigued scientists. Although tardigrades are active only when surrounded by a film of water, they can enter latent states in response to desiccation (anhydrobiosis), temperature (cryobiosis), low oxygen (anoxybiosis), and salinity changes (osmobiosis). Cryptobiotic states aid in dispersal.**

## Introduction

The phylum Tardigrada is often classified as one of the ''lesser-known'' groups of protostomes. Although much has been learned about these organisms, as evidenced by publications of international symposia (Higgins, 1975; Węglarska, 1979; Nelson, 1982*a*; Bertolani, 1987*a*; McInnes and Norman, 1996; Greven, 1999; Kristensen, 2001), the tardigrades present an exceptional opportunity for teaching and research, especially in the areas of development, evolution, and ecology. These micrometazoans may play an important role in the elucidation of metazoan phylogeny, particularly with respect to the evolution of the arthropods. Tardigrades have a ubiquitous distribution, being found in a diversity of niches in terrestrial, freshwater, and marine environments throughout the world, ranging from the abyss in the deep sea to the highest mountains (Ramazzotti and Maucci, 1983; Kinchin, 1994).

### *General morphology*

Small but complex organisms, tardigrades have a bilaterally symmetrical body with four pairs of lobopodous legs usually terminating in claws and/or sucking disks. Mature adults average 250–500 μm, with body lengths ranging from 50 μm in juveniles to over 1,200 μm in adults (although adults of some marine species may be less than 100 μm). They have a complete digestive system and a hemocoel-type of fluid-filled body cavity that functions in circulation and respiration. Reproductive modes include sexual reproduction and parthenogenesis (Bertolani, 1987*b*; Bertolani and Rebecchi, 1999; Rebecchi and Bertolani, 1988; Rebecchi and Nelson, 1998; Rebecchi *et al.*, 2000), but much remains to be known about development (Nelson, 1982*b*; Eibye-Jacobsen, 1996/97, 1997). The nervous system consists of a lobed dorsal brain and ventral nerve cord with fused paired ganglia (Dewel and Dewel, 1996; Dewel *et al.*, 1999; Wiederhöft and Greven, 1996, 1999).

### *Major taxa*

Based on morphological characters, the phylum is divided into two major classes: Heterotardigrada and Eutardigrada. A third class, Mesotardigrada, based on a single species, *Thermozodium esakii* Rahm, is of dubious status. (The type specimens of *T. esakii* no longer exist and the type locality, a hot spring in Japan, was destroyed in an earthquake. Subsequent searches for the species have been fruitless.)

The class Heterotardigrada is comprised of two orders: Arthrotardigrada and Echiniscoidea. The arthrotardigrades are marine species (with one exception) and usually have a median cirrus on the head and telescopic legs with 4–6 digits (toes) that have complex claws and/or sucking disks; the echiniscoids are primarily terrestrial species with an armored cuticle (having thickened plates), but there are some marine and freshwater unarmored species with telescopic legs bearing up to 13 claws. The class Eutardigrada includes the unarmored orders Apochela (terrestrial) and Parachela (primarily terrestrial and freshwater, with a few marine species); their legs terminate in claws without digits (Schuster *et al.*, 1980). (See Table 1 for classification.)

### *Taxonomic characters*

The two main classes are separated on the basis of taxonomic characters of the claws and/or sucking disks, cuticle, cephalic appendages, buccal apparatus,

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TABLE 1. *Subdivision of the Phylum Tardigrada with Habitat Classifications (Nelson, 2001).*

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|---|---|
| I. Class Heterotardigrada | |
| Order Arthrotardigrada | Marine (many families and genera), except *Styraconyx hallasi* (freshwater) |
| Order Echiniscoidea | Marine, freshwater, terrestrial |
| Family Echiniscoididae | Marine; genera *Echiniscoides* and *Anisonyches* |
| Family Oreellidae | Terrestrial; genus *Oreella* |
| Family Carphaniidae | Freshwater; genus *Carphania* |
| Family Echiniscidae | Terrestrial, except for a few species of *Echiniscus, Hypechiniscus*, and *Pseudechiniscus* occasionally in freshwater |
| II. Class Mesotardigrada (dubious) | |
| Order Thermozodia | |
| Family Thermozodiidae | Hot spring; genus *Thermozodium* |
| III. Class Eutardigrada | |
| Order Parachela | |
| Family Macrobiotidae | Terrestrial and freshwater; genera *Adorybiotus, Calcarobiotus, Dactylobiotus, Macrobiotus, Macroversum, Minibiotus, Murrayon, Pseudodiphascon, Pseudohexapodibius, Richtersius, Xerobiotus* |
| Family Calohypsibiidae | Terrestrial; genera *Calohypsibius, Haplohexapodibius, Haplomacrobiotus, Hexapodibius, Parhexapodibius* |
| Family Eohypsibiidae | Terrestrial and freshwater; genera *Eohypsibius, Amphibolus* |
| Family Microhypsibiidae | Terrestrial; genera *Microhypsibius, Fractonotus* |
| Family Hypsibiidae | Marine: genus *Halobiotus, Ramajendas* (1 sp.), and a few *Isohypsibius*. Terrestrial and freshwater: genera *Acutuncus, Astatumen, Diphascon, Doryphoribius, Eremobiotus, Hebesuncus, Hypsibius, Isohypsibius, Itaquascon, Fujiscon, Mesocrista, Mixibius, Paradiphascon, Parascon, Platicrista, Pseudobiotus, Ramajendas, Ramazzottius, Thulinia* |
| Family Necopinatidae | Terrestrial; genus *Necopinatum* |
| *Incertae sedis* | Terrestrial; genus *Apodibius* |
| Order Apochela | |
| Family Milnesiidae | Terrestrial; *Milnesium, Limmenius, Milnesioides* |

and reproductive structures. The class Heterotardigrada is characterized by the presence of cephalic, trunk and leg appendages; gonopore separated from anus; Malpighian tubules lacking; placoids consisting of three $CaCO_3$ elements or three delicate, bar-shaped cuticular structures (Ramazzotti and Maucci, 1983; Kristensen, 1987). In contrast, the class Eutardigrada has cephalic papillae only in the Apochela (non-homologous to the cephalic appendages in heterotardigrades); gonopore and anus combined to form a cloaca; Malpighian tubules present; placoids consisting of three sets of thickened cuticular structures (Ramazzotti and Maucci, 1983; Schuster *et al.*, 1980; Guidi and Rebecchi, 1996; and others). For a discussion of tardigrade morphology, taxonomic characters, and ecology (primarily terrestrial and freshwater), see Dewel *et al.*, 1993; Dewel and Dewel, 1997; Kinchin, 1994; Nelson, 1982*b*, 2001; Nelson and Higgins, 1990; Nelson and Marley, 2000; Ramazzotti and Maucci, 1983.

## EVOLUTION

Arthropod phylogeny has been the subject of considerable debate for years. The Onychophora and Tardigrada have been discussed as enigmatic groups that may shed light on early arthropod radiation (Giribet *et al.*, 1996). Doyère (1840) first assigned the name Tardigrada to the taxon, and Ramazzotti (1962) recognized the tardigrades as a phylum in his first monograph. In the past, tardigrades have been associated with various taxonomic groups including arthropods and various aschelminths (reviewed in Ramazzotti and Maucci, 1983). Recent molecular studies with 18S rRNA, however, have indicated that they are a monophyletic sister group of the arthropods (Garey *et al.*, 1996, 1999; Giribet *et al.*, 1996), although one study was inconclusive (Moon and Kim, 1996). Most morphological studies (*e.g.*, Dewel and Dewel, 1997; Eernisse *et al.*, 1992; Nielsen, 1995; Nielsen *et al.*, 1996) agree that there is a close relationship between tardigrades and arthropods. In addition, the evidence for a clade of molting animals, the Ecdysozoa, provides support for the close relationship between tardigrades and arthropods (Aguinaldo *et al.*, 1997), as well as an explanation of the morphological characters that tardigrades share with some of the ''aschelminths'' such as nematodes (Garey *et al.*, 1996). Morphological analyses that include fossil lobopods and arthropods suggest that tardigrades branch between the Onychophora and Euarthropoda and probably derived from an anomalocariid-like member of the euarthropod stem group (Dewel and Dewel, 1996, 1997; Dewel *et al.*, 1999).

The marine heterotardigrades, order Arthrotardigrada, have the greatest number of plesiomorphic characters and thus have been considered the sister group for all other tardigrades. Within the eutardigrades, the order Apochela, family Milnesiidae, is considered to be the sister group to the other eutardigrades (Garey *et al.*, 1999). The 18S rRNA gene phylogeny is consistent with previous hypotheses of tardigrade evolutionary relationships based on morphology. However, further molecular studies of additional genes and genera of both heterotardigrades (especially marine species) and eutardigrades will be required to elucidate the phylogeny within the phylum. Cladistic analyses of combined morphological and molecular data have not yet been performed on the major tardigrade groups. The small genome size of tardigrades and the homogeneity of genome size among tardigrade taxa could be related to the high degree of specialization of the phylum in morphology and life style (Garagna *et al.*, 1996).

One difficulty that may be encountered by molecular biologists is the verification of tardigrade species. Specimens obtained from Carolina Biological Supply Company (North Carolina, USA) labeled ''*Milnesium* and other species'' were a mixture of *Milnesium* and *Macrobiotus* (both eutardigrades, but one in the order Apochela and one in Parachela) as well as the heterotardigrade *Echiniscus*. Specimens marketed as ''*Hypsibius* sp.'' by Ward's, Inc. (Massachusetts, USA) were actually *Thulinia stephaniae*. In addition to misidentifying specimens, commercial suppliers may change their sources, resulting in different species present in the samples. The importance of verification by tardigrade taxonomists of specimens used in molecular studies can not be overstated.

## COLLECTION AND EXTRACTION TECHNIQUES

Marine tardigrades are collected in core samples in intertidal sediments, scrapings of barnacles and seaweeds on intertidal rocks, and dredged sediments from subtidal substrates at various depths. To remove tardigrades from the substrate, samples can be treated with $MgCl_2$ as a narcotizing agent and subsequently fixed in buffered formaldehyde (Renaud-Mornant, 1988). Alternatively, samples can be briefly shocked with freshwater, which is then decanted into a fine-mesh (30–40 μm) net, ''Higgins' mermaid bra'' (Kristensen and Higgins, 1984*a*, *b*). Samples can also be ''bubbled'' with an aquarium airstone; the meiofauna is collected with a piece of paper (*e.g.*, coarse paper towel) laid on the water surface, which is then rinsed through a 30–40 μm net with seawater. To remove tardigrades from barnacles, the barnacles can be crushed in seawater in a Petri dish under a dissecting scope and the specimens removed from the sutures of the barnacle plates (Kristensen and Hallas, 1980).

Qualitative collections of tardigrades from aquatic habitats with sandy bottoms can be made by stirring the sand in a container of water and decanting immediately after the sand settles (Schuster *et al.*, 1977). The decanted water containing the tardigrades is passed through a sieve (U.S. Standard No. 325) with pores of about 44 μm, and the specimens are rinsed from the screen into a sample jar. Artificial substrates in wire baskets placed in the water for an extended period of time (60 days) have also been used successfully (Kathman and Nelson, 1987).

Modifications of techniques to extract soil nematodes by centrifugation in water, sucrose, or Ludox AM™ (DuPont product, colloidal silica, Sigma-Aldrich Chemical Co.) have been used to remove tardigrades from both aquatic and terrestrial habitats (Hallas, 1975; Bertolani, 1982; McInnes and Ellis-Evans, 1987). A watery suspension of the sample sediment or vegetation is prepared and filtered through two stacked sieves with mesh openings of 500 μm or greater (top sieve) and 44 μm (bottom sieve). The sediment remaining on the fine-mesh sieve is centrifuged with water at about 3,000 rpm for 5 min. The supernatant is then poured through the fine-mesh sieve, and the remaining sediment is centrifuged with Ludox or a sucrose solution (density 1.18) at about 5,000 rpm for 1 min. The supernatant is immediately rinsed through the fine-mesh sieve with running tap water, and the residue is either fixed with boiling alcohol or washed into a Petri dish for examination under a dissecting microscope.

Collections of tardigrades from vegetation (mosses, lichens, algae) can be made by placing the plant material in a container of water for several hours. The sample is agitated vigorously, the plants are removed and squeezed over the container, and after the material has settled, the excess water is decanted. The remaining sediment is filtered through sieves.

## ECOLOGY

### *Habitats*

Although all individuals require water to be active, the environments in which tardigrades live are generally divided into (1) marine and estuarine, (2) freshwater, and (3) terrestrial habitats. The distinction between freshwater and terrestrial species is sometimes unclear because some tardigrades can live in a wide range of moisture regimes. Often they are referred to as ''limno-terrestrial'' species.

Marine tardigrades inhabit all seas from the intertidal to the subtidal zones, down to the abyss (−4,690 m in the Indian Ocean) (Renaud-Mornant, 1982, 1988). They exhibit high species richness although populations tend to be small and patchy in distribution. Since the publication of the monograph by Ramazzotti and Maucci (1983), many new species and genera have been described (Villora-Moreno and Grimaldi, 1996). The low number of species in most marine genera is indicative of their ancient origin and their morphological diversity. In the intertidal zone on sandy shores, some species are typically found in the interstitial community at different depths and in different parts of the intertidal. On rocky shores, tardigrades (especially species of *Echiniscoides*) are found in algae and in acorn barnacles (Kristensen and Hallas, 1980; Miller and Kristensen, 1999; Grimaldi *et al.*, 2000). During low tide, they may be capable of limited cryptobiosis or crawl into spaces that retain water. In the subtidal zone, marine tardigrades are present in all types of substrates with morphological adaptations in response to different subtidal environmental requirements (Bello and de Zio Grimaldi, 1998; D'Addabbo Gallo *et al.*, 1987; Grimaldi *et al.*, 1983; Renaud-Mornant, 1982; Renaud-Mornant and Pollock, 1971; Villora-Moreno and Grimaldi, 1996; Kristensen and Neuhaus, 1999;

Kristensen and Higgins, 1984*a*, *b*). Some species are semibenthic in the water column, while others are mud and deep-sea ooze dwellers or epibenthic on subtidal algae, coral, and other organisms. The marine eutardigrade *Halobiotus crispae* Kristensen, closely related to freshwater species of the genus *Isohypsibius*, possesses giant Malpighian tubules for osmoregulation (Møbjerg and Dahl, 1996). This species is probably secondarily adapted to the marine environment (Crisp and Kristensen, 1983).

Freshwater tardigrades are found in lakes, ponds, streams, springs, and temporary ponds (Schuster *et al.*, 1977; Bertolani, 1982; Kathman and Nelson, 1987; Nelson *et al.*, 1987; Van Rompu *et al.*, 1992; Strayer *et al.*, 1994; Nelson, 2001). Hydrophilous tardigrades are ''distinctly aquatic'' species that live only in permanent freshwater habitats. They are benthic organisms, crawling on vegetation and in the interstitial spaces of sandy substrates. Occasionally specimens are collected in plankton nets, often during periods of high water flow. Most species live in the littoral zone; however, some individuals have been collected from lakes up to 150 m deep (Ramazzotti and Maucci, 1983). Benthic algal mats in maritime Antarctic lakes and pools are productive habitats for tardigrades (Everitt, 1981; McInnes and Ellis-Evans, 1987, 1990; McInnes and Pugh, 1999). Cryoconite holes in glaciers, formed when heat is absorbed by surface accumulation of dark dust, also provide a specialized habitat for rotifers and tardigrades, which feed on the microflora living there (Dastych, 1993; De Smet and Van Rompu, 1994; Grøngaard *et al.*, 1999). Hygrophilous tardigrades, which inhabit moist mosses, and eurytopic species, which live in a wide range of moisture conditions, are often found in aquatic habitats. For a review of the biology and ecology of freshwater tardigrades, see Nelson and Marley (2000) and Nelson (2001).

Most tardigrade species are terrestrial and live in moist habitats, such as soil and leaf litter or among mosses, lichens, liverworts, and cushion-shaped flowering plants (Ramazzotti and Maucci, 1983; Kinchin, 1994). To survive, they must have alternate wet and dry periods as well as sufficient food and oxygen. Although tardigrades are most commonly collected from mosses or lichens (McInnes, 1994), the tardigrade community in leaf litter from beech forests is characterized by high diversity and large numbers of individuals (Guidetti, 1998; Guidetti *et al.*, 1999). Similarly, soil-inhabiting tardigrades exhibit high species diversity and high densities (often as high as dominant soil microarthropods) and are associated with specific habitat preferences (Bertolani and Rebecchi, 1996; Hallas and Yeates, 1972; Ito, 1999; Manicardi and Bertolani, 1987; Nelson and Higgins, 1990).

### *Biogeography*

Due to the paucity of data and the uncertainty of identifications, the biogeography of the Tardigrada remains relatively unknown (McInnes, 1994; McInnes and Pugh, 1998), in spite of its worldwide distribution as a phylum. Many species with broad ecological requirements are considered to be cosmopolitan, whereas others with more narrow tolerances are rare or endemic. They may be found from the Arctic to the Antarctic and from the deep sea (−4,690 m in the Indian Ocean) to the slopes of the highest mountains (above 6,000 m in the Himalayas) (Ramazzotti and Maucci, 1983). Species with wide distributions, probably due to their ability to undergo cryptobiosis, are commonly considered cosmopolitan; however, these species, such as *Macrobiotus hufelandi*, *Minibiotus intermedius*, *Diphascon scoticum*, and *Milnesium tardigradum*, may actually be species complexes (Bertolani and Rebecchi, 1993; Claxton, 1998, 1999; Pilato, 1987). The homothermic springs in Greenland have provided a number of endemic species (Kristensen, 1982*a*). The data are still too scarce and scattered to determine definitive biogeographical distributions of tardigrade species.

### *Cryptobiosis*

All tardigrades are aquatic, regardless of their specific habitat, since they require a film of water surrounding the body to be active. They are capable, however, of entering a latent state (cryptobiosis) when environmental conditions are unfavorable, *e.g.*, freezing, desiccation, low oxygen tensions, and salinity variations. Crowe (1975) identified five types of latency: encystment, anoxybiosis, cryobiosis, osmobiosis, and anhydrobiosis. When a tardigrade is in a latent state, metabolism, growth, reproduction, and senescence are reduced or cease temporarily, and resistance to environmental extremes such as cold, heat, drought, chemicals, and ionizing radiation is increased. Latent states thus have a significant impact on the ecological role of the organism. (See review by Pilato, 1979; Wright *et al.*, 1992; Kinchin, 1994.)

Encystment commonly occurs in freshwater tardigrades living in permanent pools and canal locks, although cysts can also be formed by tardigrades inhabiting soil and moss (Westh and Kristensen, 1992). Encystment is not obligatory in the life cycle of a tardigrade, and reasons for emergence are not fully known. Although the cysts are drought-resistant, they are not anhydrobiotic; they are not able to withstand high temperatures, as in anhydrobiotes, because of their high water content. Cysts can survive for over a year in nature without totally depleting food reserves (Westh and Kristensen, 1992).

Anoxybiosis is a cryptobiotic state induced by low oxygen tension in the environmental water. Some species can survive up to 5 days in anoxybiosis; however, intertidal *Echiniscoides* specimens have survived for months in vials of sea water with decaying barnacles. In contrast, strictly aquatic species live only from a few hours to 3 days in the asphyxial state. Prior to this time limit, specimens can be revived after a few minutes to several hours by the addition of oxygen to the water (Wright *et al.*, 1992).

Induced by low temperatures, cryobiosis is a type of cryptobiosis that enables tardigrades to survive freezing and thawing, allowing limno-terrestrial tardigrades to be common in polar regions and high mountains (Sømme, 1996; McInnes and Pugh, 1998). (See Westh *et al.*, 1991; Westh and Kristensen, 1992; Ramløv and Westh, 1992, for in-depth information.)

Osmobiosis is a form of cryptobiosis induced by elevated osmotic pressures. Some intertidal marine species and euryhaline limno-terrestrial species can tolerate variations in salinity. However, most freshwater and terrestrial tardigrades form contracted tuns (barrel-shaped resistant forms) in various salt solutions (Wright *et al.*, 1992).

Anhydrobiosis, a type of cryptobiosis induced by evaporative water loss, occurs in eggs, juveniles, and adults of terrestrial eutardigrades and echiniscids. (See Wright *et al.*, 1992; Crowe *et al.*, 1992; Kinchin, 1994; Sømme, 1996, for reviews.) Trehalose and glycerol serve as membrane protectants during desiccation. True freshwater tardigrades are considered to lack the ability to survive dehydration and undergo anhydrobiosis, but experimental evidence is sparse. Individuals and eggs in the anhydrobiotic state may be dispersed by winds, even over great distances (Bertolani *et al.*, 1990; Pugh and McInnes, 1998).

*Life history characteristics and molting*

Molting, which usually requires 5–10 days, occurs periodically throughout the life of the tardigrade (Walz, 1982). During the molt, the old cuticle, including the claws and the lining of the foregut and hindgut, is shed. While in the ''simplex'' stage, which is characterized by the absence of sclerified parts of the buccal-pharyngeal apparatus, the animal cannot feed (Walz, 1982). Although growth is more rapid during the earlier molts, molting and growth continue even after sexual maturity (Nelson, 1982*b*). Generally, body length increases with each molt until maximum size is attained. Defecation and oviposition may also be associated with molting.

Life histories of certain tardigrade species have been reviewed by Walz (1982), Nelson (1982*b*), Ramazzotti and Maucci (1983), and Kinchin (1994). Based on frequency distributions of body length and buccal length, the number of molts has been estimated to range from 4 to 12, although there are problems inherent in the method (Morgan, 1977; Ramazzotti and Maucci, 1983; Kinchin, 1994). Considerable variation and overlapping of the stages may occur within a species.

The lifespan of active tardigrades (excluding cryptobiotic periods, explained in the section on latent states) has been estimated to be from 3–30 mo (Franceschi *et al.*, 1962/63; Ramazzotti and Maucci, 1983). The total lifespan, from hatching until death, may be greatly extended by periods of latent life (encystment and anhydrobiosis). Since aquatic tardigrades have little or no ability to undergo anhydrobiosis, the period of active life generally corresponds to that of total life. Ramazzotti and Maucci (1983) concluded that freshwater species of *Macrobiotus* and *Hypsibius* live about one to two years; whereas moss-inhabiting species of the same genera average 4–12 yr.

Cyclomorphosis, an annual cycle of morphological change in individuals, has been documented in the marine eutardigrade *Halobiotus crispae* (Kristensen, 1982*b*), in a moss-dwelling species of *Amphibolus* (Rebecchi and Bertolani, 1994), and in a cryoconite-dwelling species of *Hypsibius* (Dastych, 1993). Cyclomorphosis is an adaptation for survival during harsh environmental conditions and short breeding seasons. For example, in Greenland, *Halobiotus crispae* alternates between two morphs (pseudosimplex stages). The winter morph (pseudosimplex 1) is an inactive hibernating stage that is sexually immature. The individuals are gregarious, gathering in large numbers in protected areas to survive freezing temperatures in winter. The entire population undergoes synchronous development of the gonads so that the active summer morphs become sexually mature and breed at the same time (Kristensen, 1982*b*). The occurrence of cyclomorphosis in freshwater species has not been investigated.

*Population and community interactions*

Population densities of tardigrades are highly variable; however, neither minimal nor optimal conditions for population growth are known. (See review in Kinchin, 1994.) Changes in tardigrade population densities have been correlated with a variety of environmental conditions, including temperature and moisture (Franceschi *et al.*, 1962/63; Morgan, 1977; Briones *et al.*, 1997), air pollution (Steiner, 1994*a*, *b*, *c*, 1995), and food availability (Hallas and Yeates, 1972). Considerable variation in both population density and species diversity occurs in adjacent, seemingly identical, microhabitats. In general, patchiness is a common characteristic of tardigrade populations, necessitating replicate samples for obtaining valid data for ecological studies.

Other factors such as competition, predation, and parasitism may also play a role in determining population density and diversity. Predators include nematodes, other tardigrades, mites, spiders, springtails, and insect larvae; parasitic protozoa and fungi often infect tardigrade populations (Ramazzotti and Maucci, 1983). ''Ecosystem grazers'' such as freshwater crustaceans, earthworms, and arthropods also ablate tardigrade populations. In turn, tardigrades use their buccal apparatus to feed upon detritus or a variety of organisms, including bacteria, algae, protozoans, and other meiofauna. The buccal apparatus consists of a buccal tube, a pair of piercing stylets, and a muscular sucking pharynx. Gut contents often contain chloroplasts or other components of cells of algae, mosses, or lichens. The larger species of terrestrial *Macrobiotus* and *Milnesium* have been observed preying on protozoans, nematodes, rotifers, and smaller species of eutardigrades (*e.g.*, *Diphascon* and *Hypsibius*), even sucking in the entire organism. Rotifer jaws and tardigrade claws and buccal apparati have been observed in the guts of these predaceous tardigrades. It is assumed that the type of buccal apparatus is correlated with the type of food consumed; however, little is known of the specific feeding requirements of either marine or limno-terrestrial species, and further research is obviously needed in this area.

## CONCLUSIONS

Despite their overall abundance and cosmopolitan distribution, the Tardigrada have been relatively neglected by invertebrate zoologists. Because of difficulties in collecting and culturing the organisms and their apparent lack of economic importance to humans, our knowledge of tardigrades has lagged that of other groups. However, their importance in elucidating the phylogeny of the Metazoa, particularly the arthropods, has recently increased interest in this group. In addition, their development and ecology are poorly understood, and proper training of taxonomists skilled in identifying tardigrade species is essential for systematic, ecological, and molecular analyses.

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