CHAPTER 5-1
TARDIGRADE SURVIVAL

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Figure 1. *Dactylobiotus* sp. on the green alga *Spirogyra*. Photo by Yuuji Tsukii, with permission.

Tardigrades – Water Bears

Tardigrades (*tardus* = slow, *gradus* = step, or slow walkers), also known as water bears or moss piglets, are close relatives of the arthropods (Garey *et al*. 1996, 1999; Giribet *et al*. 1996).

Water bears resemble small bears (0.1-1 mm), complete with claws, but a few too many legs (4 pairs) (Figure 1). They are either armored (**Heterotardigrada**) or unarmored (**Eutardigrada**). The aquatic ones are usually a translucent white, whereas the terrestrial ones are often colored. Each of the eight legs has claws, which, when combined with their slow gait, makes them look very much like miniature polar bears with some extra legs. The very common *Macrobiotus hufelandi* (Figure 2) lumbers along at a maximum of 17.7 cm h^{-1} (Ramazzotti & Maucci in Mach 2010). Tardigrades are just the right size to move among the bryophyte leaves, they lumber along slowly like bears, and they are downright cute!

Tardigrades, comprising about 900 species (Garey *et al*. 2008), can be found in marine, aquatic, and terrestrial habitats (Goeze 1773; May 1948; Greven 1980; Maucci 1986; Kinchin 1994). On land they frequently live in association with bryophytes (Figure 3; Figure 4) and lichens (Mihelčič 1967; Mehlen 1969; Utsugi 1984; Meininger *et al*. 1985; Mancardi 1988; Szymanska 1994; Bertolani & Rebecchi 1996; Tarter *et al*. 1996; Miller

1997; Jerez Jaimes *et al*. 2002; Boeckner *et al*. 2006; Bartels *et al*. 2009; Meyer & Hinton 2009; Rossi *et al*. 2009; Simmons *et al*. 2009). In water, algae, as well as bryophytes, provide homes.
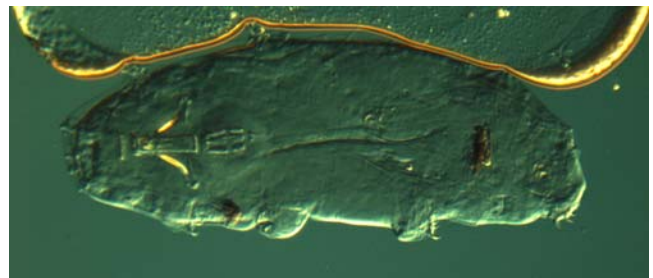
Figure 2. *Macrobiotus hufelandi*, a common tardigrade that is among those inhabiting mosses. Photo by Paul Bartels, with permission.

These terrestrial tardigrades depend on the water drops that adhere to mosses and liverworts (Hingley 1993) and are therefore often termed **limnoterrestrial** (living in terrestrial habitats, but requiring a water film). Aquatic bryophytes can also house tardigrades (Hallas 1975; Kinchin 1987b, 1988; Steiner 1994a, b), as do the algae. However, of the ~1000 tardigrades reviewed by Guidetti

and Bertolani (2005) and Garey *et al.* (2008), only 62 were truly aquatic. The others depend on water associated with the interstitial spaces of terrestrial algae, lichens, bryophytes, soil, and leaf litter. Water bears are found in habitats from hot springs to layers under the ice (in cryoconite holes in glaciers) and occupy every continent of the world.

Figure 3. This tardigrade resided among the leaves of the moss *Hypopterygium arbuscula* (Figure 4). Photo courtesy of Filipe Osorio.

Figure 4. *Hypopterygium arbuscula*, a known bryophyte habitat for tardigrades in Chile. Photo by Juan Larrain through Creative Commons.

Most of the terrestrial tardigrades are bryophyte inhabitants (Nelson 1991a). These terrestrial bryophyte taxa have a life span ranging 3-4 months (Franceschi *et al.* 1962-1963), 3-7 months for *Macrobiotus hufelandi* (Figure 2; Morgan 1977), up to about 3 months for roof-moss-dwelling *Echiniscus testudo* (Figure 5; Morgan 1977), to about 2 years (Altiero & Rebecchi 2001) of active life (not counting dormant periods). The bryophyte-inhabiting taxa are more common in temperate and polar zones than in the tropics (Nelson 1991a). Some, as for example *Echiniscus testudo* (Figure 5), live almost exclusively on bryophytes (Corbet & Lan 1974).

Despite their cosmopolitan distribution (Romano 2003), broad habitat requirements, and relative visibility (compared to protozoa, for example), the tardigrades remain poorly known. As late as 1985, Hidalgo and Coombs reported that 16 states in the USA had no records of tardigrades. Species not previously described are easily discovered by those who know where to look for them.

The environmental conditions that affect tardigrades are likewise poorly studied (Guil *et al.* 2009), despite the extensive studies on a few species that have become travellers of the universe in space. Among those conditions

that have been studied, altitudinal gradients and micro-environmental variables (including substrate choice among vegetation, bryophytes, and leaf litter) seem to have gotten the bulk of the attention (Guil *et al.* 2009). Guil and coworkers found a unimodal distribution relative to altitude for species richness, but it was closely tied to habitat variation. The best predictor of the distribution of tardigrades in the Sierra de Guadarrama Mountain Range, Spain, was bioclimatic classification. Soil, climate, vegetation structure, and leaf litter type worked both separately and in combination to determine species richness, explaining nearly 60% of the species richness in micro-scale plots. Abundance, on the other hand, was significantly influenced only by soil composition and leaf litter type. The macro-environmental gradients seemed to be unimportant in determining abundance (*e.g.* Figure 6).
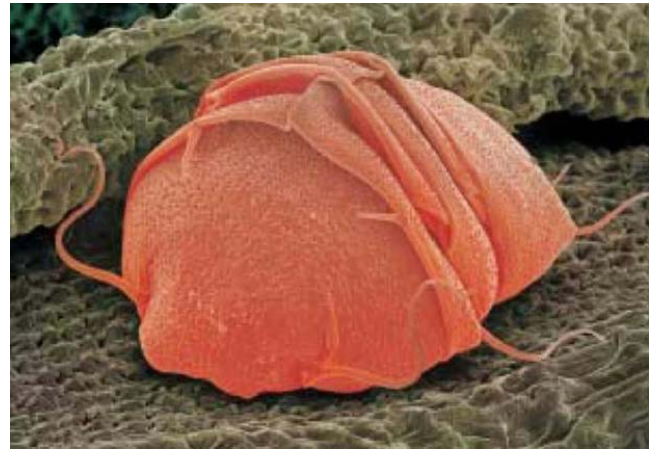

Figure 5. *Echiniscus testudo* dormant stage (tun), demonstrating the rigid nature of its armor that prevents it from extensive changes in size. Photo by Power & Syred through Creative Commons.
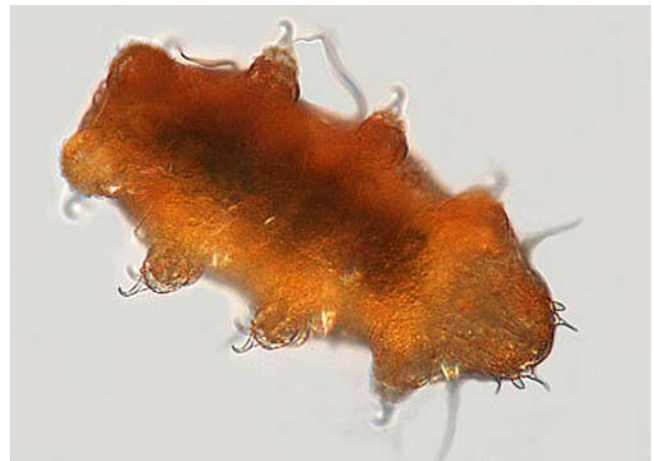
Figure 6. *Echiniscus* species (*E. testudo* occurs almost exclusively on bryophytes) seem to be unresponsive to moisture changes. Photo by Martin Mach, with permission.

Suitability of Bryophytes as Habitat

The importance of bryophytes as a tardigrade habitat is evident by the number of publications on "moss" tardigrade fauna: Mihelčič 1967; Hallas 1975; Pilato & Sperlinga 1975; Morgan 1976; Bruegmann 1977; Morgan 1977; Maucci 1978, 1980; Bertolani 1983, 2001; Binda 1984;

Utsugi 1984; Meininger *et al*. 1985; Hofmann 1987; Hofmann & Eichelberg 1987; Kinchin 1987a, b, 1988, 1994; Meininger & Spatt 1988; Mancardi 1988; Bertolani *et al*. 1990; Tarter & Nelson 1990; Kathman & Cross 1991; Nelson 1991a, b; Utsugi & Ohyama 1991; Moon *et al*. 1994; Szymanska 1994; Miller & Heatwole 1995; Adkins & Nelson 1996; Tarter *et al*. 1996; Hooie & Davison 2001; Guidetti & Jönsson 2002; Jönsson 2003; Meyer *et al*. 2003; Hooie 2005), to name a few. It appears that when tardigrade lovers want to collect a lot of them, they collect bryophytes and lichens – or just bryophytes (generally lumped into "mosses").

Unfortunately, the authors rarely name the bryophytes from which their prizes were extracted. However, some evidence suggests that little specificity exists for bryophyte species, and lichens are as suitable as bryophytes, with no apparent differences in tardigrade species (Meyer & Hinton 2007). I have to wonder, however, why reports on tardigrades from liverworts are so scant (Figure 7). Perhaps it is just as suggested to me by Łukasz Kaczmarek, that most zoologists do not understand the differences between mosses and liverworts. (Neither do my students when they begin looking at them.)
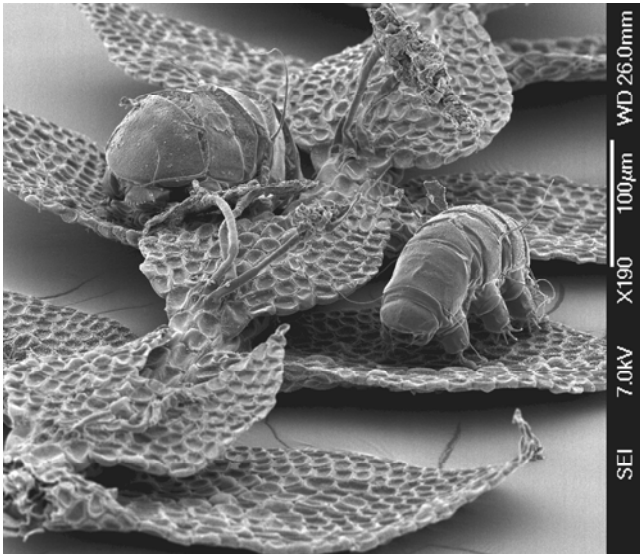

Figure 7. SEM view of tardigrades on the lower sides of leaves of a leafy liverwort. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Ramazzotti and Maucci (1983) considered mosses suitable habitat based on three needs of the **limnoterrestrial** tardigrades:

1. a structure that allows sufficient oxygen diffusion
2. the ability to undergo alternate periods of wetting and drying resulting from solar radiation and wind
3. a medium that contains sufficient food.

Based on these criteria, bryophytes are particularly good habitats for tardigrades in several ways (Ramazzotti & Maucci 1983; Claps & Rossi 1984; Adkins & Nelson 1996). Their structure permits sufficient oxygen diffusion, both in aquatic and terrestrial habitats. Bryophytes experience drying, which most do slowly, permitting the tardigrades likewise to dry slowly, and both have a tolerance to dehydration that permits them to survive adverse conditions (Kinchin 1994). Furthermore, the

tardigrades have a prolonged life span when it is interrupted by such a dormancy period. And bryophytes contain food items, such as algae, protozoa, and nematodes, as well as the bryophytes themselves, sufficient for the tardigrades. Most likely, the small chambers among the bryophyte branches also afford protection from larger would-be predators. And when fragments of bryophytes disperse, they may carry tardigrades with them.

It is the interstitial water of bryophytes that provides the suitable habitat for tardigrades (Hallas 1975). This water is typically found in leaf sheaths of bryophytes. Hallas investigated the drying of "cushions" of **Hypnum cupressiforme** (Figure 8). It required 19 hours for the moss to dry to a stable weight. The water retention relative to the weight of the cushion decreased linearly with the density of the cushion. However, the rate of drying can change with the temperature, saturation of the air, and air movement. He concluded that all compartments dry at the same rate, independent of the initial water content of each pocket, and the small variation in drying time is of no consequence for the tardigrades and other inhabitants. The tardigrades were concentrated in the living, chlorophyllous layer (compared to deeper, senescent layers) where there was more moisture. The water pockets connect vertically from one leaf to the next, but only connect horizontally when it rains. Therefore, horizontal migration of the tardigrades is only possible when the moss becomes saturated during rainfall. In **H. cupressiforme** (Figure 8), such bridges are formed only when rainfall exceeds 3 mm. Hallas considered that nighttime and morning dew were not sufficient for the most common tardigrade [**Macrobiotus hufelandii** (Figure 2) – a species that comprised 91% of the 386 tardigrades] to become active, suggesting that it would take ten times that amount of water to bring the moss to saturation levels.

Figure 8. Mat of **Hypnum cupressiforme**. Photo by Dick Haaksma, with permission.

But bryophytes do pose their problems for the tiny tardigrades. These animals are quite light weight, so imagine their struggle to control their movements when they encounter fully hydrated bryophytes with a continuous bath of water surrounding them. Greven and Schüttler (2001) observed these slow-moving creatures [**Macrobiotus** sp., **Echiniscus testudo** (Figure 5) on **Encalypta streptocarpa** [=*E. contorta*] (Figure 9) when the bryophyte was fully hydrated. The poor bears could barely

move and had difficulty maintaining the direction of their movements in the water. They could easily become dislodged by rainwater unless they are able to nestle in a leaf axil or other protected niche. And that is often a good place to look for them.

Figure 9. *Encalypta streptocarpa*, a tardigrade habitat that can be difficult to navigate when it is fully hydrated. Photo by Michael Lüth, with permission.

On the other hand, *Polytrichastrum* [=*Polytrichum*] *formosum* (Figure 10) did not sustain a continuous water film and the tardigrades seemed also unable to move in this "dry" habitat (Greven & Schüttler 2001). Rather, they seemed confined to the leaf axils, where water collected. As water receded, the animals ceased movement and formed a **tun** (protective dormant stage of tardigrade that is altered both chemically and physically) right there, permitting it to survive without water for up to 10 years (Jönsson & Bertolani 2001)! Perhaps tardigrades were the inspiration for the Rip Van Winkle story.

Figure 10. *Polytrichastrum formosum*, a moss that does not maintain a water film and is thus a poor tardigrade habitat. Photo by Des Callaghan, with permission.

Moisture seems to be the greatest determinant of species distribution among bryophytes. Richness among epiphytic bryophytes in the Cincinnati, Ohio, USA area was greatest in areas of high humidity (Meininger *et al*. 1985). Hofmann and Eichelberg (1987) found that the tardigrades lacked correlation with bryophyte species but that their distribution could be predicted by the degree of moisture they prefer. It is therefore not surprising that some bryophytes housed no tardigrades.

Tardigrades in association with roads along the Alaska pipeline demonstrate a moisture relationship (Meininger & Spatt 1988). Dust resulting from gravel roads associated with the pipeline alters the habitat for both mosses and tardigrades. Those tardigrades living among mosses near roads were species adapted to xeric habitats. These species typically fed on fungi and algae, whereas those farther from the road were more likely to be omnivores or carnivores, presumably because they had more freedom to move about in a somewhat more moist environment.

Adaptations of Tardigrades

One might ask if these bryophyte-dwelling creatures have any special adaptations that permit them to live where they do. Their greatest adaptation is that they live in a habitat that permits them to dry slowly and go into a dormant state, as we will discuss shortly – a kind of behavioral/physiological adaptation. In fact, it appears that limnoterrestrial species actually require a habitat where they have dormant periods. And for many, the stylets permit them to suck the contents out of bryophyte cells, among other things, making them one of the few organisms specially adapted for obtaining nutrients from bryophytes.

Like insects, tardigrades have chitin, in this case in the innermost layer of the cuticle. The chitinous armor of some terrestrial tardigrades (heterotardigrades) may slow drying and offer protection from damage while dry. Of course small size is essential for living in the miniature world of bryophytes. And their claws (Figure 11-Figure 13) may permit them to clamber about more easily among the leaves and branches of the bryophytes. But Bertolani and Biserov (1996) consider that the reduction of claws on the fourth pair of legs is an adaptation to moving among the interstitial spaces in the soil. Does this same adaptation pertain to those among bryophytes?

Figure 11. Claws on four of the eight legs of *Echiniscoides sigismundi* (a tidal zone species). Photo by Martin Mach, with permission.

Figure 12. Claws of a tardigrade that is most likely *Cornechiniscus cornutus* (a bryophyte-dweller). Photo by Martin Mach, with permission.

Figure 13. Claws of *Echiniscus* sp., a genus with many bryophyte-dwelling species. Photo by Martin Mach, with permission.

Their light weight facilitates tardigrade dispersal. Their bodies are flexible, permitting them to nestle in leaf axils or move in small spaces. But most of these as adaptations to the bryophyte habitat are speculation. There have been no tests to determine if any of these traits actually increases their survival among bryophytes compared to other habitats. Some very interesting experiments could be designed.

Let's examine one of the bryophyte-dwelling tardigrades as an example of potential adaptations. Martin Mach (The Water Bear) found *Cornechiniscus cornutus* (Figure 14) among bryophytes on a mountain top in Hungary. This cute little bear has two horns on its head (Figure 15) and a nice salmon color. But it is slow and clumsy, out-classed by the faster-moving and more abundant *Ramazzottius* (formerly *Hypsibius*) *oberhaeuseri* (Figure 25). Do such ornamentations as horns and hairs help to reduce predation in this habitat? Is that an advantage to offset the slower movement? Does the bright color protect the water bear from UV damage, especially while it is dry?
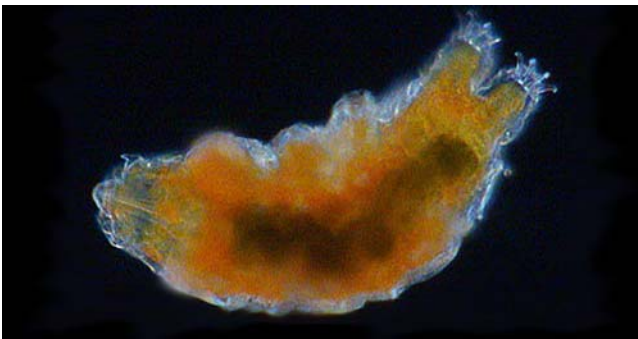

Figure 14. *Cornechiniscus cornutus*. Photo by Martin Mach, with permission.

Survival of Hazardous Conditions

The biggest hazard a bryophyte imposes on a tardigrade is intermittent desiccation. But in addition to that desiccation, the organism may be subjected to high or low temperatures, low oxygen conditions, and UV light for prolonged periods. With little ability to move elsewhere, it needs some other type of protection.

Figure 15. *Cornechiniscus cornutus* showing one of its two head horns. Photo by Martin Mach, with permission.

Aquatic organisms rarely need to be concerned with desiccation. However, if an animal is to survive among terrestrial bryophytes, it must be prepared for drying when the bryophyte dries out, and many of the tardigrade habitats are in dry places, including **cryptogamic crusts** (assemblages of Cyanobacteria, algae, lichens, & mosses) in the prairie and desert, and among epiphytes on trees. These bring with them the very hazards mentioned above – UV light in the absence of water for protection, and extremes in temperature. And the watery body must be hydrated for oxygen to enter it.

To unravel the relative importance of these stressors related to desiccation, Wright (1991) studied fifteen species of tardigrades and their responses to insolation, elevation, standardized desiccation rate, and hydration capacity of the plant substrate. There was considerable variation in ecotype among seven species with xeric associations. *Macrobiotus hufelandi* (Figure 2) and *Hypsibius dujardini* (Figure 16), both hygrophilic species, are absent from habitats that desiccate rapidly. On the other hand, the xerophiles *Milnesium tardigradum* (Figure 51) and *Ramazzottius oberhaeuseri* (Figure 25) avoid locations with high insolation and rapid desiccation rate, but also avoid poorly drained sites and sites with prolonged hydration. Despite these differences, *Milnesium tardigradum* often associates with the two *Hypsibius* species and may use them for food. The lack of association among *Macrobiotus hufelandi*, *Paramacrobiotus* (formerly *Macrobiotus*) *richtersi* (Figure 17), and *Hypsibius prosostomus* may be due to competitive exclusion.
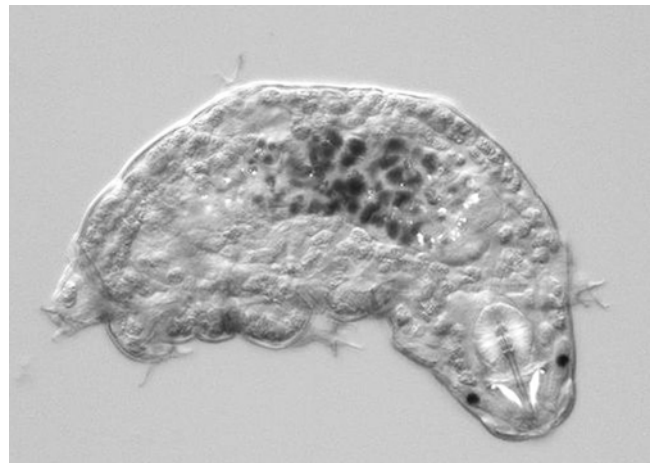

Figure 16. *Hypsibius dujardini* with the alga *Chlorococcum* in its gut. Photo by Willow Gabriel through EOL Creative Commons.

Figure 17. *Paramacrobiotus richtersi*. Photo by Science Photo Library through Creative Commons.

Physical Adaptations

The soft-bodied tardigrades appear to have few structural adaptations to survive drought. Some, like *Echiniscus*, have long hairs (Figure 18-Figure 19), but the hairs are so few that one can hardly imagine they are of any help to reduce water loss or protect the dry animal. Hmmm...What might their function be? Or are they a non-detrimental left-over? One interesting idea is that they might serve as straws to draw water into the body – a hypothesis requiring both experimentation and TEM examination. But suppose that is true. It could be a way to cause water to enter without drowning the animal – *i.e.*, permitting maintenance of an air layer on the surface. On the other hand, they could serve as fine hairs to collect dew and fog on their surface and direct it to the skin, thus increasing surface area and collection surface for the water. Or the hairs cold act like whiskers on a cat, facilitating navigation among the mosses. More interesting experiments are needed!

Figure 18. *Echiniscus trisetosus*, illustrating the sparse but long hairs and plates of armor. Photo by Łukasz Michalczyk and Łukasz Kaczmarek, with permission.

Oxygen availability can be a problem, and for this reason the tardigrades avoid dense bryophytes and usually remain in the top few centimeters of soil where more oxygen is available (Ramazzotti & Maucci 1983). Hence, another possibility for the long hairs is that they could be tactile extensions to warn of an environment that is too tight, or chemical sensors to aid in finding food or sensing predators – or sensing a low-oxygen environment. Hypothesis testing is needed!

Figure 19. Two of the long hairs of *Echiniscus*. Photo by Martin Mach, with permission.

Echiniscus viridianus (Figure 20) seems to do well among bryophytes. It was originally described from mosses in Alabama USA (20 individuals) and later collected in New Mexico and the Azores Islands, again among mosses (Pilato *et al*. 2007). As in other members of the genus, this species bears armor and long hairs.

Figure 20. *Echiniscus viridianus*. Photo by Paul J. Bartels, with permission.

Spines/hairs and body armor may offer a bit more protection. Some bryophyte-dwelling species such as *Cornechiniscus cornutus* (Figure 21) and some members of the genera *Echiniscus* (Figure 22-Figure 23) and *Ramazzottius* (Figure 24-Figure 25) (and others) have "**armor**" on their bodies that is somewhat leathery. I am aware of no studies that demonstrate the ability of the armor to reduce water loss, but it would appear to be a good possibility. Other possible advantages of this armor-like **cuticle** may include protection from fungi and other pathogens and some kinds of predators, particularly while in cryptobiosis, and it most likely would afford limited UV protection. How little we know!
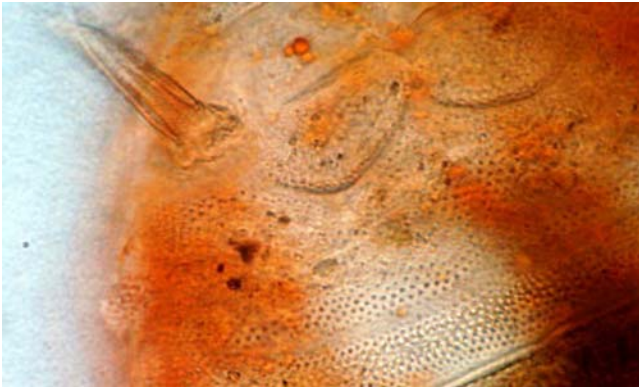
Figure 21. *Cornechiniscus cornutus* showing armor. Photo by Martin Mach, with permission.

Figure 22. *Echiniscus* sp. posterior dorsal side showing a type of armor. Photo by Martin Mach, with permission.
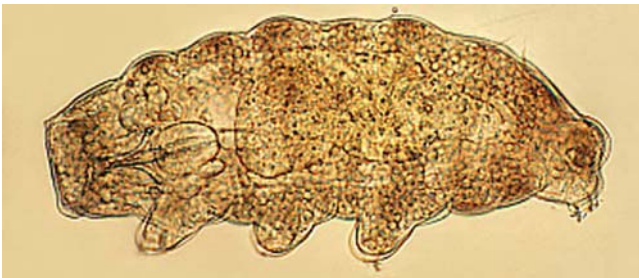

Figure 23. Tardigrade sp. showing a type of armor. Photo by Martin Mach, with permission.
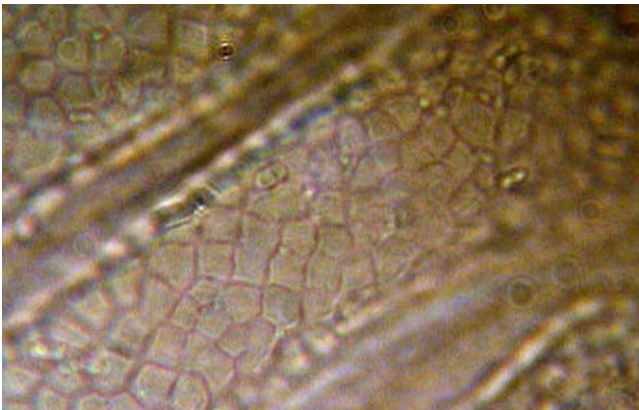

Figure 24. Armor on *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.

Figure 25. *Ramazzottius oberhaeuseri*, a tardigrade with armor. Photo by Martin Mach, with permission.

Pigments

Terrestrial tardigrades come in green, brown, yellow, orange, pink, red, purple, or black, whereas aquatic ones are white (Hebert 2008). Bonifacio *et al*. (2012) pointed out that despite the many studies on tardigrades relative to space travel, little is known about the nature or function of their pigments. They described the carotenoid nature of the pigments and the ability of these pigments to decrease under high oxidative stress. They hypothesized that these pigments had an anti-oxidant function and could possibly protect the animals during extreme environmental conditions.

It is possible that the wonderful colors of some tardigrades (Figure 26-Figure 27) are adaptations against UV damage to DNA, especially during prolonged periods in a cryptobiotic state. Such pigmentation advantages have been demonstrated in bryophytes (Martínez Abaigar & Olivera 2007) and copepods (Byron 1982), so it is reasonable to expect them to serve similar functions in tardigrades, particularly in those more open habitats such as **cryptogamic crusts**. It would be an interesting study to examine the relationship of color with habitat in tardigrades. I am aware of no such study, but Martin Mach (pers. comm. 18 October 2012) pointed me to the publication of Ernst Marcus (1929). Marcus suggested that pigments were a response to UV light. He cited as support the findings that pigmentation varies with winter vs summer UV radiation. Experiments are needed to support this hypothesis, and one must wonder how this relates to those living among bryophytes. Marcus pointed out that insolation does not penetrate well into the moss colony, making bright pigments unnecessary for these tardigrades.

Physiological Adaptations

Light Response

Tardigrades have a pair of eyes, although at times they may be "ghost eyes" (Figure 28) that cannot be seen

through ordinary observation (Mach 2012). These eyes respond to light, and at least in **Macrobiotus hufelandi** (Figure 2) the response changes with size and age (Beasley 2001). The smaller, younger size group had a significantly negative response to light. Beasley hypothesized that this behavior serves to conserve body moisture in small individuals that have a larger surface area to volume ratio than do larger ones. The response is not a phototaxis (directional response to light), but rather was photokinesis (non-directed, random movement), resulting in either an increase in speed or a change in direction when exposed to light. Such behavior would seem to support finding a "safe" place away from light.
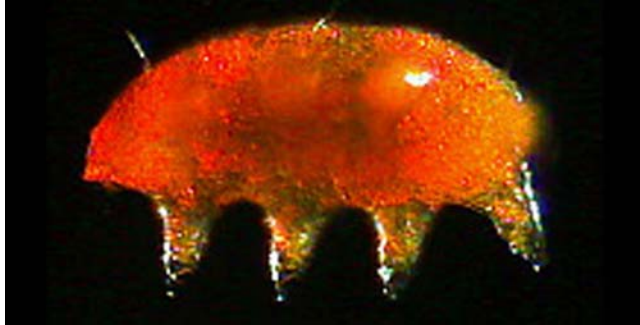

Figure 26. Adult **Echiniscus** sp., demonstrating one of the bright colors found in tardigrades. Photo by Martin Mach, with permission.

Figure 27. **Cornechiniscus cornutus**, a bryophyte-dwelling "horned" species that exhibits brilliant colors that could afford UV protection. Photo by Martin Mach, with permission.

Figure 28. Ghost eyes of **Ramazzottius oberhaeuseri**. Photo by Martin Mach, with permission.

Cryptobiosis

Albert Szent-Gyorgyi, a 20th Century Hungarian biochemist, once stated "Water is life's mater and matrix, mother and medium. There is no life without water." In their cryptobiotic state, tardigrades come close to disproving that statement. Anthony van Leuwenhoek first described cryptobiosis in 1702, but it was not until 1959 that Keilin coined the term **cryptobiosis** (Wright 2001). Cryptobiosis is a reversible ametabolic state that can be induced by dehydration and cooling, and possibly osmotic stress and anoxia.

Metazoans such as tardigrades use **glycerol** and the disaccharide sugars **sucrose** and **trehalose** (Wright 2001) as protectants. Before entering the cryptobiotic state, these substances must be synthesized from glycogen reserves, hence requiring a preparatory period. Certain behavioral adaptations may help to delay the desiccation, thus permitting these compounds to be synthesized.

One of the factors that contributes to the tolerance of desiccation is the ability to reduce surface area during tun formation (Wright 1991), hence slowing the process. Those that are most desiccation tolerant have the greatest infolding. This means those species with thick dorsal plates (Figure 29) are at a disadvantage. As Wright showed for **Echiniscus testudo** (Figure 5), there is very little surface area reduction possible.
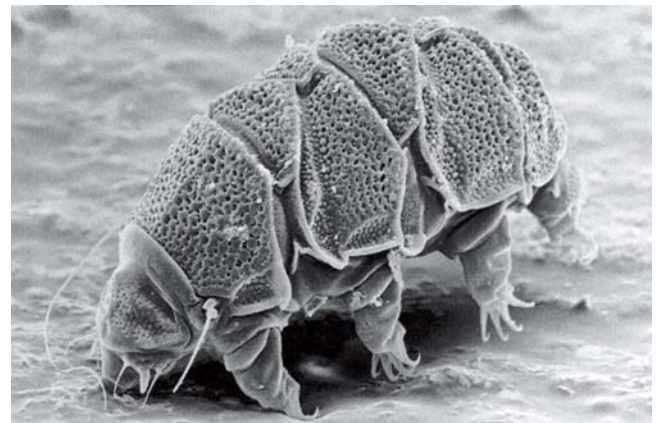

Figure 29. **Echiniscus mauccii** showing the plates that make shrinkage during desiccation all but impossible. Photo by Diane Nelson and Paul Bartels, with permission.

Moss cushions help to make survival of this cryptobiotic state possible. The small spaces among mosses hold static air that can slow the dispersion of water vapor (Wright 1989). This permits the slow drying that is necessary for survival of the tardigrade in the desiccated state. Mosses in exposed positions may desiccate rapidly. Some mosses [*e.g.* **Polytrichum** (Figure 30), **Dawsonia** spp. (Figure 31)] are able to slow this process by folding their leaves (van Zanten 1974).

Wright experimented with tardigrades on mosses in their natural habitat. **Eutardigrada** species were hydrated at least 24 hours before the experiments. As expected, there is considerable variation among species in their ability to tolerate desiccation. But they also differ in the lethal humidities (53-78%) for initial desiccation. Those species that are best able to tolerate rapid initial drying are also those most able to acquire tolerance to low humidities of 25-31% following drying in high humidity.

Figure 30. ***Polytrichum formosum*** showing open leaves (left & right) and folded leaves (center). Photo by Michael Lüth, with permission.

Figure 31. ***Dawsonia superba***, a moss that seems unfavorable for tardigrade feeding and rolls its leaves when dry. Photo by Jan-Peter Frahm, with permission.

Typically, tardigrades are desiccated in 80% relative humidity (Wright 1991). In this condition, they dehydrate rapidly, then abruptly reduce water loss (the **permeability slump**). This slump occurs in both live animals prior to tun formation and in extended dead animals, so it is not a physiological phenomenon. This slump permits the animals to retain considerable water in their desiccated state. Crowe (1972) examined the humidity effects on *Paramacrobiotus areolatus* (Figure 32). He found that at humidities lower than 70% this species became flattened and crumpled. Above this level, dehydrating animals form tuns. This appears to be an active process that is not as effective in anesthetized animals. Tuns of active animals lose water at only 0.3 times the rate of anaesthetized animals. The anaesthetized animals reach moisture equilibration with the environment within one hour, whereas tuns do not reach that equilibrium within 100 hours. In dry air, tardigrades can reach as little as 2-3% water content without dying if they are able to dry properly.

Literally meaning "hidden life," **cryptobiosis** is a state of suspended animation in which the organism is able to survive unfavorable conditions while expending little energy. During that state, the organism does not feed,

reproduction stops, and metabolism is extremely reduced and may possibly even cease. For the **limnoterrestrial** (living in water films on land) tardigrade, it appears to be an essential part of survival and life, and it stops the aging clock.

Figure 32. Head region of ***Paramacrobiotus*** [=*Macrobiotus*] ***areolatus***. The bulbous oval to the right of the three filaments (stylets and buccal tube) is the pharynx. Photo by Martin Mach, with permission.

Despite the apparent absence of structural adaptations, desiccated tardigrades, like their mossy habitats, have great survival capabilities. They have two forms of **dormancy**: **cryptobiosis** and **encystment** (Guidetti *et al.* 2006). The cryptobiosis of tardigrades is exhibited in several forms:

- **anhydrobiosis** (induced by loss of water)
- **cryobiosis** (induced by declining temperatures)
- **anoxybiosis** (induced by insufficient oxygen)
- **osmobiosis** (induced by loss of water due to higher external salt concentrations) (Bertolani *et al.* 2004).

To be active, tardigrades must stay in a water film in order to breathe (Bordenstein 2008). But in a cryptobiotic state, as discussed below, tardigrades can survive not only desiccation, but temperatures as low as 0.05K (-272.95ºC) for 20 hours or -200ºC for 20 months (Miller 1997). They have even survived 151ºC for a few minutes (Lindahl & Balser 1999). They become active again after living with 0% hydration (Lindahl & Balser 1999). This desiccated dormant state also permits them to survive pressures of 6000 atmospheres (Seki & Toyoshima 1998), *i.e.* six times the pressure of the deepest part of the oceans! Yet they can also survive the vacuum and UV radiation of space (Jönsson *et al.* 2008), a feat not known for any other animal. The ability of tardigrades to undergo **cryptobiosis** is more widely known than their encystment behavior. True cryptobiotic states are survived as a **tun** (Figure 33-Figure 43). The tardigrades will be the ones to survive when everything else is deceased.

Tun Formation

When they undergo desiccation, the **tardigrades** form a **tun** (Figure 33-Figure 43) (Lindahl & Balser 1999). The **tun** is a barrel-shaped, dry, dormant tardigrade. Tuns are

formed in the process of entering true cryptobiosis, *i.e.*, in anhydrobiosis, osmobiosis, and cryobiosis, but not in anoxybiosis. Although the stimulus differs among these, each ultimately involves the loss of free water.
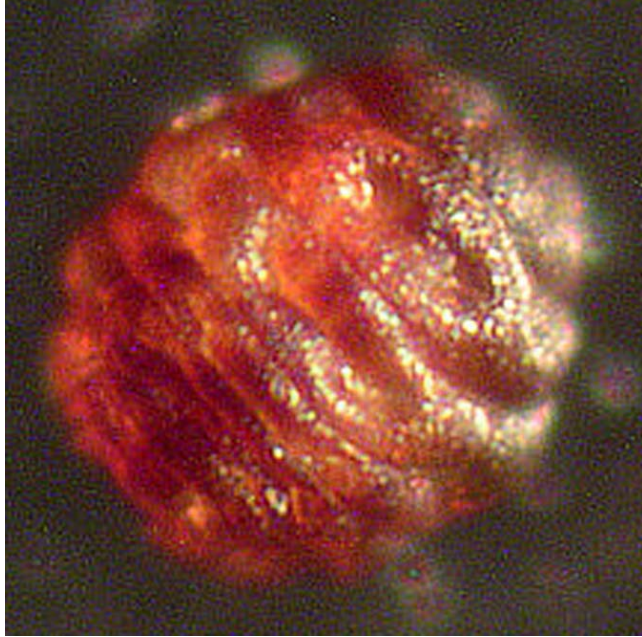

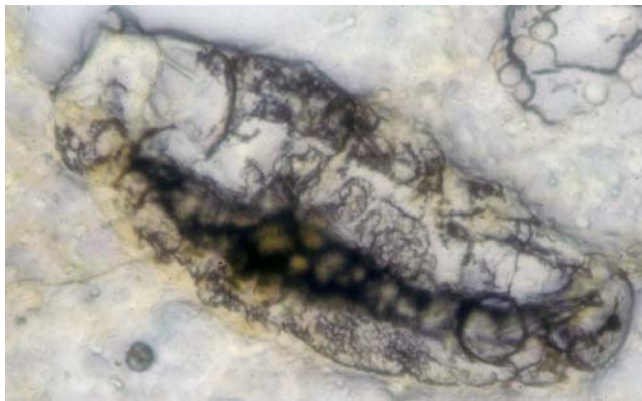Figure 33. Tun of ***Ramazzottius oberhaeuseri***. Photo by Martin Mach, with permission.

Figure 34. **Tardigrade** tun – water bear in a state of anhydrobiosis. Photo by Janice Glime.
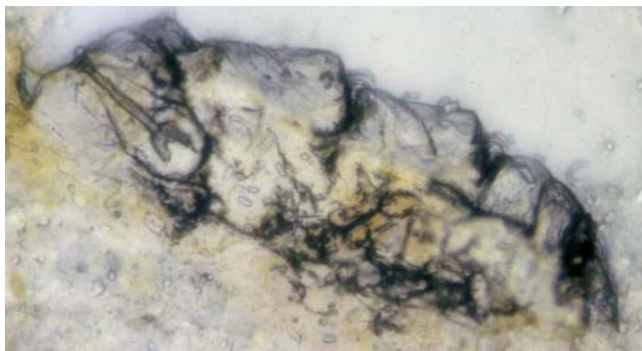
Figure 35. **Tardigrade** tun – water bear in a state of anhydrobiosis. Note the buccal apparatus (resembles a tuning fork on left end). Photo by Janice Glime.

Figure 36. Tun of ***Hypsibius*** sp. Photo by Martin Mach, with permission.

Figure 37. Tun of ***Echiniscus*** sp. Photo by Martin Mach, with permission.

Figure 38. Tun of ***Echiniscus*** sp. on moss leaf. Photo by Martin Mach, with permission.

Figure 39. Multiple tuns of ***Echiniscus*** sp. on a single moss leaf. Photo by Martin Mach, with permission.

Figure 40. Tun of *Echiniscus* sp. on moss leaf. Photo by Martin Mach, with permission.

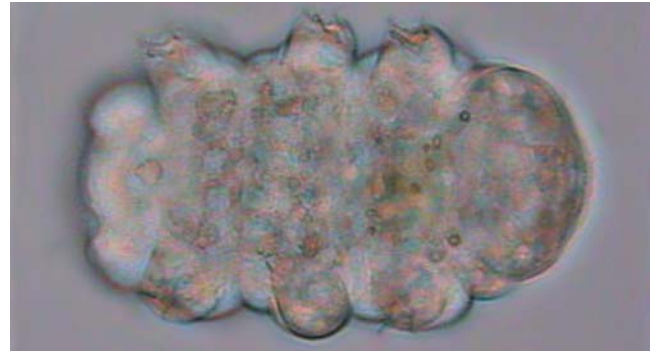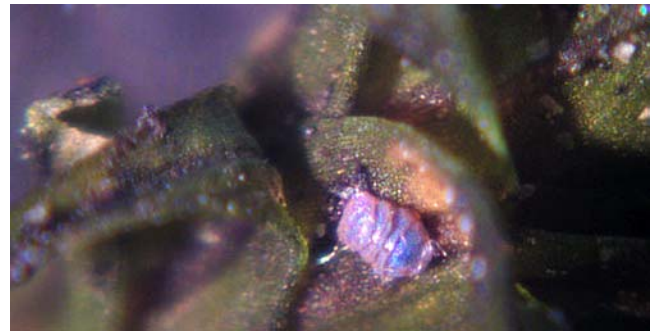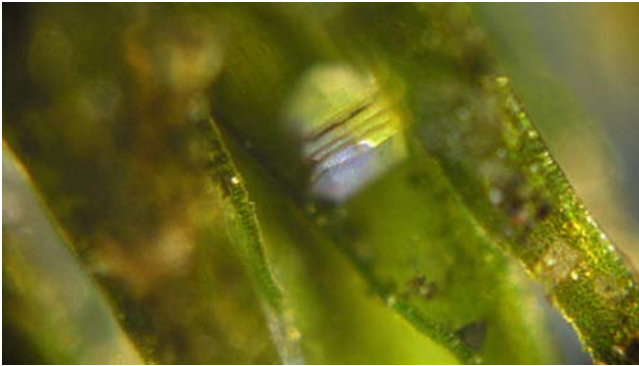Figure 41. Tun of *Echiniscus* sp. on a moss leaf. Photo by Martin Mach, with permission.
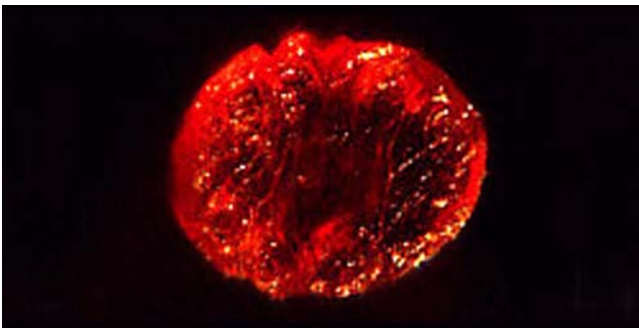

Figure 42. Tun of *Echiniscus* sp. Photo by Martin Mach, with permission.
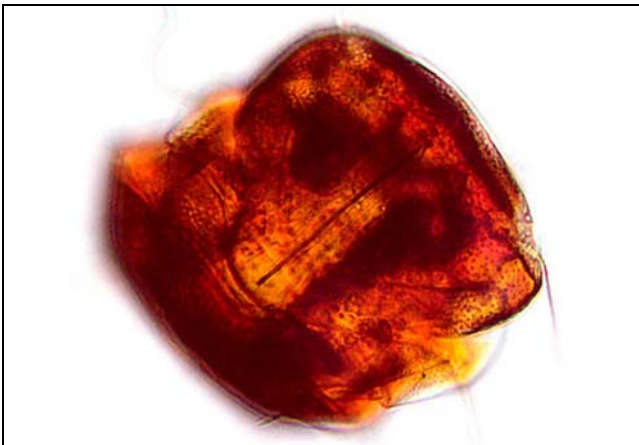

Figure 43. Tun of *Echiniscus* sp. Photo by Martin Mach, with permission.

This tun is a little ball in which the tardigrade can survive 0% relative humidity! However, it only requires a reduction to 70-95% humidity to trigger the **tun** formation,

a resting form in a cryptobiotic state in which the tardigrade appears to be dead (Crowe 1972). During tun formation, loss of free and bound water is greater than 95% (Bertolani *et al.* 2004). The body folds and the appendages are withdrawn (Lindahl & Balser 1999). Wax is extruded onto the surface and most likely reduces water loss (Wright 1988a, b). Those tardigrades with the most variability in the thickness of this **cuticle**, making them more pliable, are those able to have the greatest surface area reduction when they form tuns (Wright 1988a, 1989). The thin areas would permit greater infolding. Lipids of the inner cuticle are thickest in the species that are best able to tolerate rapid drying. Crowe (1972) demonstrated that the cuticle of *Paramacrobiotus areolatus* (Figure 32) is removed from air contact during tun formation and becomes less permeable to water. Crowe likewise suggested that this loss of permeability might be due to a lipid phase change,.

Despite the waxy cuticular protection, the water content is reduced to less than 1% (Lindahl & Balser 1999) and the tun becomes shrivelled and wrinkled (Hingley 1993). *Echiniscus testudo* (Figure 5), an armored tardigrade, has much thicker **dorsal** (back) plates, apparently compensating for its limited ability to reduce surface area as it is drying (Wright 1988a, 1989).

The tardigrade bodies synthesize cell protectants such as **trehalose**, **glycerol**, and **heat shock proteins** that contribute to successful recovery from the tun state (Wright 1989). Trehalose is typically found in high concentrations in animals in a state of cryptobiosis (Crowe & Crowe 1984). Trehalose is able to bond with DPPC and alter the spacing of polar head groups, apparently stabilizing the dry cell membranes. Hengherr *et al.* (2008b) determined that levels of trehalose varied considerably among species. In fact, they detected no changes in trehalose levels in any Heterotardigrada, and *Milnesium tardigradum* (Figure 51) apparently had no trehalose at all. They did demonstrate that tardigrade embryos can accumulate high levels of trehalose, seemingly explaining the high level of desiccation tolerance in that life cycle stage.

Tun formation is essential to tardigrade survival under desiccating conditions. For *Paramacrobiotus areolatus* (Figure 44), and probably most tardigrades, if the humidity is low (<70%) or **anoxic** (lacking oxygen) during its desiccation, it is unable to form a tun and cannot be revived (Crowe 1972). It must have sufficient energy (requiring oxygen), hydration, and time to enter the tun stage.
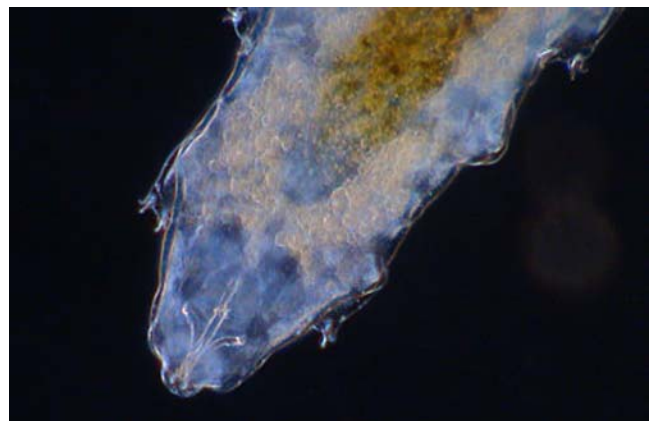

Figure 44. *Paramacrobiotus areolatus*. Photo by Martin Mach, with permission.

Tardigrades revive (Figure 45) almost as quickly as a moss when water returns (Crowe & Higgins 1967), in as little as 4 minutes (Hingley 1993), or several hours, depending on how long they have been dehydrated (Lindahl & Balser 1999). One marine tardigrade has been induced to alternate between a cryptobiotic state and activity on a 6-hour cycle.
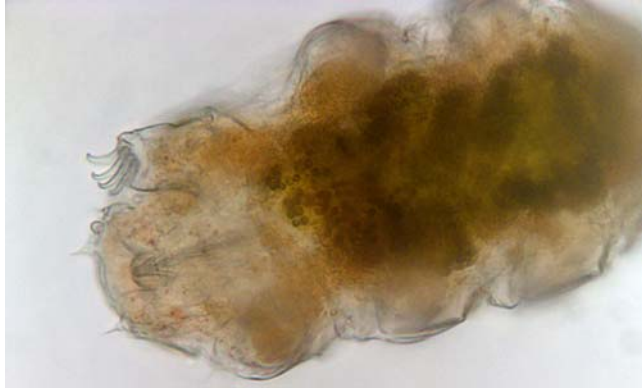

Figure 45. *Echiniscus* sp. rehydrated after four years of desiccation. Photo by Martin Mach, with permission.

Dangers in a Tun

One concern that comes to mind is the possible damage that could happen to these organisms while in the tun stage. I am reminded of the frozen frogs and toads during the winter. They are very susceptible to physical damage if they are disturbed. I would think an animal such as the amphibians hiding under a rock or clump of moss would experience no more physical abuse than the tiny tardigrade among the moss leaves. Ice crystals could poke holes in cells, larger animals could eat them, or they could get knocked off into a hole where conditions were not favorable to their maintenance and survival. I have to wonder just what dangers these dormant organisms do face, and how many actually survive these in the wild to become once again active. It seems we currently have no idea.

Certain dangers include cell degradation and DNA damage. As the tardigrades exist longer and longer, they accumulate cell degradation and DNA damage (Rebecchi *et al*. 2009b), ultimately accumulating too much for successful repair. Hence, the tun does not completely protect them, and chances of survival decrease with time.

Effects of Size

Jönsson *et al*. (2001) found that size influenced survival of cryptobiotic tardigrade species, but that direction of influence differed among species. The common ***Ramazzottius oberhaeuseri*** (300 μm length; Figure 46) had a much higher survival rate (66%) (Figure 47) than did ***Richtersius coronifer*** (40%) (up to 1 mm length; Figure 48). ***Ramazzottius oberhaeuseri*** (Figure 46) has a high ability to retain water, perhaps with a physiological adaptation to reduce water loss in this smaller organism with a high surface area to volume ratio.

Within ***Richtersius coronifer*** (Figure 48), large individuals were less likely to survive cryptobiosis than medium-sized ones (Figure 49); reproductive state had no effect (Jönsson & Rebecchi 2002). Better energetic

conditions increased survival. Jönsson and Rebecchi suggested that larger organisms had greater energy constraints when entering and leaving anhydrobiosis, decreasing survival rate.

Figure 46. ***Ramazzottius oberhaeuseri***. Photo by Martin Mach, with permission.
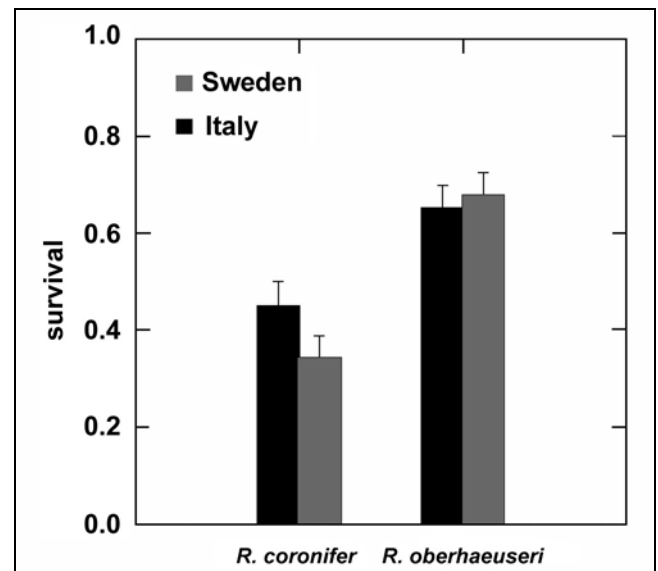

Figure 47. Comparison of survival during encystment for ***Richtersius coronifer*** and ***Ramazzottius oberhaeuseri*** from Italy and Sweden. Vertical line represents standard error. Redrawn from Bertolani et al. 2004, based on Jönsson et al. 2001.
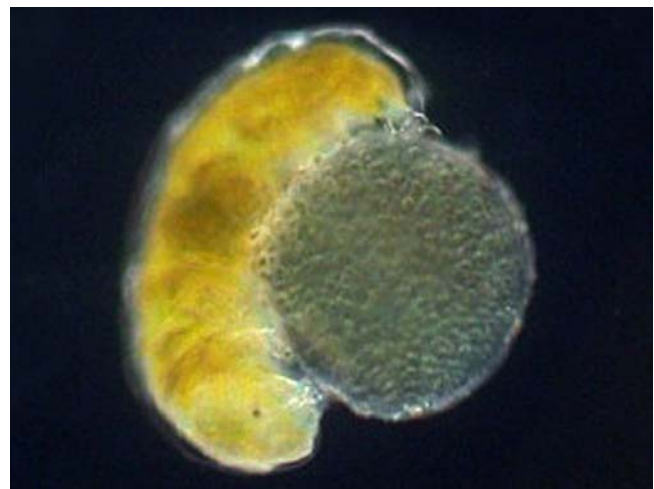

Figure 48. ***Richtersius coronifer***, clinging to an algal cell. Photo by Martin Mach, with permission.
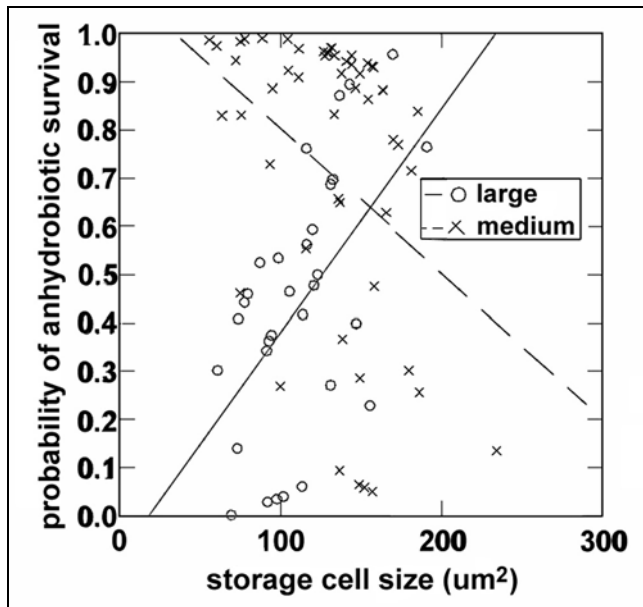
Figure 49.   Probability of survival from anhydrobiosis for large and medium-sized **Richtersius coronifer** as a function of storage cell size. Probability is based on the predicted values from a logistic regression model, using buccal tube length, category, storage cell size, and interaction between the last two categories. Redrawn from Jönsson & Rebecchi 2002, in Bertolani *et al*. 2004.

Jönsson and Rebecchi (2002) likewise found that medium-sized tardigrades had a better chance of survival than did large ones in **Richtersius coronifer** (Figure 48). Large storage cell size was an important parameter to predict greater survival in the large tardigrades (Figure 49).

Reuner *et al*. (2010) described the storage cells as free-floating cells in **Milnesium tardigradum** (Figure 51), **Paramacrobiotus tonollii** (Figure 50), and **Macrobiotus sapiens** that apparently store and release energy as glycogen, protein, and fat. These stores provide energy during cryptobiosis. Storage cell size did not relate to body size, except that the largest tardigrade, **Milnesium tardigradum** (Figure 51), also had the largest storage cells. After seven days of anhydrobiosis (tun stage resulting from desiccation), this species had decreased cell size, but the other two species did not. Food sources used in the study did not seem to affect cell size.
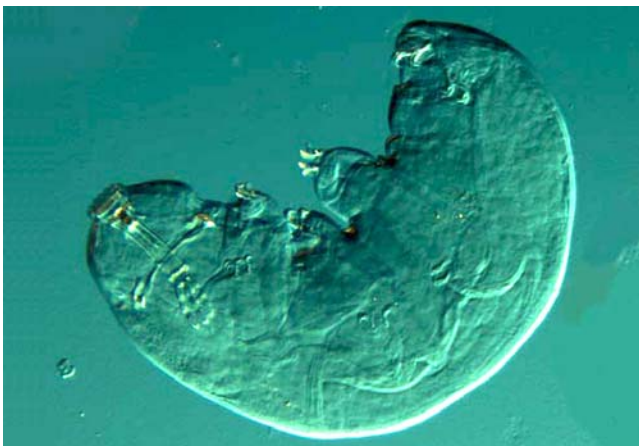

Figure 50. **Paramacrobiotus tonollii**. Photo by P. J. Bartels, with permission.

Figure 51.   **Milnesium tardigradum**, a large tardigrade. Photo by Yuuji Tsukii, with permission.

Longevity

Tardigrades are often credited with century-long survival in a cryptobiotic state. This is due to the report that one herbarium specimen of a moss housed a tardigrade that began cellular activity after 120 years of being dry in the herbarium (Franceschi 1948; Brusca & Brusca 1990; Jönsson & Bertolani 2001)! But, sadly, this record has been called into question, and the tardigrade never fully recovered despite its cellular activity. At the very best, even this faint degree of survival is probably a rare occurrence (see Jönsson & Bertolani 2001). Jönsson and Bertolani (2001) reviewed the evidence and considered that ten years is a more realistic estimate of survival time for tardigrades in a cryptobiotic state.

Rebecchi *et al*. (2008) decided to test this claim of longevity further, using five species of tardigrades from lichens. They collected wet lichens with active tardigrades and permitted them to dry in the ambient conditions of the lab. Among these, **Ramazzottius oberhaeuseri** (Figure 25), **Echiniscus testudo** (Figure 5), and **E. trisetosus** (Figure 18), species that also occur on bryophytes, were sufficiently abundant to permit statistical conclusions. At the beginning of the experiment 91% of **R. oberhaeuseri** and 72% of **Echiniscus** spp. were active. **Ramazzottius oberhaeuseri** (Figure 25) survived up to 1604 days, whereas **Echiniscus** spp. lived only 1085 days. Nevertheless, this may not reflect going into the tun stage under natural conditions, which is likely to be slower among mosses. This could be particularly important for tardigrades that increase trehalose levels.

To test the **longevity** of tuns vs **eggs** under anhydrobiosis, Guidetti and Jönsson (2002) examined 63 different moss samples from stored collections, ranging in anhydrobiotic state 9-138 years. Eggs survived longer than dry adults (tuns), with those of **Ramazzottius oberhaeuseri** (Figure 25) surviving nine years. Much more work is needed to determine what factors account for such differences in survivorship and how it relates to individual species and habitats. The ability to survive unfavorable conditions permits the tardigrades to live in such places as **Grimmia pulvinata** tufts (Figure 52) on house roofs (Corbet & Lan 1974) or among branches of the epiphyte **Orthotrichum cupulatum** (Figure 53) (Jönsson *et al*. 2001).

Figure 52. *Grimmia pulvinata*, a moss that can support tardigrade communities on roofs. Photo by Michael Lüth, with permission.

Figure 53. *Orthotrichum cupulatum*, an acrocarpous moss that provides habitats for tardigrades. Photo by Michael Lüth, with permission.

Like the rotifers, tardigrades suspend their aging clock while they are dormant (Hengherr *et al* 2008a). *Milnesium tardigradum* (Figure 51) that was subjected to alternating periods of drying and activity exhibited similar longevity of active periods to that of animals of the species that had not experienced dry periods.

Ramazzotti and Maucci (1983) estimated that freshwater species such as those of *Hypsibius* (Figure 54) and *Macrobiotus* (Figure 55) live about 1-2 years. Terrestrial bryophyte-inhabiting species of the same genera live much longer, averaging 4-12 years. This extended life is due largely to their periods of cryptobiosis, during which the biological clock stops.
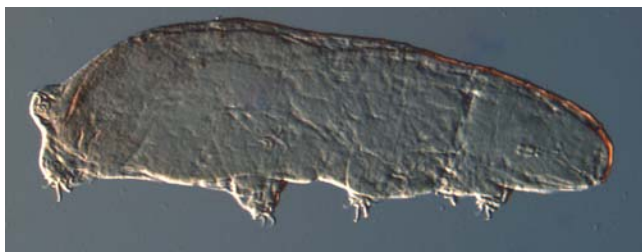

Figure 54. *Hypsibius convergens*, a common bryophyte inhabitant. Photo by Paul Bartels, with permission.

Figure 55. *Macrobiotus marlenae*, a terrestrial species known from mosses on rock. Photo by Martin Mach, with permission.

Dangers and Protective Mechanisms

One contributing factor in their survival of drying is the ability of tardigrades to alter their cell membranes (Brave New Biosphere 1999). They replace the water in the cell membranes with sugar, thus preventing radiation from causing ionization. Like the nematodes and rotifers, some tardigrades prepare for desiccation by producing **disaccharide** sugars, including **trehaloses** (Bordenstein: Tardigrades; Westh & Ramløv 1991). Disaccharides like trehalose and **sucrose**, as well as **glycerol**, are used as membrane protectants by metazoans such as tardigrades, whereas plants typically use **oligosaccharides** such as **stachyose** and **raffinose** (Wright 2001).

This water replacement by sugars also protects invertebrates during freezing because crystallization cannot occur (Brave New Biosphere 1999). The accumulation of trehalose of 0.1-2.3% of dry weight occurred within 5-7 hours during desiccation in *Richtersius coronifer* (Figure 48; Westh & Ramløv 1991). This accumulation was reversed within 6 hours upon rehydration. Both water loss and sugar replacement prevent the rupture of the cell membrane that would result in death. But trehalose has multiple properties that help to stabilize desiccated cells (Table 1).

Table 1. Properties of trehalose that benefit dehydrating cells. From Watanabe 2006.

Non-reducing activity
Low tendency to crystallize
Stable glass formation
High vitrification temperature
High ability of water replacement
Structuring activity of intracellular water with HSP
Stabilization of dry membranes
Antioxidant activity of protein and fatty acids
Free-radical scavenger

Nevertheless, tardigrades accumulate trehalose at the low end of the scale for anhydrobiotic organisms – about 2% (Watanabe 2006). This lower level in tardigrades and absence of trehalose in rotifers is coupled with their ability to enter anhydrobiosis within one hour, whereas organisms with larger accumulations (up to 40%) can take at least two days.

Trehalose is not a cure-all for desiccation effects in tardigrades. High temperatures and high humidity may lead to destruction of trehalose (Rebecchi *et al.* 2009a). In other cases, or in consort, oxidative damage may occur. Using *Paramacrobiotus richtersi* (Figure 17) as an experimental organism, Rebecchi *et al.* demonstrated that DNA changes can occur during desiccation. Neumann *et al.* (2009) likewise demonstrated a slight increase in DNA damage during drying, but they also found that DNA damage increased with duration of anhydrobiosis. Furthermore, high temperatures and relative humidity have negative effects on both survival and time to recover after rehydration, with effects increasing with duration of exposure. One reason for this is that damages are not repaired during anhydrobiosis and therefore accumulate with time.

Anhydrobiosis

The most common of the cryptobiotic states is **anhydrobiosis** (state of dormancy brought on by dehydration). In their state of **anhydrobiosis**, tardigrades can remain inactive during unfavorable conditions such as prolonged dryness (Kinchin 1987b). **Anhydrobiosis** is usually restricted to animals less than 1 mm in length (Watanabe 2006). Hence, some invertebrates are only able to enter this state during early developmental stages. Tardigrades and rotifers, being less than 1 mm when fully developed, are able to do so at any developmental stage.

In order to survive anhydrobiosis, tardigrades must dry very slowly (Hingley 1993; Collins & Bateman 2001). To form the tun, they must retract their head, legs, and hind end, forming a rounded tun, thus reducing surface area. In this state of anabiosis, they are able to withstand extremes of temperature and desiccation. Nevertheless, water arouses them in as little as four minutes.

It appears that continuously hydrated conditions may be detrimental to the survival of tardigrades (Jönsson 2007). Using bryophyte populations from Island Öland, Sweden, Jönsson subjected the tardigrades to two treatments of 6-month duration over an 18-month period. These experimental treatments increased hydration, decreased hydration, or remained as controls. The total population was significantly smaller (barely so) under increased hydration. But effects were not the same for all tardigrades. *Richtersius coronifer* (Figure 48) and *Echiniscus spiniger* failed to respond to the treatment, whereas *Milnesium tardigradum* (Figure 51) declined under increased hydration. But even *Richtersius coronifer* experienced reduction in the density of eggs (Figure 56-Figure 57) under the watering treatment. Hydration did not significantly increase density in any of the tardigrades. This adds further support to the idea that periods of dormancy (cryptobiosis) are necessary to increase longevity of the tardigrade. This would, in turn, increase variability of conditions, offering an array of conditions for reproduction.

Richtersius coronifer (Figure 48) can increase its survival rate by forming **aggregates**, a mechanism barely known for tardigrades but common in nematodes (Ivarsson & Jönsson 2004). The clustering reduces exposed surface area and thus slows drying. It is possible that this is used more in tardigrades than is realized; its use among bryophyte fauna is as yet unknown.

Figure 56. Egg of *Richtersius coronifer*. Photo by Martin Mach, with permission.
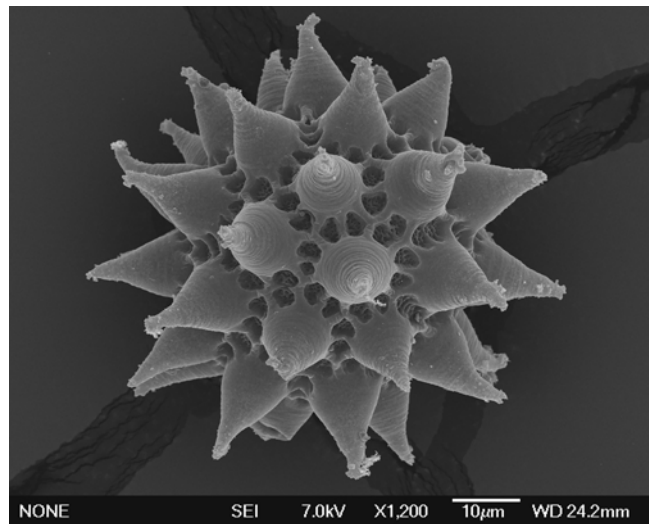

Figure 57. *Macrobiotus magdalenae* egg showing the highly decorated nature that is typical of eggs laid free from the **exuvia** (shed body shells). In this state the organism can survive as well as in a tun. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Schill and Fritz (2008) examined the desiccation tolerance of the embryonic stages of *Milnesium tardigradum* (Figure 58), a potential means of surviving desiccation that has been neglected. They rehydrated this species after treatment at eight different humidity levels (10, 20, 31, 40, 54, 59, 72, 81%). They found that the less developed stages were more susceptible to desiccation damage. In the first three days of development, low humidity caused a decrease in hatching rates following rehydration. Later stages fared better, but when older embryos were dried fast at low humidity levels, development was delayed and hatch rates were lower after rehydration. Nevertheless, older embryos fared better than younger ones.

Even in 2011, Schokriae *et al.* still considered the survival mechanisms of tardigrades to be poorly understood. They considered the possibility that heat shock proteins (HSPs) might protect them from irreversible aggregation and degradation during anhydrobiosis. They found that *Milnesium tardigradum* (Figure 51) had HSPs in several major chaperone families, suggesting the ability

of the tardigrade to use these for protection when dehydrated.

Figure 58. *Milnesium tardigradum*, a bryophyte dweller whose younger stages are the most susceptible to desiccation. Photo by Yuuji Tsukii, with permission.

The cryptobiotic state of anhydrobiosis has a significant impact on the ecological role of the tardigrades. It affects their role in the food chain, their ability to disperse, and their survival through a longer period of time (see reviews by Pilato 1979; Wright *et al.* 1992; Kinchin 1994). Bryophytes often play a significant role in achieving that state.

Osmobiosis

Osmobiosis is a special case of cryptobiosis that permits some species to tolerate high salinity and to form a tun (Lindahl & Balser 1999). It is initiated when the animal experiences an external salt concentration that is higher than that inside the organism. However, for tardigrades, while possible, osmobiosis is typically not necessary as most tardigrades already have a high salt tolerance.

Anoxybiosis

Anoxybiosis is another special case where the tardigrade has the ability to survive low oxygen (Lindahl & Balser 1999). Tardigrades are very sensitive to changes in oxygen tension, and prolonged reduction of oxygen leads to **osmoregulatory** failure.

Anoxybiosis is not a true state of cryptobiosis and does not involve tun formation (Figure 59). Unlike true cryptobiosis, anoxybiosis involves the uptake of water. The lack of oxygen results in the inability to control osmosis, causing water to enter the cells in excess. The animals become turgid, immobile, and retain fully extended bodies that are perfectly bilaterally symmetrical (Figure 60). Even animals in a molt can enter anoxybiosis (Figure 61).

Figure 59. *Macrobiotus hufelandi* male in anoxybiotic state, showing lack of tun formation. Photo by Martin Mach, with permission.
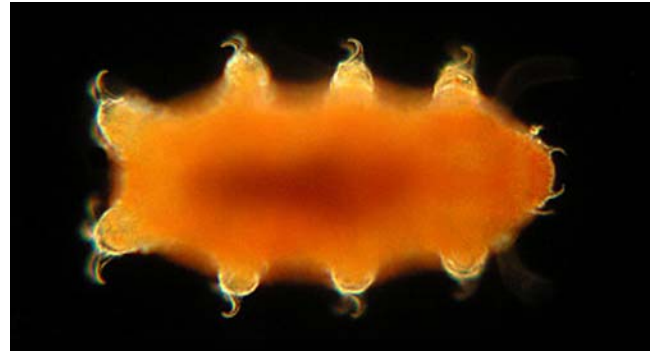
Figure 60. Tardigrade showing **anoxybiosis**, where water has entered through the cuticle by osmosis and caused swelling and turgidity. Note the extended legs and perfectly symmetrical body. The animal cannot move in this state. Photo by Martin Mach, with permission.

Figure 61. Tardigrade induced into **anoxybiosis** during its molt. Photo by Martin Mach, with permission.

Revival to normal state (Figure 62) relates to the duration of the dormant state. However, the success of that recovery is controversial (Wright *et al.* 1992), with some researchers finding that they can survive for only 3-4 days (Crowe 1975) and others finding survival of *Echiniscoides* (a tidal zone genus) up to six months in closed vials (Kristensen & Hallas 1980).

Figure 62. This tardigrade was caught by low oxygen during molt and entered **anoxybiosis**. Here it has recovered and is moving within the swollen cuticle to complete its molt. Photo by Martin Mach, with permission.

Cryobiosis

Cryobiosis is another special case of cryptobiosis that results when the temperature decreases and the water in the cells has frozen (Wikipedia: Cryptobiosis 2009). Molecular mobility stops (Wikipedia: Cryptobiosis 2009), permitting the tardigrades to survive very low temperatures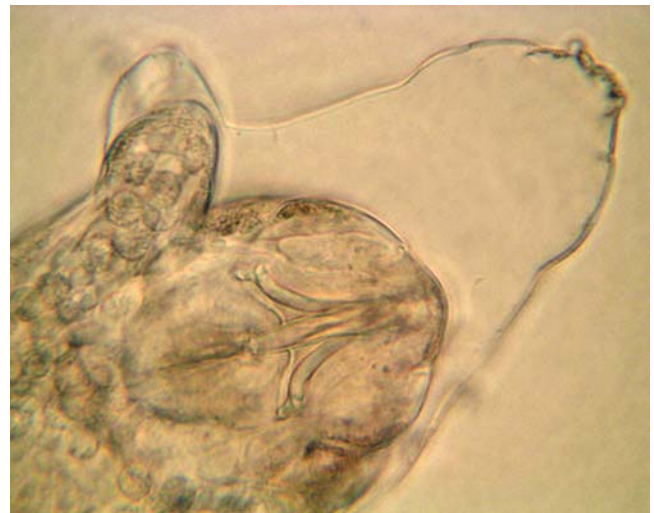 (Westh *et al.* 1991; Westh & Kristensen 1992; Ramløv & Westh 1992; Sømme 1996; McInnes & Pugh 1998). They do this by actually freezing, but the freezing is ordered (Lindahl & Balser 1999) and the result once again is a tun.

Cryobiosis in tardigrades differs from anhydrobiosis (Wright 2001). First of all, tun formation in not necessary for survival. The important process is that intracellular freezing must be avoided. But tardigrades, unlike many arthropods, are freeze-tolerant. This can be accomplished by **ice-nucleating proteins** that are located outside the cells. These proteins act like water magnets, drawing water out of the cell. Also unlike many other arthropods, the extracellular freezing, promoted by the ice-nucleating proteins, occurs at temperatures near 0°C. In *Richtersius coronifer* (Figure 48), **trehalose** synthesis is not part of this process, although there may be other cryoprotectants in the cell. The details of freeze protection are not fully understood, but the loss of water from the cells may cause the cells to become "unfreezable." Glycerol contributes as an antioxidant. Being imbedded in ice probably also prevents oxidation damage. In this cryobiotic state, the tardigrades can survive for decades.

Tardigrades often experience wide temperature fluctuations while in an active state. In particular, they can be subjected to subzero temperatures. Their ability to tolerate these sub-zero conditions requires either tolerance of freezing body water or having a mechanism to lower the freezing point. Hengherr *et al.* (2009) subjected nine species from polar, temperate, and tropical regions to cooling by 9, 7, 5, 3, and 1°C h^{-1} down to -30°C, then returning them to ambient temperature at a rise of 10°C h^{-1}. Survival was better at fast and slow cooling rates, with low survival rates at intermediate cooling rates. Hengherr *et al.* suggested that this relationship may indicate a physical effect during fast cooling and possible synthesis of **cryoprotectants** during slow cooling. The increased survival with slower cooling indicates that tardigrades protect their cellular structure from freezing injury without altering their freezing temperature.

As indicated above, at least some protection seems to be accomplished by using **ice-nucleating proteins** in the body fluids (Westh *et al.* 1991). Such proteins serve as centers for crystal formation, a technique used to make snow for ski hills. This cryoprotective mechanism permits tardigrades to survive rapid freezing and thawing cycles such as those experienced in the Arctic and Antarctic. Usually this type of protection means that the nucleating centers are small, permitting only small crystals to form, consequently reducing damage to the cell membranes.

The ice-nucleating activity in the body fluid from *Richtersius coronifer* (Figure 48) is reduced by 50% following *ca* 7x10^3 times dilution (Westh *et al.* 1991). Heating to temperatures above 68°C induces an abrupt decrease in the activity, suggesting that the nucleators are proteinaceous.

Westh and Kristensen (1992) examined *Richtersius coronifer* (Figure 48) and *Bertolanius* [=*Amphibolus*]

nebulosus (see Figure 63) and compared their cryoprotective strategies. *Richtersius coronifer* (Figure 48) lives in drought-resistant mosses and overwinters in a frozen or dry state (cryptobiosis). *Bertolanius nebulosus*, on the other hand, lives among moist mosses and algae and spends its winter frozen in a cyst or as eggs. Both species can supercool to as low as -7°C. But these two species have distinctly different heat stability, resulting from differences in ice-nucleating proteins. In both cases, ice formation is rapid, but crystallization most likely stops within a minute of nucleation. This protects the cells from damage caused by large, sharp crystals. Nevertheless, ice constitutes 80-90% of the body water. Winter acclimatization of *R. coronifer* results in a 10% lower ice formation than summer acclimatization. The thaw point was unaffected by winter vs summer, suggesting that there is no accumulation of low molecular weight cryoprotective substances.
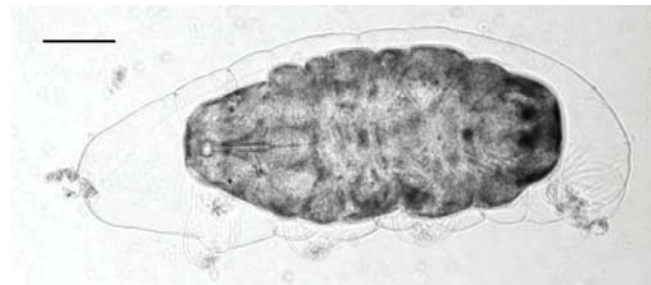
Figure 63. *Bertolanius volubilis* type A cyst. Photo by Roberto Bertolani from Bertolani *et al.* 2004, with permission.

Despite their seeming indestructibility, not all tardigrade individuals fare well at low temperatures, and some species fare better than others. Bertolani *et al.* (2004) demonstrated this for three species of tardigrades (Figure 64). *Ramazzottius oberhaeuseri* (Figure 25) seems to be almost indestructible down to -80°C, whereas *Hypsibius dujardini* (Figure 16) had only 20% survival at that temperature. In fact, it had less than 80% survival at -9°C.
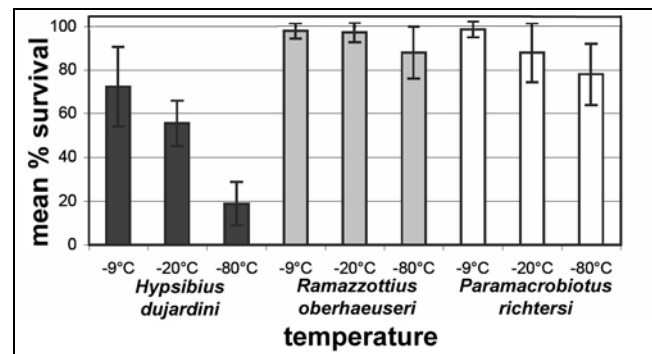
Figure 64. Comparison of survival of three bryophyte-dwelling tardigrades subjected to sub-zero temperatures. Redrawn from Bertolani *et al.* 2004.

Holmstrup *et al.* (2002) considered the problem of low temperatures in soil environments. Most of the work had been done on insects, but tardigrades are ideal for this kind of study. Insects often have the ability to supercool and to retain water in a dry environment. But non-insect soil invertebrates have resistance to desiccation that is orders of

magnitude less than that of terrestrial insects. This suggests that the other invertebrates may use an entirely different mechanism for surviving desiccation and cooling. Dehydration results because the water vapor pressure of supercooled water is higher than that of ice under the same temperature. A drop in temperature of even a few degrees of supercooling will cause considerable water loss. This loss continues until the vapor pressure of body fluids and that of surrounding ice are equal. When this is achieved, there is no longer any danger of ice formation in the tissues, permitting the invertebrate to survive at subzero temperatures. Holmstrup *et al.* (2002) showed that soil invertebrates do not use supercooling to survive. Instead, they dehydrate, changing their body-fluid melting point to that of the ambient temperature. This mechanism works even in the extreme cooling conditions and rapid rates seen in the polar soils.

Tardigrades live in many habitats that experience such cold temperatures. The widely fluctuating temperatures of the polar regions subject them to extremes while they are still in the active state. Thus they must either have the ability to tolerate the freezing of their body tissues or a means to lower their tissue freezing point (Hengherr *et al.* 2009).

Temperature

Rebecchi *et al.* (2009) pointed out that most stress studies on tardigrades had involved terrestrial tardigrades. Hence, they examined the limnic boreo-alpine species ***Borealibius zetlandicus*** for its stress responses. This species is able to survive freezing in lab experiments by entering a cryptobiotic state. Faster cooling rates lead to greater death from freezing. It furthermore is able to survive relatively "hot" water, having an experimental heat-shock LT50 of 33.0 ± 0.5°C. On the other hand, no members of this species were able to survive desiccation in the experiments. It is likely that in its natural habitat it is able to survive drying conditions by behavior – finding locations among moss leaves or in other plants or in muds where it does not dry as completely.

Ramløv and Westh (2001) studied ***Richtersius coronifer*** (Figure 48) and found that when this species enters anhydrobiosis it accumulates the disaccharide **trehalose**, with the maximum content of 2.3% of the dry weight. This species was able to survive temperatures up to 70°C in a moss cushion for an hour with no effect on survival rate. However, above that temperature, survival decreased rapidly, with no survival at 100°C. Ramaløv and Westh suggested that heat shock proteins may be involved in the high temperature survival.

Diapause (Encystment)

Tardigrades are especially endowed with the physiological ability to survive. They are among the few organisms that can use both **anhydrobiosis** and **diapause** (**encystment**) as a means of dormancy to survive unfavorable conditions (Guidetti *et al.* 2008). Diapause is common among aquatic tardigrades, but there are some terrestrial species that experience diapause (Westh & Kristensen 1992; Nelson 2002). Whereas cryptobiosis is well studied, the role of diapause (encystment) is not well known in tardigrades. It appears that it is not an essential part of the life cycle – only a means to survive some unfavorable conditions.

Węglarska (1957) found that ***Dactylobiotus dispar*** (Figure 65-Figure 68) was induced to encyst by environmental conditions that gradually became worse. Interestingly, when there was a rapid change to poor conditions, this tardigrade went into **anoxybiosis**. When a tardigrade is about to encyst, it ingests large amounts of food that is stored in the body cavity cells (Nelson 1991a). The remaining material in the gut is defecated.

Figure 65. ***Dactylobiotus*** sp. Photo by Yuuji Tsukii, with permission.

Encystment is more complex than **tun** formation (Bertolani *et al.* 2004). The **cysts** are ovoid and are composed of a series of cuticles that surround the sleeping animal (Figure 69; Guidetti *et al.* 2006). They are described as resembling an onion or a Matrioshka Russian doll.
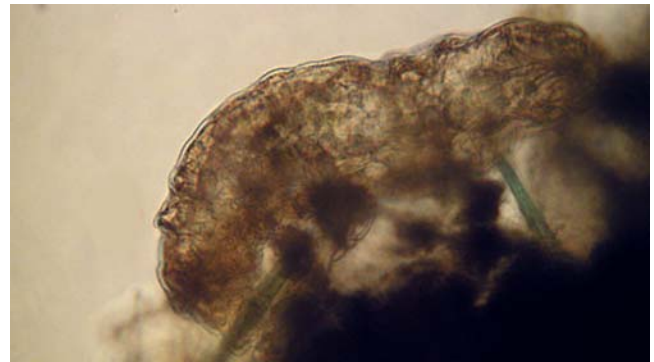

Figure 66. ***Dactylobiotus dispar***. Photo by Martin Mach, with permission.

Figure 67. Eggs of ***Dactylobiotus dispar***. Photo by Martin Mach, with permission.

During encystment, new cuticular structures are synthesized (Guidetti *et al.* 2006). Encystment starts with the discharge of the sclerified portions of the buccal-pharyngeal apparatus without the loss of cuticle. Rather, they produce two or three new cuticles. In *Bertolanius* [=*Amphibolus*] *volubilis* (Figure 69), the new cuticle is similar to that found on the non-encysted organisms, whereas in *Dactylobiotus parthenogeneticus* (Figure 70-Figure 72) the ultrastructure of the new cuticle differs. The tardigrade retracts within the cuticle (Nelson 1991a).
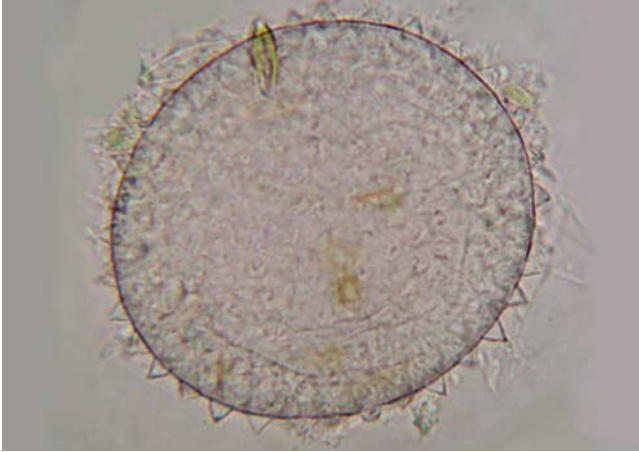
Figure 68. Egg of *Dactylobiotus dispar*. Photo by Martin Mach, with permission.

Tardigrade encystment is known for only a few species, although it may be more widespread than is currently known. There are at least three types of cysts (Guidetti *et al.* 2006). *Bertolanius volubilis* has two types (Figure 69); *Dactylobiotus parthenogeneticus* (Figure 70-Figure 72) exhibits only one. Having two types of cysts in the same species seems to be a terrestrial character (Bertolani *et al.* 2004). Type 2 cysts have an additional layer of cuticle compared to type 1 cysts. Although only a few species have been described, it appears that a type 1 cyst never shows a modified buccal-pharyngeal apparatus, whereas a type 2 cyst does.
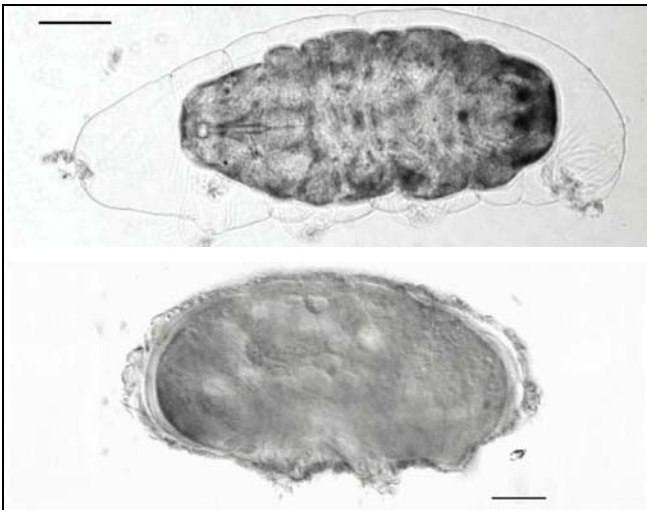
Figure 69. **Upper:** Type 1 cyst. **Lower:** Type 2 cyst (surrounded by several layers of cuticle), both of *Bertolanius volubilis*. Photos by Roberto Bertolani in Bertolani *et al.* 2004, reproduced with permission.

Figure 70. *Dactylobiotus* sp., a member of a genus with only one type of diapause. Photo by Yuuji Tsukii, with permission.

Figure 71. *Dactylobiotus* sp., a tardigrade with only one type of diapause cyst. Photo by Martin Mach, with permission.
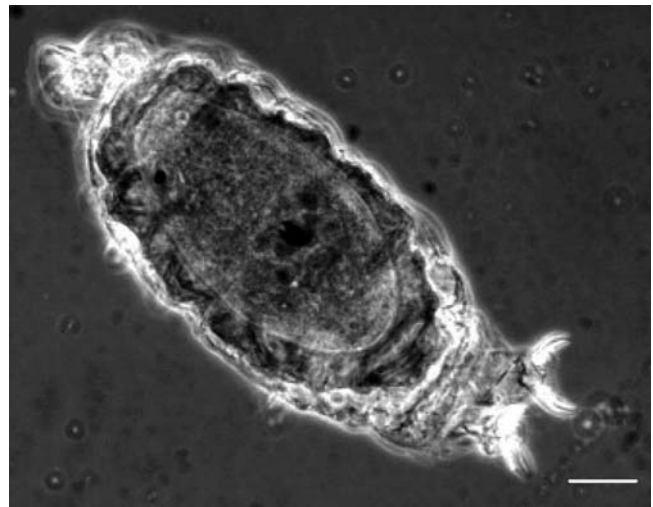
Figure 72. *Dactylobiotus* sp. cyst. Photo by Roberto Bertolani in Bertolani *et al.* 2004, reproduced with permission.

Conditions that cause emergence from the cysts are not understood. Unlike those in an anhydrobiotic state, the encysted tardigrades are not drought-resistant. Nor can they withstand high temperatures, because they have continuous water content. Nevertheless, the cysts can survive in nature for more than a year on their food reserves (Westh & Kristensen 1992).

Using the tardigrade **Bertolanius volubilis** (Figure 69) from the mosses **Racomitrium sudeticum** (Figure 73) and **R. elongatum** (Figure 74) on sandstone in the Northern Apennines of Italy, Guidetti *et al*. (2008) examined the factors involved in the inducement of diapause. They learned that in **B. volubilis** the type of diapause cysts produced in April differed from those produced in November. The April cysts are produced during a warm season, whereas the other type is present during the cold season. Temperature is responsible for induction, maintenance, and termination of the cyst. Both exogenous (temperature) and endogenous (physiological) factors serve as stimuli.

Figure 73. **Racomitrium sudeticum**, where **Bertolanius volubilis** in the Northern Apennines of Italy undergoes diapause, forming spring cysts that differ from winter cysts. Photo by Michael Lüth, with permission.

Figure 74. **Racomitrium elongatum**, a moss habitat in the Northern Apennines of Italy where **Bertolanius volubilis** makes different cysts in spring and winter. Photo by Michael Lüth, with permission.

Eggs

Eggs that are laid externally are typically ornamented (Figure 75-Figure 76) (Nelson 1991a). These may be laid singly or in groups.

Figure 75. Egg of a tardigrade, a stage that helps it survive desiccation. Photo by Martin Mach, with permission.

As already noted, eggs can provide a long-lasting escape from unfavorable conditions. At least some tardigrades can produce both **subitaneous** (non-resting) and **resting eggs** (Bertolani *et al*. 2004). Altiero *et al*. (2009) examined the eggs of **Paramacrobiotus richtersi** (Figure 17) and found that the percentage of hatching was high (75-93%), but that four different patterns were discernible. Subitaneous eggs hatched in 30-40 days. Delayed hatching eggs hatched in 41-62 days. Some eggs required 90 days or more if the culture was wet and 13% of these (**diapause resting eggs**) required a dry period followed by rehydration. The remainder (87% of this last >90-day category) never hatched. They considered this variable hatching time to be a form of **bet-hedging**.
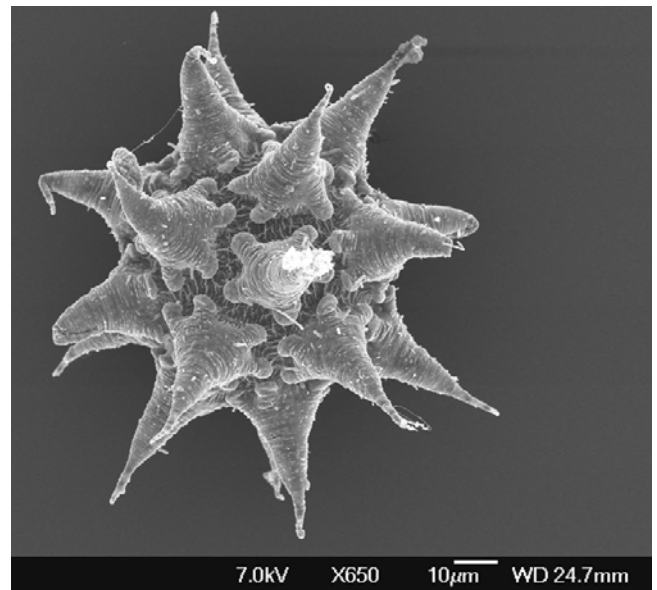

Figure 76. **Macrobiotus szeptyckii** egg showing the highly decorated surface of eggs laid free from the exuvia. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Migration?

Anhydrobiosis is not the only strategy available to organisms to escape drying conditions. Some organisms migrate to deeper levels of the moss or soil to escape

drought. However, it appears that this option might not be available to many of the slow-moving tardigrades.

Wright (1991) found that those tardigrades living in the interstices of the moss habitat migrate vertically to the **soil C-zone** (layer just above bedrock) as a means of escaping or slowing desiccation. The exception to this behavior is *Echiniscus testudo* (Figure 5).

Nelson and Adkins (2001) examined this depth relationship in cushions of the moss *Schistidium rivulare* (=*Grimmia alpicola;* Figure 77)*.* They found that among five species, only one (*Echiniscus viridissimus*) was more frequent in the top layer, regardless of the wet or dry condition of the moss. (Hmmm... Could the green that gives it its name indicate it has a photosynthetic symbiont that requires light, or just a penchant for green food?)

Nelson and Adkins (2001) concluded that none of the *Schistidium* (Figure 77) inhabitants used migration as a means to escape reduction in moisture. They speculated that for tardigrade inhabitants of xeric mosses, there was no advantage to migration. Rather, they stayed put and went into a state of anhydrobiosis in both upper and lower layers.

Figure 77. *Schistidium rivulare*, a moss where excessive hydration can cause death to its tardigrade inhabitants. Photo by Michael Lüth, with permission.

Summary

Tardigrades (water bears) are common in both aquatic and terrestrial bryophytes. The land dwellers require a water film and thus are called **limnoterrestrial** tardigrades. Despite their worldwide distribution, they are not well known.

The bryophyte habitat offers sufficient oxygen, wetting and drying, sufficient food, a dispersal vehicle, and protection. Moisture is probably the most important factor in their distribution. Species of bryophytes do not seem to affect the types of tardigrades species.

Tardigrades are adapted to the bryophyte habitat by their small size, stylets that permit sucking contents from bryophyte cells, flexible bodies, and a very responsive life cycle. Colored **pigments** in some may offer UV protection, especially during dry periods. Tardigrades can **encyst** or go into a **cryptobiotic** state as a **tun**. Cysts may differ between summer and winter. Tardigrades must dry slowly to survive the cryptobiotic state. While in it, they are resistant to high and low temperature extremes, absence of water, extreme pressure, vacuum, and radiation. **Anhydrobiosis** is induced by diminishing hydration; **cryobiosis** is induced by low temperatures near 0ºC; **osmobiosis** is induced by a change in salinity; **anoxybiosis** is induced by low oxygen. Tardigrades form **trehaloses** that protect the cell membranes while dehydrated or at low temperatures. They typically can survive about 10 years in the tun, but one specimen resumed physiological activity after 120 years on a herbarium moss specimen, then died. Nevertheless, **DNA damage** accumulates during cryptobiosis; survival seems to be based on **DNA repair**. Furthermore, high temperatures and high humidity destroy trehalose.

Another means of long-term survival is by producing resistant eggs. Variable hatching times may provide a form of **bet-hedging** in some species.

Literature Cited

Adkins, R. and Nelson, D. R. 1996. Tardigrade distribution within a moss cushion. [Abstract]. Bull. Assoc. S. E. Biol. 43: 147.

Altiero, T. and Rebecchi, L. 2001. Rearing tardigrades: Results and problems. Zool. Anz. 240: 217-221.

Altiero, T., Bertolani, R., and Rebecchi, L. 2009. Hatching phenology and resting eggs in *Paramacrobiotus richtersi* (Eutardigrada, Macrobiotidae). J. Zool. (in press).

Bartels, P., Pilato, G., Lisi, O., and Nelson, D. R. 2009. *Macrobiotus* (Eutardigrada, Macrobiotidae) from the Great Smoky Mountains National Park, Tennessee/North Carolina, USA (North America): Two new species and six new records. Zootaxa 2022: 45-57.

Beasley, C. W. 2001. Photokinesis of *Macrobiotus hufelandi* (Tardigrada, Eutardigrada). Zool. Anz. 240: 233-236.

Bertolani, R. 1983. Tardigardi muscicoli delle dune costiere Italiane, con descrizione di una nuova specie. Atti Soc. Tosc. Sci. Nat. Mem., Ser. B 90: 139-148.

Bertolani, R. 2001. Evolution of the reproductive mechanisms in tardigrades – a review. Zool. Anz. 240: 247-252.

Bertolani, R. and Biserov, V. I. 1996. Leg and claw adaptations in soil tardigrades, with erection of two new genera of Eutardigrada, Macrobiotidae: *Pseudohexapodibus* and *Xerobiotus*. Invert. Biol. 115: 299-304.

Bertolani, R. and Rebecchi, L. 1996. The tardigrades of Emilia (Italy). II. Monte Rondinaio. A multihabitat study on a high altitude valley of the northern Apennines. Zool. J. Linn. Soc. 116: 3-12.

Bertolani, R., Guidetti, R., Jönsson, K. I., Altiero, T., Boschini, D., and Rebecchi, L. 2004. Experiences on dormancy in tardigrades. J. Limnol. 63: 16-25.

Bertolani, R., Rebecchi, L., and Beccaccioli, G. 1990. Dispersal of *Ramazzottius* and other tardigrades in relation to type of reproduction. Invert. Repro. Devel. Rehovot 18(3): 153-157.

Binda, M. G. 1984. Notizie sui tardigradi dell'Africa Meridionale con descrizione di una nuova specie di *Apodibius* (Eutardigrada). [Remarks on some species of tardigrades from Southern Africa and description of *Apodibius nuntius* n. sp.]. Animalia 11(1-3): 5-15.

Boeckner, M., Collins, M., Finney-Crawley, J., and Bateman, L. 2006. The bryofauna of remote coastal Labrador: Including a review of current Canadian records. Zootaxa 1105: 1-16.

Bonifacio, A., Sergo, V., Guidett, R., Altiero, T., and Rebecchi, L. 2012. Origin, nature and function of pigments in tardigrades: a label-free Ramam imaging study on a living multicellular organism. Accessed 20 May 2012 at <http://www.cognicao.pt/AbstractsECSBM/index_arquivos/089_Bonifacio.pdf>.

Bordenstein, Sarah. 2008. Tardigrades (Water Bears), Microbial Life, Educational Resources. Accessed on 9 February 2008 at <http://serc.carleton.edu/microbelife/topics/tardigrade/>.

Brave New Biosphere. 1999. The Why Files. University of Wisconsin. Accessed on 7 January 2008 at <http://whyfiles.org/022critters/meiofauna.html>.

Bruegmann, L. 1977. Zur Verteilung einiger Schwermetalle in der Ostsee – eine Uebersicht. [The distribution of heavy metals in the Baltic – a review.]. Acta Hydrochem. Hydrobiol. 5: 3-21.

Brusca, R. C. and Brusca, G. J. 1990. Invertebrates. Sinauer Associates, Sunderland, Massachusetts.

Byron, E. R. 1982. The adaptive significance of calanoid copepod pigmentation: A comparative and experimental analysis. Ecology 63: 1871-1886.

Claps, M. C. and Rossi, G. C. 1984. Contribucion al conocimiento de los tardigrados de Argentina. 4. [Contribution to the knowledge of tardigrades from Argentina. 4.]. Acta Zool. Lilloana 38(1): 45-50.

Collins, M. and Bateman, L. 2001. The ecological distribution of tardigrades in Newfoundland. Zool. Anz. 240: 291-297.

Corbet, S. A. and Lan, O. B. 1974. Moss on a roof and what lives in it. J. Biol. Ed. 5: 153-160.

Crowe, J. H. 1972. Evaporative water loss by tardigrades under controlled relative humidities. Biol. Bull. 142: 407-416.

Crowe, J. H. 1975. The physiology of cryptobiosis in tardigrades. Memorie dell' Istituto Italiano di Idrobiologia 1975: 37-59.

Crowe, J. H. and Crowe, L. M. 1984. Preservation of membranes in anhydrobiotic organisms: The role of trehalose. Science 223: 701-703.

Crowe, J. H. and Higgins, R. P. 1967. The revival of *Macrobiotus areolatus* Murray (Tardigrada) from the cryptobiotic state. Trans. Amer. Microsc. Soc. 86: 286-294.

Franceschi, T. 1948. Anabiosi nei tardigdi. Boll. Mus. Ist. Biol. Univ. Genova 22: 47-49.

Franceschi, T., Loi, M. L., and Pierantoni, R. 1962-1963. Risultati di una prima indagine ecologica condotta su popolazioni di Tardigradi. Bollettino dei Musei e degli Istituti Biologici dell'Universita di Genova 32: 69-93.

Garey, J., Krotec, M., Nelson, D., and Brooks, J. 1996. Molecular analysis supports a tardigrade - arthropod association. Invert. Biol. 115: 79-88.

Garey, J. R., McInnes, S. J., and Nichols, P. B. 2008. Global diversity of tardigrades (Tardigrada) in freshwater. Develop. Hydrobiol. 198: 101-106.

Garey, J., Nelson, D., Mackey, L. and Li., J. 1999. Tardigrade phylogeny: Congruency of morphological and molecular evidence. In: Greven, H. (ed.). Proceedings of the Seventh International Symposium on the Tardigrada, August 1997, Düsseldorf, Germany. Zool. Anz. 238: 205-210.

Giribet, G., Carranza, S., Baguñá, J., Riutort, M., and Ribera, C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Molec. Biol. Evol. 13: 76-84.

Greven, H. 1980. Die Bärtierchen. In: Die Neue Brehm Bücherei 537: 1-101. Ziemsen-Verlag, Wittenberg Lutherstadt.

Greven, H. and Schüttler, L. 2001. How to crawl and dehydrate on moss. Zool. Anz. 240: 341-344.

Goeze, J. A. E. 1773. Herrn Karl Bonnets Abhandlungen aus der Insektologie aus d. Franz. übers. u. mit einigen Zusätzen hrsg. v. Joh. August Ephraim Goeze Gebauer, Halle.

Guidetti, R. and Bertolani, R. 2005. Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. Zootaxa 845: 1-46.

Guidetti, R. and Jönsson, K. I. 2002. Long-term anhydrobiotic survival in semi-terrestrial micrometazoans. J. Zool. 257: 181-187.

Guidetti, R., Boschini, D., Altiero, T., Bertolani, R., and Rebecchi, L. 2008. Diapause in tardigrades: A study of factors involved in encystment. J. Exper. Biol. 211: 2296-2302.

Guidetti, R., Boschini, D., Rebecchi, L., and Bertolani, R. 2006. Encystment processes and the "Matrioshka-like stage" in a moss-dwelling and in a limnic species of eutardigrades (Tardigrada). Hydrobiologia 558: 9-21.

Guil, N., Hortal, J., Sánchez-Moreno, S., and Machordom, A. 2009. Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment. Landscape Ecol. 24: 375-390.

Hallas, T. E. 1975. Interstitial water and Tardigrada in a moss cushion. Ann. Zool. Fenn. 12: 255-259.

Hebert, Paul D. N. 2008. Tardigrada. The Encyclopedia of Earth. Accessed on 27 January 2010 at <http://www.eoearth.org/article/Tardigrada>.

Hengherr, S., Brummer, F., and Schill, R. O. 2008a. Anhydrobiosis in tardigrades and its effects on longevity traits. J. Zool. 275: 216-220.

Hengherr, S., Heyer, A. G., Köhler, H.-R., and Schill, R. O. 2008b. Trehalose and anhydrobiosis in tardigrades – evidence for divergence in responses to dehydration. FEBS J. 275: 281-288.

Hengherr, S., Worland, M. R., Reuner, A., Brümmer, F., and Schill, R. O. 2009. Freeze tolerance, supercooling points

and ice formation: Comparative studies on the subzero temperature survival of limno-terrestrial tardigrades. J. Exper. Biol. 212: 802-807.

Hidalgo, H. and Coombs, D. 1985. Tardigrada from Missouri. Trans. Kansas Acad. Sci. 88: 121-134.

Hingley, M. 1993. Microscopic Life in *Sphagnum*. Illustrated by Hayward, P. and Herrett, D. Naturalists' Handbook 20. [i-iv]. Richmond Publishing Co. Ltd., Slough, England, 64 pp. 58 fig. 8 pl. (unpaginated).

Hofman, I. 1987. Habitat preference of the most frequent moss-living Tardigrada in the area of Giessen (Hessen). In: Bertolani, R. (ed.). Biology of Tardigrades. Selected Symposia and Monographs U. Z. I., Mucchi, Modena Italia, pp. 211-216.

Hofmann, I. and Eichelberg, D. 1987. Faunistisch-oekologische Untersuchungen zur Habitat-praeferenz moosbewohnender Tardigraden. [Ecological investigations of the habitat preference of moss-inhabiting tardigrades.]. Zool. Beitr. 31(1): 61-76.

Holmstrup, M., Bayley, M., and Ramløv, H. 2002. Supercool or dehydrate? An experimental analysis of overwintering strategies in small permeable arctic invertebrates. Proc. Natl. Acad. Sci. 99: 5716-5720.

Hooie, Amanda. 2005. Tardigrade diversity in the moss *Ulota crispa* from tree canopies of the Great Smoky Mountains National Park. Accessed on 14 May 2005 at <http://www2.una.edu/pdavis/Tardigradepres_files/frame.htm>.

Hooie, A. K. and Davison, P. G. 2001. Tardigrade diversity in the moss *Ulota crispa* from tree canopies in the Great Smoky Mountains National Park - A preliminary report. Abstract. J. Alabama Acad. Sci. 72: 91.

Ivarsson, J. and Jönsson, K. I. 2004. Aggregation effects on anhydrobiotic survival in the tardigrade *Richtersius coronifer*. J. Exper. Zool. 301A: 195-199.

Jerez Jaimes, J. H., Narváez, E. X., and Restrepo, R. 2002. Tardígrados en musgos de la Reserva el Diviso (Santander, Colombia). Revista Colombiana de Entomología 28(2): 199-206.

Jönsson, K. I. 2003. Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography 26: 356-364.

Jönsson, K. I. 2007. Long-term experimental manipulation of moisture conditions and its impact on moss-living tardigrades. J. Limnol. 66(Suppl. 1): 119-125.

Jönsson, K. I. and Bertolani, R. 2001. Facts and fiction about long-term survival in tardigrades. J. Zool. 255: 121-124.

Jönsson, K. I. and Rebecchi, L. 2002. Experimentally induced anhydrobiosis in the tardigrade *Richtersius coronifer*: Phenotypic factors affecting survival. J. Exper. Zool. 293: 578-584.

Jönsson, K. I., Borsari, S., and Rebecchi, L. 2001. Anhydrobiotic survival in populations of the tardigrades *Richtersius coronifer* and *Ramazzottius oberhaeuseri* from Italy and Sweden. Zool. Anz. 240: 419-423.

Jönsson, K. I., Rabbow, E., Schill, R. O., Harms-Ringdahl, M., and Petra Rettberg, P. 2008. Tardigrades survive exposure to space in low Earth orbit. Current Biol. 18: R729-R731 (9 September 2008).

Kathman, R. D. and Cross, S. F. 1991. Ecological distribution of moss-dwelling tardigrades on Vancouver Island, British Columbia, Canada. Can. J. Zool. 69: 122-129.

Kinchin, I. M. 1987a. The moss ecosystem. School Sci. Rev. 68: 499-503.

Kinchin, I. M. 1987b. The moss fauna 1: Tardigrades. J. Biol. Ed. 21: 288-290

Kinchin, I. M. 1988. The tardigrade fauna of moss cushions. SSR Science Notes June, 1988, pp. 733-737.

Kinchin, I. M. 1994. The Biology of Tardigrades. Blackwell Publishing Co., London, 186 pp.

Kristensen, R. M. and Hallas, T. E. 1980. The tidal genus *Echiniscoides* and its variability with erection of Echiniscoididae fam. n. (Tardigrada). Zool. Scripta 9: 113-127.

Lindahl, Karen and Balser, Susie. 1999. Tardigrade Facts. Illinois Wesleyan University. Accessed on 29 April 2009 at <http://www.iwu.edu/~tardisdp/tardigrade_facts.html>.

Mach, Martin. 2010. The Water Bear web base Issue 3. Accessed on 27 January 2010 at <http://www.baertierchen.de/wb_sep00.html>.

Mach, Martin. 2012. The Water Bear Web Base. Issue 36. Accessed 21 May 2012 at <http://www.baertierchen.de/wb_jun03.html>.

Mancardi, G. C. 1988. Two new species of soil moss eutardigrades (Tardigrada) from Canada. Can. J. Zool. 67: 2282-2285.

Marcus, E. 1929. Tardigrada. Akademische Verlagsgesellschaft, Leipzig, 608 pp.

Martínez Abaigar, J. and Olivera, E. 2007. Climate change and ultraviolet radiation: Effects on stream bryophytes. Field Bryol. 91: 35-36.

Maucci, W. 1978. Moss-dwelling Tardigrada of Turkey, 3. Boll. Mus. Civ. Stor. Nat. Verona 5: 111-140.

Maucci, W. 1980. Analisi preliminare di alcuni dati statistici sulla ecologia dei Tardigradi muscicoli. [Some statistical data about ecology of moss-dwelling Tardigrada.]. Boll. Mus. Civ. Stor. Nat. Verona 7: 1-47.

Maucci, W. 1986. Tardigrada. Fauna d'Italia. Calderini, Bologna, 388 pp.

May, R.-M. 1948. La Vie des Tardigrades. Gallimard, Paris, 121 pp, 40 plates.

McInnes, S. and Pugh, P. 1998. Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. J. Biogeogr. 25: 31-36.

Mehlen, R. H. 1969. Tardigrada: Taxonomy and distribution in Costa Rica. Trans. Amer. Microsc. Soc. 88: 498-505.

Meininger, C. A. and Spatt, P. D. 1988. Variations of tardigrade assemblages in dust-impacted Arctic mosses. Arct. Alp. Res. 20: 24-30.

Meininger, C. A., Uetz, G. W., and Snider, J. A. 1985. Variation in epiphytic microcommunities (tardigrade-lichen-bryophyte assemblages) of the Cincinnati, Ohio area. Urban Ecol. 9(1): 45-62.

Meyer, H. A. and Hinton, J. G. 2007. Limno-terrestrial Tardigrada of the Nearctic realm. J. Limnol. 66(Suppl. 1): 97-103.

Meyer, H. A. and Hinton, J. G. 2009. The Tardigrada of southern Africa, with the description of *Minibiotus harrylewisi*, a new species from KwaZulu-Natal, South Africa (Eutardigrada: Macrobiotidae). African Invertebrates 50: 255-268.

Meyer, H. A., Hinton, J. G., and Trahan, K. 2003. Spatial variability of tardigrade populations in leaf litter, moss, and lichens. Abstracts of the 88th meeting of the Ecological Society of America, Savannah, GA, 3-8 August 2003. Accessed on 3 July 2004 at <http://abstracts.co.allenpress.com/pweb/esa2003/document/?ID=26552>.

Mihelčič, F. 1967. Baummoose und Flechten als Lebenstätten fur Tardigraden. Carinthia II 77: 227-236.

Miller, W. R. 1997. Tardigrades: Bears of the moss. Kans. School Nat. 43(3): 3-15.

Miller, W. R. and Heatwole, H. 1995. Tardigrades of the Australian Antarctic Territories: the Mawson Coast, East Antarctica. Invert. Biol. 114: 27-38.

Moon, S. Y., Kim, W., and Bertolani, R. 1994. *Doryphoribius koreanus*, new species of Tardigrada from Korea. Proc. Biol. Soc. Washington, DC 107: 514-516.

Morgan, C. I. 1976. Studies on the British tardigrade fauna. Some zoogeographical and ecological notes. J. Nat. Hist. 10: 607-632.

Morgan, C. I. 1977. Population dynamics of two species of Tardigrada, *Macrobiotus hufelandii* (Schultze) and *Echiniscus* (*Echiniscus*) *testudo* (Doyère), in roof moss from Swansea. J. Anim. Ecol. 46: 263-279.

Nelson, D. R. 1991a. Tardigrada. Chapt. 15. In: Thorp, J. H. and Covich, A. P. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, New York, pp. 501-521.

Nelson, D. R. 1991b. A new species of *Diphascon* from New Brunswick, Canada (Tardigrada). Can. J. Zool. 69: 1911-1915.

Nelson, D. R. 2002. Current status of the Tardigrada: Evolution and ecology. Integrat. Compar. Biol. 42: 652-659.

Nelson, D. R. and Adkins, R. G. 2001. Distribution of tardigrades within a moss cushion: Do tardigrades migrate in response to changing moisture conditions? Zool. Anz. 240: 493-500.

Neumann, S., Reuner, A., Brummer, F. and Schill, R. O. 2009. DNA damage in storage cells of anhydrobiotic tardigrades. Comparative Biochemistry and Physiology, Part A 153: 425-429.

Pilato, G. 1979. Correlations between cryptobiosis and other biological characteristics in some soil animals. Boll. Zool. 46: 319-332.

Pilato, G. and Sperlinga, G. 1975. Tardigradi muscicoli di Sardegna. [Moss-inhabiting Tardigrada from Sardinia.]. Animalia 2(1-3): 79-90.

Pilato, G., Fontoura, P., and Lisi, O. 2007. Remarks on the *Echiniscus viridis* group, with the description of a new species (Tardigrada, Echiniscidae). J. Limnol., 66(Suppl. 1): 33-39.

Ramazzotti, G. and Maucci, W. 1983. The phylum Tardigrada – 3rd edition: English translation by C. W. Beasley. Mem. Ist. Ital. Idrobiol. Dott. Marco de Marchi 41: 1B680.

Ramløv, H. and Westh, P. 1992. Survival of the cryptobiotic eutardigrade *Adorybiotus coronifer* during cooling to –196°C: Effect of cooling rate, trehalose level, and short term acclimation. Cryobiology 29: 125-130.

Ramløv, H. and Westh, P. 2001. Cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*: Tolerance to alcohols, temperature and de novo protein synthesis. Zool. Anz. 240: 517-523.

Rebecchi, L., Boschini, D., Cesari, M., Lenicioni, V., Bertolani, R., and Guidetti, R. 2009a. Stress response of a boreo-alpine species of tardigrade, *Borealibius zetlandicus* (Eutardigrada, Hypsibiidae). J. Limnol. 68: 64-70.

Rebecchi, L., Cesari, M., Altiero, T., Frigieri, A., and Guidetti, R. 2009b. Survival and DNA degradation in anhydrobiotic tardigrades. J. Exper. Biol. 212: 4033-4039.

Rebecchi, L., Guidetti, R., Borsari, S., Altiero, T., and Bertolani, R. 2008. Dynamics of long-term anhydrobiotic survival of lichen-dwelling tardigrades. Hydrobiologia 558: 23-30.

Reuner, A., Hengherr, S., Brümmer, F., and Schill, R. O. 2010. Comparative studies on storage cells in tardigrades during starvation and anhydrobiosis. Uncorrected proof. Accessed on 14 February 2010 at <http://www.actazool.org/site_media/onlinefirst/downloadable_file/2009/11/23/Manuscript_ReviewIV.pdf>.

Romano, F. A. III. 2003. On water bears. Florida Entomol. 86: 134-137.

Rossi, G., Claps, M., and Ardohain, D. 2009. Tardigrades from northwestern Patagonia (Neuquén Province, Argentina) with the description of three new species. Zootaxa 2095: 21-36.

Schill, R. O. and Fritz, G. B. 2008. Desiccation tolerance in embryonic stages of the tardigrade. J. Zool. 276: 103-107.

Schokraie, E., Hotz-Wagenblatt, A., Warnken, U., Frohme, M., Dandekar, T., Schill, R. O., and Schnölzer, M. 2011. Investigating heat shock proteins of tardigrades in active versus anhydrobiotic state using shotgun proteomics. J. Zool. Syst. Evol. Res. 49 (Suppl 1): 111–119.

Seki, K. and Toyoshima, M. 1998. Preserving tardigrades under pressure. Nature 395: 853-854.

Simmons, B. L., Wall, D. H., Adams, B. J., Ayres, E., Barrett, J. E., and Virginia, R. A. 2009. Terrestrial mesofauna in above- and below-ground habitats: Taylor Valley, Antarctica. Polar Biol. 32: 1549-1558.

Sømme, L. 1996. Anhydrobiosis and cold tolerance in tardigrades. Eur. J. Entomol. 93: 349-357.

Steiner, W. 1994a. The influence of air pollution on moss-dwelling animals: 1. Methodology and composition of flora and fauna. Rev. Suisse Zool. 101: 533-556.

Steiner, W. A. 1994b. The influence of air pollution on moss-dwelling animals: 2. Aquatic fauna with emphasis on Nematoda and Tardigrada. Rev. Suisse Zool. 101: 699-724.

Szymanska, B. 1994. The Tardigrada from the Axel Heiberg Island and the associate bryophyte species. Folia Entomol. Hung. 55: 359-368.

Tarter, D. C. and Nelson, D. R. 1990. An altitudinal comparison of the tardigrade fauna (Phylum: Tardigrada) from mosses on Spruce Mountain, West Virginia. 51st Annual Meeting of the Association of Southeastern Biologists, Baltimore, MD, USA, 18-20 Apr 1990. (World Meeting Number 902 5017).

Tarter, D. C., Nelson, D. R., and Midkiff, E. F. 1996. New distributional records, including two state records, of tardigrades (Phylum: Tardigrada) from mosses in the Monongahela National Forest, West Virginia. [Abstract]. Bull. Assoc. S. E. Biol. 43: 147.

Utsugi, K. 1984. Tardigrades found in the mosses of cities of Japan. Zoological Society of Japan, 55th Annual Meeting, Morioka, Japan, 27-29 Sep 1984. (World Meeting Number 843 5010).

Utsugi, K. and Ohyama, Y. 1991. Tardigrades in King George Island (Antarctica). Zool. Sci. 8: 1198.

Watanabe, M. 2006. Anhydrobiosis in invertebrates. Appl. Entomol. Zool. 41: 15-31.

Węglarska, B. 1957. On the encystation in Tardigrada. Zool. Poloniae 8: 315-325.

Westh, P. and Kristensen, R. 1992. Ice formation in the freeze-tolerant eutardigrades *Adorybiotus coronifer* and *Amphibolus nebulosus* studied by differential scanning calorimetry. Polar Biol. 12: 693-699.

Westh, P. and Ramløv, H. J. 1991. Trehalose accumulation in the tardigrade *Adorybiotus coronifer* during anhydrobiosis. J. Exper. Zool. 258: 303-311.

Westh, P., Kristiansen, J., and Hvidt, A. 1991. Ice-nucleating activity in the freeze-tolerant tardigrade *Adorybiotus coronifer*. Compar. Biochem. Physiol. A: Physiol. 99: 401-404.

Wikipedia: Cryptobiosis. 2009. Accessed on 2 February 2010 at <http://en.wikipedia.org/wiki/Cryptobiosis>.

Wright, J. C. 1988a. Structural correlates of permeability and tun formation in tardigrade cuticle: An image analysis study. J. Ultrastruc. Molec. Struc. Res. 101: 23-39.

Wright, J. C. 1988b. The tardigrade cuticle. I. Fine structure and the distribution of lipids. Tissue Cell 20: 745-758.

Wright, J. C. 1989. Desiccation tolerance and water-retentive mechanisms in tardigrades. J. Exper.. Biol. 142: 267-292.

Wright, J. C. 1991. The significance of four xeric parametres in the ecology of terrestrial Tardigrada. J. Zool. 224: 59–77.

Wright, J. C. 2001. Cryptobiosis 300 years on from van Leeuwenhoek: What have we learned about tardigrades? Zool. Anz. [J. Compar. Zool.] 240: 563-582.

Wright, J. C., Westh, P., and Ramløv, H. 1992. Cryptobiosis in Tardigrada. Biol. Rev. 67: 1-29.

Zanten, B. O. van. 1974. The hygroscopic movement of the leaves of *Dawsonia* and some other Polytrichaceae. Bull. Soc. Bot. Fr., Colloque Bryol. 121: 63-66.

Glime, J. M. 2017. Tardigrade Reproduction and Food. Chapt. 5-2. In: Glime, J. M. Bryophyte Ecology. Volume 2. Bryological   5-2-1
Interaction. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated
21 April 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>.

CHAPTER 5-2
TARDIGRADE REPRODUCTION
AND FOOD

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Figure 1. ***Echiniscus***, a parthenogenetic genus with at least 146 described species (Guidetti & Bertolani 2005). This genus is common on bryophytes and reproduces there. Photo by Martin Mach, with permission.

Life Cycle and Reproductive Strategies

One means by which organisms survive in such changeable habitats as bryophytes is by progressing to a different life cycle stage to wait out the storm – or lack of one. Tardigrades are especially adept at this, as seen in Chapter 5-1 (diapause and cryptobiosis). In tardigrades, diapause and cryptobiosis can occur at any time and developmental stage. Here we will look at reproduction and its role in further providing an escape route, at least for the species, if not the individual, an even that often occurs on bryophytes (Figure 1).

Hofmann (1987) considers that tardigrades must be able to reproduce quickly and in sufficient numbers when conditions are favorable because their life style is one of intermittent activity and inactivity, the latter in either a state of dormancy or cryptobiosis. This constraint of brief reproductive periods and the necessity for a few individuals to have sufficient offspring makes them **r-strategists**. They lack a defined carrying capacity and the population density is dependent upon the length of time since establishment in that location.

Life history of tardigrades can differ among species, presumably providing somewhat different adaptive strategies. For example, ***Paramacrobiotus tonolii*** (Figure 2) requires 16 days for its embryonic development whereas

Macrobiotus sapiens requires only 12 days (Lemloh *et al.* 2011). ***Paramacrobiotus tonollii*** is larger than ***M. sapiens*** but the latter has a longer life span of 83 days.
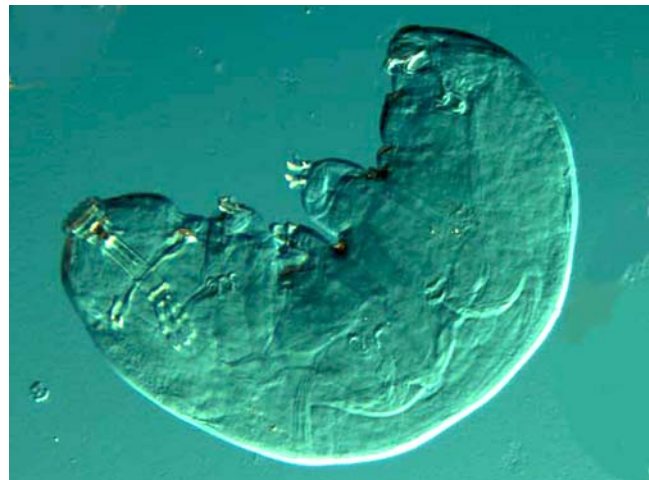

Figure 2. ***Paramacrobiotus tonollii***. Photo by Paul Bartels, with permission.

Reproductive Strategies and Habitat

Reproductive mechanisms do differ among tardigrades in different habitats (Bertolani 2001). Those living among bryophytes, as well as those of freshwater, leaf litter, and soil, commonly are **parthenogenetic** (Figure 1), or more rarely **hermaphrodites** that self-fertilize. Marine species, on the other hand, have separate sexes. Bertolani hypothesized that organisms living in isolated and unstable habitats (including bryophytes) have evolved **cryptobiosis**, **parthenogenesis**, **self-fertilization**, and **passive dispersal**, benefitting them in their challenging living conditions.

Passive wind dispersal of tardigrades with mosses is already known and may be their primary dispersal strategy (Pilato 1979). As is common among many mosses, those tardigrades that have **parthenogenesis** (equivalent to vegetative reproduction in mosses, *i.e.* reproduction without males) do not also have self-fertilization. These reproductive strategies, as in bryophytes, permit tardigrades to reproduce when only one individual, or its egg (Figure 3), arrives to colonize a new location.

Figure 3. Eggs of a species of *Dactylobiotus*. The clustering of eggs may be beneficial in protecting each other, but their inherent resistance to almost everything suggests that is probably not important. Photo by Yuuji Tsukii, with permission.

Dispersal in tardigrades seems independent of the tardigrade because it is passive dispersal. But Bertolani *et al.* (1990) considered that the differences in distribution and frequency of members of the genus *Ramazzottius* relate to their differences in reproductive modes. In their study of *Ramazzottius* species on bryophytes and lichens, they found that the sex ratio was strongly influenced by the type of reproduction, but also differed when samples were collected from tree trunks and limited rocky areas versus extensive rocky outcrops. Bertolani *et al.* found that bryophyte-dwelling *Ramazzottius* tardigrades from tree trunks or slightly rocky areas exhibited parthenogenesis and absence of male tardigrades. Among the rocky outcrops, there were always at least some males, although some parthenogenesis still occurred. Males are only useful if there is sufficient opportunity for contact with females. Perhaps the rocky outcrops provided less of a labyrinth and permitted the needed contact? Eggs provide light-weight, windborne propagules to disperse the species (Figure 3).

[To clarify for botanists, some references tend to use the term egg for the zygote and sometimes even the developing organism (embryo) until it has hatched, like the hatching of a bird egg. Since I found the term egg used in my references, I shall use egg here as well.]

Eggs

Depending on the species, there are two ways tardigrade eggs (including embryos) may be deposited. Some lay free eggs on their substrate (Figure 3), but others deposit them in the shed **exuvia** of a molt (Mach: The Water Bear; Figure 4).
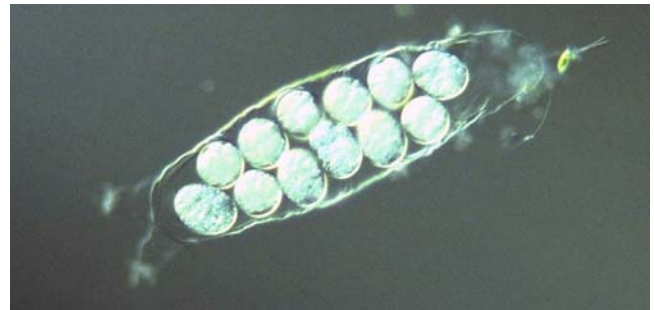
Figure 4. A number of eggs can develop within one shed **exuvia**, as shown here for *Hypsibius* sp. Photo by Yuuji Tsukii, with permission.

Macrobiotus hufelandi (Figure 5) has two sexes – males do exist (Figure 5) (Mach 2010). It is one of the species having free eggs (Figure 6). Eggs deposited outside the exuviae generally have decorative processes (Figure 7) (Mach 2010). Kinchin (1994) suggests that the functions of the egg processes include anchorage of the egg to a substrate or a transporting medium, defensive structure against being eaten by other animals, water reservoir which slows down the desiccation process, and regulation of gas exchange between egg and environment.
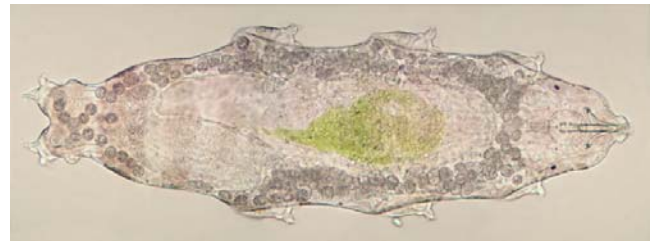
Figure 5. *Macrobiotus hufelandi* male. Photo by Martin Mach, with permission.

Figure 6. Egg of *Macrobiotus hufelandi*, demonstrating the decorative processes on this free-egg deposit. Photo by Martin Mach, with permission.
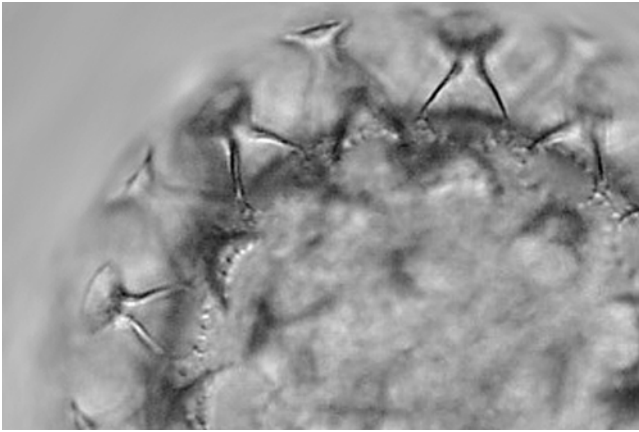
Figure 7. Egg of *Macrobiotus* sp., demonstrating the highly decorative surface that is typical of eggs laid free of the organism. Photo by Martin Mach, with permission.

When healthy adult tardigrades discard their outer covering, many taxa deposit eggs in these shed **exuviae** (outer "skins") (Figure 8-Figure 11) (Bertolani *et al*. 2009). The eggs may be few or many (up to 30-40) and may differ even within the same species, as can be seen for *Milnesium tardigradum* in Figure 9-Figure 11 (Altiero *et al*. 2006). The number of eggs depends on the species, but also on the nutritional status of the individual female (Mach: The Water Bear). And it seems that some bears may even ingest their own eggs to improve their nutritional status.

Egg development is poorly known. In *Paramacrobiotus* [=*Macrobiotus*] *richtersi* (Figure 12) it can be prolonged to 90 days or more if the eggs undergo desiccation and become resting eggs (Altiero *et al*. 2009). The non-resting (**subitaneous**) eggs may hatch in as little as 30-40 days.

Figure 8. These eggs reside in the shed exuvial "armor" of the parent and permit the tardigrade species to survive winter and desiccation. Photo by Martin Mach, with permission.

Figure 9. Individual of *Milnesium tardigradum* with eleven eggs in the shed exuvia. Photo by Martin Mach, with permission.

Figure 10. Individual of *Milnesium tardigradum* with only three large eggs in the exuvia. Photo by Martin Mach, with permission.

Figure 11. Developed eggs of *Milnesium tardigradum* with the buccal apparatus visible, indicating nearness to maturity. Photo by Martin Mach, with permission.

Figure 12. *Paramacrobiotus richtersi*. Photo from Science Photo Library, through Creative Commons.

The eggs generally develop within the exuvia until the fully-formed tardigrade is ready to leave the egg, as shown here for individuals in the genus *Echiniscus* (Figure 13-Figure 19), requiring several weeks for completion (Mach: The Water Bear). The eggs are able to survive the same drying conditions as the adult; development stops during that dry state. The young tardigrades resemble the adults (Figure 20-Figure 23), but are smaller, requiring a series of molts as they grow. Growth occurs by cell enlargement rather than by addition of cells. Since the eggs often remain in the exuvia until they hatch (Figure 21), size would tend to reduce wind-dispersal of the pollen-grain-sized eggs except when they are dispersed along with a substrate such as mosses.
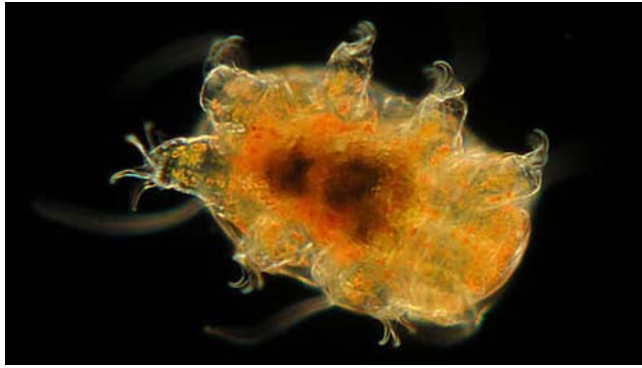
Figure 13. *Echiniscus* adult. Photo by Martin Mach, with permission.

Figure 14. *Echiniscus* exuvia with eggs (embryos) after first division. Photo by Martin Mach, with permission.

Figure 15. *Echiniscus* embryo after two divisions. Photo by Martin Mach, with permission.

Figure 16. Multicellular *Echiniscus* embryo Photo by Martin Mach, with permission.

Figure 17. Morula stage in the embryo development of *Echiniscus*. Photo by Martin Mach, with permission.

Figure 18. This mature "egg" of *Echiniscus* can be found among bryophytes, and its smooth surface is typical of eggs that are kept within the exuvia. Note the buccal apparatus that signifies its late developmental stage. Photo by Martin Mach, with permission.

In soil-dwelling *Paramacrobiotus richtersi* (Figure 12; also a known bryophyte dweller), temperature played a role in rate of development, survival rate, body growth, and generation time (Figure 24; Hohberg 2006). On the other hand, hatching time, first to fourth molts, and maturation time were dependent upon body size alone.
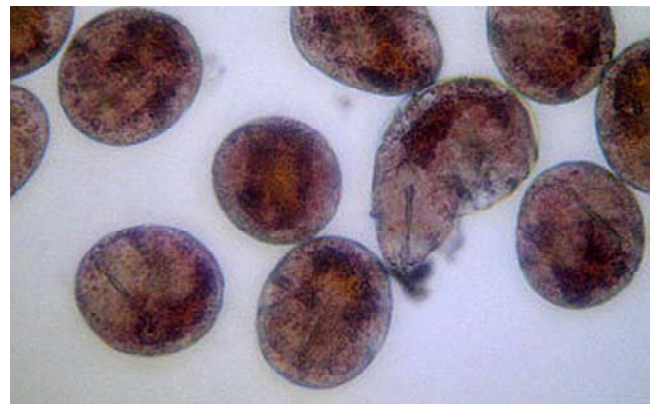
Figure 19. *Echiniscus* hatching from its eggs. Photo by Martin Mach, with permission.
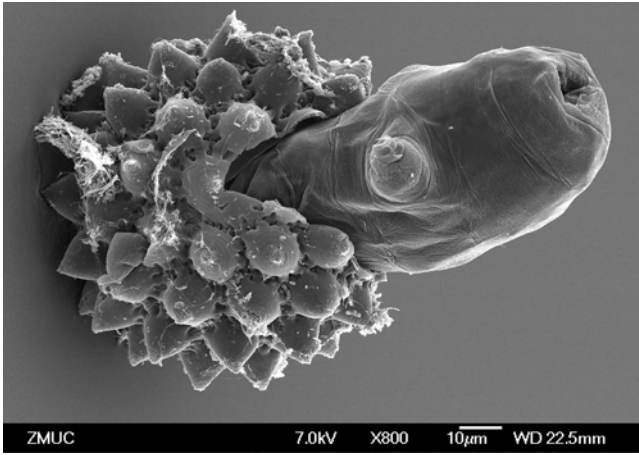
Figure 20. Moss-dweller *Macrobiotus derkai* hatching. from a free "egg." Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.
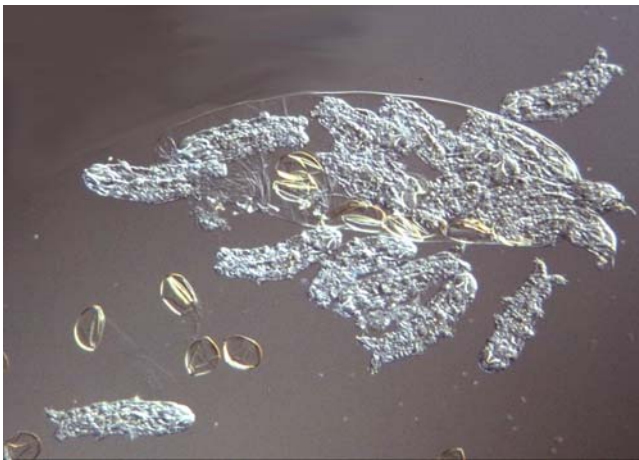
Figure 21. Despite the large number of eggs/embryos, it appears that most of them are successful in hatching into young tardigrades, as seen here for a species of *Hypsibius*. Photo by Yuuji Tsukii, with permission.
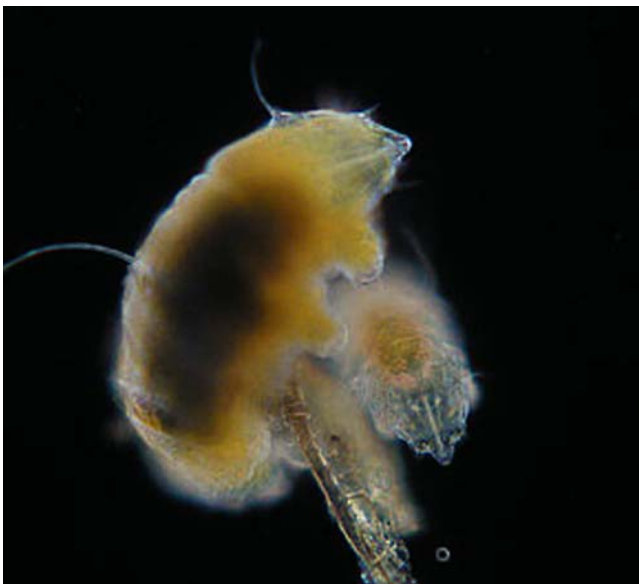
Figure 22. *Echiniscus* young and old. Note the long "hairs" extending from the body, giving the genus its name. Photo by Martin Mach, with permission.

Figure 23. *Echiniscus* young. Photo by Martin Mach, with permission.

Some tardigrades have found another safe site for their eggs. They can use the capsule of a moss as an egg depository (Mach: The Water Bear; Figure 25-Figure 26).
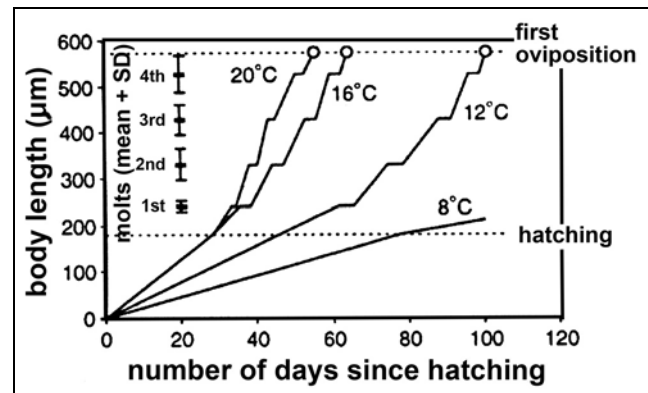
Figure 24. Effects of temperature on the development of *Paramacrobiotus richtersi* (Figure 12), starting with the day the tardigrades hatched. Body lengths are for hatching and first oviposition only. Redrawn from Hohberg (2006).
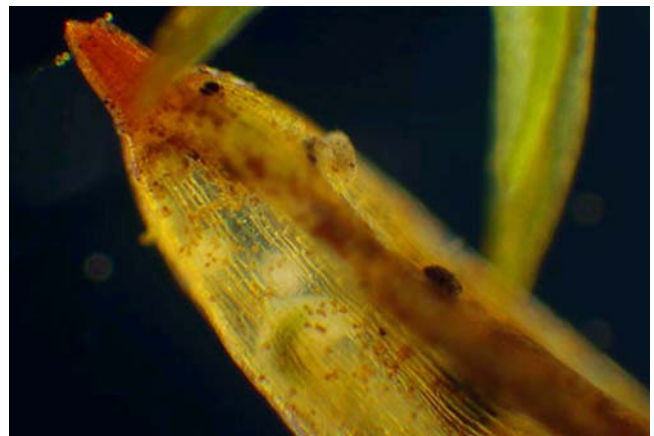
Figure 25. Moss capsule with tardigrade (with green gut) and two white eggs. Photo by Martin Mach, with permission.
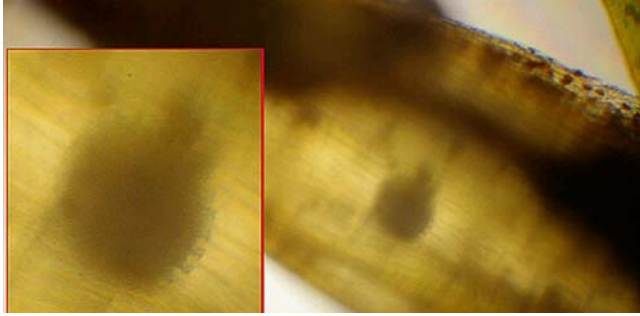
Figure 26. This egg is protected by a moss capsule. Based on the decorations on the eggs, they appear to be close to *Macrobiotus hufelandi*. Photos by Martin Mach, with permission.

Molting

Like its relatives in the Arthropoda, the tardigrade must **molt** (Figure 27-Figure 35). This process usually requires 5-10 days and occurs several times throughout its life (Walz 1982), including after sexual maturity while the body is still increasing in size (Nelson 1982). During molting, the old cuticle, claws, and lining of the fore- and hindgut are shed (Figure 28), causing a stage known as the **simplex** stage (Figure 35). Lacking its sclerified parts of the buccal-pharyngeal apparatus, the tardigrade cannot feed. It appears that tardigrades molt 4-12 times during their 3-30 months of active lives (Nelson 2002).

Figure 27. *Milnesium tardigradum* as it recedes from its cuticle in preparation for molting. Note the dark brown eggs that will soon be left behind. Photo by Martin Mach, with permission.

Figure 28. *Milnesium tardigradum* emerging from its exuvia during molting, leaving its claws, eggs, and various other parts behind. Photo by Martin Mach, with permission.
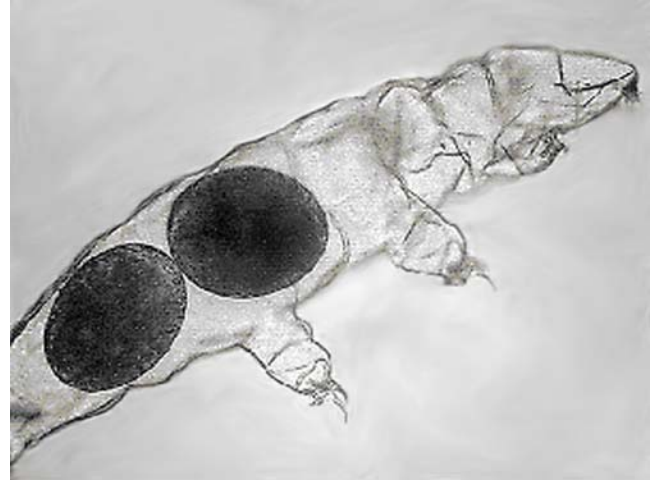
Figure 29. Eggs in the shed exuvia of *Milnesium tardigradum*. Photo by Martin Mach, with permission.

Figure 30. *Milnesium tardigradum* eggs in its shed exuvia. Photo by Martin Mach, with permission.

Cyclomorphosis

It appears that **cyclomorphosis** (annual cycle of morphological change) occurs in tardigrades, although it has been demonstrated in only a few species. It has been documented in the marine species *Halobiotus crispae* (Kristensen 1982; Halberg *et al*. 2009). Likewise, Dastych (1993) demonstrated cyclomorphosis in a cryoconite-dwelling species of **Hypsibius** (Figure 31), and in a bryophyte dweller. Furthermore, Rebecchi and Bertolani (1994) did demonstrate it for one species in the genus **Bertolanius** [=*Amphibolus*] (Figure 33), which does have moss-dwelling species.

Kristensen (1982) studied the marine *Halobiotus crispae* cycle and found two morphs. In winter there is a **pseudosimplex** stage that hibernates and is sexually immature. These winter forms gather in large **aggregations** in protected areas where the aggregations increase chances for survival of the freezing temperatures. The population experiences synchronous development of gonads, hence all reaching sexual maturity and breeding simultaneously. But the cycle for other taxa and habitats, including bryophytes, remains to be explored (Nelson 2002).
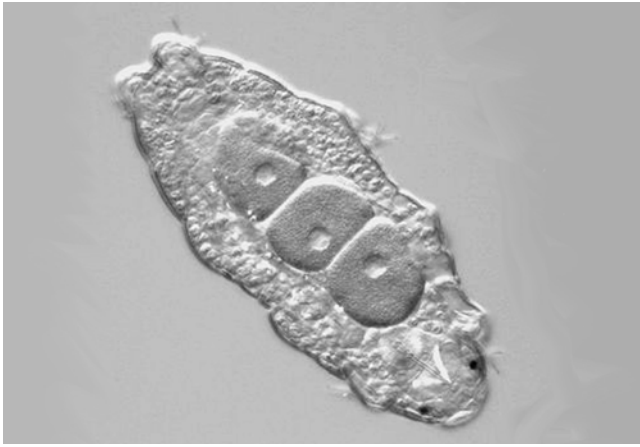
Figure 32. *Hypsibius dujardini* with 3 oocytes. Photo by Willow Gabriel, through EOL Creative Commons.
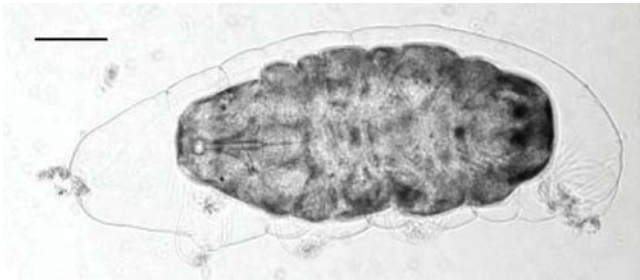
Figure 33. *Bertolanius volubilis* cuticle with a type A cyst inside. Photo by Roberto Bertolani, with permission.

Figure 34. *Ramazzottius oberhaeuseri* completing its molt out of its old cuticle. The emerging organism will remain in this **simplex** stage until it rebuilds its cuticular parts. Photo by Martin Mach, with permission.

Bryophytes as Food Reservoirs

Bryophyte-dwelling tardigrades include both bryophyte-eating tardigrades and those with a variety of other feeding strategies, including carnivory. The tardigrade has a specially adapted pair of **stylets** (Figure 49) and a muscular pharynx (Figure 50-Figure 52) that produces a suction into the gut, permitting the tardigrade to suck fluids from the interior of a bryophyte or algal cell (Figure 53) or even small animals such as rotifers (Figure 54) and nematodes [Tardigrada (Water Bears) 2005]. In the family Echiniscidae (Figure 36), a common family on

bryophytes, the stylet may be very long, permitting penetration of the thick cellulose walls of bryophytes. For example, *Echiniscus testudo* (Figure 37) feeds primarily on bryophytes (Morgan 1977). *Diphascon* (Figure 52), also a bryophyte dweller, has a flexible buccal tube with spiral rings resembling the extension on a vacuum cleaner. Small bryophyte dwellers may subsist on diatoms and bacteria that live epiphytically among the bryophytes [Bartels 2005; Tardigrada (Water Bears) 2005].

Figure 35. *Pseudobiotus* sp. shedding its cuticular exuvia and leaving its eggs/embryos behind. Photo by Paul Davison, with permission.

Figure 36. *Echiniscus perviridis* with green color, most likely due to its vegetarian diet. *Echiniscus testudo* is known to feed primarily on bryophytes. Photo by Łukasz Kaczmarek, with permission.

Figure 37. *Echiniscus testudo* tun. Photo by Power & Syred through Creative Commons.

Schill *et al.* (2011) consider the bryophytes to be a "rich food supply for both carnivorous and herbivorous species." These food sources include nematodes, rotifers, plant cells, algae (Figure 38), yeast, and bacteria, and for some, bryophytes. Schill and coworkers conducted a genetic tracer study (rbcL) on the guts of tardigrade species from various sites in Europe that demonstrated the presence of mosses from the **Erpodiaceae [*Aulacopilum hodgkinsoniae*, *Venturiella sinensis* (Figure 39)] and Pottiaceae [*Syntrichia* (=*Tortula*) *obtusissima* (Figure 40)]** in the guts of field-collected *Macrobiotus sapiens*, **Grimmiaceae** [*Grimmia elongata* (Figure 41), *Coscinodon cribrosus* (Figure 42), *Schistidium strictum* (Figure 43)] from *Macrobiotus persimilis* and *Echiniscus granulatus*, and the green alga *Trebouxia* (Figure 44) from *Richtersius coronifer* (Figure 38). For *Macrobiotus sapiens* they found no rbcL sequence demonstrating presence of the families **Pottiaceae** or **Orthotrichaceae**. It appears that *Macrobiotus sapiens* will only eat these latter two moss families when **Grimmiaceae** is not available, or that others had been digested completely before samples were extracted. The small tardigrade stylet makes it difficult for them to obtain cell contents from the moss genera *Polytrichum* (Figure 45), *Dicranum* (Figure 46), *Leucobryum* (Figure 47), and *Racomitrium* (Figure 48). Digestion in tardigrades is aided by the gut *p*H, with the foregut having an acidic environment and the midgut having a basic environment (Marcus 1928).

Figure 40. *Syntrichia obtusissima* showing hair points. Photo by Claudio Delgadillo, with permission.

Figure 38. *Richtersius coronifer*, clinging to an algal cell. Photo by Martin Mach, with permission.

Figure 39. *Venturiella sinensis*, a moss eaten by *Macrobiotus sapiens*. Photo from Digital Museum, Hiroshima University, with permission, with permission.

Figure 41. *Grimmia elongata*, a moss eaten by *Macrobiotus persimilis* and *Echiniscus granulatus*. Photo by Michael Lüth, with permission.

Figure 44. *Trebouxia*, a lichen symbiont that appeared in the guts of field collected **Macrobiotus persimilis** and **Echiniscus granulatus**. Photo by Yuuki Tsukii, with permission.

Figure 42. **Coscinodon cribrosus**, a moss that is suitable habitat and food for **Macrobiotus persimilis** and **Echiniscus granulatus**. Photo by Michael Lüth, with permission.

Figure 43. **Schistidium strictum**, a moss that is eaten by **Macrobiotus persimilis** and **Echiniscus granulatus**. Photo by Jan-Peter Frahm, with permission.

Figure 45. **Polytrichum commune**, a moss with thick leaves that make feeding by tardigrades difficult. Photo by Michael Lüth, with permission.

Figure 46. **Dicranum scoparium**, a moss with leaves that seem to make feeding by tardigrades difficult. Photo by Janice Glime.

Figure 47. *Leucobryum glaucum*, showing thick leaves that make tardigrade feeding difficult. Photo by James K. Lindsey, with permission.

Figure 48. *Racomitrium macounii* ssp *macounii*, a moss with leaves that seem to make feeding by tardigrades difficult, in Europe. Photo by Michael Lüth, with permission.
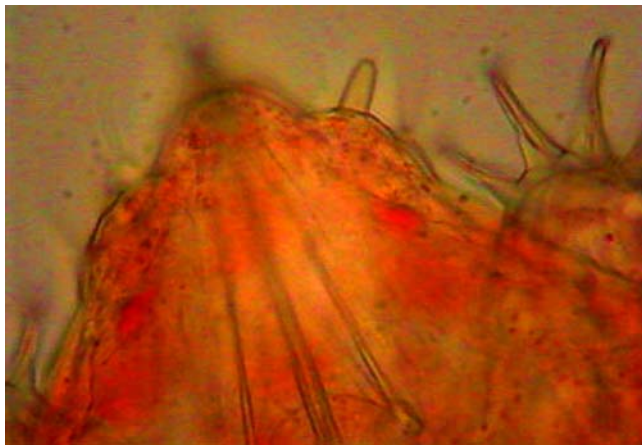
Figure 49. *Echiniscus* with the stylets protruding (out of focus). Photo by Martin Mach, with permission.

Tardigrades even consume smaller tardigrades. Larger species such as those of *Macrobiotus* (Figure 5) and *Milnesium* (Figure 9, Figure 51) consume smaller members such as *Diphascon* (Figure 52) and *Hypsibius* (Figure 64), as exhibited by remains of claws and buccal apparati (Figure 57) in the gut (Nelson 2002). Large carnivorous Eutardigrada such as *Paramacrobiotus richtersi* (Figure 12), *Milnesium tardigradum* (Figure 9, Figure 10, Figure 51, Figure 54), and *Bertolanius*

nebulosus are widespread in many habitats, including bryophytes [Tardigrada (Water Bears) 2005]. They eat nematodes, rotifers, and smaller tardigrades, but still use the stylet to suck out cell contents. Suzuki (2003) reared *Milnesium tardigradum* from the moss *Bryum argenteum* (Figure 55), using only rotifers [*Lecane inermis*, common in wet *Sphagnum* (Miller 1931)] as food.

Figure 50. This tardigrade has the stylets withdrawn into its head. The pharynx is in the center behind the stylets. Photo by Paul Davison, with permission.

Figure 51. "Head" region of *Milnesium tardigradum* showing the pharynx. Photo by Martin Mach, with permission.
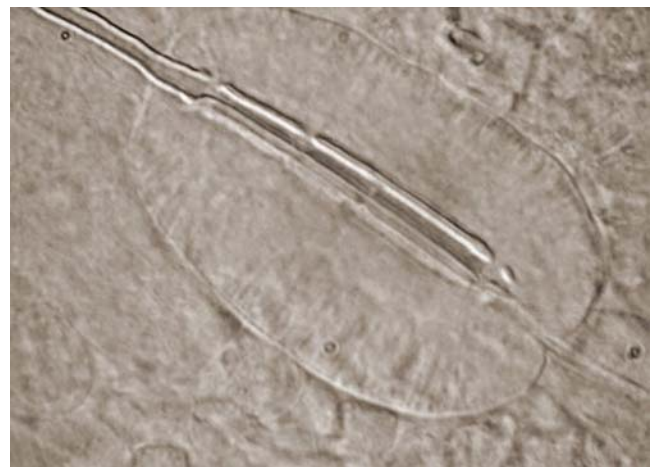
Figure 52. Pharynx (oval) of *Diphascon*, the organ that produces the suction for the stylets. Photo by Martin Mach, with permission.

Figure 53. The green in this tardigrade is likely to be algae or moss. Photo by Paul Davison, with permission.
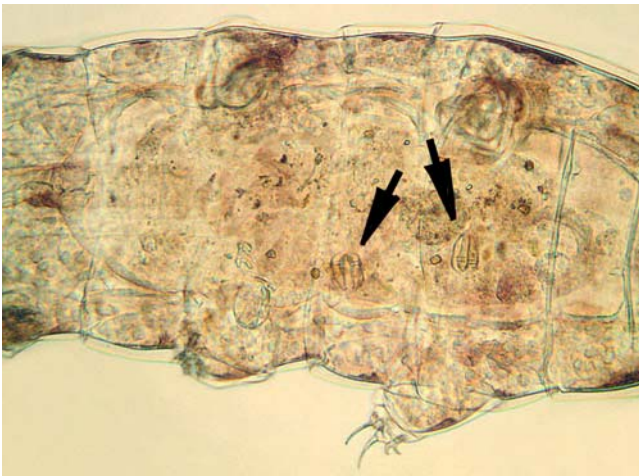
Figure 54. *Milnesium* with the mastax of rotifers visible in the gut (black arrows). Photo by Martin Mach, with permission.

Figure 55. *Bryum argenteum*, a moss known to house *Milnesium tardigradum*. Photo by Michael Lüth, with permission.

Suren (1988) attempted to determine the importance of bryophytes as food vs. simply substrate by using artificial mosses in high alpine New Zealand streams. When artificial structures were used, similar communities of invertebrates developed, but tardigrades appeared to be affected negatively by the absence of the bryophytes, a difference Suren interpreted to reflect a loss of bryophytes as a food source. It seems to be one of the few animals specifically adapted to obtaining the good stuff from the insides of the cells of bryophytes. Its stylets (Figure 49-Figure 50) serve as a miniature needle and straw to puncture the cell and suck the nutrients from it. The pharynx (Figure 52) serves as a pump to draw fluids in through the stylets (Tardigrades, Bears of the Moss).

It appears that eating bryophytes requires more than just the equipment to suck the good stuff out of the cell. The excretory system seems also to be altered. Węglarska (1990) found that in four genera of tardigrades, those that live among bryophytes have larger excretory organs relative to body size than do the freshwater species. The purpose of this added size remains a mystery.

Ramazzotti and Maucci (1983) suggested that excretion probably occurs in four ways in tardigrades. At molting it occurs through the salivary glands. Likewise, when the cuticle is shed it removes accumulated excretory granules. It can occur through the wall of the midgut. And in the eutardigrades, it occurs through excretory glands. There is no study to determine how these various mechanisms might relate to a diet of bryophytes.

Role in Food Web

As seen above, tardigrades typically are either plant eaters or are carnivorous (Garey *et al*. 2008), including protozoa, nematodes, and rotifers (Figure 54), but also consume bacteria and fungi (Kinchin 1988). As noted in the earlier chapter on nematodes, they can be predators on nematodes that live in the same clump of moss (Sánchez-Moreno *et al*. 2008), making them important consumers and often the top carnivore.

Both *Paramacrobiotus* [=*Macrobiotus*] *richtersi* (Figure 12) and *Macrobiotus harmsworthi* (Figure 56-Figure 57) caused significant declines in the nematode populations, thus regulating the food web. In fact, a single *P. richtersi* dined on an average of 61 nematodes in a day! Unlike many of the slow-walking water bears, these carnivorous water bears are able to move swiftly to attack and devour their prey (Kristensen & Sørensen 2005).

Davison (2005) reports that tardigrades lumber across the substrate, swinging their heads back and forth in search of food. When he offered them nematodes and rotifers, the tardigrades made no attempt to eat them. When he offered them a larger choice, the annelid *Lumbriculus* sp., a genus with known members that inhabit mosses, he found that they immediately approached it and began eating it.
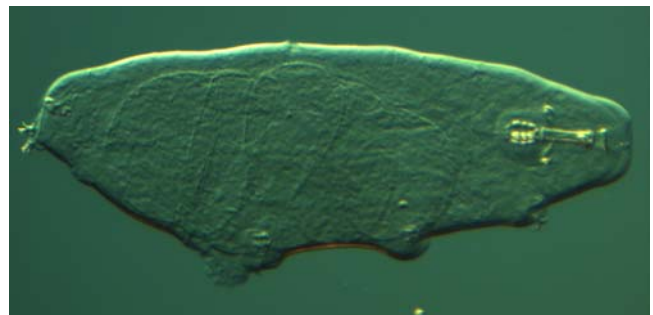
Figure 56. *Macrobiotus harmsworthi*, a nematode predator. Photo by Paul J. Bartels, with permission.
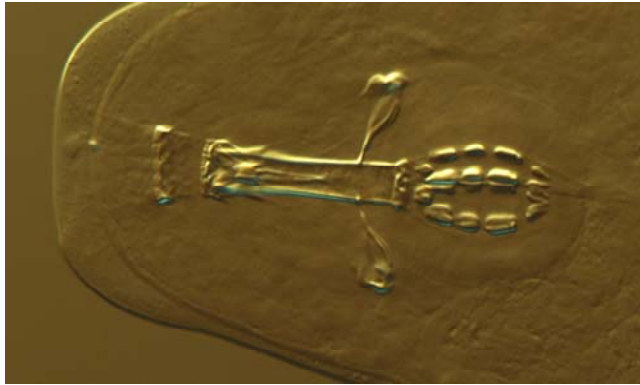
Figure 57. *Macrobiotus harmsworthi* buccal apparatus. Photo by Paul Bartels, with permission.

Tardigrade specialists have assumed that the **buccal apparatus** (Figure 58-Figure 60) indicates characteristics of the food, but no studies exist on the relationships of buccal apparati among the limnoterrestrial taxa (Nelson 2002).
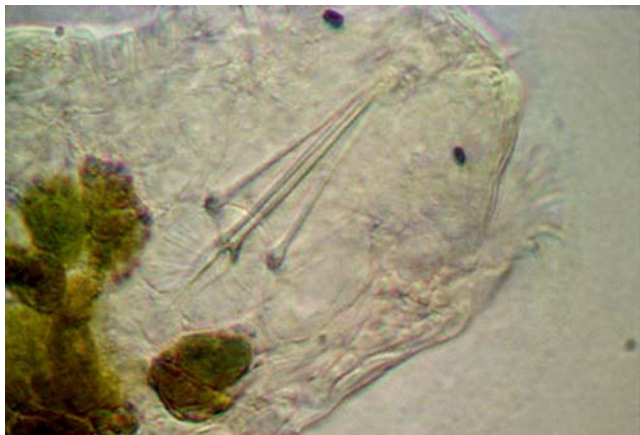
Figure 58. The three "filaments" and pharynx are the buccal apparatus of this *Echiniscoides sigismundi*. The **pharynx** resembles a pair of kidneys in contact with the three filaments. The outer two filaments are the stylets; the inner one is the buccal tube. The gut contains algal or plant material that has been ingested by this tidal zone species. Photo by Martin Mach, with permission.

Figure 59. The three "filaments" and oval behind them are the buccal apparatus of *Paramacrobiotus* [=*Macrobiotus*] *areolatus*. The bulbous oval to the right of the three filaments (stylets and buccal tube) is the pharynx. Photo by Martin Mach, with permission.

Figure 60. *Dactylobiotus dispar* has a buccal apparatus similar to that of *Paramacrobiotus areolatus*. Photo by Martin Mach, with permission.

But tardigrades can have their predators too. Snails that live among the moss leaves could enjoy a meal of tardigrades (Fox 1966). The land snail *Bulimulus guadalupensis* (Figure 61) from Puerto Rico had evidence that all life cycle stages of the tardigrade *Echiniscus molluscorum* (see Figure 62) live in its feces (Fox & Garcia-Moll 1962). It is not clear if these passed unharmed through the gut or if they took advantage of the feces as a food source after defecation. It is even possible that eggs passed through the gut and hatched in the feces.

Figure 61. The land snail *Bulimulus guadalupensis* is a known predator on moss-dwelling tardigrades. Photo by Gary Rosenberg at <www.discoverlife.org>.

Tardigrades have smaller predators as well. The fungus *Ballocephala pedicellata* (Figure 63) is known from the tardigrades *Hypsibius dujardini* (Figure 64) and *Diphascon pingue* complex (Figure 65) living in the moss *Atrichum angustatum* (Figure 66) (Pohlad & Bernard 1978). In this study, tardigrades with the fungus were only

present in January and February in the collecting area in southeastern USA.

Figure 62. Armor of *Echiniscus* sp. Material such as this is easy to identify in the feces of predators. Photo by Martin Mach, with permission.

Figure 63. *Ballocephala sphaerospora* zygospores in a tardigrade. Photo by George Barron, with permission.

Figure 64. *Hypsibius dujardini*, a host for the fungus *Ballocephala pedicellata*. Photo from Rpgch Wikimedia Commons.

Figure 65. *Diphascon pingue*. Photo by Michael Collins, with permission.

Figure 66. *Atrichum angustatum*, a habitat for tardigrades, and in winter, their parasitic fungus *Ballocephala pedicellata*. Photo by Michael Lüth, with permission.

Summary

Life cycle stages often provide a means of surviving changes in the environment. Bryophyte-dwelling tardigrades are usually **parthenogenetic**. They rarely are **hermaphrodites**, and parthenogenetic individuals do not self-fertilize.

Tardigrade species may either lay free eggs or deposit them inside the exuvia as it is shed. External eggs are usually highly decorated, whereas those laid inside the exuvia tend toward smooth. The number of eggs varies up to 40, with the number depending on the species and nutritional status. Time required for development of the fertilized egg may be up to 90 days. Variability in development time permits **bet-hedging**. Temperature affects development rate, survival rate, and body growth, as well as affecting generation time. Young tardigrades resemble the adults and continue to grow by cell enlargement. Molting permits the tardigrade to expand its size and requires 5-10 days during which the tardigrade cannot eat and is less protected. Some species have more than one morph, where the winter morph may form aggregations that enhance survival of freezing conditions.

> Tardigrades consume algae, bryophytes, fungi, protozoa, nematodes, rotifers, and smaller tardigrades. In many cases this is accomplished using a **stylet** that forms a straw for sucking cell contents. They suck in their prey with the pair of stylets, with the muscular pharynx producing suction. For whatever reason, bryophyte dwellers also have larger excretory organs than do tardigrades of other substrata.
>
> They play an important role in regulating the food web of bryophytes in some circumstances. Tardigrades are subject to predation by snails and even larger tardigrades. Fungi may extract nutrition from them.

Acknowledgments

Literature Cited

Altiero, T., Rebecchi, L., and Bertolani, R. 2006. Phenotypic variations in the life history of two clones of *Macrobiotus richtersi* (Eutardigrada, Macrobiotidae). Hydrobiologia 558: 33-40.

Altiero, T., Bertolani, R., and Rebecchi, L. 2009. Hatching phenology and resting eggs in *Paramacrobiotus richtersi* (Eutardigrada, Macrobiotidae). J. Zool. (in press).

Bartels, P. 2005. "Little known" water bears? ATBI Quart. 6(2): 4.

Bertolani, R. 2001. Evolution of the reproductive mechanisms in tardigrades – a review. Zool. Anz. 240(3-4): 247-252.

Bertolani, R., Altiero, T., and Nelson, D. R. 2009. Tardigrada (water bears). Chapter 188. In: Likens, G. E. (Ed.). Vol. 2. Encyclopedia of Inland Waters. Elsevier, Oxford, pp. 443-465.

Bertolani, R., Rebecchi, L., and Beccaccioli, G. 1990. Dispersal of Ramazzottius and other tardigrades in relation to type of reproduction. Invert. Repro. Dev. 18: 153-157.

Dastych, H. 1993. Redescription of the cryoconital tardigrade *Hypsibius klebelsbergi* Mihelčič, 1959, with notes on the microslide collection of the late Dr. F. Mihelčič. Veröff. Mus. Ferdinandeum 73: 5-12.

Davison, P. G. 2005. Feeding water bears: A simple activity to connect the public with microorganisms. ATBI Quarterly 6(2): 5.

Fox, I. 1966. On the significance of tardigrades in the digestive tract of snails. Proc. First Internat. Congr. Parasitol. 2: 1064.

Fox, I. and Garcia-Moll, I. 1962. *Echiniscus molluscorum*, new tardigrade from the feces of the land snail, *Bulimulus exilis* (Gmelin) in Puerto Rico (Tardigrada: Scutechiniscidae). J. Parasitol. 48(2): 177-181.

Garey, J. R., McInnes, S. J., and Nichols, P. B. 2008. Global diversity of tardigrades (Tardigrada) in freshwater. Develop. Hydrobiol. 198: 101-106.

Guidetti, R. and Bertolani, R. 2005. Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. Zootaxa 845: 1-46.

Halberg, K. A., Persson, D., Ramløv, H., Westh, P., Møbjerg Kristensen, R., and Møbjerg, N. 2009. Cyclomorphosis in Tardigrada: Adaptation to environmental constraints. J. Exper. Biol. 212: 2803-2811.

Hofmann, I. 1987. Habitat preference of the most frequent moss-living Tardigrada in the area of Giessen (Hessen). In: Bertolani, R. (ed.). Selected Symposia and Monographs U.Z.I., 1, Mucchi, Modena, pp. 211-216.

Hohberg, K. 2006. Tardigrade species composition in young soils and some aspects on life history of *Macrobiotus richtersi* J. Murray, 1911. Pedobiologia 50: 267-274.

Kinchin, I. M. 1988. The tardigrade fauna of moss cushions. SSR Science Notes June, 1988, pp. 733-737.

Kinchin, I. M. 1994. The Biology of Tardigrades. Blackwell Publishing Co., London, 186 pp.

Kristensen, R. 1982. The first record of cyclomorphosis in Tardigrada based on a new genus and species from Arctic meiobenthos. Z. Zool. Syst. Evol.-forsch 20: 249-270.

Kristensen, Reinhardt Møbjerg and Sørensen, Martin Vinther. 2005. Tardigrada (Water Bears) Accessed on 16 January 2010 at <http://www.answers.com/topic/tardigrada-water-bears-biological-family>.

Lemloh, M.-L., Brümmer, F., and Schill, R. O. 2011. Life-history traits of the bisexual tardigrades *Paramacrobiotus tonollii* and *Macrobiotus sapiens*. J. Zool. Syst. Evol. Res. 49(suppl 1): 58-61.

Mach, Martin. 2010. The Water Bear web base Still Images I. Overview. Accessed on 27 January 2010 at <http://www.baertierchen.de/tardigrada.html>.

Marcus, E. 1928. Spinnentiere oder Arachnoidea IV: Bärtierchen (Tardigrada). Springer, Jena.

Miller, H. M. 1931. Alternation of generations in the rotifer *Lecane inermis* Bryce: I. Life histories of the sexual and non-sexual generations. Biol. Bull. 60: 345-381.

Morgan, C. I. 1977. Population dynamics of two species of Tardigrada, *Macrobiotus hufelandii* (Schultze) and *Echiniscus* (*Echiniscus*) *testudo* (Doyère), in roof moss from Swansea. J. Anim. Ecol. 46: 263-279.

Nelson, D. R. 1982. Developmental biology of the Tardigrada. In: Harrison, F. and Cowden, R. (eds.). Developmental Biology of Freshwater Invertebrates. Alan R. Liss, New York, pp. 363-368.

Nelson, D. R. 2002. Current status of the Tardigrada: Evolution and ecology. Integrat. Compar. Biol. 42: 652-659.

Pilato, G. 1979. Correlations between cryptobiosis and other biological characteristics in some soil animals. Boll. Zool. 46: 319-332.

Pohlad, B. R. and Bernard, E. C. 1978. A new species of Entomophthorales parasitizing tardigrades. Mycologia 70: 130-139.

Ramazzotti, G. and Maucci, W. 1983. The phylum Tardigrada – 3rd edition: English translation by C. W. Beasley. Mem. Ist. Ital. Idrobiol. Dott. Marco de Marchi 41: 1B680.

Rebecchi, L. and Bertolani, R. 1994. Maturative pattern of ovary and testis in eutardigrades of freshwater and terrestrial habitats. Invert. Reprod. Dev. 26: 107-118.

Sánchez-Moreno, S., Ferris, H., and Guil, N.. 2008. Role of tardigrades in the suppressive service of a soil food web. Agric. Ecosyst. Environ. 124: 187-192.

Schill, R. O., Jönsson, K. I., Fannkuchen, M., and Brümmer, F. 2011. Food of tardigrades: a case study to understand food choice, intake and digestion. J. Zool. Syst. Evol. Res. 49(suppl 1): 66-70.

Suren, A. M. 1988. Ecological role of bryophytes in high alpine streams of New Zealand. Internat. Ver. Theor. Angew. Limnol. 23: 1412-1416.

Suzuki, A. C. 2003. Life History of *Milnesium tardigradum* Doyère (Tardigrada) under a Rearing Environment. Zool. Sci. 20: 49-57. Tardigrada (Water Bears). 2005. Based on Animal Classification. Grzimek's Animal Life Encyclopedia, Vol. 2. The Gale Group, Inc. Accessed on 27 February 2010 at Answers.com <http://www.answers.com/topic/tardigrada-water-bears-biological-family>.

Tardigrada (Water Bears). 2005. Accessed 14 June 2010 at < http://www.answers.com/topic/tardigrada-water-bears-biological-family>.

Walz, B. 1982. Molting in Tardigrada. A review including new results on cuticle formation in *Macrobiotus hufelandi*. In: Nelson, D. R. (ed.). Proceedings of the Third International Symposium on the Tardigrada, August 3–6, 1980, Johnson City, Tennessee. East Tennessee State University Press, Johnson City, pp. 129-147.

Węglarska, B. 1990. Morphology of excretory organs in Eutardigrada. Acta Biol. Cracow Ser. Zool. 31: 63-70.

CHAPTER 5-3
TARDIGRADE HABITATS

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CHAPTER 5-3
TARDIGRADE HABITATS

Figure 1. *Echiniscus* sp., member of a genus that is common on bryophytes. Photo by Martin Mach, with permission.

Bryophyte Habitats

Tardigrades exist in both aquatic and terrestrial habitats worldwide, and in both cases can be found with bryophytes (Figure 1) (and lichens and leaf litter) (Utsugi *et al*. 1997). The tropics seem unfavorable (Mathews 1938), perhaps for the same reasons that temperate stream bryophytes are uncommon in lowland tropical waters – they are warm and wet at the same time, encouraging bacterial and fungal growth.

Most of the more than 900 known tardigrade species are **limnoterrestrial** (Garey *et al* 2008). That is, they live in a thin surface film of water, most commonly on bryophytes, lichens, algae, and other plants. They can only remain active while this film of water exists. Of the 910 species reviewed by Garey *et al*. (2008), only 62 species, in 13 genera, are truly aquatic and unknown from limnoterrestrial habitats. Nevertheless, many of the limnoterrestrial species can at least occasionally be found in freshwater. In these terrestrial habitats, pH of the substrate, oxygen tension, moisture content of the bryophyte, thickness of the bryophyte mat or cushion, and altitude (and its attendant conditions) all contribute to the habitat distribution.

I have taken the liberty of changing the word "moss," used in many tardigrade studies, to "bryophyte." I have learned from one of my kind tardigrade reviewers that people who study tardigrades often do not understand leafy liverworts and lump them into mosses. Hence, unless I could determine that the researcher definitely had in mind only mosses (and not also liverworts), I used the term bryophytes. I also learned that many ecologists include mosses in the category of "soil"! Others include them in "litter." While this lumping can be a useful concept for some aspects of functional ecology, one needs to be aware of it when searching for bryological literature or interpreting soil literature.

Tardigrades are especially common on tree bark bryophytes (**epiphytes**), presumably due to having similar tolerances to drying (Crum 1976). They are known from all seven continents and up to 6600 m altitude in the Himalayas (Ehrenberg 1859 in Fontoura *et al*. 2009). Collins and Bateman (2001) examined factors affecting tardigrade distribution in Newfoundland and learned that in this case altitude and type of bedrock were important in determining tardigrade distribution. Moisture and rate of drying further defined their distribution. And in some cases, competitive exclusion or interspecific competition seemed to be determining factors for community composition.

Specificity

Species assemblages seem to be similar throughout the world. Is this due to lack of taxonomic understanding or to widespread dispersal? In the following sections we will examine what we know about factors affecting tardigrade communities under different circumstances.

It appears that many tardigrades have little preference for bryophytes versus lichens (Meyer & Hinton 2007). But even those cryptogams, supporting their wet-dry requirements, are usually not unique habitats for the tardigrades, with the same species of bryophytes and lichens also present in soil, leaf litter, and additional habitats. Several studies have attempted to show any species preferences for bryophytes, but typically with no success (Kathman & Cross 1991; Miller & Heatwole 1995; Meyer & Hinton 2007). Further evidence of non-specificity is in their distribution. Meyer and Hinton (2007) report that the Nearctic realm shares 82 species of tardigrade with the Neotropical realm. Everything is everywhere! On the other hand, 30% of the Nearctic species are known from only one site. One of the problems in describing the tardigrade habitat is that substrate records are inconsistent or absent for many collections.

But some studies have indicated that lichens and mosses may be preferred over other substrata. Working in China, Beasley *et al*. (2006) found 18 species of tardigrades from three provinces, primarily in lichens and bryophytes.

One of the most common tardigrades among bryophytes is *Milnesium tardigradum* (Figure 21) and the less common *Macrobiotus hibiscus*. Hinton and Meyer (2008) reported these among liverworts (*Jungermannia* sp.; Figure 2) in a suburban lawn in central Georgia (USA).

Figure 2. *Jungermannia atrovirens*, member of a genus where tardigrades have been found in a lawn in Georgia, USA. Photo by Michael Lüth, with permission.

Liverworts are rarely mentioned in tardigrade studies. However, Hinton and Meyer (2007) reported *Echiniscus virginicus* and *Milnesium tardigradum* from liverworts. In their study, they collected handfuls of mosses, liverworts, and lichens from 54 parishes in Louisiana, USA. They found 51 species in the region: 19 in Texas, 16 in Louisiana, 10 in Mississippi, 33 in Alabama, 3 in Georgia, and 15 in Florida. Of these 51 cryptogam dwellers, 20 are widely distributed in the region and 18 are

apparently cosmopolitan. Hinton and Meyer suggested that *Biserovus bindae*, *Echiniscus cavagnaroi*, *Echiniscus kofordi*, *Hexapodibius christenberryae*, *Minibiotus fallax*, and *Parhexapodibius pilatoi*, along with a new variant of *Macrobiotus* cf. *hufelandi*, may represent a distinctive southeastern USA regional fauna living among cryptogams. On the other hand, *Echiniscus cavagnaroi* and *Echiniscus kofordi* are known only in the Neotropical Region and in the Galapagos Islands, but *Minibiotus fallax* was described in Australia. These three species are frequently encountered in mosses and lichens.

Habitat Differences

Several tardigrade researchers have considered five types of bryophyte habitats (Mihelčič 1954/55, 1963; Ramazzotti 1962; Hofmann 1987):

- bryophytes that are submerged
- bryophytes that are permanently moist
- bryophytes growing in shady places and rarely dry
- bryophytes that dry out frequently and receive direct sunlight regularly
- bryophytes that are extremely exposed and often dry for a longer period.

Ito (1999) was able to identify six groupings of tardigrades, based on habitat preference, in his altitudinal study on Mt. Fuji, Japan. Kaczmarek *et al*. (2011) likewise found altitudinal relationships in Costa Rica. They furthermore found a higher diversity among mosses than among lichens or liverworts, although they admitted to a possible bias due to unequal sampling. Tardigrades from these substrates were most common from 2000 to 2400 m asl and above 3200 m asl.

Tardigrades do not have much control over their dispersal, typically depending on dispersal of the substrate. This may help to explain the observations on two morphotypes of the moss *Grimmia*. In this case, the tardigrade distribution was very patchy. There were no differences in distribution patterns on the two *Grimmia* morphotypes, despite their representation of different moisture conditions (Bettis 2008). Bettis suggested that the greater rainfall during the winter of observation might account for the lack of difference. But tardigrades are well known for their great tolerance of extremes, so their greatest limitation may be dispersal.

Acid or Alkaline?

Bartels (2005) reported greater diversity in limestone habitats than elsewhere. It appears that acid can be an uncomfortable or lethal milieu. *Hypsibius dujardini* (Figure 13) had reduced activity after only five minutes at pH 3 and died at pH 2.8. Even at pH 4.0, it had reduced activity after 30 hours.

But in Giessen, Germany, Hofmann (1987) found a somewhat different relationship. The four most abundant species [*Macrobiotus hufelandi* (Figure 7), *Ramazzottius* (formerly in *Hypsibius*) *oberhaeuseri* (Figure 3), *Milnesium tardigradum* (Figure 21), and *Echiniscus testudo* (Figure 4)] had similar preferences for alkaline substrata, but the remaining species, as a group, had a preference for the acidic habitats, thus presenting greater tardigrade species diversity among the acid substrata and the mosses that inhabited them.

Figure 3. *Ramazzottius oberhaeuseri*, a bryophyte dweller that cannot tolerate constant moisture. Photo by Martin Mach, with permission.

Hingley (1993) found only two genera in her acid peatland studies: *Diphascon* (*D. scoticum*) (Figure 29) and *Macrobiotus* (Figure 6). To that Pilato (2009) added *Bindius triquetrus* from *Sphagnum* (Figure 5) in Sicily. Distribution is patchy (Romano *et al.* 2001), requiring greater sampling effort. One must wonder, is the paucity of reports of aquatic tardigrades on bryophytes a realistic representation of a meager aquatic fauna, or are there simply too few studies that have looked for them?
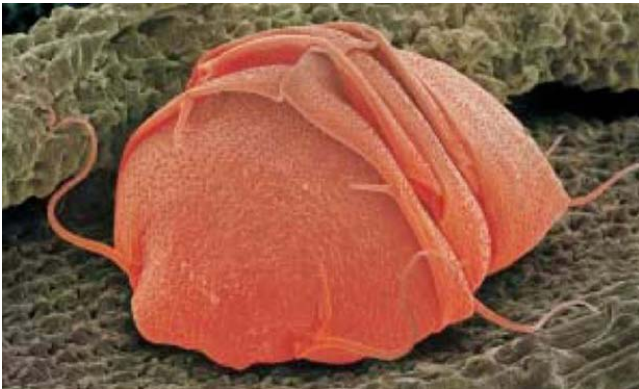
Figure 4. *Echiniscus testudo* tun on a bryophyte leaf. Photo by Power & Syred, through Creative Commons.

Figure 5. *Sphagnum fuscum*, a species that forms hummocks where a tardigrade could find moisture but usually avoid being flooded. Photo by Michael Lüth, with permission.

Altitude

Many researchers have shown a relationship between altitude and the distribution of tardigrades (Rodriguez-Roda 1951; Nelson 1973, 1975; Ramazzotti & Maucci 1983; Dastych 1985, 1987, 1988; Beasley 1988), suggesting that species richness increases with altitude. Bertolani and Rebecchi (1996) found that some species were typical of high altitudes or latitudes. Some researchers have even classified the tardigrades based on

altitudinal groupings (lowland, upland, montane, etc.) (Ramazzotti & Maucci 1983; Dastych 1987, 1988). Collins and Bateman (2001) found that in Newfoundland, Canada, the lowland class could be further divided into locations up to 100 m and those above (101-200 m).

Table 1 compares the altitudinal abundance of 45 species of tardigrades associated with bryophytes on mountains in British Columbia, Canada (~48-60ºN), with those of **riparian** epiphytes (inhabiting trees on banks of natural water courses) in Alabama, USA (~33ºN). Although the latitudes are quite different, six species are common to both), but six species differ. The three most abundant Alabama species were common to both, but the very common *Macrobiotus hufelandi* (Figure 7) was absent in the Alabama collections. These data suggest that there may be more than just chance determining the species and abundance differences.

But not all altitudinal studies have supported these conclusions (*e.g.* Kathman & Cross 1991). It is interesting that Meininger and Spatt (1988) found that altitude was not influential in determining distribution and abundance of moss-dwelling tardigrades in Alaska, USA. Likewise, Guil *et al.* (2009) found that the altitudinal differences could be explained by differences in soil, climate, vegetation structure, and litter type. Nevertheless, Ramazzotti and Maucci (1983) considered certain species to occur only above 500 m. This may simply be a lack of sufficient collecting – they claimed that *Macrobiotus harmsworthi* (Figure 6) was one of these "montane" species, but Dastych (1985) later reported it from locations between 0 and 1100 m altitude on Spitsbergen Island, Norway. Furthermore, Dastych (1980, 1988), showed a large correlation between Tardigrada species and altitude in the Tatra Mountains in Poland. Certainly latitude must be considered in making comparisons of altitude. And local moisture regimes are likely to play a major role in altitudinal relationships.
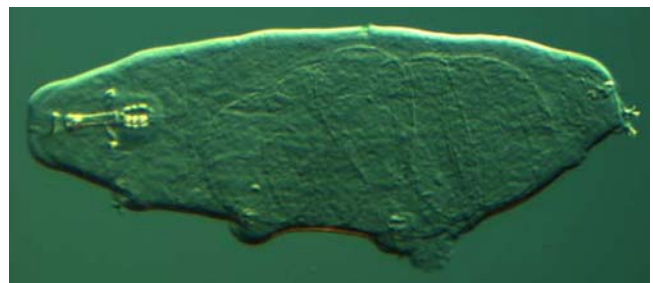
Figure 6. *Macrobiotus harmsworthi*, a common tardigrade on bryophytes and elsewhere. Photo by Paul J. Bartel, with permissions.
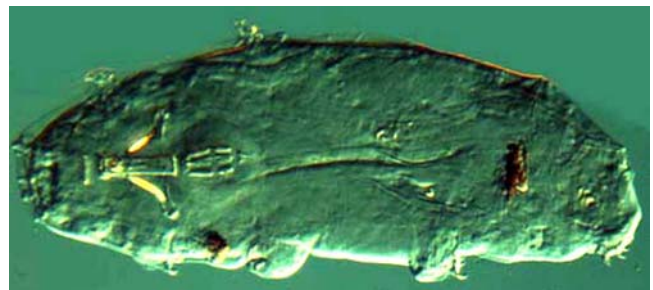
Figure 7. *Macrobiotus hufelandi*, one of the most abundant tardigrades on bryophytes. Photo by Paul J. Bartels, with permission.

Table 1. Altitudinal distribution of numbers of tardigrades in eleven bryophyte samples each, from six altitudes on five mountains on Vancouver Island, British Columbia, Canada, from Kathman & Cross 1991, and from 108 riparian epiphytic bryophyte samples representing 6 sites at Choccolocco Creek, Alabama, USA, from Romano *et al*. 2001. Those highlighted in grey occur in both sites.

	Altitude (m)						
	150	450	750	1050	1350	>1525	riparian
Astatumen trinacriae							3
Bertolanius [=*Amphibolus*] *weglarskae*	0	0	0	0	0	2	
Calohypsibius ornatus	0	3	10	4	2	3	
Diphascon [=*Hypsibius*] *scoticum*	22	18	11	13	30	16	
Diphascon belgicae	1	0	0	6	2	1	
Diphascon iltisi	0	1	0	0	0	0	
Diphascon modestum	12	0	1	4	0	0	
Diphascon nodulosum	14	4	1	16	17	26	
Diphascon pingue sl	70	318	45	7	40	27	
Diphascon prorsirostre	4	38	16	3	8	5	
Diphascon recamieri	49	47	2	3	13	1	
Echiniscus cf. *arctomys*							28
Echiniscus horningi	2	1	1	3	3	5	
Echiniscus mauccii	6	3	0	3	1	7	16
Echiniscus quadrispinosus	0	0	2	14	4	1	
Echiniscus sp. n.							24
Echiniscus wendti	2	3	3	0	38	3	
Hypechiniscus gladiator	0	0	0	0	0	0	
Hypsibius convergens	199	203	188	78	54	26	
Hypsibius dujardini							1
Insuetifurca arrowsmithi	0	0	40	0	1	0	
Isohypsibius lunulatus	6	9	0	0	0	0	
Isohypsibius sattleri	96	49	28	4	8	0	
Isohypsibius woodsae	0	0	0	2	0	0	
Itaquascon pawlowskii	1	0	0	2	0	1	
Macrobiotus crenulatus	1	0	0	0	0	0	
Macrobiotus echinogenitus (Figure 8)	0	0	10	79	48	0	737
Macrobiotus harmsworthi	177	459	284	44	8	10	
Macrobiotus hufelandi	3039	1710	2061	1116	1586	662	
Macrobiotus islandicus							1
Macrobiotus lazzaroi	10	0	0	0	0	0	
Macrobiotus sp. A	1	0	0	0	0	0	
Mesocrista spitzbergensis	5	1	0	2	2	0	
Milnesium tardigradum	21	24	2	0	2	4	87
Minibiotus cf. *intermedius*							27
Minibiotus intermedius	2	1	12	3	0	0	
Murrayon hibernicus	0	0	0	14	1	0	
Paramacrobiotus [=*Macrobiotus*] *areolatus*	31	16	0	0	0	0	476
Paramacrobiotus[=*Macrobiotus*] *richtersi*	0	0	0	0	0	1	4
Platicrista cheleusis	8	1	2	13	10	13	
Pseudechiniscus goedeni	0	5	0	0	0	2	
Pseudechiniscus juanitae	0	0	3	0	0	0	44
Ramazzottius baumanni	18	44	8	7	5	3	
Ramazzottius oberhaeuseri	11	2	0	1	1	0	
Testechiniscus laterculus	0	0	0	0	39	0	
SUM OF INDIVIDUALS	3808	2960	2730	1421	1923	819	1448
NUMBER OF SPECIES	27	23	21	24	24	22	12

Figure 8. *Macrobiotus echinogenitus*, a tardigrade living on riparian bryophytes at Choccolocco Creek Alabama, USA. Photo by Paul J. Bartels, with permission.

Using PCA analysis, Kathman and Cross (1991) also reported no relationship between altitude and abundance on Vancouver Island, British Columbia, on the western coast of Canada (Table 1 suggests a decreasing trend in number of individuals might be present). Collins and Bateman (2001) later reported that altitude was one of the major determining factors in tardigrade distribution in Newfoundland, eastern coast of Canada. Rodriguez-Roda (1951 in Kathman & Cross 1991) found that altitude had a distinct effect on the abundance of tardigrades in Spain, with numbers increasing with altitude and reaching a maximum between 1000 and 2000 m. Dastych (1980) likewise found that tardigrades in the Tatra Mountains of Poland increased with altitude, again with the maximum numbers between 1000 and 2000 m. In one of his later studies, Dastych (1985) reported a seemingly opposite effect, demonstrating that the number of species and individuals decreased with increasing altitude in West Spitsbergen, Norway. The differences between studies may be a matter of scale and the fact that only some species are affected by altitude within the study range, but moisture regimes are likely to differ as well.

Nelson (1973, 1975) found that only seven of the 21 bryophyte-dwelling species on Roan Mountain, Tennessee, USA, were affected by altitude. Bertrand (1975) found three altitudinal groups in the Aigoual Mountains of France. Beasley (1988) divided the tardigrades from 1052-3567 m in New Mexico, USA, into altitudinal ranges. Ito (1999) examined tardigrades at 17 stations on Mt. Fuji, Japan, finding little distributional pattern related to altitude (950-2380 m). Rather, the distribution related to habitat.

Collins and Bateman (2001) found that tardigrades of Newfoundland, Canada, were affected by both altitude (Table 2) and type of bedrock, but that moisture content and rate of desiccation of the mosses and lichens where they live also contributed to their distributional pattern.

Table 2. Decline in number of species with increasing altitude in Newfoundland. From Collins and Bateman 2001.

Altitude	Total number of species found at each altitude	Mean number of tardigrade species at each site
0->100 m	28	2.75
101->200 m	15	1.75
200+ m	8	1.88

Differences in techniques, lack of or differences in statistical analyses to support purported differences, and misidentification could contribute to the apparent differences in relationships among these studies, but moisture regimes most likely play a major role. In some cases, competitive exclusion appears to play a role (Collins & Bateman 2001). Nevertheless, it is likely that the effect of altitude, if it exists, depends in part on both latitude and scale.

Polar Bryophytes

Because of their relative abundance, and the predominance of mosses and lichens in the Antarctic flora, we have learned some interesting aspects of their faunal ecology and physiology there.

As early as 1976, 23 species of tardigrades were known from Antarctica (Jennings 1976). That's not bad for that early date in a place with limited vegetation, harsh climate, and limited opportunity for collecting, not to mention the distance to be travelled for colonization. Most of these tardigrade species have worldwide distribution (Venkataraman 1998). In the short Antarctic summers, the tardigrades multiply quickly, using parthenogenesis.

Unlike most habitats elsewhere, the tardigrades in the Antarctic moss turf do exhibit a vertical zonation pattern. Schwarz *et al.* (1993) found that protozoa, rotifers, nematodes, and tardigrades dominated the moss-dominated flushes at Canada Glacier, southern Victoria Land, Antarctica. Mites were of less importance. These invertebrates occurred in the range of 5 to 10.83 mm depth in the moss clumps. Post melt samples had a greater percentage of all groups of organisms in the upper 5 mm of mosses compared to those at that depth range in the pre-melt samples, suggesting either migration or rapid reproduction once melting occurred.

Venkataraman (1998), in clumps of **Bryum argenteum** (Figure 9) from continental Antarctica, found that the tardigrades only live down to 15 cm depth in the 30 cm deep turfs. If they prefer to eat rotifers, they can only find those down to 10 cm. Their slow movement could limit the distance they can reasonably move downward and still return for food in a timely manner. Ramazzotti (1972) estimated that tardigrades could travel an average of 17.7 cm h^{-1}, seemingly enough speed to travel another 10 cm, but perhaps not in the cold and not during the daytime feeding period when downward movement would be most beneficial to avoid drying.

Temperature may play a role in the zonation of these Antarctic bryophytes. Bryophyte temperatures in the Antarctic can differ considerably from those of the air and may provide a warm refuge for activity even on cold days. Bryophytes exhibit a sharp temperature zonation on sunny days when there is no snow cover (Jennings 1979). The surface is subject to evaporative cooling in the polar winds while the moss layer immediately below that interface is quiet and often dark in color, absorbing the heat like a black body, as seen by the temperatures shown in Figure 10. Hence, in summer the moss turf has temperatures much higher than that of the air and at the beginning and end of the summer season the temperatures fluctuate around freezing for a considerable time, even if the mosses are snow-covered.

Sohlenius and Boström (2006) described tardigrade communities from moss cushions on four ice-free mountains (nunataks) in Antarctica. Tardigrades occurred in 32% of the 91 samples of mosses. No invertebrates at all occurred in 8% of the samples. They considered stochastic processes (random events) accounted for the uneven colonization of the moss cushions, most likely as a result of random dispersal. They suggested that nematodes seem to offer competition, whereas the rotifers seem to provide a food choice.

Figure 9. *Bryum* sp. cushions in Antarctica, demonstrating the deep turfs that can house tardigrades. Photo courtesy of Catherine Beard.

Figure 11. *Richtersius coronifer*, a tardigrade that survives Arctic winters on mosses in a frozen state. Photo by Martin Mach, with permission.

Tardigrades manage to survive the extreme cold of the long Antarctic winter as a **tun** (see Chapter 4-6). But other physiological adaptations are needed to combat the extremes of temperature that can be experienced in a single Antarctic day (see for example Figure 10).

Both **Bertolanius nebulosus** and **Richtersius coronifer** (Figure 11) endure ice formation as they proceed through the onset of freezing temperatures (Westh & Kristensen 1992). Both are able to supercool to -6 or -7ºC before they succumb to freezing. These two tardigrades are common in Polar areas, as well as elsewhere. **Richtersius coronifer** (Figure 11) spends its Arctic winters in drought-resistant mosses as a frozen or dry individual. **Bertolanius nebulosus** has adopted a somewhat different strategy, spending its cold period in moist mosses and algae as a frozen cyst, or occasionally as an egg or adult.

It appears that adapted tardigrades can survive extreme conditions for a long time. Newsham *et al*. (2006) experimented in a way that might be considered cruelty to animals (but not unlike Mother Nature herself). They partially dried a bit of the leafy liverwort **Cephaloziella varians** from Rothera Point on the Wright Peninsula, Adelaide Island, western Antarctic Peninsula, then stored it frozen at -80ºC for six years and two months. They then rapidly thawed the liverwort at 10ºC. You guessed it! Tardigrades survived, along with nematodes and a bdelloid rotifer. Only two individuals [**Diphascon** sp. (see Figure 12), **Hypsibius** cf. **dujardini** (Figure 13)] out of fifteen tardigrades (13%) made it, but that is still remarkable! The eleven individuals of **Macrobiotus furciger** (Figure 14) and one of **Echiniscus** sp. did not. Nematodes fared a bit better, with 31% survival out of 159 individuals.
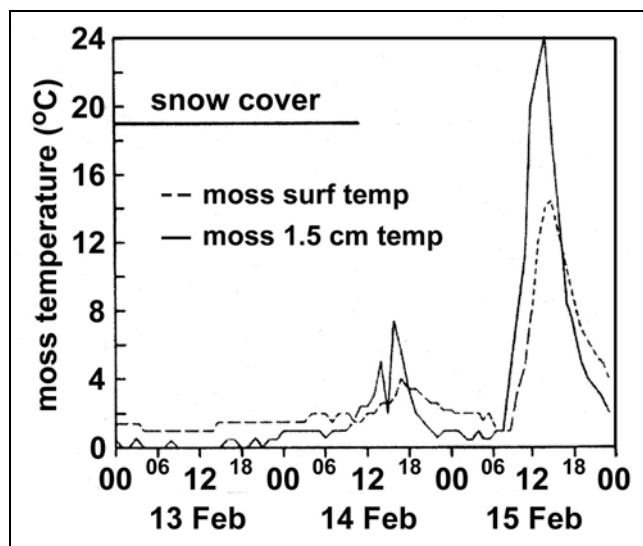

Figure 10. Comparison of moss surface temperature with that of 1.5 cm depth in moss cover on Signy Island in the Antarctic on three days in February, 1973. Redrawn from Jennings 1979.

Figure 12. **Diphascon scoticum**, a moss-dwelling representative of a genus in which one member survived storage at -80ºC for six years! Photo by Łukasz Kaczmarek, with permission.

Figure 13. *Hypsibius dujardini*, a species that survived -80ºC for six years. Photo by Rpgch, through Wikimedia Commons.

Figure 14. *Macrobiotus furciger*. Photo by Smithsonian Institution, through EOL Creative Commons.

Sømme and Terje Meier (1995) examined *Echiniscus jenningsi* (Figure 15)*, Macrobiotus furciger* (Figure 14), and *Diphascon chilenense* from Müihlig-Hofmannfjella, Dronning Maud Land, Antarctica to ask similar winter survival questions. They compared both hydrated and dehydrated individuals. After 600 days at -22ºC, both hydrated and dehydrated tardigrades had high survival rates. After 3040 days, the dry individuals still had a high rate of survival. However, at -80ºC hydrated *Echiniscus jenningsi* (Figure 15) did not fare as well as the others, decreasing in survivorship as time increased from 7 to 150 days. At -180ºC, all hydrated individuals of these three species rapidly died, but all dehydrated species had good survivorship after 14 days at -180ºC.
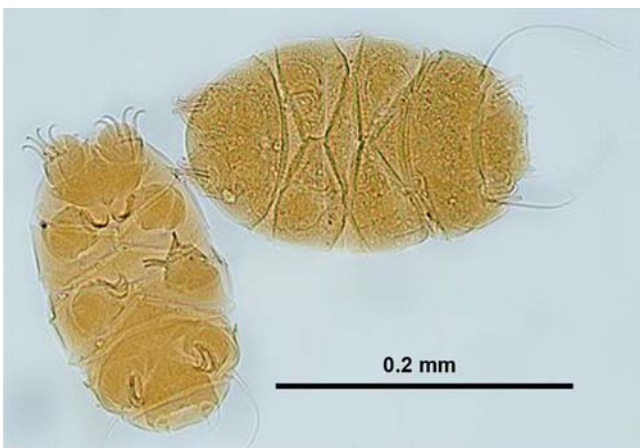

Figure 15. *Echiniscus jenningsi*. Photo by Smithsonian Institution, through EOL Creative Commons.

It is incredible that some Antarctic tardigrades can survive temperatures as low as -80ºC in a **hydrated** state (Sømme& Meier 1995; Sømme, 1996)! The ability to survive short periods in a hydrated condition just below a freezing temperature is important to survival in bryophyte clumps that regularly warm in the day and freeze at night. Ice crystals on the bryophytes would most likely help to dehydrate the tardigrades as temperatures plummeted to well below freezing, facilitating their survival during the long and very cold winters. Furthermore, the ability to survive low temperatures for years would permit tardigrades to survive dispersal across the ice or remain viable within it until a suitable habitat or conditions are reached.

But how do the rapidly changing temperatures of the environment affect the ability of the tardigrade to move about on the bryophyte to seek food? The **beneficial acclimation hypothesis** (BAH) predicts that animals will have their best performance at the temperature to which they are acclimated. Li and Wang (2005) tested this hypothesis with the moss-dwelling species *Macrobiotus hufelandi* (Figure 7, Figure 19), collected from the Qinling Mountains in central China. They acclimated the water bears to 2 and 22ºC for two weeks. Using walking speed and percentage of time moving, they compared performance at the acclimation temperature with that at the alternate temperature. They found that both walking speed and percentage of time moving was significantly faster when the tardigrades were kept at their acclimation temperature than when they were placed at the higher or lower experimental temperature.

But in the Antarctic, we have seen that such extreme temperature fluctuations within a single day are not unusual. Could this be a threat to the water bears, who must find food, often adhering bacteria and algae, on the moss? And others eat nematodes and other moving targets. One factor to consider is that in the experiments of Li and Wang, only 1.5 minutes acclimation were provided at the new temperature before measurements began, lasting another 3-5 minutes. This seems unrealistic as a representation of nature. The next question to ask is how fast can the tardigrades acclimate to a new temperature?

Danger may lurk among the Antarctic bryophytes. Gray *et al.* (1982) isolated eighteen taxa of predaceous fungi from among Antarctic mosses and soil samples. Among these eight different trapping mechanisms were present. The fungus *Monacrosporium ellipsosporum* seemed to be associated primarily with calcicolous mosses. Although these are nematode-trapping fungi, they may also catch the occasional tardigrade. More importantly, it indicates that the moss habitat is suitable for parasitic fungi that might attack other invertebrate groups such as tardigrades.

Miller *et al.* (1996) actually looked at the role of bryophytes vs other cryptogamic substrata in harboring tardigrades on the Windmill Islands in East Antarctica. *Pseudechiniscus suillus* (Figure 16), *Macrobiotus* sp. (see Figure 7, Figure 19), *Hypsibius antarcticus*, *Ramajendas frigidus*, *Diphascon chilenense*, and *Diphascon pingue* (Figure 17) occurred among mosses and lichens. Three of these had positive associations with each other and with bryophytes: *Pseudechiniscus suillus*, *Hypsibius*

antarcticus, and *Diphascon chilenense*. These three species had a strong negative association with algae and lichens.
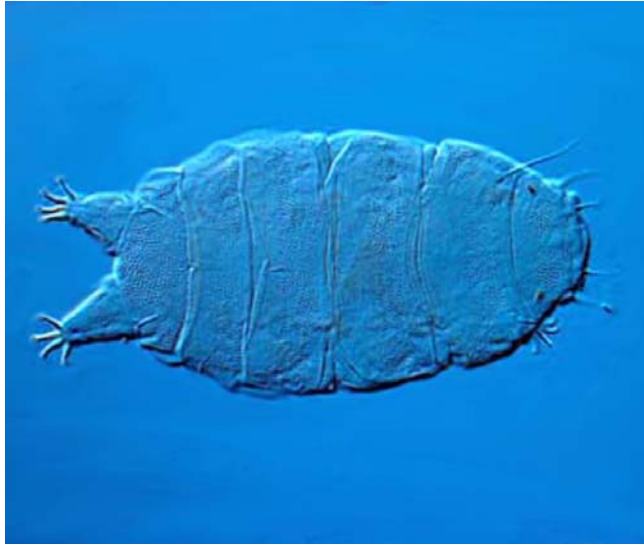
Figure 16. *Pseudechiniscus suillus* group, an Antarctic bryophyte dweller that avoids lichens. Photo by P. J. Bartels, with permission.

Figure 17. *Diphascon pingue*, a moss and lichen dweller Swedish forests and in the Antarctic. Photo by Michael Collins, with permission.

Forest Bryophytes

Forests have a much more tempered climate than the Antarctic. Trees reduce the rate of water loss and shade the bryophytes and their fauna from the heat of the bright sun.

Jönsson (2003) examined bryophytes in Swedish forests and found sixteen species of tardigrades, five of which were previously undescribed for that region [*Murrayon dianeae* (Figure 36), *Isohypsibius sattleri*, *Platicrista angustata* (Figure 18), *Diphascon belgicae*, *D. pingue* (Figure 17)]. Jönsson found that the pine forest had the most species compared to clearcut areas, but that abundance differed little from that of clearcut areas. Of the sixteen species of tardigrades recorded, the cosmopolitan *Macrobiotus hufelandi* (Figure 19) was by far the most abundant. The weft growth form seemed to harbor more tardigrades than did other bryophyte growth forms.

Figure 18. *Platicrista angustata*. Photo by Michael Collins, with permission.

Figure 19. *Macrobiotus hufelandi*. Photo by Martin Mach, with permission.

Schuster and Greven (2007) conducted a 54-month study of the tardigrade fauna of the moss *Rhytidiadelphus squarrosus* in the Black Forest of Germany. They found 24 species, dominated by *Macrobiotus hufelandi* (56%), *M. richtersi* (18%), and *Diphascon pingue* (12%). Diversity tended to be higher in winter, but the three dominant species generally declined in winter and increased from spring until fall. Rainfall, humidity. and temperature seemed to play a major role in changes in seasonal abundance.

Epiphytes

Whereas forest floor bryophyte dwellers are protected by snow in winter, bryophytes on trees (**epiphytes**) are often above the winter snow level. In summer they have intermittent wet and dry periods and in winter they often have exposures to extreme temperatures, lacking the protective cover of snow. In the Cincinnati, Ohio, USA area, bark-inhabiting bryophytes provide homes to numerous tardigrades, with the greatest species richness in environs of high humidity and clean air (Meininger *et al.* 1985). Hence, cities afford a less hospitable environment due to the lower humidity and decreased air quality.

Despite their seemingly unfriendly habitat, epiphytic bryophytes are particularly suitable as a tardigrade habitat. Indeed, this habitat seems to house the most species. The frequency of wetting and drying of these bryophytes seems to be most suitable to the tardigrade life cycle. Richness seems to run about 4-16 species. Hooie and Davison (2001; Hooie Tardigrade diversity) found the following tardigrades associated with the epiphytic moss *Ulota crispa* (Figure 20) on four tree species (*Acer saccharum*, *Acer rubrum*, *Betula lenta*, *Tilia americana*) in the Great Smoky Mountains National Park, USA:

> ***Echiniscus*** cf. ***oihonnae***
> ***Echiniscus virginicus***
> ***Hypechiniscus gladiator***
> ***Macrobiotus hufelandi*** (Figure 19)
> ***Milnesium tardigradum*** (Figure 21)
> ***Minibiotus*** cf. ***pustulatus***
> ***Paramacrobiotus tonollii*** (Figure 23)
> ***Pseudechiniscus*** (Figure 22)

Figure 21. *Milnesium tardigradum*. A cosmopolitan bryophyte inhabitant. Photo by Martin Mach, with permission.

Figure 22. *Pseudechiniscus juanitae*. Photo by Paul J. Bartels, with permission.

Figure 20. *Ulota crispa*, an epiphytic moss that houses a number of tardigrade species. Photo by Michael Lüth, with permission.

In a study of riparian tardigrades, Romano *et al*. (2001) reported on the tardigrades on epiphytic bryophytes in Alabama, USA. Using 108 samples, they extracted 1588 tardigrades from three tree species on six sampling dates. Like Riggin (1962) for forest bryophytes, Romano *et al*. (2001) found that *Macrobiotus* species (Figure 6-Figure 8) were the most abundant (1358 of the 1588 tardigrades, 86%). They found no differences among tree species, bryophyte species, or seasons, but there were site differences, possibly suggesting dispersal limitations. Although relative humidity and temperature did not seem to influence abundance, precipitation did. Interestingly, as precipitation increased, the number of tardigrades decreased. Beasley (1981) found that higher humidity resulted in lower tardigrade abundance in the Caribbean National Rain Forest at Luquillo, Puerto Rico. This further supports the hypothesis that periods of anhydrobiosis are required in the life of a tardigrade and that lack of them shorten the length of life. However, if only active periods are considered, there may be little difference.

Figure 23. *Paramacrobiotus tonollii*, a tardigrade known from *Ulota crispa* in the Smoky Mountains. Photo by Martin Mach, with permission.

Briones *et al*. (1997) suggested that during periods of high precipitation the film of water surrounding the bryophytes may become anoxic, killing the tardigrades. This could especially be a problem in the riparian zone, where the bryophytes, and hence the tardigrades, were under water during several collection periods. Diversity of tardigrades was somewhat low in the Alabama, USA,

riparian sites (Table 1), with only twelve species overall (Romano *et al*. 2001). Mosses included **Anomodon** (Figure 24), **Leucodon** (Figure 25), and **Schwetschkeopsis** (Figure 26), all epiphytes.

Figure 24. **Anomodon rugelii**, an epiphytic moss. Photo by Michael Lüth, with permission.

Figure 25. **Leucodon sciuroides**, an epiphytic moss that compresses and curls its branches upward when it dries. Photo by Michael Lüth, with permission.

Figure 26. **Schwetskeopsis fabronia** , an epiphyte from Asia and North America, and home for tardigrades. Photo by Misha Ignatov, with permission.

As in the Alabama study, Jerez Jaimes (2002) found a low diversity of only seven species on the moss *Calymperes palisotii* (Figure 27) on six species of trees on the campus of the University of Puerto Rico at Mayagüez. The highest species richness was on *Mangifera indica* (mango) and *Swietenia macrophylla* (mahogany).

When collections came from trees and shrubs from all 67 counties in Florida, only 20 species of tardigrades were found (Meyer 2006, 2008). Meyer also found no specificity for a particular bryophyte (47 species included) or ecological region, but there was specificity for bryophytes as opposed to foliose lichens.

There seemed to be an avoidance of one moss species, **Aulacomnium heterostichum** (Figure 28), with no tardigrades occurring on it (Meyer 2008). Bartels and Nelson (2006), working in the Great Smoky Mountains National Park, USA, increased the number of known species in the park from three to 42 from multiple substrates, a further testimony to how little known these organisms are. Bartels and Nelson found that more tardigrades occurred in bryophytes at breast height on a tree than at the tree bases, perhaps again relating to longer or more frequent dry periods.

Diphascon [=*Hypsibius*] **scoticum** (Figure 29), a very common tardigrade, inhabits mosses on logs (Cushman, pers. comm. 1970). It would be interesting to compare the log-dwelling tardigrade taxa with those living on epiphytic bryophytes of the same species. Presumably, the log habitat would have longer moist periods. Based on the findings discussed above of Bartels and Nelson (2006) and Romano *et al.* (2001), one might expect more on the epiphytes, where alternating wet and dry periods might fit better with the apparent dormancy requirements of the tardigrades.

Figure 27. **Calymperes palisotii**, a moss that had the lowest tardigrade diversity on the University of Puerto Rico campus, Mayagüez. Photo by Claudio Delgadillo Moya, with permission.

Figure 28. *Aulacomnium heterostichum*, a moss that seems to be avoided by tardigrades. Photo by Janice Glime.

Figure 30. *Dactylobiotus* [=*Macrobiotus*] *dispar*. Photo by Martin Mach, with permission.

Figure 29. *Diphascon scoticum*, a very common tardigrade, one that has been found among mosses on logs. Photo by Paul J. Bartels, with permission.

Figure 31. *Dactylobiotus* [=*Macrobiotus*] *dispar*. Photo by Martin Mach, with permission.

Aquatic

Few truly aquatic tardigrades seem to exist. Garey *et al*. (2008) reported that of the more than 900 species of tardigrades they reviewed, most live in the water film on the surface of bryophytes, lichens, algae, and other photosynthetic organisms. Of their 910 species of tardigrades, only 62 (in 13 genera) were truly aquatic. In New Zealand, tardigrades represented only 2.4% of the fauna among the bryophytes of an unshaded stream in the alpine zone on South Island (Suren 1991a). In a more extensive study there, they represented only 1.2% of the invertebrates collected among mosses in 103 streams in one study (Suren 1993). Similar small numbers were present among the moss *Fontinalis antipyretica* (Figure 34) in the Czech Republic, where one stream had 1541 per mL of moss (0.6% of the fauna in the moss) and another stream had only 545 per mL (0.1%) (Vlčková *et al*. 2001/2002). In the Colorado Rocky Mountains, Elgmork and Sæther (1970) found most of the tardigrades *Dactylobiotus* cf *macronyx* (formerly *Macrobiotus macronyx*) associated with algae, but they also reported them from *Fontinalis* beds (Figure 34) and other submerged mosses.

In streams, flow velocity may cause tardigrades to seek refuge among mosses. Suren (1992) reported high densities of tardigrades (*Dactylobiotus* [=*Macrobiotus*] *dispar*; Figure 30-Figure 31) associated with mosses in alpine streams of New Zealand. In an earlier study Suren (1991b) found that the colonization of this species on artificial mosses was at a reduced density compared to that on mosses [*Fissidens rigidulus* (Figure 32), *Cratoneuropsis relaxa*, *Bryum blandum* (Figure 33)].

Figure 32. *Fissidens rigidulus* from New Zealand, a good tardigrade habitat. Photo by Bill and Nancy Malcolm, with permission.

Figure 33. *Bryum blandum* from New Zealand, where tardigrade density is greater than that on artificial mosses. Photo by Jan-Peter Frahm, with permission.

Suren (1992) reported densities of 3120 and 8160 per m² on the mosses in two trials, whereas they reached only 1760 and 1600 on the artificial substrata. He suggested that the high periphyton biomass among mosses provided a good food source that made this a good habitat for the tardigrades. This suggestion is supported by the largest percentage of variation (24.2%) being explained by the ultra-fine particulate matter (UFPOM). The abundance of tardigrades on bryophytes was 10 times that found on stream gravel.

Linhart *et al.* (2002) examined scattered clumps of the aquatic moss *Fontinalis antipyretica* (Figure 34) and found that whereas several groups of invertebrates were distributed among the clumps in relation to stream flow, this was not the case for tardigrades. Even though the researchers showed that fine organic matter trapped within the moss mat was determined by flow velocity, this did not seem to be a determining factor in tardigrade distribution.

Figure 34. *Fontinalis antipyretica* in flowing water. Photo by Michael Lüth, with permission.

In the Italian Alps *Borealibius zetlandicus* occurred on *Warnstorfia exannulata* (Figure 35; Rebecchi *et al.* 2009). This tardigrade species is known only from boreo-alpine areas, where it typically occurs in sediment, submerged aquatic mosses, or *Sphagnum* (Figure 5). But like many other tardigrades, this one has a wide habitat range, including the Barents Sea and terrestrial mosses and soil that rarely dry out. This boreal habitat distribution for this species is possible because this species is able to survive freezing. But the populations of the species studied are unable to survive desiccation.

Figure 35. *Warnstorfia exannulatus*, home for the tardigrade *Borealibius zetlandicus* in the Italian Alps. Photo by Michael Lüth, with permission.

Suren (1992) also considered the possibility that the mosses offered shelter from the rapid flow of water elsewhere. These tiny organisms are often in the leaf axils of the mosses, where they have almost no effect from the strong flow, yet the oxygen diffusion could be fairly rapid. But as yet, no data seem to support cause and effect of flow velocity and tardigrade distribution.

Living in a stream is challenging for a tardigrade. Using 22 animals, Shcherbako *et al.* (2010) found that *Milnesium tardigradum* (Figure 21) could manage in water with a mean velocity of 23.3 mm/h, moving at a mean speed of 19.8 mm/h in the light and 29 mm/h in the dark, making snails look like track stars! Bryophytes provide a safe refuge from fast-moving waters. Eles and Repas (2009) described the stream tardigrades as having faster motion and longer claws than their terrestrial counterparts.

In New Zealand, Suren (1992) found that the tardigrade *Dactylobiotus dispar* (Figure 31) represented about 6.6% of the fauna on mosses in unshaded streams compared to 0.6% on gravel. In shaded streams they occupied only 5.3% of the bryophyte fauna compared to 0.4% on gravel. But not all wet habitats seem to be very suitable for tardigrades.

Kaczmarek (pers. comm. 29 January 2010) has reminded me that most of the water-dwelling tardigrades are in fact marine. Those that are truly freshwater aquatic species live on algae or plants (including bryophytes), in the sand, or in sediments. The genus *Murrayon* (Figure 36) is unusual among the water-dwelling tardigrades in that some aquatic individuals lay their eggs in the shed shells of cladocerans (Bertolani *et al.* 2009).

Figure 36. *Murrayon dianeae*, an aquatic tardigrade. Photo by Michael Collins, with permission.

Emergent bryophytes may be especially comfortable for some species of tardigrades. One of the more "friendly" environments is in association with *Barbula* [=*Didymodon*] *tophacea* (Figure 37-Figure 38), a well-known rock-forming moss, above the wet zone.
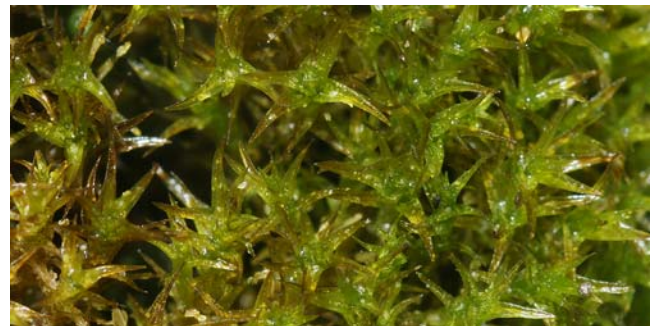

Figure 37. *Barbula* [=*Didymodon*] *tophacea*, an emergent moss known to house 84 tardigrades per gram. Photo by Barry Stewart, with permission.

The aquatic habitat is also sometimes hospitable to hygrophilous species that are more typical among moist mosses or species with a wide tolerance range for moisture conditions (Nelson & Marley 2000). On the other hand, *Hypsibius dujardini* (Figure 13) is known from moist mosses, but it is primarily aquatic (McFatter *et al.* 2007). *Parhexapodibius pilatoi* was found among mosses on a stream bank in central Georgia (McFatter *et al.* 2007), but otherwise is known only from Michigan (Bernard 1977).

Figure 38. *Barbula* [=*Didymodon*] *tophacea*, an emergent moss showing the numerous possibilities for resting in leaf axils. Photos by Michael Lüth, with permission.

Dry Habitats

Although collectors looking for a rich bryophyte flora would most likely ignore the desert, some bryophytes depend on its predominately dry nature. The cryptogamic crust of prairies and deserts has its own tardigrade fauna (Brantley & Shepherd 2002). In this habitat of bryophytes, lichens, Cyanobacteria, and algae, long dry periods are guaranteed. The occasional wet periods make it a suitable tardigrade habitat.

As already discussed, Meininger and Spatt (1988), working along Dalton Highway in the tundra adjacent to the trans-Alaska Pipeline, found that road dust had a major impact on both the mosses and the tardigrades. The mosses near the road represented xerophytic species tolerant of high calcium. Consequently, the tardigrades likewise were taxa tolerant of drier conditions. Because of the moisture limitations on other kinds of taxa, the tardigrades near the road were mostly fungivorous and algal feeders; those farther from the road, where *Sphagnum* (Figure 5) was able to grow, tended to be more omnivorous and carnivorous. These habitat differences caused differences in tardigrade fauna between roadside bryophytes and more distant *Sphagnum* species.

Vertical and Horizontal Distribution

It seems likely that some vertical distribution within the bryophyte mat should occur. These could be defined by light levels. The presence of eyespots (Figure 41) in at least some members of Tardigrada was reported by Greven (2007), with responses to light varying from none to both positive and negative. Beasley (2001) reported negative photokinesis in the common tardigrade *Macrobiotus hufelandi* (Figure 19), a common moss dweller. Rather than being attracted to or from the light, they increased

their rate of movement. Since light indicates sun intensity, it also is an indicator of the likelihood of drying, making the response to move quickly away from light an adaptive one. Vertical differences in tardigrade distribution are known from soil (Leetham *et al.* 1982). Nevertheless, as noted elsewhere, there seems to be little evidence for vertical position differences or migration of tardigrades in mosses; only one tardigrade (*Echiniscus viridissimus*) seems more common near the upper portion of the moss (Nelson & Adkins 2001). Wright (1991) found that in xeric habitats this species does not migrate vertically to the C zone as the moss dehydrates, even though other species do at the same time.

Data from the Antarctic suggest that temperature may play a role in the vertical positioning of tardigrades there. On Signy Island, 80% of the tardigrades occurred in the upper 6 cm of moss, and usually 70% were in the top 3 cm (Jennings 1979). One factor that contributes to this limited distribution is that the turf below 7-8 cm is **anaerobic** (lacking oxygen), making it inhospitable for the tardigrades. In moss-dominated flushes near the Canada Glacier in southern Victoria Land, Antarctica, the invertebrates, including tardigrades, occurred at a mean depth ranging 5-10.83 mm (Schwarz *et al.* 1993). As discussed above, the relative number of organisms increased near the surface in post-melt mosses. This is not necessarily a direct temperature response; it could result from changes in light or humidity associated with the melt.

Schuster *et al.* (2009) examined the microclimate within a cushion of the moss *Rhytidiadelphus loreus* (Figure 39). They found that the deep layers had lower daytime and higher nighttime temperatures than **ambient** (in this case, air temperature). Oxygen was similar throughout the cushion, but CO_2 increased greatly with depth. The six species of tardigrades were concentrated in the green-brown layer of the moss. The authors suggested that light and oxygen had little impact on the distribution but that CO_2 kept the tardigrades from occupying lower positions and that temperature might cause migrations within the upper portion.

Figure 39. *Rhytidiadelphus loreus*. Photo by Michael Lüth, with permission.

Differences in horizontal distribution may be the result of microhabitat differences such as shade vs sun or distance from water. But they can also be a simple result of passive, random dispersal and the slow-moving nature of the animal. Degma *et al.* (2011) sampled *Hypnum cupressiforme* (25 samples; Figure 40) to try to determine

the causes of horizontal positioning of tardigrades on that species. They found 224 tardigrades in the species *Milnesium tardigradum* (Figure 41), *Hypsibius convergens* (Figure 42), *H. microps*, *Diphascon pingue* (Figure 17), *Astatumen trinacriae* (Figure 43), *Macrobiotus hufelandi* (Figure 7), and *Minibiotus* sp. (Figure 44) They found no significant moisture gradient among the moss plants. The distribution of the tardigrade species was aggregated, but the number of species (richness) was random. There was no relationship of tardigrade species distribution to moisture.

But even these species may not be able to tolerate desiccation. *Macrobiotus hufelandi* (Figure 7) and *Hypsibius dujardini* (Figure 13) are unable to live in habitats that desiccate quickly, perhaps explaining their association with the slow-drying bryophytes (Wright 1991). Other bryophyte dwellers [*Milnesium tardigradum* (Figure 41) and *Ramazzottius* (formerly *Hypsibius*) *oberhaeuseri* (Figure 3)] may not tolerate constant moisture, but these two species avoid high insolation and rapid desiccation, again making bryophytes a suitable habitat.

Figure 40. *Hypnum cupressiforme*, home of seven species of tardigrades. Photo by Michael Lüth, with permission.
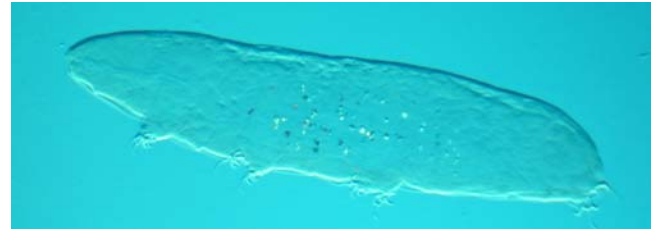
Figure 43. *Astatumen trinacriae*. Photo by Paul J. Bartels, with permission.
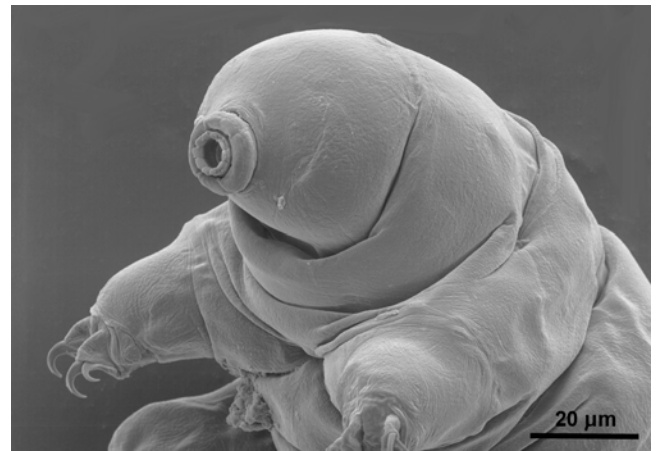
Figure 44. *Minibiotus intermedius*. Photo by William Miller, through Flickr.

Figure 41. Eyespots of *Milnesium tardigradum*. Photo by Martin Mach, with permission.

Competition and food relations are often determinants of the species assemblages. For example, *Milnesium tardigradum* (Figure 41) may be found with two *Hypsibius* species that it can use for food (Wright 1991). Competition may account for the negative associations among *Macrobiotus hufelandi* (Figure 7), *Paramacrobiotus richtersi* (Figure 45), and *Isohypsibius prosostomus* (see Figure 46) in xeric habitats.

Figure 42. *Hypsibius convergens*. Photo by Łukasz Kaczmarek, with permission.

Figure 45. *Paramacrobiotus richtersi*. Photo through Creative Commons.
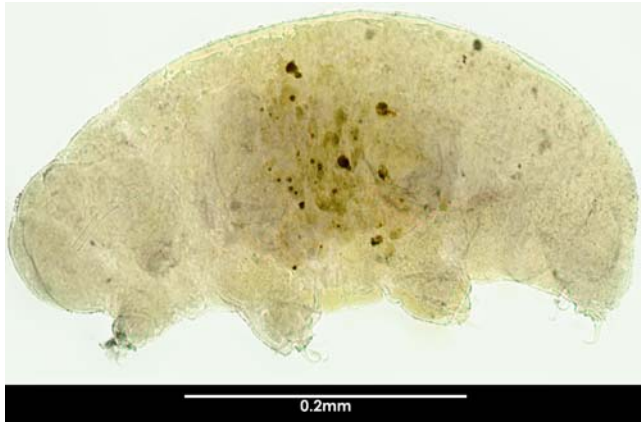
Figure 46. *Isohypsibius asper*. Photo by Smithsonian Institution through EOL Creative Commons.

Summary

Tardigrades live on both terrestrial and aquatic bryophytes, with the lowest numbers in the tropics. Epiphytic mosses seem to be especially suitable for them. Altitude is influential on species and numbers in some cases, but in others researchers were unable to find any differences. There are indications that the greatest numbers on higher mountains occur at mid elevations. Latitude, scale, and moisture availability most likely play a role.

Numbers of species in most studies are modest. In forests, studies reported herein range 7-42 species. In one study, the number of species was greater in the pine forest, but the number of individuals differed little from that of clearcut areas. *Macrobiotus hufelandi* seems to be the most common species just about everywhere. The most common genera are *Echiniscus*, *Macrobiotus*, *Diphascon*, and *Hypsibius*, and the genera recently segregated from them. Tardigrades comprised only 1.2% of the invertebrates in an expansive New Zealand study and Hingley found only two taxa in peatlands. Dry habitats may pose food limitations; constantly wet ones may be unfavorable to their longevity.

Although mosses get wet and dry on top first, it appears that tardigrades have little ability to migrate and do not even seem to be arranged in vertical assemblages. But, they have eyespots, indicating that light may play some role in their locations.

Acknowledgments

Literature Cited

Bartels, P. 2005. "Little known" water bears? ATBI Quart. 6(2): 4.

Bartels, P. J. and Nelson, D. R. 2006. A large-scale, multihabitat inventory of the phylum Tardigrada in the Great Smoky Mountains National Park, USA: A preliminary report. Hydrobiologia 558: 111-118.

Beasley, C. W. 1981. Some Tardigrada from Puerto Rico. Tex. J. Sci. 33: 9-12.

Beasley, C. W. 1988. Altitudinal distribution of Tardigrada of New Mexico with the description of a new species. Amer. Midl. Nat. 120: 436-440.

Beasley, C. W. 2001. Photokinesis of *Macrobiotus hufelandi* (Tardigrada, Eutardigrada). Zool. Anz. 240: 233-236.

Beasley, C. W., Kaczmarek, Ł., and Michalczyk, Ł. 2006. New records of tardigrades from China, with zoogeographical remarks. Biol. Lett. 43(1): 13-20.

Bernard, E. C. 1977. A new species of *Hexapodibius* from North America, with a redescription of *Diphascon belgicae* (Tardigrada). Trans. Amer. Microsc. Soc. 96: 476-482.

Bertolani, R. and Rebecchi, L. 1996. The tardigrades of Emilia (Italy). II. Monte Rondinaio. A multihabitat study on a high altitude valley of the northern Apennines. Zool. J. Linn. Soc. 116: 3-12.

Bertolani, R., Altiero, T., and Nelson, D. R. 2009. Tardigrada (water bears). Chapter 188. In: Likens, G. E. (ed.). Volume 2. Encyclopedia of Inland Waters. Elsevier, Oxford, pp. 443-465.

Bertrand, M. 1975. Répartition des tardigrades "terrestres" dans le massif de l'Aigoual. Vie Milieu 25: 283-298.

Bettis, C. J. 2008. Distribution and abundance of the fauna living in two *Grimmia* moss morphotypes at the McKenzie Table Mountain Preserve, Fresno County, California. M.S. Thesis, California State University, Fresno, CA, 65 pp.

Brantley, S. and Shepherd, U. 2002. Microarthropods on different types of cryptobiotic crusts in pinyon-juniper habitat. Abstracts of the 87th Annual Meeting of the Ecological Society of America and the 14th Annual International Conference of the Society for Ecological Restoration, 4-9 August, 2002, Tucson, AZ.

Briones, M. J. I., Ineson, P., and Piearce, T. G. 1997. Effects of climate change on soil fauna; responses of enchytraeids, Diptera larvae and tardigrades in a transplant experiment. Appl. Soil Ecol. 6: 117-134.

Collins, M. and Bateman, L. 2001. The ecological distribution of tardigrades in Newfoundland. Zool. Anz. 240: 291-297.

Crum, H. 1976. Mosses of the Great Lakes Forest, revised edition. Contributions from the University Herbarium, Univ. Mich., Ann Arbor. Vol. 10, 404 pp.

Dastych, H. 1980. Tardigrades from the Tatra National Park. Polska Akademia Nauk Zaklad Zoologii Systematycznej i Doswiadczalnej. Monografie Fauny Polski Vol. 9. 232. pp.

Dastych, H. 1985. West Spitsbergen Tardigrada. Acta Zool. Cracov. 28(3): 169-214.

Dastych, H. 1987. Altitudinal distribution of Tardigrada in Poland. In: Bertolani, R. (ed.). Selected Symposia and Monographs U.Z.I., 1, Mucchi, Modena, pp. 45-56.

Dastych, H. 1988. The Tardigrada of Poland. Monografie Fauny Polski. Vol. 16. Institute of Systematics and Evolution of

Animals, Polish Academy of Sciences, Kraków, Poland, pp. 1-255.

Degma, P., Bertolani, R., and Guidetti, R. 2010. Actual checklist of Tardigrada species (Ver. 11:26-01-2010). Accessed 18 June 2010 at <http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf>.

Degma, P., Stanislav, K., and Sabatovicova, L. 2011. Horizontal distribution of moisture and Tardigrada in a single moss cushion. J. Zool. Syst. Evol. Res. 49 (Suppl 1): 71-77.

Eles, A. and Repas, L. 2009. New dates and new methods for research of freshwater Tardigrada fauna of city Szekesfehervar. In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009, pp. 56-57.

Elgmork, K. and Sæther, O. R. 1970. Distribution of invertebrates in a high mountain brook in the Colorado Rocky Mountains. Univ. Colorado Studies Ser. Biol. 31: 3-55.

Fontoura, P., Pilato, G., Morais, P., and Lisi, O. 2009. *Minibiotus xavieri*, a new species of tardigrade from the Parque Biológico de Gaia, Portugal (Eutardigrada: Macrobiotidae). Zootaxa 2267: 55-64.

Garey, J. R., McInnes, S. J., and Nichols, P. B. 2008. Global diversity of tardigrades (Tardigrada) in freshwater. Hydrobiologia 595: 101-106.

Gray, N. F., Wyborn, C. H. E., and Smith, R. I. L. 1982. Nematophagous fungi from the maritime Antarctic. Oikos 38: 194-201.

Greven, H. 2007. Comments on the eyes of tardigrades. Arthropod Struc. Dev. 36: 401-407.

Guil, N., Hortal, J., Sánchez-Moreno, S., and Machordom, A. 2009. Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment. Landscape Ecol. 24: 375-390.

Hingley, M. 1993. Microscopic Life in *Sphagnum*. Naturalists' Handbook 20. [i-iv]. Richmond Publishing Co. Ltd., Slough, England, pp. 1-64. 58 fig. 8 pl.

Hinton, J. G., and Meyer, H. A. 2007. Distribution of limnoterrestrial Tardigrada in Georgia and the Gulf Coast states of the United States of America with ecological remarks. J. Limnol. 66(Suppl. 1): 72-76.

Hinton, J. G. and Meyer, H. A. 2008. Tardigrades from Fayette County, Georgia. Georgia J. Sci. 66: 30-32.

Hofmann, I. 1987. Habitat preference of the most frequent moss-living Tardigrada in the area of Giessen (Hessen). In: Bertolani, R. (ed.). Selected Symposia and Monographs U.Z.I., 1, Mucchi, Modena, pp. 211-216.

Hooie, A. K. and Davison, P. G. 2001. Tardigrade diversity in the moss *Ulota crispa* from tree canopies in the Great Smoky Mountains National Park – A preliminary report. J. Alabama Acad. Sci. 72: 91. Abstract.

Ito, M. 1999. Ecological distribution, abundance and habitat preference of terrestrial tardigrades in various forests on the northern slope of Mt. Fuji, central Japan. Zool. Anz. 238: 225-234.

Jennings, P. G. 1976. The Tardigrada of Signy Island, South Orkney Islands, with a note on the Rotifera. Brit. Antarct. Surv. Bull 44: 1-25.

Jennings, P. G. 1979. The Signy Island terrestrial reference sites: X. Population dynamics of Tardigrada and Rotifera. Brit. Antarct Surv. Bull. 47: 89-105.

Jerez Jaimes, J. H., Narváez, E. X., and Restrepo, R. 2002. Tardígrados en musgos de la Reserva el Diviso (Santander, Colombia). Revista Colombiana de Entomología 28(2): 199-206.

Jönsson, K. I. 2003. Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography 26: 356-364.

Kaczmarek, L., Gołdyn, B., Wełnicz, W., and Michalczyk, L. 2011. Ecological factors determining Tardigrada distribution in Costa Rica. J. Zool. Syst. Evol. Res. 49: 78-83.

Kathman, R. D. and Cross, S. F. 1991. Ecological distribution of moss-dwelling tardigrades on Vancouver Island, British Columbia, Canada. Can. J. Zool. 69: 122-129.

Leetham, J. W., McNary, T. J., Dodd, J. L., and Lauenroth, W. K. 1982. Response of soil nematodes, rotifers and tardigrades to three levels of season-long sulfur dioxide exposures. Water Air Soil Pollut. 17: 343-356.

Li, X.-C. and Wang, L.-Z. 2005. Effects of temperature and thermal acclimation on locomotor performance of *Macrobiotus hufelandi* Schultze (Tardigrada: Macrobiotidae). Current Zool. (formerly Acta Zoologica Sinica) 51: 516-520.

Linhart, J., Vlcková, S., and Uvíra, V. 2002. Moss-dwelling meiobenthos and flow velocity in low-order streams. Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium (2001-2002) Biologica 39/40: 111-122.

Mathews, G. B. 1938. Tardigrada of North America. Amer. Midl. Nat. 19: 619-627.

McFatter, M. M., Meyer, H. A., and Hinton, J. G. 2007. Nearctic freshwater tardigrades: A review. In: Pilato, G. and Rebecchi, L. (guest eds.). Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 84-89.

Meininger, C. A. and Spatt, P. D. 1988. Variations of tardigrade assemblages in dust-impacted Arctic mosses. Arct. Alp. Res. 20: 24-30.

Meininger, C. A., Uetz, G. W., and Snider, J. A. 1985. Variation in epiphytic microcommunities (tardigrade-lichen-bryophyte assemblages) of the Cincinnati, Ohio area. Urban Ecol. 9(1): 45-62.

Meyer, H. A. 2006. Small-scale spatial distribution variability in terrestrial tardigrade populations. Hydrobiologia 558: 133-139.

Meyer, H. A. 2008. Distribution of tardigrades in Florida. Southeast. Nat. 7: 91-100.

Meyer, H. A. and Hinton, J. 2007. Limno-terrestrial Tardigrada of the Nearctic Realm. In: Pilato, G. and Rebecchi, L. (eds.). Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 97-103.

Mihelcic, F. 1954/55. Zur Ökologie der Tardigraden. Zool. Anz. 153: 250-257.

Mihelcic, F. 1963. Moose als Lebensstätten für Tardigraden. Der Schlern 37: 179-181.

Miller, W. R. and Heatwole, H. 1995. Tardigrades of the Australian Antarctic Territories: The Mawson Coast, East Antarctica. Invert. Biol. 114(1): 27-38.

Miller, W. R., Miller, J. D., and Heatwole, H. 1996. Tardigrades of the Australian Antarctic Territories: The Windmill Islands, East Antarctica. Zool. J. Linn. Soc. 116: 175-184.

Nelson, D. R. 1973. Ecological distribution of tardigrades on Roan Mountain, Tennessee – North Carolina. Ph. D. dissertation, University of Tennessee, Knoxville.

Nelson, D. R. 1975. Ecological distribution of Tardigrada on Roan Mountain, Tennessee – North Carolina. In: Higgins, R. P. (ed.). Proceedings of the First International

Symposium on Tardigrades. Mem. Ist. Ital. Idrobiol., Suppl. 32: 225-276.

Nelson, D. R. and Adkins, R. G.   2001.   Distribution of tardigrades within a moss cushion: Do tardigrades migrate in response to changing moisture conditions? Zool. Anz. 240: 493-500.

Nelson, D. R. and Marley, N. J. 2000. The biology and ecology of lotic Tardigrada. Freshwat. Biol. 44: 93-108.

Newsham, K. K., Maslen, N. R., and McInnes, S. J.   2006. Survival of Antarctic soil metazoans at -80ºC for six years. CryoLetters 27(5): 269-280.

Pilato, G.   2009.   Bindius triquetrus gen. nov. sp. nov. (Eutardigrada, Hypsibiidae) from Sicily (Italy).   Zootaxa 2058: 62-68.

Ramazzotti, G. 1962. Il Phylum Tardigrada. Mem. Ist. Ital. Idrobiol. 14: 1-595.

Ramazzotti, G.   1972.   Phylum Tardigrada (Seconda edizione aggionata). Vol. II. Mem. Ist. ital. Idrobiol. 28: 1-732.

Ramazzotti, G. and Maucci, W. 1983. Il Phylum Tardigrada. [The phylum Tardigrada - 3rd edition: English translation by C. W. Beasley.]. Mem. Ist. Ital. Idrobiol. 41: 1-1011.

Rebecchi, L., Boschini, D., Cesari, M., Lencioni, V., Bertolani, R., and Guidetti, R. 2009. Stress response of a boreo-alpine species of tardigrade, *Borealibius zetlandicus* (Eutardigrada, Hypsibiidae). J. Limnol., 68(1): 64-70.

Riggin, G. T. Jr. 1962. Tardigrada of Southwest Virginia: With the Addition of a Description of a New Marine Species from Florida.   Technical Bull. 152, Virginia Agricultural Experiment Station, Blacksburg, VA. 145 pp.

Rodrigues-Roda, J. 1951. Alguños datos sobre la distribucion de los Tardigrados españoles. Bol. Real Soc. Esp. Hist. Nat. Secc. Biol. 49: 75-83.

Romano, F. A. III., Barreras-Borrero, B., and Nelson, D. R. 2001. Ecological distribution and community analysis of Tardigrada from Choccolocco Creek, Alabama. Zool. Anz. 240: 535-541.

Schuster, R. and Greven, H.   2007.   A long-term study of population dynamics of tardigrades in the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst. J. Limnol. 66: 141-151.

Schuster, R., Spiertz, I., Lösch, R., and Greven, H.   2009. Microclima and vertical distribution of tardigrades in a moss cushion.   In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada.   Conference Guide. Tübingen, Germany, 3-6 August 2009, p. 59.

Schwarz, A.-M. J., Green, J. D., Green, T. G. A., and Seppelt, R. D. 1993. Invertebrates associated with moss communities at

Canada Glacier, southern Victoria Land, Antarctica. Polar Biol. 13: 157-162.

Shcherbako, D., Schill, R. O., Brümmer, F., Nissen, M., and Blum, M. 2010. Movement behaviour and video tracking of *Milnesium tardigradum* Doyère, 1840 (Eutardigrada Apochela).   Contrib. Zool. 79: 33-38.

Sohlenius, B. and Boström, S.   2006.   Patch-dynamics and population structure of nematodes and tardigrades on Antarctic nunataks. Eur. J. Soil Biol. 42(Suppl. 1): S321-S325.

Somme, L.   1996.   Anhydrobiosis and cold tolerance in tardigrades. Eur. J. Entomol. 93: 349-357.

Somme, L. and Terje Meier, T.   1995.   Cold tolerance in Tardigrada from Dronning Maud Land, Antarctica. Polar Biol. 15: 1432-2056.

Suren, A. M. 1991a. Bryophytes as invertebrate habitat in two New Zealand alpine streams. Freshwat. Biol. 26: 399-418.

Suren, A. M.   1991b.   Assessment of artificial bryophytes for invertebrate sampling in two New Zealand alpine streams. N. Z. J. Marine Freshwat. Res. 25: 101-112.

Suren, A. M. 1992. Enhancement of invertebrate food resources by bryophytes in New Zealand alpine headwater streams. N. Z. J. Marine Freshwat. Res. 26: 229-239.

Suren, A. 1993. Bryophytes and associated invertebrates in first-order alpine streams of Arthur's Pass, New Zealand. New Zeal. J. Marine Freshwat. Res. 27: 479-494.

Utsugi, K., Hiraoka, T., and Nunomura, N.   1997.   On the relations between tardigrade fauna and bryophyte flora in Toyama Prefecture. Bull. Toyama Sci. Mus. 20: 57-71.

Venkataraman, K. 1998. Studies on Phylum Tardigrada and Other Associated Fauna, South Polar Skua and Bird and Mammal Logging During 1994 - 1995 Expedition. Fourteenth Indian Expedition to Antarctica, Scientific Report, 1998.   Department of Ocean Development, Technical Publication No. 12: 221-243.

Vlcková, S., Linhart, J., and Uvíra, V. 2001/2002. Permanent and temporary meiofauna of an aquatic moss Fontinalis antipyretica Hedw. Acta Univers. Palack. Olom. Biol. 39-40: 131-140.

Westh, P. and Kristensen, R.-M.   1992.   Ice formation in the freeze-tolerant eutardigrades *Adorybiotus coronifer* and *Amphibolus nebulosus* studied by differential scanning calorimetry. Polar Biol. 12: 693-699.

Wright, J. C. 1991. The significance of four xeric parameters in the ecology of terrestrial Tardigrada. J. Zool. 224: 59-77.

Glime, J. M. 2017. Tardigrades: Species Relationships. Chapt. 5-4. In: Glime, J. M. Bryophyte Ecology. Volume 2. Bryological Interaction. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 21 April 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>.

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CHAPTER 5-4
TARDIGRADES: SPECIES RELATIONSHIPS

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TARDIGRADES: SPECIES RELATIONSHIPS

Figure 1. SEM of tardigrades on a leafy liverwort.   Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Species Relationships

Tardigrades occur on both mosses and liverworts (Figure 1). Since bryophytes vary widely in structure, compactness, and moisture-holding nature, one would expect that some bryophytes would be more suitable for tardigrades than others, causing specificity. But is that really the case?

Although Hofmann and Eichelberg (1987), in Lahnau near Giessen, Germany, found a correlation between species of tardigrade and degree of moisture in their preferred mosses, there seemed to be no example of a single species of tardigrade preferring a single species of moss. It appeared that species of bryophyte was not an important factor for most tardigrades.

A number of studies name the bryophytes where the tardigrades have been found, but quantitative approaches are limited. For example, Degma (2006) found **Echiniscus reticulatus** on the moss **Ctenidium molluscum** (Figure 2) and **Testechiniscus spitsbergensis** from the mosses **Tortella tortuosa** (Figure 3), **Ctenidium molluscum** (Figure 2), **Distichium capillaceum** (Figure 4), and **Ditrichum flexicaule** (Figure 5-Figure 6) in Slovakia.

Baxter (1979) did find differences in the tardigrades on several moss species in Ireland. These represented different growth forms as well as habitats. Some of their more specific finds include stream bank mosses that had **Diphascon oculatum** (Figure 7). **Polytrichum** (Figure 8), with its more open structure, had **Diphascon scoticum** (Figure 9). **Hypsibius dujardini** (Figure 1) was abundant, accompanied by **Isohypsibius tuberculatus**, on the turfs of **Rhytidiadelphus squarrosus** (Figure 10).

Figure 2. *Ctenidium molluscum*, a moss that is home to *Echiniscus reticulatus*, among others. Photo by Michael Lüth, with permission.

Figure 3. *Tortella tortuosa*, a Slovakian habitat for *Testechiniscus spitsbergensis*. Photo by Michael Lüth, with permission.

Figure 4. *Distichium capillaceum*, a known tardigrade habitat. Photo by Michael Lüth, with permission.

Figure 5. *Ditrichum flexicaule*, a habitat for *Testechiniscus spitsbergensis*. Photos by Michael Lüth, with permission.

Figure 6. View inside cushion of *Ditrichum flexicaule*, a habitat for *Testechiniscus spitsbergensis*. Photos by Michael Lüth, with permission.

Figure 7. *Diphascon oculatum*, an inhabitant of streambank mosses. Photo by Björn Sohlenius, Swedish Museum of Natural History, with permission.

Figure 8. *Polytrichum*, a moss with spreading leaves that provide limited tardigrade habitat. Photo by Michael Lüth, with permission.
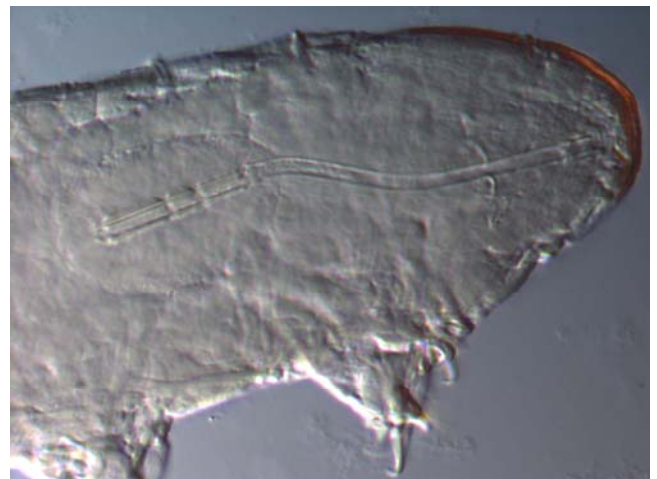

Figure 9. *Diphascon scoticum*, a tardigrade that is able to inhabit *Polytrichum*. Photo by Paul J. Bartels, with permission.

Figure 10. *Rhytidiadelphus squarrosus*, where Baxter (1979) found *Isohypsibius tuberculatus* and *Diphascon scoticum*. Photo by Michael Lüth, with permission.

Figure 11. *Lembophyllum divulsum*, a home for 16 tardigrade species in New Zealand. Photo by Li Zhang, with permission.

Figure 12. *Hypnum cupressiforme*, the moss with the most tardigrade species in the New Zealand study by Horning *et al*. (1978), shown here on rock and as a pendant epiphyte. Photos by Michael Lüth, with permission.

Horning *et al*. (1978) examined the tardigrades on 21 species of mosses in New Zealand and listed the tardigrade species on each (Table 1). Some moss species clearly had more tardigrade species than others, ranging from 1 on *Syntrichia rubra* to 17 on *Hypnum cupressiforme* (Figure 12). *Lembophyllum divulsum* (Figure 11) had 16 species.

Hopefully lists like the one provided by Horning *et al*. (1978) will eventually permit us to determine the characteristics that foster tardigrade diversity and abundance. Perhaps the moss *Hypnum cupressiforme* (Figure 12) had the most tardigrade species among the mosses in New Zealand because of its own wide habitat range there. However, Degma *et al*. (2005) found that distribution of the number of tardigrade species on this moss in their Slovakia sites was random, as supported by a Chi-square goodness of fit test. But this still does not preclude the assertion that its ubiquitous nature on a wide range of habitats in New Zealand may account for the greater number of species of tardigrades on *Hypnum cupressiforme* in the New Zealand study.

A kind of vertical zonation occurs among tardigrades on trees that is the reverse of that sometimes found within a moss cushion. In the Great Smoky Mountains National Park, the number of tardigrade species among epiphytes at breast height was greater than the number of species found at the base (Bartels & Nelson 2006). This may relate to the need for dry periods, but it could also relate to differences in predators and possibly even to dispersal patterns.

In their study of Chinese mosses Beasley and Miller (2007) found that **Heterotardigrada** (armored tardigrades) were better represented than were **Eutardigrada** (unarmored tardigrades), a factor the authors attribute to the **xerophilic** moss samples and the locality, which has hot, dry summers, very cold, dry winters, low summer rainfall, and high winds (Fullard 1968). The Heterotardigrada have armor, which may account for their ability to withstand the dry habitat. These tardigrades also have **cephalic** (head) appendages with a sensorial function, a character lacking in the Eutardigrada, but so far their function has not been related to a bryophyte habitat. Beasley and Miller found little specificity, but most of the mosses were xerophytic and exhibited similar moisture requirements. They did find that *Echiniscus testudo* (Figure 13) occurred on a wider variety of mosses than did other tardigrade species.

On Roan Mountain in Tennessee and North Carolina, Nelson (1973, 1975) found no specificity among 21 tardigrade species on 25 bryophyte species. Hunter (1977) in Montgomery County, Tennessee, and Romano *et al*. (2001) in Choccolocco Creek in Alabama, USA, again were unable to find any dependence of tardigrades upon a particular species of bryophyte in their collections.

Table 1. Tardigrade species found on the most common moss taxa in New Zealand. From Horning *et al*. 1978.

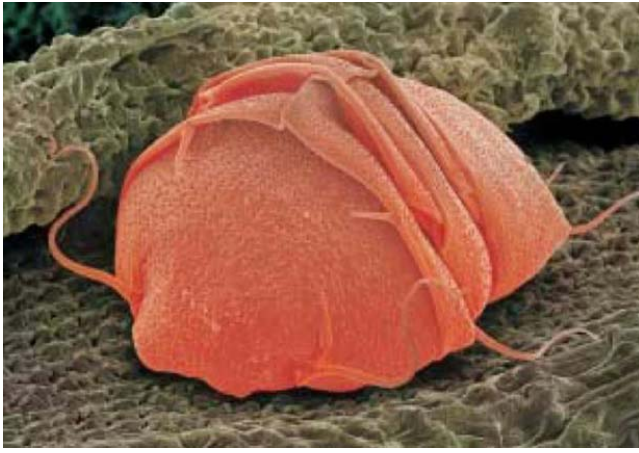| Moss taxon	Species	Moss taxon	Species
Breutelia elongata	*Macrobiotus hibiscus*		*Macrobiotus furciger*
	Macrobiotus liviae		*Macrobiotus hibiscus*
	Milnesium tardigradum		*Macrobiotus liviae*
	Minibiotus intermedius		*Macrobiotus recens*
Breutelia pendula	*Diphascon prorsirostre*		*Macrobiotus subjulietae*
	Diphascon scoticum		*Milnesium tardigradum*
	Doryphoribius zyxiglobus		*Minibiotus intermedius*
	Hypechiniscus exarmatus		*Paramacrobiotus areolatus*
	Macrobiotus hibiscus		*Pseudechiniscus novaezeelandiae*
	Macrobiotus liviae		*Pseudechiniscus juanitae*
	Milnesium tardigradum	*Macromitrium erosulum*	*Macrobiotus furciger*
Bryum campylothecium	*Hypsibius convergens*		*Macrobiotus hibiscus*
	Isohypsibius sattleri		*Macrobiotus liviae*
	Minibiotus intermedius		*Pseudechiniscus juanitae*
Bryum dichotomum	*Hypsibius wilsoni*	*Macromitrium longipes*	*Doryphoribius zyxiglobus*
	Macrobiotus coronatus		*Hypsibius convergens*
	Macrobiotus liviae		*Macrobiotus recens*
Bryum truncorum	*Diphascon chilenense*		*Minibiotus intermedius*
	Diphascon scoticum	*Porotrichum ramulosum*	*Diphascon alpinum*
	Isohypsibius sattleri		*Diphascon scoticum*
	Isohypsibius wilsoni		*Doryphoribius zyxiglobus*
	Macrobiotus coronatus		*Echiniscus bigranulatus*
	Macrobiotus furciger		*Hypsibius convergens*
	Macrobiotus liviae		*Macrobiotus anderssoni*
	Macrobiotus recens		*Macrobiotus coronatus*
	Paramacrobiotus areolatus		*Macrobiotus furciger*
	Paramacrobiotus richtersi		*Macrobiotus hibiscus*
	Ramazzottius oberhaeuseri		*Macrobiotus liviae*
Dicranoloma billardieri	*Hypechiniscus exarmatus*		*Macrobiotus rawsoni*
	Macrobiotus hibiscus		*Minibiotus aculeatus*
Dicranoloma grossialare	*Diphascon prorsirostre*		*Pseudechiniscus lateromamillatus*
	Hypechiniscus exarmatus		*Pseudechiniscus novaezeelandiae*
	Hypsibius dujardini		*Pseudechiniscus juanitae*
	Isohypsibius cameruni	*Racomitrium crispulum*	*Calohypsibius ornatus*
	Isohypsibius sattleri		*Diphascon alpinum*
	Limmenius porcellus		*Echiniscus quadrispinosus*
	Macrobiotus anderssoni		*Echiniscus zetotrymus*
	Macrobiotus hibiscus		*Hebesuncus conjungens*
	Macrobiotus liviae		*Hypsibius convergens*
	Milnesium tardigradum		*Isohypsibius wilsoni*
	Pseudechiniscus novaezeelandiae		*Macrobiotus anderssoni*
Dicranoloma menziesii	*Macrobiotus hibiscus*		*Macrobiotus coronatus*
	Macrobiotus liviae		*Macrobiotus furciger*
	Paramacrobiotus areolatus		*Macrobiotus hibiscus*
Dicranoloma robustum	*Echiniscus bigranulatus*		*Macrobiotus orcadensis*
	Macrobiotus anderssoni		*Milnesium tardigradum*
	Macrobiotus furciger		*Oreella minor*
	Macrobiotus liviae		*Paramacrobiotus areolatus*
	Milnesium tardigradum		*Pseudechiniscus juanitae*
	Pseudechiniscus juanitae	*Racomitrium lanuginosum*	*Diphascon scoticum*
Dicranoloma trichopodum	*Echiniscus quadrispinosus*		*Echiniscus quadrispinosus brachyspinosus*
	Echiniscus q. brachyspinosus		*Echiniscus vinculus*
	Macrobiotus furciger		*Hebesuncus conjungens*
	Pseudechiniscus lateromamillatus		*Macrobiotus furciger*
Hypnum cupressiforme	*Diphascon alpinum*		*Milnesium tardigradum*
	Diphascon bullatum		*Minibiotus intermedius*
	Echiniscus quadrispinosus		*Oreella mollis*
	Echiniscus spiniger		*Pseudechiniscus juanitae*
	Hypsibius dujardini	*Racomitrium ptychophyllum*	*Echiniscus quadrispinosus*
	Macrobiotus anderssoni		*Echiniscus velaminis*
	Macrobiotus coronatus		*Hebesuncus conjungens*
	Macrobiotus furciger		*Hypechiniscus exarmatus*
	Macrobiotus hibiscus		*Hypsibius dujardini*
	Macrobiotus liviae		*Macrobiotus furciger*
	Macrobiotus recens		*Milnesium tardigradum*
	Milnesium tardigradum		*Minibiotus intermedius*
	Oreella mollis		*Oreella mollis*
	Paramacrobiotus areolatus	*Syntrichia princeps*	*Hypsibius convergens*
	Pseudechiniscus novaezeelandiae		*Ramazzottius oberhaeuseri*
	Pseudechiniscus juanitae		*Isohypsibius wilsoni*
	Ramazzottius oberhaeuseri		*Macrobiotus coronatus*
Lembophyllum divulsum	*Diphascon alpinum*		*Macrobiotus recens*
	Doryphoribius zyxiglobus		*Milnesium tardigradum*
	Hypsibius convergens		*Pseudechiniscus novaezeelandiae*
	Isohypsibius sattleri	*Syntrichia rubra*	*Diphascon scoticum*
	Macrobiotus anderssoni	*Tortula subulata* var. *serrulata*	*Diphascon scoticum*
	Macrobiotus coronatus		*Paramacrobiotus areolatus*

Figure 13. ***Echiniscus testudo*** tun. Photo by Power & Syred through Creative Commons.

Hofmann developed a preference coefficient:

$$P_n = (T_n/S_n) \sum_{i=1}^{n} 100(T_i/S_i)$$

where　P = preference index for category n of observed factor

n = index of observed category

T = number of tardigrade populations of a single species

S = number of samples in category

The preference indices will add up to 100%. The categories can be the five bryophyte habitat groups listed by Mihelčič 1954/55, 1963; Ramazzotti 1962, and Hofmann 1987 or other groupings defined for the purpose.

Table 2. Distribution of tardigrades on specific mosses in Xinjiang Uygur Region, China, based on herbarium specimens. From Beasley & Miller 2007.

tardigrade	numb/samples	moss
Bryodelphax asiaticus	1/1	*Pseudoleskeella catenulata*
Cornechiniscus holmeni	18/5	*Grimmia tergestina*
		Mnium laevinerve
		Schistidium sp.
Echiniscus blumi	4/4	*Abietinella abietina*
		Schistidium sp.
Echiniscus canadensis	82/7	*Grimmia laevigata*
		Grimmia ovalis
		Grimmia tergestina
Echiniscus granulatus	8/3	*Grimmia longirostris*
		Schistidium trichodon
		Schistidium sp.
Echiniscus testudo	11/4	*Grimmia anodon*
		Grimmia longirostris
		Grimmia tergestina
		Lescuraea incurvata
		Pseudoleskeella catenulata
		Schistidium sp.
Echiniscus trisetosus	33/5	*Abietinella abietina*
		Grimmia ovalis
		Pseudoleskeella catenulata
Macrobiotus mauccii	2/2	*Schistidium* sp.
Milnesium asiaticum	10/4	*Grimmia anodon*
		Grimmia tergestina
		Grimmia ovalis
		Schistidium sp.
Milnesium longiungue	4/2	*Grimmia laevigata*
		Grimmia ovalis
Milnesium tardigradum	5/4	*Grimmia tergestina*
		Grimmia ovalis
		Orthotrichum sp.
Paramacrobiotus alekseevi	5/4	*Brachythecium albicans*
		Schistidium sp.

Table 3. Preference of moss species by tardigrades, using five moss species plus the remaining species combined (total = 43 species) as the habitat categories, based on 106 samples from Giessen, Germany (Hofmann 1987).

	Ceratodon purpureus	*Grimmia pulvinata*	*Bryum argenteum*	*Syntrichia ruralis*	*Syntrichia montana*	Other
samples (%)	19	9	7	7	6	52
Macrobiotus hufelandi	16	18	18	18	21	8
Ramazzottius oberhaeuseri	18	27	29	17	0	8
Milnesium tardigradum	13	23	15	20	23	6
Echiniscus testudo	11	20	20	9	34	6
mean	14.5	22.0	20.0	16.0	19.5	7.0
empty samples	25	7	9	9	11	38

Figure 14. ***Macrobiotus hufelandi***. Photo by Martin Mach, with permission.
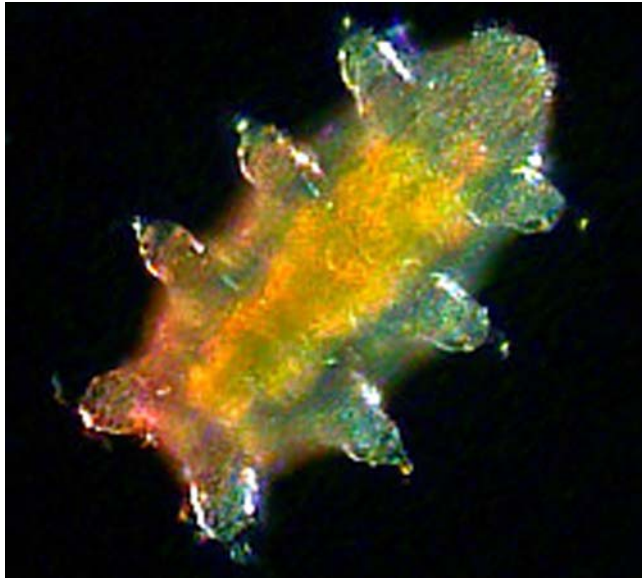
Figure 15. Adult *Echiniscus* sp.. Photo by Martin Mach, with permission.

Kathman and Cross (1991) found that species of bryophyte had no influence on the distribution or abundance of tardigrades from five mountains on Vancouver Island, British Columbia, Canada. In fact, Kathman and Cross (1991) were unable to find any correlation with altitude or aspect throughout a span from 150 to 1525 m. They concluded that it was the presence of bryophyte that determined tardigrade presence, not the species of bryophyte, altitude, or locality. Despite a lack of specificity among the tardigrades, 39 species inhabited these 37 species of mountain bryophytes, comprising 14,000 individuals. Several researchers contend that any terrestrial species of tardigrade can be found on any species of moss, given the "appropriate microhabitat conditions" (Bertrand 1975; Ramazzotti & Maucci 1983). If these tardigrade bryophyte specialists find no differences among the bryophytes, can we blame the ecologists for lumping all the bryophytes in their studies as well?

In collections from Giessen, Germany, the most common tardigrade species, the cosmopolitan *Macrobiotus hufelandi* (Figure 14), had no preference for any moss species (Hofmann 1987). But lack of influence of bryophyte species may not always be the case. Hofmann (1987) used a preference index to show that four out of sixteen tardigrades from Giessen had distinct preferences among five moss species and that they seemed to prefer cushion mosses over sheet mosses. Also contrasting with the above researchers, Bertolani (1983) found that there seemed to be a species relationship between tardigrades and coastal dune mosses. It is possible that this is again related to moisture. The moisture relationship might also explain why mosses on rotten logs seem to have few tardigrades. Could it be that they are too wet for too long?

Meyer (2006a, b, 2008) found 20 species of tardigrades among 47 species of mosses, liverworts, lichens, and ferns in Florida. There were some tardigrade species that were significantly associated with either mosses or lichens, but, as in most other studies, there was no convincing evidence for associations with any plant species substratum. Despite the lack of substrate specificity, there were three significant negative associations and one positive association between species of tardigrades. Likewise, in Georgia and the Gulf Coast, USA, Hinton and Meyer (2007) found *Milnesium tardigradum* (Figure 16), *Minibiotus intermedius* (Figure 39), and *Minibiotus furcatus* among mosses, whereas *Echiniscus cavagnaroi*, *E. kofordi* (see Figure 15), and *Minibiotus fallax* were in both mosses and lichens.
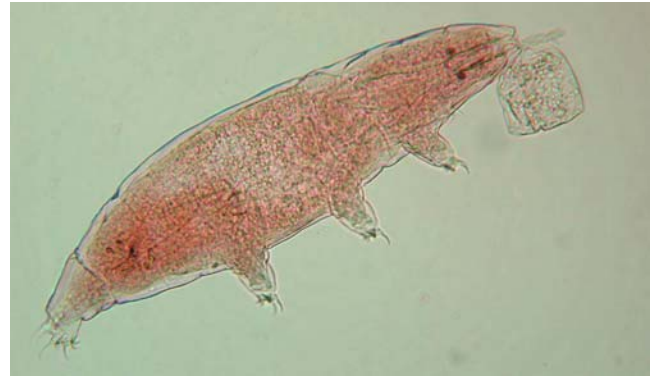

Figure 16. *Milnesium tardigradum*, an inhabitant of both mosses and liverworts. Photo by Björn Sohlenius, Swedish Museum of Natural History.

Growth Forms

There is some indication that species differences may exist, based on growth form. The bryophyte form can affect the moisture-holding capacity and rate of loss of moisture. That foregoing evidence suggests that the moisture-holding capacity of cushion mosses was probably a desirable trait in that habitat. On the other hand, Beasley (1990) found that more samples of clubmosses (Lycopodiaceae – tracheophytes) (75%) had tardigrades than did mosses (46%) or liverworts (0%) in Gunnison County, Colorado.

There seems to be a preference for cushions among the most common species [*Macrobiotus hufelandi* (Figure 14), *Ramazzottius oberhaeuseri* (Figure 17), *Milnesium tardigradum* (Figure 16), and *Echiniscus testudo* (Figure 13)] (Hofmann 1987). But the less frequent species are commonly found among sheet mosses. The ubiquitous *Macrobiotus hufelandi* seems to have no preference for moss shape.

Figure 17. *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.

Jönsson (2003), working in the forests of Sweden, found that wefts had more tardigrades than other moss forms. Kathman and Cross (1991) likewise found that tardigrades from Vancouver Island were more common on weft-forming mosses than on turfs, suggesting that the thick carpets of the wefts were more favorable habitat than

the thinly clustered turfs with their thick rhizoidal mats and attached soil. Contrasting with some of these findings, and the preference for cushion mosses in the study by Hofmann (1987), Diane Nelson (East Tennessee State University, Johnson City, pers. comm. in Kathman & Cross 1991) found no preference for sheet or cushion mosses in her Roan Mountain, Virginia, USA study. Rather, those tardigrades were more common in thin, scraggly mosses or in small tufts than in thick cushion mosses.

Sayre and Brunson (1971) compared tardigrade fauna on mosses in 26 North American collections from a variety of habitats and substrata (Figure 18). They found that mosses of short stature in the **Thuidiaceae** (Figure 19) and **Hypnaceae** (Figure 20) had the highest frequencies of tardigrades. Other moss-dwellers were found in fewer numbers on members of the moss families **Orthotrichaceae** (epiphytic and rock-dwelling tufts; Figure 21), **Leucobryaceae** (cushions on soil and tree bases; Figure 22), **Polytrichaceae** (tall turfs on soil; Figure 23), **Plagiotheciaceae** (low mats on soil and tree bases; Figure 24), and **Mniaceae** (mats & wefts on soil; Figure 25).

Figure 20. *Hypnum revolutum* (Hypnaceae), representing a family that includes low-stature mosses that had among the highest frequencies of tardigrades in 26 North American collections (Sayre & Brunson 1971). Photo by Michael Lüth, with permission.
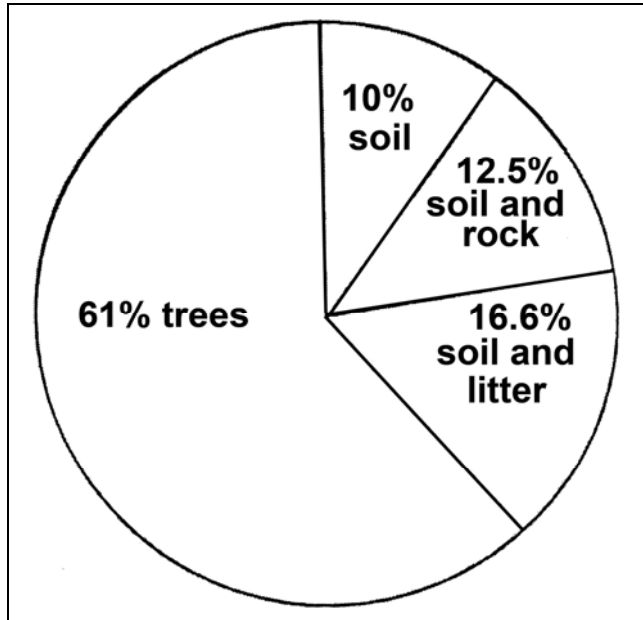
Figure 18. Relative frequency of tardigrades on bryophytes of various North American substrata. Redrawn from Sayre & Brunson 1971.

Figure 21. *Orthotrichum pulchellum*, an epiphytic moss in the **Orthotrichaceae**. This family is among those with lower numbers of tardigrades in the North American study of Sayre & Brunson (1971) compared to families of mat-forming species. Photo by Michael Lüth, with permission.

Figure 22. *Leucobryum glaucum*, a cushion moss in the **Leucobryaceae**. This family of mosses had lower numbers of tardigrades than those found in the mat-forming mosses in 26 North American collections (Sayre & Brunson 1971). Photo by Michael Lüth, with permission.

Figure 19. *Thuidium delicatulum* (Thuidiaceae), a low-stature moss that is a good tardigrade habitat. Photo by Michael Lüth, with permission.

Figure 23. *Polytrichum juniperinum*, a moss in the Polytrichaceae. This family of mosses tends to have low numbers of tardigrades (Sayre & Brunson 1971). The tardigrades do live among them often nestle in the leaf bases where water evaporates more slowly. Photo by Michael Lüth, with permission.

Figure 24. *Plagiothecium denticulatum*, a low-growing soil moss in Plagiotheciaceae, a family with limited numbers of tardigrade dwellers (Sayre & Brunson 1971). The flattened growth habit provides few protective chambers, perhaps accounting for the lower numbers. Photo by Michael Lüth, with permission.

Figure 25. *Plagiomnium cuspidatum*, a soil moss in the Mniaceae, a family with limited numbers of tardigrade dwellers (Sayre & Brunson 1971). The spreading nature of the vertical shoots and the flattened nature of the horizontal shoots would most likely not provide many protective chambers for the tardigrades. Photo by Michael Lüth, with permission.

Collins and Bateman (2001), studying tardigrade fauna of bryophytes in Newfoundland, Canada, found that rate of desiccation of the mosses affected distribution of tardigrades, and this suggests that bryophyte species and growth forms that dehydrate quickly should have fewer individuals and probably different or fewer species than those that retain water longer. In different climate regimes, that rate will differ. This may explain a preference for cushions in some locations and not in others. Data are needed on humidity within the various growth forms of bryophytes, correlated with tardigrade densities, to try to explain why different growth forms seem to be preferred in different locations.

Liverworts

I would expect liverworts, with their flat structure, to have at least some differences in tardigrade communities (Figure 1). But reports on liverwort inhabitants are limited, at least in part due to lack of knowledge about bryophytes on the part of the tardigrade specialists and an equal lack of knowledge of tardigrades by bryologists. Hinton and Meyer (2009) found two species of tardigrades [*Milnesium tardigradum* (Figure 16) and *Macrobiotus hibiscus*], both also common among mosses, in samples of the liverwort *Jungermannia* sp. (Figure 26). In the Gulf Coast states, USA, Hinton and Meyer (2007) found *Echiniscus virginicus* among liverworts.

Figure 26. The leafy liverwort *Jungermannia sphaerocarpa*, representing a genus from which tardigrades are known. Photo by Michael Lüth, with permission.

Liverworts may actually house some interesting differences as a result of their underleaves (Figure 27) and flattened growth form (Figure 28). In their New Zealand study, Horning *et al*. (1978) found that among the liverworts (Table 4), *Porella elegantula* (Figure 27) had the most species (16). The folds and underleaves of this genus form tiny capillary areas where water is held, perhaps accounting for the large number of species. Interestingly, the tardigrade *Macrobiotus snaresensis* occurred on several liverwort species [4 *Lophocolea* species, *Plagiochila deltoidea* (Figure 29)], but did not appear in any moss collections. Of 150 liverwort samples (26 species), 27% had tardigrades, with a total of 16 species, mean of 2.8 species, range 1-9. In 107 samples of foliose lichens, 60.7% had tardigrades, mean 2.2 species, range 1-11.

Figure 27. **Porella elegantula**, showing the underleaves and folds that create numerous capillary spaces. Photo by Jan-Peter Frahm, with permission.
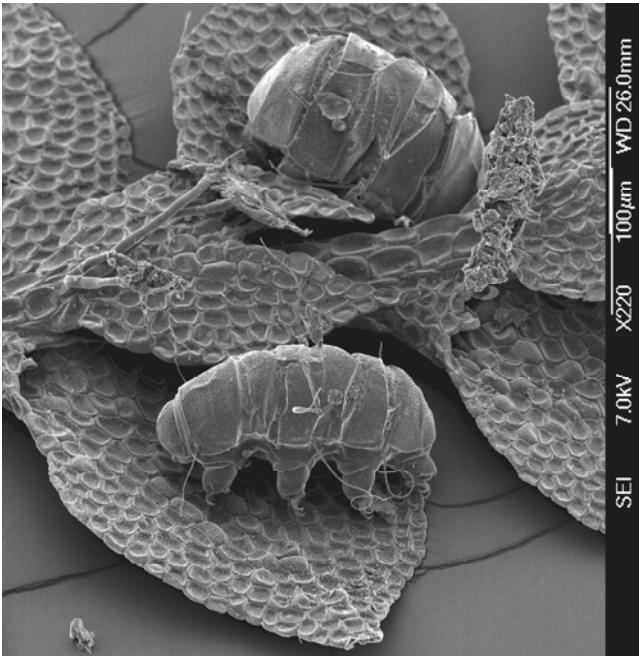

Figure 28. Underside of leafy liverwort with two tardigrades. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Figure 29. **Plagiochila deltoidea**, a leafy liverwort that forms large patches in wet ground in New Zealand. This is a known habitat for tardigrades. Photo by Clive Shirley, Hidden Forest <http://www.hiddenforest.co.nz>, with permission.

It appears that at least some other researchers have paid attention to liverworts. Christenberry (1979) found **Echiniscus kofordi** and **E. cavagnaroi** on liverworts in Alabama, USA. Hinton and Meyer (2009) found **Milnesium tardigradum** (Figure 16) and **Macrobiotus hibiscus** in a liverwort sample from Georgia, USA. Michalczyk and Kaczmarek (2006) found a new species, **Paramacrobiotus magdalenae** (Figure 30, Figure 31), on liverworts in Costa Rica. Newsham *et al.* (2006) identified the tiny leafy liverwort **Cephaloziella varians** and used it to experiment on the effects of low temperature storage on tardigrades and other Antarctic invertebrates.
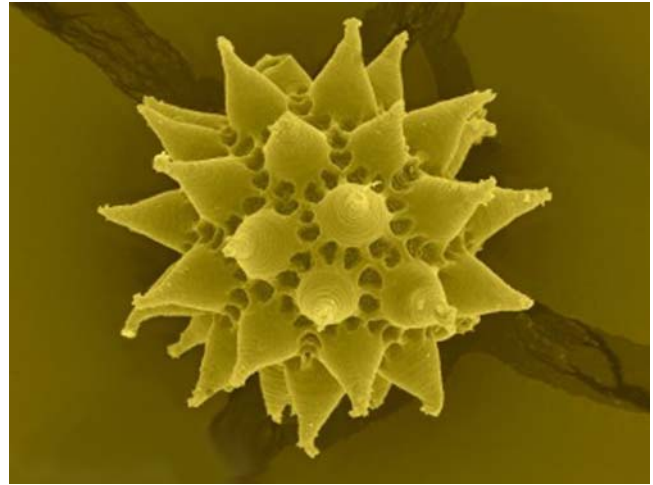
Figure 30. **Paramacrobiotus magdalenae** egg. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.
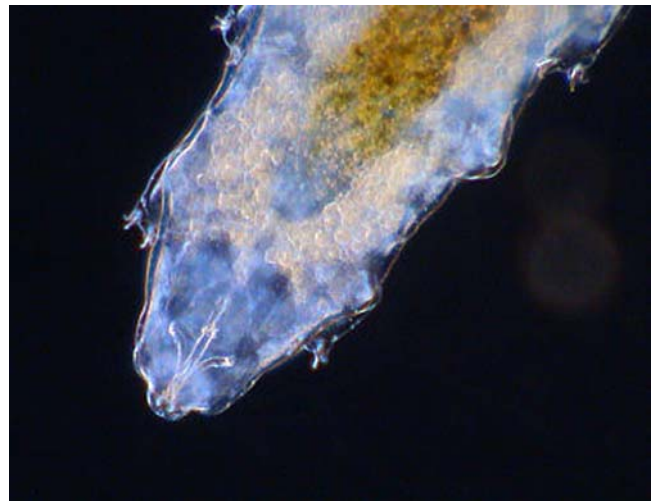
Figure 31. **Paramacrobiotus areolatus**. Photo by Martin Mach, with permission.

Just what do we mean by "appropriate habitat conditions"? The bryophytes only occur in conditions that are appropriate for them, hence defining the conditions for the tardigrades. And the bryophytes create habitat conditions of moisture due to their morphology and substrate preference. Lack of species preference in many studies may result from methods that were insensitive to subtle differences or that failed to control for microhabitat differences. Usually no statistical tests were employed, sample sizes were small, and enumeration was often simple presence/absence data.

Table 4. Species of tardigrades found on 13 liverwort species in New Zealand and surrounding islands. From Horning *et al.* 1978.

Liverwort Species	Tardigrade Species	Liverwort Species	Tardigrade Species
Lophocolea innovata	*Macrobiotus snaresensis*		*Macrobiotus anderssoni*
Lophocolea. minor	*Macrobiotus snaresensis*		*Macrobiotus liviae*
Lophocolea. subporosa	*Macrobiotus snaresensis*		*Macrobiotus recens*
Lophocolea semiteres	*Diphascon chilenense*		*Macrobiotus snaresensis*
	Macrobiotus coronatus	*Plagiochila fasciculata*	*Diphascon scoticum*
Lophocolea subporosa:	*Diphascon scoticum*		*Macrobiotus furciger*
	Hypsibius dujardini	*Plagiochila obscura*	*Macrobiotus coronatus*
	Macrobiotus snaresensis		*Macrobiotus liviae*
Lophocolea sp.	*Macrobiotus liviae*		*Pseudechiniscus juanitae*
Metzgeria decipiens	*Echiniscus spiniger*	*Plagiochila strombifolia*	*Macrobiotus anderssoni*
	Isohypsibius sattleri		*Macrobiotus furciger*
	Paramacrobiotus areolatus)	*Porella elegantula*	*Doryphoribius zyxiglobus*
	Macrobiotus furciger		*Echiniscus vinculus*
	Macrobiotus coronatus		*Diphascon alpinum*
	Minibiotus intermedius		*Diphascon bullatum*
	Macrobiotus liviae		*Diphascon prorsirostre*
	Macrobiotus snaresensis		*Hypsibius convergens*
	Milnesium tardigradum		*Isohypsibius sattleri*
	Pseudechiniscus novaezeelandiae		*Macrobiotus anderssoni*
Metzgeria decrescens	*Diphascon scoticum*		*Macrobiotus furciger*
	Macrobiotus recens		*Macrobiotus coronatus*
	Macrobiotus snaresensis		*Macrobiotus hibiscus*
	Milnesium tardigradum		*Minibiotus intermedius*
Plagiochila deltoidea	*Echiniscus bigranulatus*		*Minibiotus aculeatus*
	Hypechiniscus exarmatus		*Macrobiotus liviae*
	Hypsibius convergens		*Milnesium tardigradum*
	Isohypsibius cameruni		*Pseudechiniscus novaezeelandiae*

Substrate Comparisons

Meyer (2006b) extended the comparison of substrata in Florida, USA, to include not only liverworts, mosses, and foliose lichens, but also ferns. He found 20 species of tardigrades on 47 species of plants and lichens. They found that some species were positively associated with mosses or with foliose lichens, but as in most other studies, there was no association with any particular plant or lichen species.

Guil *et al*. (2009a) reviewed tardigrades and their habitats (altitude, habitat characteristics, local habitat structure or dominant leaf litter type, and two bioclimatic classifications), including bryophytes and leaf litter at various elevations. They were able to show some habitat preference. Species richness was most sensitive to bioclimatic classifications of macroenvironmental gradients (soil and climate), vegetation structure, and leaf litter type. A slight altitude effect was discernible. These relationships suggest that differences among bryophyte species should exist where bryophyte species occupy different environmental types or maintain different microenvironments within a habitat. But it also suggests that within the same habitat, bryophytes of various growth forms should provide different moisture regimes, hence creating species relationship differences.

In a different study in the Iberian Peninsula (extreme southwestern Europe), Guil *et al*. (2009b) found that leaf litter habitats showed high species richness and low abundances compared to rock habitats (mosses and lichens), which had intermediate species richness and high abundances. Tree trunk habitats (mosses and lichens) showed low numbers of both richness and abundances. One might conclude that the moisture of these habitats is the overall determining factor, and this should coincide with bryophyte species groups on the large scale.

Miller *et al*. (1996) found six species of tardigrades in lichen and bryophyte samples on ice-free areas at Windmill Islands, East Antarctica. The tardigrade species **Diphascon chilenense** (see Figure 32), **Acutuncus antarcticus** (formerly **Hypsibius antarcticus**; see Figure 33), and **Pseudechiniscus juanitae** (=**Pseudechiniscus suillus**; Figure 34) showed a positive association with bryophytes and a negative association with algae and lichens.
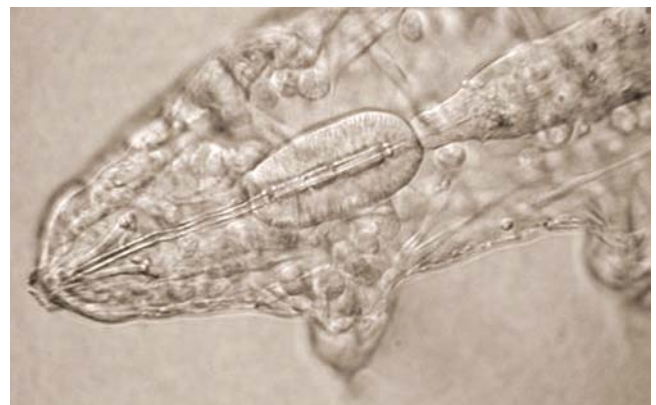
Figure 32. **Diphascon** sp., member of one of the most common bryophyte-dwelling genera. Photo by Martin Mach, with permission.

Meyer and Hinton (2007) reviewed the Nearctic tardigrades (Greenland, Canada, Alaska, continental USA, northern Mexico). They found that one-third of the species occur in both cryptogams (lichens and bryophytes) and soil/leaf litter (Table 5). Few tardigrades occurred exclusively in soil/leaf litter habitats. Although many

occurred among both bryophytes and lichens, 18 species occurred only in bryophytes. It is likely that bryophytes offer a better moisture environment, but this has not been tested.

Figure 33. *Hypsibius*. Photo by Yuuji Tsukii, with permission.

Figure 34. *Pseudechiniscus juanitae*. Photo by Paul J. Bartels, with permission.

Table 5. Comparison of tardigrades inhabiting their primary substrates in the Nearctic realm. Only species present on that substrate in at least three sites are included. From Meyer & Hinton 2007.

Substrate category	number of species
Cryptogams only	64
Both cryptogams and soil/leaf litter	27
Soil/leaf litter only	3
Both bryophyte and lichen	50
Bryophyte only	18
Lichen only	5

Beasley (1990) conducted a similar study in Colorado, USA. Out of 135 samples of liverworts, mosses, lichens, and club mosses (Lycopodiaceae), they found 20 species in 55 samples. There were no tardigrades on liverworts (!), but they were on 46% of mosses and 43% of lichens. The big surprise is that 75% of the clubmosses had tardigrades.

In the Great Smoky Mountains National Park, Bartels and Nelson (2006) found that the number of species differed little among the substrates they sampled (soil, lichen, moss, & stream habitats). Whereas it is not unusual for the soil, lichens, and mosses to have similar fauna and richness, it seems a bit unusual for the stream habitat to be as rich. *Amphibolus* cf. *weglarskae* and *Diphascon* cf. *ramazzottii* were the only species found only on bryophytes among those four substrates.

Horning *et al*. (1978) collected from soil, fungi, algae, bryophytes, lichens, marine substrata, freshwater substrata, and litter in New Zealand and surrounding islands. From bryophyte and lichen habitats, they found that all 14 of the most abundant species occurred in at least three of the five "plant" categories (three lichen forms, liverworts, and mosses). Among these, the highest occurrence was among mosses. Although *Milnesium tardigradum* (Figure 16) was slightly more abundant on lichens than on mosses, the combined numbers on mosses and liverworts was still higher. Horning *et al*. identified the bryophytes and lichens and presented the species of tardigrades on each (Table 1, Table 4, Table 6). In 559 moss samples, 45.8% had tardigrades, mean of 1.8 species, range 1-8 (Table 1). Of 55 species of tardigrades known for New Zealand, 45 occurred on mosses.

Finding New Species

The common appearance of tardigrades among bryophytes causes those who seek to describe new taxa to go first to the mossy habitats. In this spirit, Kaczmarek and Michalczyk (2004a) found the new species of moss-dwelling *Doryphoribius quadrituberculatus* in Costa Rica. From mosses in China they described the new species *Bryodelphax brevidentatus* (Kaczmarek *et al.* 2005) and *B. asiaticus* (Figure 35; Kaczmarek & Michalczyk 2004b), as did Li and coworkers for *Echiniscus taibaiensis* (Wang & Li 2005), *Isohypsibius taibaiensis* (Li & Wang 2005), *Isohypsibius qinlingensis* (Li *et al.* 2005a), *Pseudechiniscus papillosus* (Li *et al.* 2005b), *Pseudechiniscus beasleyi*, *Echiniscus nelsonae*, and *E. shaanxiensis* (Li *et al.* 2007), and Tumanov (2005) for *Macrobiotus barabanovi* and *M. kirghizicus*. Pilato and Bertolani (2005) described *Diphascon dolomiticum* from Italy.
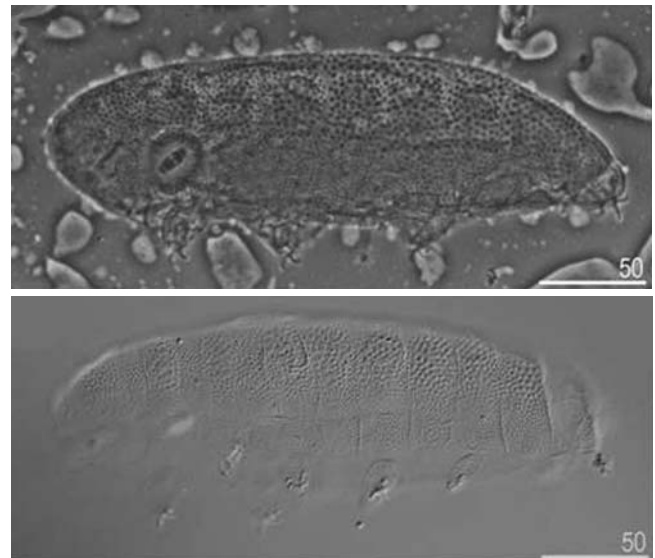

Figure 35. *Bryodelphax asiaticus*. Photo through Creative Commons.

Table 6. Comparison of numbers of individuals and percentage of individuals of each of 14 tardigrade species on liverworts, mosses, and lichens in collections from New Zealand and surrounding islands. The remaining ones were on other non-plant substrata. Number of samples is in parentheses. From Horning *et al.* 1978.

	n	liverworts % (150)	mosses % (559)	lichens % (239)
Pseudechiniscus novaezeelandiae	46	8.70	56.50	23.90
Pseudechiniscus juanitae	43	6.98	44.19	27.91
Macrobiotus harmsworthi	89	5.62	55.06	34.83
Macrobiotus hibiscus	90	7.78	60.00	17.78
Minibiotus intermedius	65	7.69	41.54	32.30
Milnesium tardigradum	143	7.69	35.66	37.06
Hypsibius dujardini	32	10.53	50.00	2.63
Paramacrobiotus areolatus	58	3.45	60.34	18.97
Echiniscus bigranulatus	18	5.56	38.89	38.89
Hypechiniscus gladiator	21	19.05	61.90	9.50
Diphascon scoticum	35	11.43	65.71	11.43
Macrobiotus liviae	72	8.33	56.94	18.06
Macrobiotus anderssoni	63	11.11	42.86	22.22
Macrobiotus furciger	89	12.36	50.56	22.47

New species from South Africa are no surprise, as enumeration of small organisms in that country is barely out of its infancy. Kaczmarek and Michalczyk (2004c) described the new species **Diphascon zaniewi** in the Dragon Mountains there. Other species found there were more cosmopolitan: **Hypsibius maculatus** (previously known only from Cameroon and England), **H. convergens** (Figure 36), **Paramacrobiotus** *cf*. **richtersi** (Figure 37), and **Minibiotus intermedius** (Figure 38-Figure 39).

Figure 38. **Minibiotus intermedius**. Photo by William Miller through Flickr.

Figure 36. **Hypsibius convergens**, a common moss-dweller. Photo by Björn Sohlenius, Swedish Museum of Natural History, with permission.

Figure 37. **Paramacrobiotus richtersi**, a common bryophyte dweller. Photo by Science Photo Library through Creative Commons.
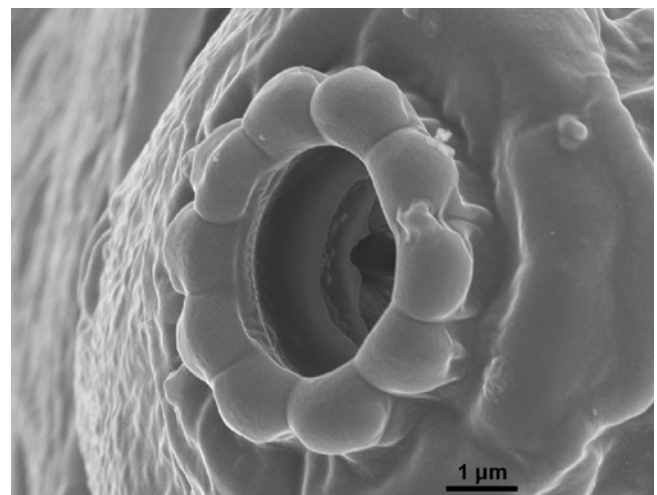

Figure 39. **Minibiotus intermedius** mouth. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Likewise, in South America, Michalczyk and Kaczmarek (2005) described **Calohypsibius maliki** as a new species from Chile; Michalczyk and Kaczmarek (2006) described **Echiniscus madonnae** (Figure 40) from

Peru, all from bryophytes. In Argentina they described *Macrobiotus szeptyckii* and *Macrobiotus kazmierskii* (Kaczmarek & Michalczyk 2009). In 2008 Degma *et al*. described another new species [*Paramacrobiotus derkai* (Figure 41)] from Chile, a country where only 29 species had previously been described.

Figure 40.　*Echiniscus madonnae*, a moss dweller from Peru. Photo by Łukasz Kaczmarek & Łukasz Michalczyk, with permission.
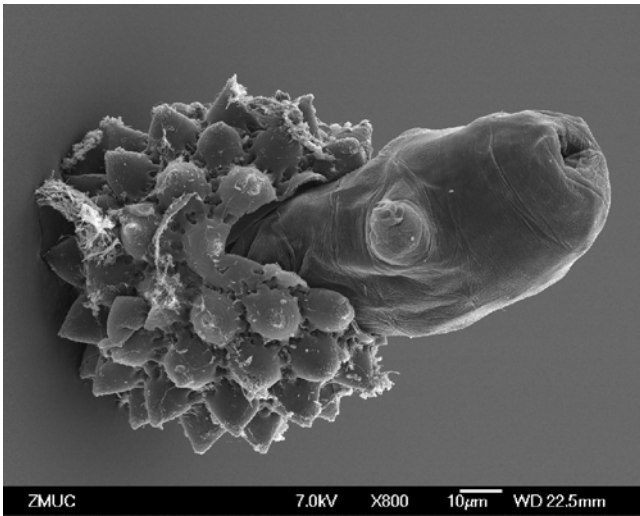

Figure 41.　*Paramacrobiotus derkai* emerging from egg. Photo by Łukasz Kaczmarek, with permission.

In Portugal, lichens and mosses provided the new species *Minibiotus xavieri* to Fontoura and coworkers (2009). In Cyprus, Kaczmarek and Michalczyk (2004d) described *Macrobiotus marlenae* (Figure 42). *Macrobiotus kovalevi* proved to be a new species from mosses in New Zealand (Tumanov 2004). Clearly, mosses have been a favorite sampling substrate for tardigrade seekers (Kaczmarek & Michalczyk 2009) and most likely hold many more undescribed species around the world.

Even when new species are collected, they are not always identified or diagnosed in a timely manner. This can result in their ultimate description from multiple locations. Such is the case for *Echiniscus viridianus* (Figure 43), a new species described by Pilato *et al*. (2007) from Alabama and New Mexico, USA, and from the Azores Islands, all from mosses.

Figure 42.　*Macrobiotus marlenae*. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Figure 43.　*Echiniscus viridianus*. Photo by Paul J. Bartels, with permission.

Summary

Most studies indicate no correlation between bryophyte species and tardigrade species. There is limited indication that cushions may have more species, but in other studies thin mats have more than cushions. Other studies indicate they are more common on weft-forming mosses than on turfs. Open mosses like *Polytrichum* seem to be less suitable as homes. There may be some specificity for liverworts rather than mosses, as for example *Macrobiotus snaresensis* in New Zealand. Unfortunately, many researchers have not identified the bryophyte taxa in tardigrade faunistic studies. A common garden study including several bryophyte species and tardigrades of the same or different species could be revealing.

send me corrections for typos in the previous online version. Thank you to my sister, Eileen Dumire, for providing the view of a novice on the readability of the original text (it has been much expanded since then). Tardigrade nomenclature is based on Degma *et al.* 2010.

Literature Cited

Bartels, P. J. and Nelson, D. R. 2006. A large-scale, multihabitat inventory of the phylum Tardigrada in the Great Smoky Mountains National Park, USA: A preliminary report. Hydrobiologia 558: 111-118.

Baxter, W. H. 1979. Some notes on the Tardigrada of North Down, including one addition to the Irish fauna. Irish Nat. J. 19: 389-391.

Beasley, C. W. 1990. Tardigrada from Gunnison Co., Colorado, with the description of a new species of *Diphascon*. Southw. Nat. 35: 302-304.

Beasley, C. W. and Miller, W. R. 2007. Tardigrada of Xinjiang Uygur Autonomous Region, China. Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 49-55.

Bertolani, R. 1983. Tardigardi muscicoli delle dune costiere Italiane, con descrizione di una nuova specie. Atti Soc. Tosc. Sci. Nat. Mem. Ser. B 90: 139-148.

Bertrand, M. 1975. Répartition des tardigrades "terrestres" dans le massif de l'Aigoual. Vie Milieu 25: 283-298.

Christenberry, D. 1979. On the distribution of *Echiniscus kofordi* and *E. cavagnaroi* (Tardigrada). Trans. Amer. Microsc. Soc. 98: 469-471.

Collins, M. and Bateman, L. 2001. The ecological distribution of tardigrades in Newfoundland. Zool. Anz. 240: 291-297.

Degma, P. 2006. First records of two Heterotardigrada (Tardigrada) species in Slovakia. Biologia Bratislava 61: 501-502.

Degma, P., Bertolani, R., and Guidetti, R. 2010. Actual checklist of Tardigrada species (Ver. 11:26-01-2010). Accessed 18 June 2010 at < http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf>.

Degma, P., Simurka, M., and Gulanova, S. 2005. Community structure and ecological macrodistribution of moss-dwelling water bears (Tardigrada) in Central European oak-hornbeam forests (SW Slovakia). Ekologia (Bratislava) 24(suppl. 2): 59-75.

Degma, P., Michalczyk, Ł., and Kaczmarek, Ł. 2008. *Macrobiotus derkai*, a new species of Tardigrada (Eutardigrada, Macrobiotidae, *huziori* group) from the Colombian Andes (South America). Zootaxa 1731: 1-23.

Fontoura, P., Pilato, G., Morais, P., and Lisi, O. 2009. Minibiotus xavieri, a new species of tardigrade from the Parque Biológico de Gaia, Portugal (Eutardigrada: Macrobiotidae). Zootaxa 2267: 55-64.

Fullard, H. 1968. China in Maps. George Philip & Sons, Ltd., London, 25 pp.

Guil, N., Hortal, J., Sanchez-Moreno, S. and Machordom, A. 2009a. Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment. Landscape Ecol. 24: 375-390.

Guil, N., Sanchez-Moreno, S., and Machordom, A. 2009b. Local biodiversity patterns in micrometazoans: Are tardigrades everywhere? System. Biodiv. 7: 259-268.

Hinton, J. G. and Meyer, H. A. 2007. Distribution of limnoterrestrial Tardigrada in Georgia and the Gulf Coast states of the United States of America with ecological remarks. In: Pilato, G. and Rebecchi, L. (guest eds.). Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 72-76.

Hinton, J. G. and Meyer, H. A. 2009. Tardigrades from Fayette County, Georgia. Georgia J. Sci. 67(2): 30-32.

Hofmann, I. 1987. Habitat preference of the most frequent moss-living Tardigrada in the area of Giessen (Hessen). In: Bertolani, R. (ed.). Biology of Tardigrades. Selected Symposia and Monographs U.Z.I., Mucchi, Modena Italia, pp 211-216.

Hofmann, I. and Eichelberg, D. 1987. Faunistisch-oekologische Untersuchungen zur Habitat-praeferenz moosbewohnender Tardigraden. [Ecological investigations of the habitat preference of moss-inhabiting tardigrades.]. Zool. Beitr. 31(1): 61-76.

Horning, D. S., Schuster, R. O., and Grigarick, A. A. 1978. Tardigrada of New Zealand. N. Zeal. J. Zool. 5: 185-280.

Hunter, M. A. 1977. A study of Tardigrada from a farm in Montgomery County, Tennessee. MS Thesis, Austin Peay State University, Clarksville, TN, 61 pp.

Jönsson, K. I. 2003. Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography 26: 356-364.

Kaczmarek, Ł. and Michalczyk, Ł. 2004a. First record of the genus *Doryphoribius* Pilato, 1969 from Costa Rica (Central America) and description of a new species *Doryphoribius quadrituberculatus* (Tardigrada: Hypsibiidae). Genus 15: 447-453.

Kaczmarek, Ł. and Michalczyk, Ł. 2004b. A new species *Bryodelphax asiaticus* (Tardigrada: Heterotardigrada: Echiniscidae) from Mongolia (Central Asia). Raffles Bull. Zool. 52: 599-602.

Kaczmarek, Ł. and Michalczyk, Ł. 2004c. Notes on some tardigrades from South Africa, with the description of *Diphascon* (*Diphascon*) *zaniewi* sp. nov. (Eutardigrada: Hypsibiidae). Zootaxa 576: 1-6.

Kaczmarek, Ł. and Michalczyk, Ł. 2004d. New records of Tardigrada from Cyprus with a description of the new species Macrobiotus marlenae (hufelandi group) (Eutardigrada: Macrobiotidae). Genus 15: 141-152.

Kaczmarek, Ł. and Michalczyk, Ł. 2009. Two new species of Macrobiotidae, *Macrobiotus szeptyckii* (*harmsworthi* group) and *Macrobiotus kazmierskii* (*hufelandi* group) from Argentina. Acta Zool. Cracoviensia, Seria B - Invertebrata 52: 87-99.

Kaczmarek, Ł., Michalczyk, Ł., and Degma, P. 2005. A new species of Tardigrada *Bryodelphax brevidentatus* sp. nov. (Heterotardigrada: Echiniscidae) from China (Asia). Zootaxa 1080: 33-38.

Kathman, R. D. and Cross, S. F. 1991. Ecological distribution of moss-dwelling tardigrades on Vancouver Island, British Columbia, Canada. Can. J. Zool. 69: 122-129.

Li, X.-C. and Wang, L.-Z. 2005. *Isohypsibius taibaiensis* sp. nov. (Tardigrada, Hypsibiidae) from China. Zootaxa 1036: 55-60.

Li, X.-C., Wang, L.-Z. and Yu, D. 2005a. *Isohypsibius qinlingensis* sp. nov. (Tardigrada, Hypsibiidae) from China. Zootaxa 980: 1-4.

Li, X.-C., Wang, L.-Z., and Yu, D. 2007. The Tardigrada fauna of China with descriptions of three new species of Echiniscidae. Zool. Stud. 46(2): 135-147.

Li, X.-C., Wang, L.-Z., Liu, Y., and Su, L.-N. 2005b. A new species and five new records of the family Echiniscidae (Tardigrada) from China. Zootaxa 1093: 25-33.

Meyer, H. A. 2006a. Interspecific association and substrate specificity in tardigrades from Florida, southeastern United States. Hydrobiologia 558: 129-132.

Meyer, H. A. 2006b. Small-scale spatial distribution variability in terrestrial tardigrade populations. Hydrobiologia 558: 133-139.

Meyer, H. A. 2008. Distribution of tardigrades in Florida. Southeast. Nat. 7: 91-100.

Meyer, H. A. and Hinton, J. G. 2007. Limno-terrestrial Tardigrada of the Nearctic realm. J. Limnol. 66(Suppl. 1): 97-103.

Michalczyk, Ł. and Kaczmarek, Ł. 2005. The first record of the genus *Calohypsibius* Thulin, 1928 (Eutardigrada: Calohypsibiidae) from Chile (South America) with a description of the new species *Calohypsibius maliki*. New Zeal. J. Zool. 32: 287-292.

Michalczyk, Ł. and Kaczmarek, Ł. 2006. Revision of the Echiniscus bigranulatus group with a description of a new species *Echiniscus madonnae* (Tardigrada: Heterotardigrada: Echiniscidae) from South America. Zootaxa 1154: 1-26.

Mihelcic, F. 1954/55. Zur Ökologie der Tardigraden. Zool. Anz. 153: 250-257.

Mihelcic, F. 1963. Moose als Lebensstätten für Tardigraden. Der Schlern 37: 179-181.

Miller, W. R., Miller, J. D., and Heatwole, H. 1996. Tardigrades of the Australian Antarctic Territories: The Windmill Islands, East Antarctica. Zool. J. Linn. Soc. 116: 175-184.

Nelson, D. R. 1973. Ecological distribution of tardigrades on Roan Mountain, Tennessee - North Carolina. Ph. D. dissertation, University of Tennessee, Knoxville.

Nelson, D. R. 1975. Ecological distribution of Tardigrada on Roan Mountain, Tennessee - North Carolina. In: Higgins, R. P. (ed.). Proceedings of the First International Symposium on Tardigrades. Mem. Ist. Ital. Idrobiol. Suppl. 32: 225-276.

Newsham, K. K., Maslen, N. R., and McInnes, S. J. 2006. Survival of Antarctic soil metazoans at -80ºC for six years. CryoLetters 27(5): 269-280.

Pilato, G. and Bertolani, R. 2005. *Diphascon* (*Diphascon*) *dolomiticum*, a new species of Hypsibiidae (Eutardigrada) from Italy. Zootaxa 914: 1-5.

Pilato, G., Fontoura, P., and Lisi, O. 2007. Remarks on the *Echiniscus viridis* group, with the description of a new species (Tardigrada, Echiniscidae). In: Pilato, G. and Rebecchi, L. (guest eds.). Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 33-39.

Ramazzotti, G. and Maucci, W. 1983. The phylum Tardigrada - 3rd edition: English translation by C. W. Beasley. Mem. Ist. Ital. Idrobiol. Dott. Marco de Marchi 41: 1B680.

Romano, F. A. III., Barreras-Borrero, B., and Nelson, D. R. 2001. Ecological distribution and community analysis of Tardigrada from Choccolocco Creek, Alabama. Zool. Anz. 240: 535-541.

Sayre, R. M. and Brunson, L. K. 1971. Microfauna of moss habitats. Amer. Biol. Teacher Feb. 1971: 100-102, 105.

Tumanov, D. V. 2004. *Macrobiotus kovalevi*, a new species of Tardigrada from New Zealand (Eutardigrada, Macrobiotidae). Zootaxa 406: 1-8.

Tumanov, D. V. 2005. Two new species of *Macrobiotus* (Eutardigrada, Macrobiotidae) from Tien Shan (Kirghizia), with notes on *Macrobiotus tenuis* group. Zootaxa 1043: 33-46.

Wang, L.-Z. and Li, X.-C. 2005. *Echiniscus taibaiensis* sp. nov. and a new record of *Echiniscus bisetosus* Heinis (Tardigrada, Echiniscidae) from China. Zootaxa 1093: 39-45.

Glime, J. M. 2017. Tardigrade Densities and Richness. Chapt. 5-5. In: Glime, J. M. Bryophyte Ecology. Volume 2. Bryological   5-5-1
Interaction. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated
21 April 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>.

CHAPTER 5-5
TARDIGRADE DENSITIES
AND RICHNESS

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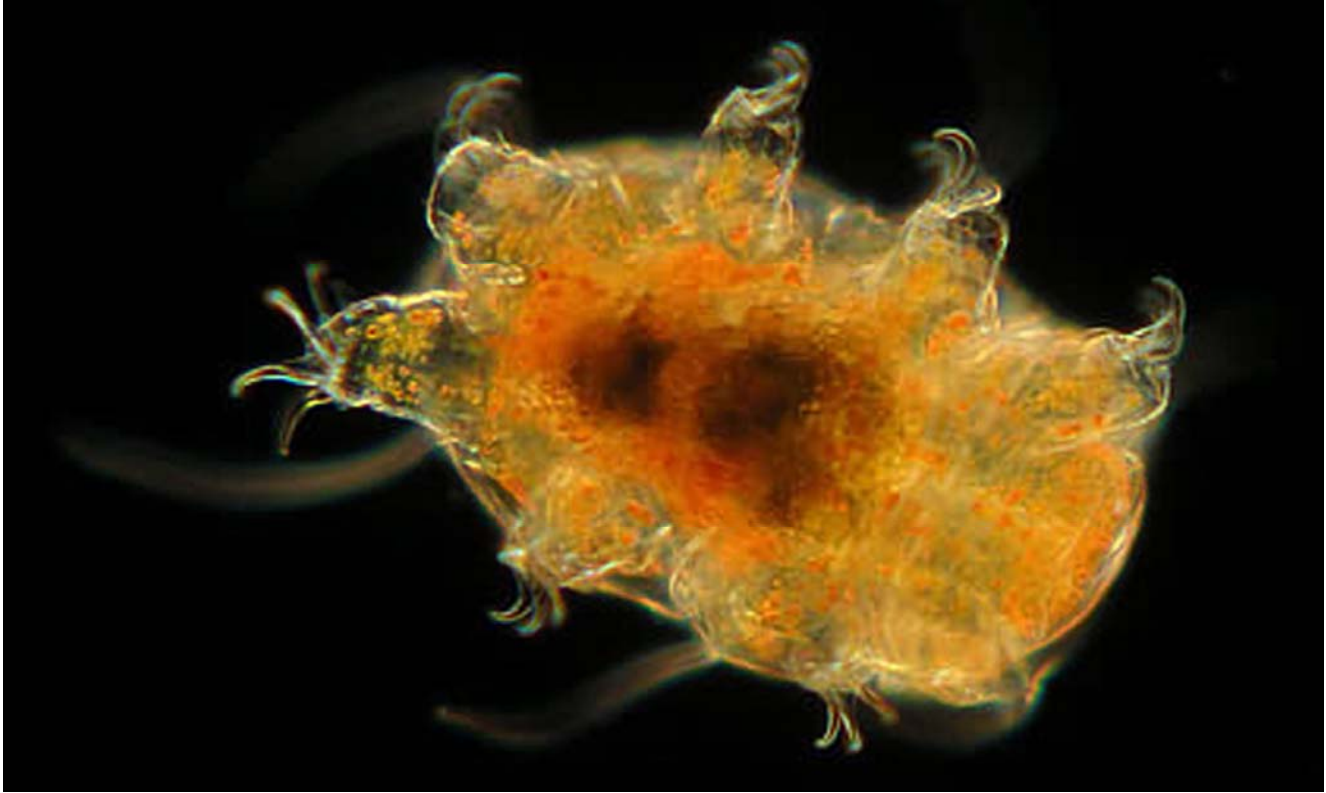
CHAPTER 5-5
TARDIGRADE DENSITIES
AND RICHNESS

Figure 1. *Echiniscus*, the genus with the most species among mosses. Photo by Martin Mach, with permission.

Densities and Richness

But just how common are these bryophyte-dwelling tardigrades (Figure 1)? I think the largest reported density I have found in the literature is 22,000 individuals per gram of dry moss (Mathews 1938), but that is an old number and may well have been replaced. These animals seem to be especially adapted for the bryophyte habitat (Jerez *et al.* 2002), achieving densities as great as 2,000,000 individuals per square meter of *Bryum argenteum* (Figure 2) (Brusca & Brusca 1990). (Is that greater than 22,000 per gram?) Nelson (2002) reminds us that densities of these animals are highly variable and conditions for optimum development of the population are unknown (see also Kinchin 1994). Factors such as temperature and moisture (Franceschi *et al.* 1962-1963; Morgan 1977; Briones *et al.* 1997), air pollution (Steiner 1994a, b, c, 1995), and food availability (Hallas & Yeates 1972) all influence population density. And it appears that random dispersal may be a major factor, since both population density and species diversity vary considerably between adjacent microhabitats that appear to be identical (Nelson 2002).

Figure 2. *Bryum argenteum* exhibiting the tight leaves that provide capillary spaces where tardigrades can enjoy prolonged water retention. Photo by Michael Lüth, with permission.

Among the more extensive studies is that of Kathman and Cross (1991) on Vancouver Island, British Columbia, Canada. They collected from mosses at six altitudes on five mountains and found 39 species among 37 moss species, with 13,696 individuals in all. However, as noted in Bertolani's (1983) study, the species of moss did not seem to be important.

Horning *et al*. (1978) collected from soil, fungi, algae, bryophytes, lichens, marine substrata, freshwater substrata, and litter in New Zealand and surrounding islands. They provide summaries of the tardigrade species from each bryophyte species. From their 1354 collections, they represented 577 terrestrial habitats. All 14 of the more abundant tardigrade species occurred in at least three of the five "plant" categories (three lichen forms, liverworts, and mosses). Among these, the highest occurrence was among mosses, except for *Milnesium tardigradum* (Figure 3), which occurred more often among lichens. They reported the number of species on each bryophyte, but not the density of individuals. As in other studies, moisture seemed to play a major role. They considered the "plant" categories, arranged from dry to moist, to be crustose lichen > fruticose lichen > foliose lichen > liverworts & mosses. The foliose lichens and mosses served as habitat for more tardigrade species than did the liverworts, crustose lichens, or fruticose lichens. Liverworts housed 30 tardigrade species on 26 liverwort species.

Figure 3. *Milnesium tardigradum*, a moss dweller that seems to have a slight preference for lichens. Photo by Martin Mach, with permission.

Roof mosses (Figure 4) have their share of tardigrade fauna; Morgan (1977) recorded densities of four tardigrade species [*Macrobiotus hufelandi* (Figure 15), *Milnesium tardigradum* (Figure 3), *Ramazzottius oberhaeuseri* (Figure 5), *Echiniscus testudo* (Figure 6)] of up to 823 individuals per gram of the mosses *Ceratodon purpureus* (Figure 7) and *Bryum argenteum* (Figure 8) on roofs in Swansea, Wales. In total, Morgan collected 32,552 tardigrades from these two mosses on just three roof locations at the University College of Swansea.

Even new species might be abundant in many parts of the world. This is an under-collected group, as suggested by finding very common species for the first time in some countries. Kristensen *et al*. (2009) found more than 200 individuals of a new species of *Bryodelphax* (see Figure 9) in a "very small moss sample." And these were cohabiting with *Macrobiotus hufelandi* (Figure 15) and *Milnesium tardigradum* (Figure 3).

Figure 4. Moss-covered roof that has been sampled along the edge. Photo by Susan Moyle-Studlar, with permission.

Figure 5. *Ramazzottius oberhaeuseri*, a common moss-dweller, including those on roofs. Photo by Martin Mach, with permission.
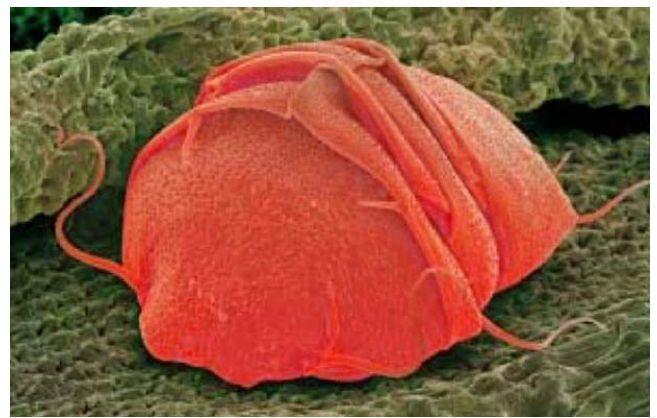

Figure 6. *Echiniscus testudo* tun on a moss leaf. Photo by Power & Syred through Creative Commons.

Figure 7. *Ceratodon purpureus*, another common roof moss that can house innumerable tardigrades. Photo by Michael Lüth, with permission.

Figure 8. *Bryum argenteum*, a common roof moss that can house innumerable tardigrades. Photo by Michael Lüth, with permission.
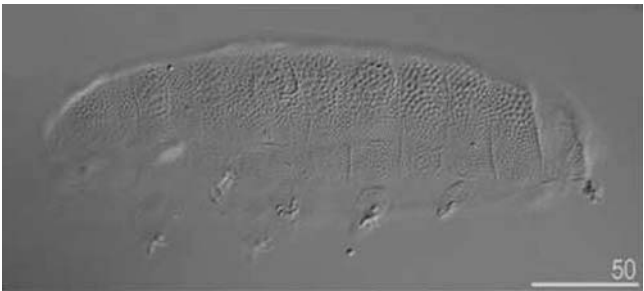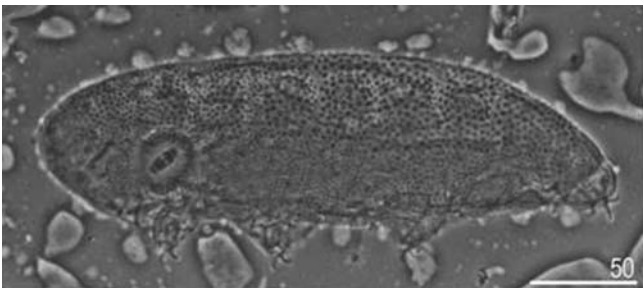
Figure 9. *Bryodelphax asiaticus*. Photo through Creative Commons.

Europe

One might expect the knowledge of European tardigrades to be the most complete, partly because the taxonomy of the bryophytes has been known longer than in many other countries, including North America, and partly because of the interest of Europeans in natural history.

Some European mosses have abundant tardigrades: *Hypnum cupressiforme* (Figure 10), *Hylocomium splendens* (=*Hypnum parietinum*) (Figure 11), and *Sanionia uncinata* (Figure 12), as well as *Grimmia* (might include *Schistidium*; Figure 13) and *Tortula* (Marcus 1928a; probably includes *Syntrichia*; Figure 14) and may contain up to 20,000 individuals per 1 g of air-dried moss (Marcus 1928b).

Figure 10. *Hypnum cupressiforme*, home of abundant tardigrades. Photo by Michael Lüth, with permission.

Figure 11. *Hylocomium splendens*, a good habitat for tardigrades. Photo by Janice Glime.

Figure 12. *Sanionia uncinata*, a suitable tardigrade habitat. Photo by Janice Glime.

Figure 13. *Grimmia elongata* cushions. Photo by Michael Lüth, with permission.

Figure 14. *Tortula intermedia* cushion. Photo by Michael Lüth, with permission.

In a boreal forest in Sweden, Jönsson (2003) found sixteen species of tardigrades on mosses, including the widespread *Macrobiotus hufelandi* (Figure 15) as the most common. Among these, five were new to Sweden. They also found that the forest tended to have more tardigrade species than did a clear-cut area, but overall abundance within a species differed little between these two habitats.
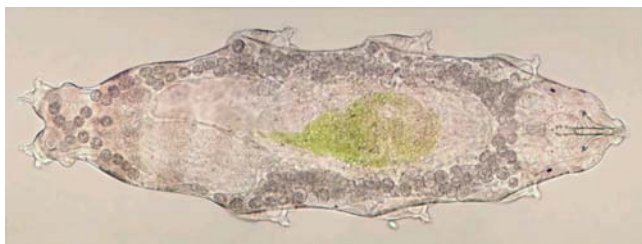

Figure 15. *Macrobiotus hufelandi*, a dominant species on *Rhytidiadelphus squarrosus* (Figure 19) in the Black Forest, Germany. Photo by Martin Mach, with permission.

In the Tihany Peninsula, Hungary, Felföldy and Iharos (1947) found modest numbers, with 38 individuals per gram of the moss *Eurhynchium swartzii* (Figure 16) and 84 per gram among clones of *Barbula* [formerly in *Didymodon*] *tophacea* (Figure 17).

Figure 16. *Eurhynchium swartzii*, a pleurocarpous moss that is known to house tardigrades. Photo by Kristian Peters through Wikimedia Commons.
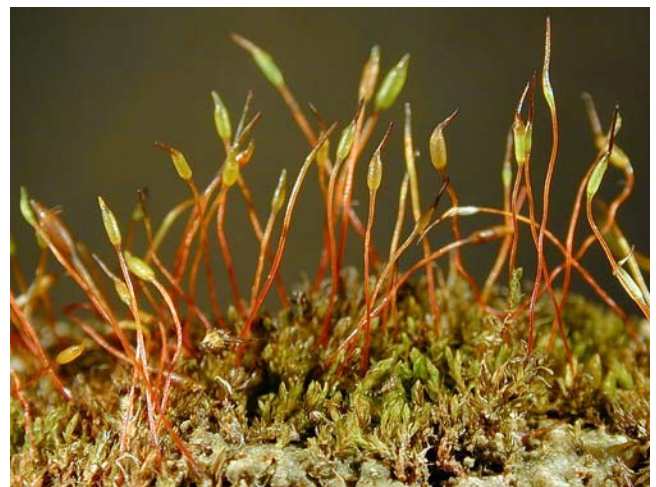
Figure 17. *Barbula tophacea*, an acrocarpous moss that houses tardigrades. Photo by Michael Lüth, with permission.

Hofmann and Eichelberg (1987) found sixteen species, including two undescribed, among mosses at Lahnau, near Giessen, Germany. Maucci (1980) collected 2686 samples of bryophytes and found 23 species of tardigrades.

In Sardinia, Pilato and Sperlinga (1975) likewise found sixteen species of tardigrades among the bryophytes. These included *Macrobiotus nuragicus* and *M. arguei* as new species. *Isohypsibius pappi*, *I. sattleri* (formerly *I. bakonyiensis*), and *Hypsibius convergens* (Figure 18) were new for Sardinia. It seems that finding new species within tardigrade communities is a fairly common occurrence.

Figure 18. *Hypsibius convergens*, one of the most common of bryophyte dwellers. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Schuster and Greven (2007) followed the inhabitants of the moss *Rhytidiadelphus squarrosus* (Figure 19) in the Black Forest in Germany for 54 months (Table 1). They uncovered 19,909 individuals comprising 24 species. The dominant species were *Macrobiotus hufelandi* (Figure 15; 56%), *Paramacrobiotus richtersi* (Figure 20; 18%), and *Diphascon pingue* (Figure 21; 12%). In contrast to the Oregon study, the highest diversity occurred in winter, whereas the number of individuals declined in winter, then increased from spring until autumn, as in Oregon. On the other hand, *D. rugosum* (Figure 22), *Hypsibius dujardini* (Figure 23), and *H*. cf. *convergens* (Figure 18) exhibited peaks in winter. Water-loving species were most numerous in the moist season, whereas **euryhydric** species increased when it was relatively dry and sunny. During the course of the 54 months, 14 of the 24 species remained, whereas species succession/change occurred among the others.
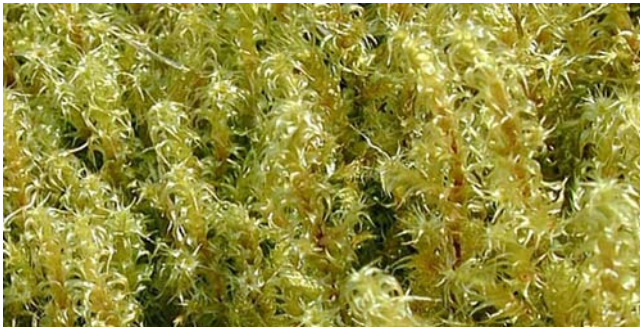

Figure 19. *Rhytidiadelphus squarrosus*, the home for 24 rotifer species in The Black Forest of Germany. Photo by Michael Lüth, with permission.

Figure 20. *Paramacrobiotus richtersi*, one of the most common and abundant of the bryophyte tardigrades. Photo by Science Photo Library through Creative Commons.

Figure 21. *Diphascon pingue*. Photo by Michael Collins, with permission.
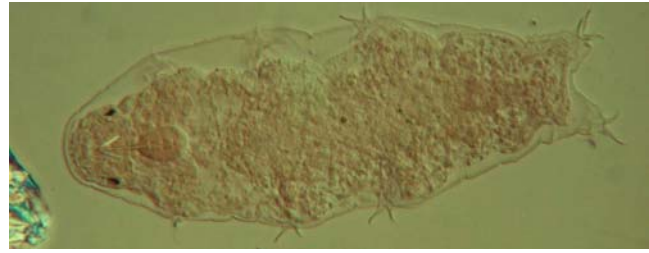
Figure 22. *Diphascon rugosum*, a tardigrade that peaks in winter in Oregon, USA. Photo by Björn Sohlenius, Swedish Museum of Natural History, with permission.

Figure 23. *Hypsibius dujardini*, a moss dweller that has its peak population in winter in the Black Forest of Germany. Photo by Bob Goldstein, with permission.

Species such as *Diphascon oculatum* (Figure 24) that had reasonable numbers on *Rhytidiadelphus squarrosus* (Figure 19), but for which no eggs were found (Schuster & Greven 2007), might deposit eggs at a different season than those sampled. It is unlikely that they would deposit eggs in a different habitat/location from that of the adults because of their limited mobility. On the other hand, rare species occurring only once, *e.g. Mesocrista spitzbergensis* (Figure 25) [note – this is a name change from *M. spitzbergense*, required to make the gender agree with that of the genus (Degma *et al.* 2010)], may have been an accidental arrival on *Rhytidiadelphus squarrosus*, or generally rare. It would be interesting to know the longevity and life cycle of rare species.

Figure 24. *Diphascon oculatum*, an inhabitant of *Rhytidiadelphus squarrosus* (Figure 19). Photo by Björn Sohlenius, Swedish Museum of Natural History, with permission.

Table 1. Comparison of total number of individuals (in order of dominance), eggs in exuviae, dominances, and frequencies for each tardigrade species collected on **Rhytidiadelphus squarrosus** (Figure 19) in the Black Forest of Germany within the investigation period of 54 months. Asterisks indicate species found at least once in each year of study. From Schuster & Greven 2007.

Species	N. individuals	Eggs / Exuviae	Dominance (%)	Frequency (%)
*Macrobiotus hufelandi (Schultze 1833)	11118	448	55.84	100
*Paramacrobiotus richtersi (Murray 1911)	3600	179	18.08	100
*Diphascon pingue sl (Marcus 1936)	2359	170	11.85	100
*Hypsibius scabropygus (Cuénot 1929)	429	15	2.15	78.5
*Macrobiotus patiens (Pilato et al. 2000)	403	7	2.02	87.9
*Hypsibius dujardini (Doyère 1840)	390	58	1.96	72.9
*Diphascon rugosum (Bartos 1935)	348	22	1.75	48.6
*Isohypsibius prosostomus (Thulin 1928)	294	29	1.48	67.3
*Hypsibius convergens (Urbanowicz 1925)	246	18	1.24	46.7
*Hypsibius pallidus (Thulin 1911)	246	13	1.24	65.4
*Hypsibius cfr. convergens	164	8	0.82	31.8
*Milnesium tardigradum (Doyère 1840)	101	4	0.51	48.6
*Diphascon oculatum (Murray 1906)	77	0	0.39	41.1
*Diphascon prorsirostre (Thulin 1928)	63	1	0.32	39.3
Isohypsibius pappi (Iharos 1966)	24	7	0.12	16.8
Hypsibius sp.	12	0	0.06	2.8
Diphascon nobilei (Binda 1969)	8	0	0.04	2.8
Minibiotus cfr. poricinctus	8	0	0.04	3.7
Minibiotus cfr. scopulus	6	0	0.03	5.6
Diphascon scoticum (Murray 1905)	5	0	0.03	2.8
Minibiotus intermedius (Plate 1888)	5	0	0.03	3.7
Diphascon bullatum (Murray 1905)	1	0	0.01	0.9
Diphascon higginsi (Binda 1971)	1	0	0.01	0.9
Mesocrista spitzbergensis (Richters 1903)	1	0	0.01	0.9
Sum	19909	979	100.00	

Figure 25. *Mesocrista spitzbergensis*, an inhabitant of *Rhytidiadelphus squarrosus*. Photo by Björn Sohlenius, Swedish Museum of Natural History, with permission.

In Scotland, Morgan (1976) found that bryophyte and lichen dwellers represented the highest number of tardigrades as well as having the greatest species diversity. In Wales, Morgan (1974) found tardigrades numbering 2287×10^3 m^{-2} among mosses. By contrast, Hallas and Yeates (1972) found only 12×10^3 m^{-2} in soil and litter in Danish forests.

Studies on abundance reveal a wide range of densities. Degma and coworkers (2003, 2006; & Pecalková 2003; *et al*. 2004, 2005) have provided us with records of tardigrades on named species of mosses in Slovakia. On **Hypnum cupressiforme** (Figure 10) in Central European

oak-hornbeam forests of Slovakia, Degma *et al*. (2005) found 3050 tardigrades [21 species in two families (**Hypsibiidae** & **Macrobiotidae**)] from 79 moss samples. As in many other studies they were unable to demonstrate any of 12 environmental variables that accounted for the distribution of the tardigrades. Rather, they found that the distribution of species was random.

Nevertheless, in his 2003 study, Degma found particular tardigrades on particular bryophytes (Figure 26-Figure 43): **Eremobiotus alicatai** on mosses **Brachythecium rutabulum** (Figure 26) and **Eurhynchium hians** (Figure 27); **Isohypsibius pappi** on these two as well as on **B. reflexum** (Figure 28), **Homalothecium sericeum** (Figure 29), **Hypnum cupressiforme** (Figure 10), **Mnium stellare** (Figure 31), and **Rhynchostegium megapolitanum** (Figure 32); **Isohypsibius josephi** on **Amblystegium serpens** (Figure 33) and **Brachythecium starkei** (Figure 34); **Diphascon iltisi** on **Campylium halleri** (Figure 35); **Astatumen trinacriae** (Figure 36) on **Brachythecium rutabulum** (Figure 26), **Homalothecium sericeum** (Figure 29), **Hypnum cupressiforme** (Figure 10), **Isothecium alopecuroides** (Figure 30), **Leskeella nervosa** (Figure 37), **Paraleucobryum longifolium** (Figure 38), and **Pterigynandrum filiforme** (Figure 39); **Isohypsibius dastychi** in unidentified moss. Degma and Pecalková (2003) reported **Diphascon belgicae** in **Brachythecium reflexum** (Figure 28); **Calohypsibius schusteri** and **Itaquascon pawlowskii** in **Hypnum cupressiforme** (Figure 10). In 2006 Degma reported **Echiniscus cf. reticulatus** on **Ctenidium molluscum** (Figure 40); **Testechiniscus**

spitsbergensis on ***Ctenidium molluscum*** (Figure 40), ***Distichium capillaceum*** (Figure 41), ***Ditrichum flexicaule*** (Figure 42), and ***Tortella tortuosa*** (Figure 43). But are these just chance findings, or is there a preference? It is interesting that all but the last three and ***Paraleucobryum longifolium*** (Figure 38) are mat-forming mosses. Was this a preference of the tardigrade or the collector? Or simply a consequence of the habitat?

Figure 26. ***Brachythecium rutabulum***, a mat-forming moss that is home to ***Astatumen trinacriae***, ***Eremobiotus alicatai***, and ***Isohypsibius pappi***. Photo by Michael Lüth, with permission.

Figure 27. ***Eurhynchium hians***, a mat-forming moss that is home to ***Eremobiotus alicatai*** and ***Isohypsibius pappi***. Photo by Michael Lüth, with permission.

Figure 28. ***Brachythecium reflexum***, a mat-forming moss that is home to ***Diphascon belgicae*** and ***Isohypsibius pappi***. Photo by Michael Lüth, with permission.

Figure 29. ***Homalothecium sericeum***, a mat-forming moss that is home to ***Astatumen trinacriae*** and ***Isohypsibius pappi***. Note the branches turned to one side. Photo by Michael Lüth, with permission.

Figure 30. ***Isothecium alopecuroides***, home to ***Astatumen trinacriae*** and ***Isohypsibius pappi***. Photo by Biopix through EOL Creative Commons.

Figure 31. ***Mnium stellares***, home to ***Isohypsibius pappi***. Photo by Michael Lüth, with permission.

Figure 32. ***Rhynchostegium megapolitanums***, home to ***Isohypsibius pappi***. Note the droplets of water adhering to the leaves, making this a good limnoterrestrial habitat. Photo by Michael Lüth, with permission.

Figure 33. *Amblystegium serpens*, home to *Isohypsibius josephi*. Photo by Michael Lüth, with permission.

Figure 34. *Brachythecium starkei*, home to *Isohypsibius josephi*. Photo by Michael Lüth, with permission.
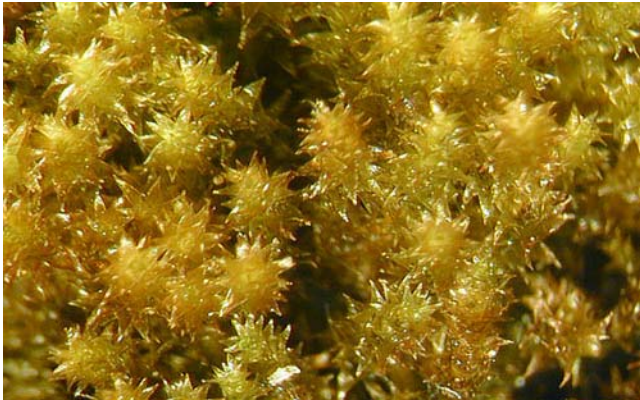
Figure 35. *Campylium halleri*, home to *Diphascon iltisi*. Photo by Michael Lüth, with permission.
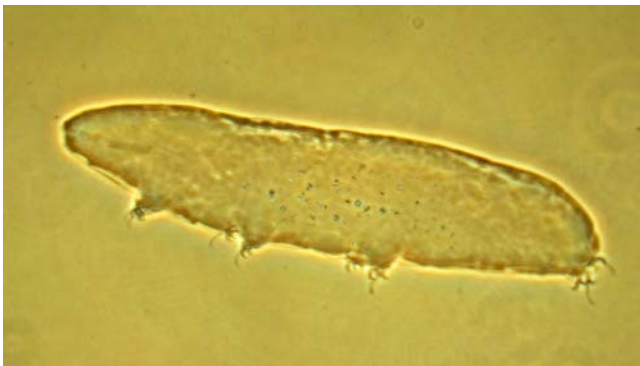
Figure 36. *Astatumen trinacriae*. Photo by Paul J. Bartels, with permission.

Figure 37. *Leskeella nervosa*, home to *Astatumen trinacriae*. Note the bulbils at the tips of branches. Photo by Michael Lüth, with permission.

Figure 38. *Paraleucobryum longifolium*, a cushion former on rocks, home to *Astatumen trinacriae*. Photo by Michael Lüth, with permission.

Figure 39. *Pterigynandrum filiforme*, home to *Astatumen trinacriae*. Photo by Michael Lüth, with permission.

Figure 40. *Ctenidium molluscum*, home to *Echiniscus cf. reticulatus* and *Testechiniscus spitsbergensis*. Photo by Michael Lüth, with permission.

Figure 41. *Distichium capillaceum*, a cushion former, home to *Testechiniscus spitsbergensis*. Photo by Michael Lüth, with permission.

Figure 42. *Ditrichum flexicaule*, exhibiting tight vertical leaves, home to *Testechiniscus spitsbergensis*. Photo by Michael Lüth, with permission.

Figure 43. *Tortella tortuosa*, a cushion former, home to *Testechiniscus spitsbergensis*. Photo by Michael Lüth, with permission.

North America

The neglect of tardigrades has not escaped North America. Meyer (2006a) lamented that only one species of tardigrade had been reported from Florida. By sampling 47 species of mosses, liverworts, lichens, and ferns from trees and shrubs in all 67 counties of Florida, he found 20 species of tardigrades. Like other studies discussed here, he could find no association between tardigrade species and any particular bryophyte or lichen species. He did, however, find differences between species occurring on lichens and mosses in general.

It is clear that neglect of the bryophyte habitat is neglect of tardigrades in general. Based on species-area curves, Bartels and Nelson (2007) estimated the greatest species richness among bryophytes in their comparison of habitats in the Great Smoky Mountains, USA, although their actual numbers showed about equal numbers of species among the terrestrial habitats:

Aquatic	29
Soil	39
Lichen	35
Moss	37
Total	140

Among the additional species most likely to contribute to the predicted number of bryophyte dwellers are a number of species found there on other substrata, that are known from bryophytes elsewhere but not found in the necessarily limited sampling in this study.

Meyer *et al.* (2003) examined populations among a variety of habitats in central Florida and Ouichita Mountains, Arkansas, USA. They found the tardigrades to be both diverse and abundant, varying greatly within the same species among mosses on different rocks and trees. For example, in an extreme case a tree exhibited three species with numerous individuals while the adjacent tree had none. Four adjacent cores yielded from 0 to 86 individuals, totalling 5 species. This type of distribution is consistent with the patchiness discussed below and supports the hypothesis of random dispersal followed by aggregation resulting from reproduction without migration.

Paul Davison (pers. comm. 21 June 2006), working in Alabama, USA, contends that tardigrades are best found on "scrappy mosses" that occur in harsh environments. These include those on the face of concrete steps or rock and concrete walls, rooftops, or bark of city trees. In fact, some researchers have suggested that the tardigrades might require a dry period during their lives to survive. Using such mosses, drying, and crumbling them through a 0.5 cm screen over a dish pan can yield as many as 70 tardigrades in just 5 mL of processed extract.

A more modest flora was in evidence in the collections from Southwestern Virginia, USA (Riggin 1962). In 434 collections of mosses and lichens, Riggin found only 694 individual tardigrades – hardly a story of high densities on a broad scale. These were represented by 26 species. *Macrobiotus* seems to be among the most common genera on bryophytes, including North American collections where Riggin found 63% of the Virginia bryophyte (moss?) and lichen collections housing members of this genus.

In a study of both the Upper and Lower Peninsulas of Michigan, USA, Meyer *et al.* (2011) revealed 28 species of tardigrades from mosses, liverworts, lichens, and leaf litter, of which 19 were from bryophytes [*Echiniscus blumi*, *E. merokensis*, *E. virginicus*, *E. wendti*, *Pseudechiniscus facettalis*, *P. suillus* (Figure 44), *Milnesium tardigradum* (Figure 3), *Hypsibius arcticus* (Figure 45), *Ramazzottius baumanni*, *R. oberhaeuseri* (Figure 5), *Diphascon alpinum*, *D. nodulosum* (Figure 46), *Astatumen trinacriae* (Figure 36), *Macrobiotus echinogenitus*, *M. hufelandi* (Figure 15), *Minibiotus intermedius* (Figure 47), *Fractonotus caelatus*, *Paramacrobiotus areolatus* (Figure 48), *P. tonollii* (Figure 49)]. Of the 28, 18 species were considered to be cosmopolitan. They found only one new

species, and it was not a bryophyte dweller. Although Ramazzotti and Maucci (1983) reported that more than ten taxa of tardigrades can often occur in a single bryophyte sample, and the range is generally 2-6, Meyer *et al.* found diversity on Michigan bryophytes to usually be at the lower end of this range.

Figure 44. *Pseudechiniscus juanitae*. Photo by Paul J. Bartels, with permission.

Figure 45. *Hypsibius arcticus*. Photo from Smithsonian Institution through EOL Creative Commons.

Figure 46. *Diphascon nodulosum*. Photo by Michael Collins, with permission.
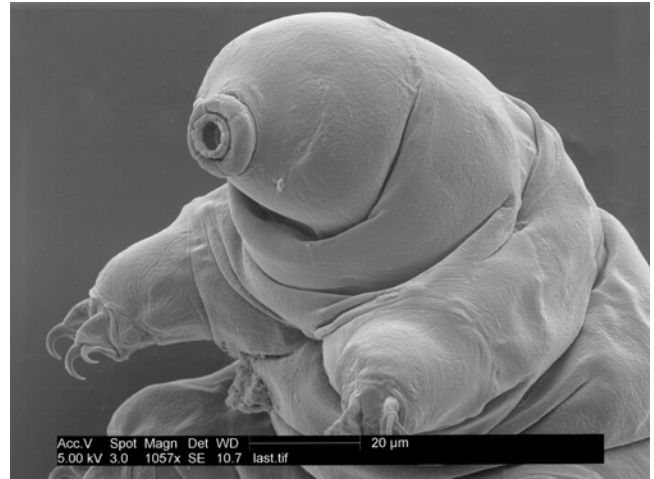
Figure 47. *Minibiotus intermedius*. Photo by William Miller through Flickr.

Figure 48. *Paramacrobiotus* [=*Macrobiotus*] *areolatus*. Photo by Martin Mach, with permission.
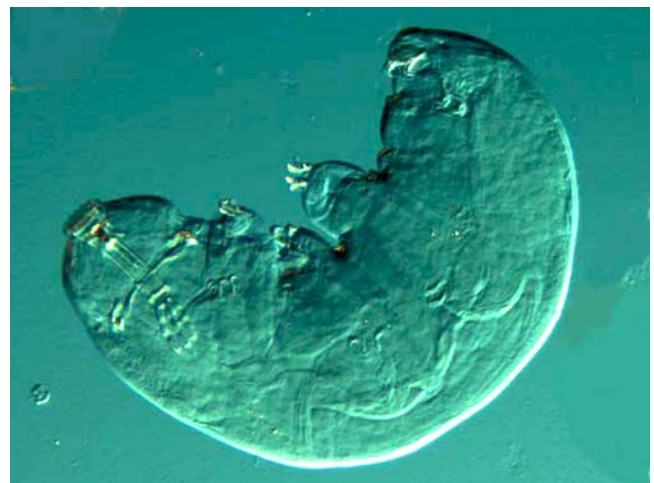
Figure 49. *Paramacrobiotus tonollii*. Photo by Paul J. Bartels, with permission.

Nelson and Hauser (2012) collected epiphytic mosses and liverworts in a natural area in Oregon, USA. Out of 1102 invertebrates collected, the tardigrades ranked second, exceeded only by the mites (Acari). They pointed out the need for water sampling (washing samples) to find tardigrades. These animals did not show up in the Berlese

extraction used by many collectors. Their collections reveal at least six or seven different taxa of tardigrades from each epiphytic moss water sample, a number that brings the patchy distribution of tardigrades into question. They considered the tardigrades to be well represented for a group with approximately 1000 species, compared to mites with approximately 50,000 species.

South America and Neotropics

Numbers of species and density varies widely among tardigrade collections. Claps *et al*. (2009) found 28 species in 10 genera in a sub-Antarctic *Nothofagus* forest (18) and plateau (13) in the Rio Negro province of Argentina. In Costa Rica, Kaczmarek *et al*. (2009, 2011) found more than 7000 tardigrade individuals in 700 samples of lichens, mosses, and liverworts. These comprised 64 species in 18 genera, but the average number of species per sample was not more than three. They found altitude to be an important factor in distribution, with the highest diversity in the range of 1400-2000 m asl (35 species, 55% frequency). Only 18 species (28% frequency) occurred in the range of 2400-2800 m asl. In the range of 2000-2400 m asl the number of individuals was high. Then at 3200 m asl the frequency (70%) and abundance increased again. Surprisingly, they found a significantly higher presence in the urban and agricultural habitats than they did in natural habitats. Although 24 species had very defined habitat preferences, with the highest frequency in humid habitats, substrate and plant type were not important in their habitat choice.

Asia

Unfortunately, much of the Asian literature is lost to the western world because of our lack of skill in reading the languages. But according to Beasley *et al*. (2006), the knowledge of tardigrades in China is meager. And ecological studies seem to be totally wanting. Many of the studies are simply reports of collections made by outsiders (*e.g.* Mathews 1937a, b; Bartos 1963; Pilato 1974; Beasley *et al*. 2006). Pilato (1974) found six species of tardigrades in Chinese bryophyte communities and identified three new species: **Bryodelphax** [=*Echiniscus*] **sinensis**, **Macrobiotus mandalaae**, and **Macrobiotus mauccii**. Yang (2002) reported on tardigrades from bryophytes in Yunnan Province. Beasley *et al*. (2006) reported only 18 species from a wide geographic range (3 provinces) in China, with 12 of these species occurring on mosses [*Echiniscus nepalensis*, *Pseudechiniscus jiroveci*, *Murrayon hibernicus*, *Hypsibius pallidus*, *Isohypsibius sattleri*, *Doryphoribius flavus*, *Diphascon pingue* (Figure 21), *Diphascon scoticum* (Figure 50), *Diphascon prorsirostre*, *Mesocrista spitsbergensis* (Figure 51), *Platicrista angustata* (Figure 52), *Milnesium tardigradum* (Figure 3)] and 1 on a liverwort [*Cornechiniscus lobatus* (see Figure 53)]. Of the 18 species reported, 8 were new to China! It is likely that a much larger fauna exists but has not been explored – or translated.

In 2007, Beasley and Miller published a list of tardigrades from Xinjiang Uygur Autonomous Region, China, based on bryophyte specimens from the Missouri Botanical Garden. They found only 78 tardigrades among the 270 specimens of bryophytes, comprising 12 species. Of these 12, 7 were new to China. Several additional

species could not be identified. The best known bryophyte dweller among these was *Milnesium tardigradum* (Figure 54). *Echiniscus testudo* (Figure 6) was found among the greatest number of bryophyte species. The majority of species were in the **Heterotardigrada**, possibly due to the higher elevation of the samples and the arid nature of the habitats.

Figure 50. *Diphascon scoticum*. Photo by Łukasz Kaczmarek, with permission.

Figure 51. *Mesocrista spitsbergensis*. Photo by Björn Sohlenius Swedish Museum of Natural History, with permission.

Figure 52. *Platicrista angustata*, a species that occurs on mosses in China. Photo by Michael Sullivan, with permission.

International knowledge of the Japanese tardigrade fauna suffers from the same language barrier. Mathews, who also named a number of Chinese taxa, reported on the Japanese tardigrades in 1936/37. More recently, Ito (1999) made an ecological study on the north slope of Mt. Fuji,

sampling soil, mosses, and lichens. The number of soil tardigrades ranged 8,050 m^{-2} to 75,500 m^{-2}. Their density was as high as the density of soil arthropods such as mites (Acari) and springtails (Collembola). A few of these showed a relationship with altitude (950-2380 m asl), but typically the dominant species for a habitat did not change much among locations. On the other hand, they changed considerably between habitats at a single location.

Figure 53. *Cornechiniscus cornutus*. Photo by Martin Mach, with permission.

The Japanese certainly have not ignored the tardigrades. They have made important contributions to the physiology (Horikawa & Higashi 2004; Horikawa *et al.* 2006) and space biology (Horikawa 2008; Ono *et al.* 2008) of these organisms. There are also good studies on the ecology of soil species. But ecological studies on bryophyte-dwelling taxa are hard to find.

Africa

Although little is known about them, Africa sports its share of moss-dwelling tardigrades. Pilato and Pennisi (1976) reported 21 species of tardigrades among the mosses in their collections from Cyrenaica (eastern coast of Libya), two of which represented the first members of their genera in Africa. A third, *Isohypsibius brulloi*, was a new species. Binda (1984) found thirteen species of moss-dwelling tardigrades in South Africa and Mozambique.

Meyer and Hinton (2009) found only nine species of tardigrades among mosses and lichens in KwaZulu-Natal, South Africa, bringing the total number of species from soil, mosses, and lichens to 61 in southern Africa. But aside from species records, tardigrade-bryophyte ecological studies seem to be rare or non-existent for Africa.

Antarctic and Arctic

Unlike Asia, Africa, and South America (McInnes 1994), tardigrades are fairly well studied in polar climates, especially in the Antarctic. In the Antarctic, bryophytes, as well as lichens and algae, provide important habitats for tardigrades, rotifers, and nematodes (Utsugi & Ohyama 1991; Sohlenius *et al.* 2004). Most invertebrates decrease in abundance as one approaches the poles, but Jennings (1979) found that tardigrades actually increase in abundance in the Antarctic tundra. Peters and Dumjahnn (1999) found 15 species in ten genera in their 249 cushion moss samples from Disko Island, West Greenland. On the

other hand, in his moss studies on the Antarctic Schirmacher Oasis, Mitra (1999) examined 36 sites and found only two tardigrade species.

Here they are also patchily distributed, nevertheless usually having the highest densities among these three groups of organisms. The ubiquitous and very common moss inhabitant, *Macrobiotus* sp., is present there, on the sub-Antarctic Marion Island (McInnes *et al.* 2001). Other tardigrades present include *Milnesium cf. tardigradum* (Figure 54) and *Echiniscus* sp. (Figure 55). Gut analysis of *M. tardigradum* revealed the presence of bdelloid rotifers and even other tardigrades (*Diphascon* sp.). Sohlenius and Boström (2006) also noted predation by tardigrades on rotifers in East Antarctica.

On the **nunataks** (mountain peaks that penetrate the ice sheet) in continental Antarctica, distribution of tardigrades is patchy, with the greatest abundance occurring within moss cushions and guana from bird colonies (Swedish Museum of Natural History 2009). Nine tardigrade taxa have been identified in the Swedish studies.

Figure 54. *Milnesium tardigradum*, a cosmopolitan moss inhabitant. Photo by Yuuji Tsukii, with permission.

Figure 55. *Echiniscus*, a ubiquitous genus that occurs on mosses in the Antarctic. Photo by Martin Mach, with permission.

On Signy Island off the coast of Antarctica, Jennings (1979) found five species of tardigrades that occurred at both of the sampling sites: *Echiniscus capillatus*, *E. meridionalis*, *Hypsibius dujardini* (Figure 23), *Diphascon alpinum*, *Diphascon pingue* *sensu lato* (Figure 21; or may be *Diphascon polare*, *D. dastychi*, or *D. victoriae*), and *Macrobiotus furciger* (Figure 56). Other less common taxa were *Diphascon scoticum* (Figure 50), *Isohypsibius renaudi* (Figure 57), and *Isohypsibius asper* (Figure 58). Jennings conducted sampling for two years and found maximum populations of $309x10^3$ m^{-2} in moss communities of *Polytrichum strictum* - *Chorisodontium aciphyllum* (Figure 59-Figure 61). In the *Calliergidium austro-*

stramineum – *Calliergon sarmentosum* – *Sanionia uncinata* communities (Figure 12; Figure 62; Figure 63) they found a maximum of 71×10^3 m^{-2}. Reproductive potential is high, with increases of 3- to 4-fold in a single year. Hallas and Yeates (1972) found they could reach as high as 10- to 20-fold increases. *Echiniscus* increased 100-fold at one Signy Island site (Jennings 1979).

Figure 56. *Macrobiotus furciger*. Photo by Smithsonian Institution through EOL Creative Commons.
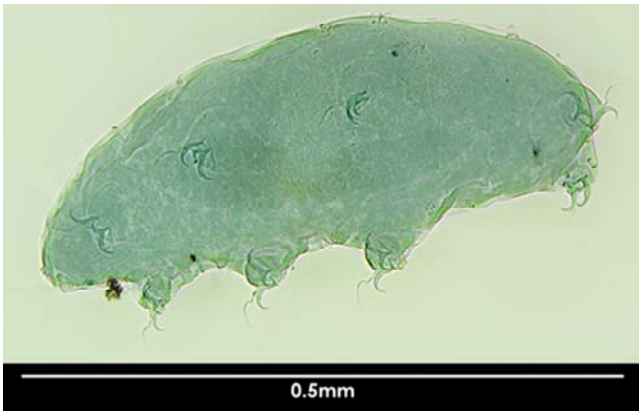

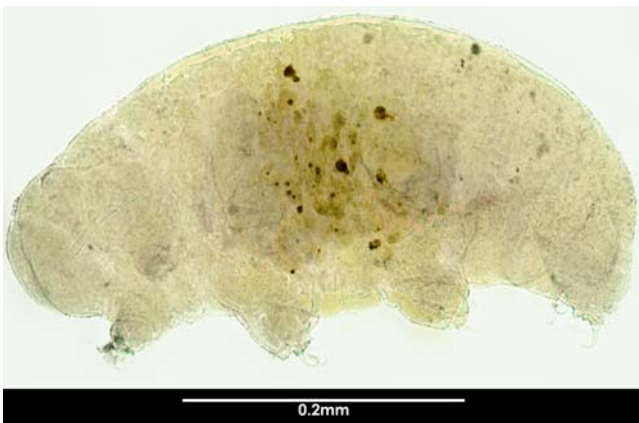Figure 57. *Isohypsibius renaudi*. Photo through EOL Creative Commons.

Figure 58. *Isohypsibius asper*. Photo by Smithsonian Institution through EOL Creative Commons.

In their Antarctic study, Utsugi and Ohyama (1989) found five species of tardigrades in 15 out of 31 samples from Ongul Island, Langhovde, Skarvsnes, Einstoingen, and Rundvagshetta, including algae, lichens, and mosses. *Hypsibius arcticus* (Figure 45) was common in all their samples. The other four species were rare.

Figure 59. *Polytrichum strictum* and *Chorisodontium aciphyllumn* in the Antarctic, where Jennings (1979) found 309×10^3 tardigrades per m^2. Photo by Tim Hooker, with permission.

In a different study on Wilkes Land, East Antarctica, Petz (1997) found tardigrades in more than 74% of the collections of fellfield mosses. These were the most abundant of the invertebrates, with 4,607 in just one gram of moss. Rotifers were the most abundant in other habitats. Ottesen and Meier (1990) likewise found that tardigrades were more abundant among mosses on South Georgia, compared to other habitats.
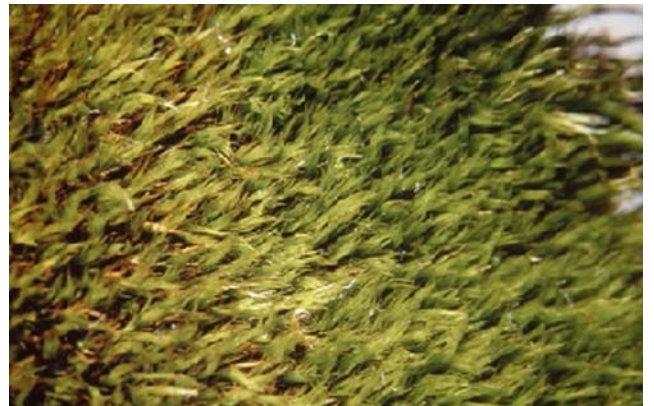

Figure 60. *Chorisodontium aciphyllumn* in the Antarctic. Photo by Tim Hooker, with permission.

Figure 61. *Polytrichum strictum*, a moss habitat in the Antarctic and other cool, wet areas. Photo by Michael Lüth, with permission.

Figure 62. *Calliergon sarmentosum*, of the *Calliergidium austro-stramineum* – *Calliergon sarmentosum* – *Sanionia uncinatus* association in the Antarctic. Photo by Michael Lüth, with permission.

Figure 63. *Sanionia uncinata*, a cosmopolitan moss that provides tardigrade habitat in the Antarctic. Photo by Jan-Peter Frahm, with permission.

Seasonal Variation

Densities may vary with seasons (Figure 66). *Hypsibius convergens* (Figure 18) exhibits temporal variation in pool and meadow moss habitats (Marcus 1929). In city mosses, the numbers of individuals of *Macrobiotus hufelandii* (Figure 15) and *Pseudechiniscus pseudoconifer* correlated with meteorological factors during a 3-month winter/early spring study (Franceschi *et al*. 1962-63). It appears that *Echiniscus* (Figure 55) and its segregate genera may commonly have seasonal variations. Jennings (1979) found that *Echiniscus* (possibly considered a segregate genus now) was the only tardigrade with seasonal variation among the eight species in his Signy Island study.

This is at least in part a reflection of changes in moisture. As already seen for *Diphascon rugosum* (Figure 22), *Hypsibius dujardini* (Figure 23), and *Hypsibius cf. convergens* (Figure 18), there were clear population peaks in winter in a carpet of the soil moss *Rhytidiadelphus squarrosus* (Figure 19) in the Black Forest, Germany (Schuster & Greven 2007). Species diversity and evenness was generally higher for the tardigrade communities in winter and least in summer (Figure 64). On the other hand, *Macrobiotus hufelandi* (Figure 15), *Diphascon pingue* (Figure 21), and to a lesser degree *Paramacrobiotus richtersi* (Figure 20), declined in winter, increasing in spring through fall (Figure 65). *Macrobiotus hufelandi* had its peaks in summer and lows in January (Schuster & Greven 2007), as shown for total tardigrades by Merrifield and Ingham (1998), but the other major species did not follow that pattern (Schuster & Greven 2007).
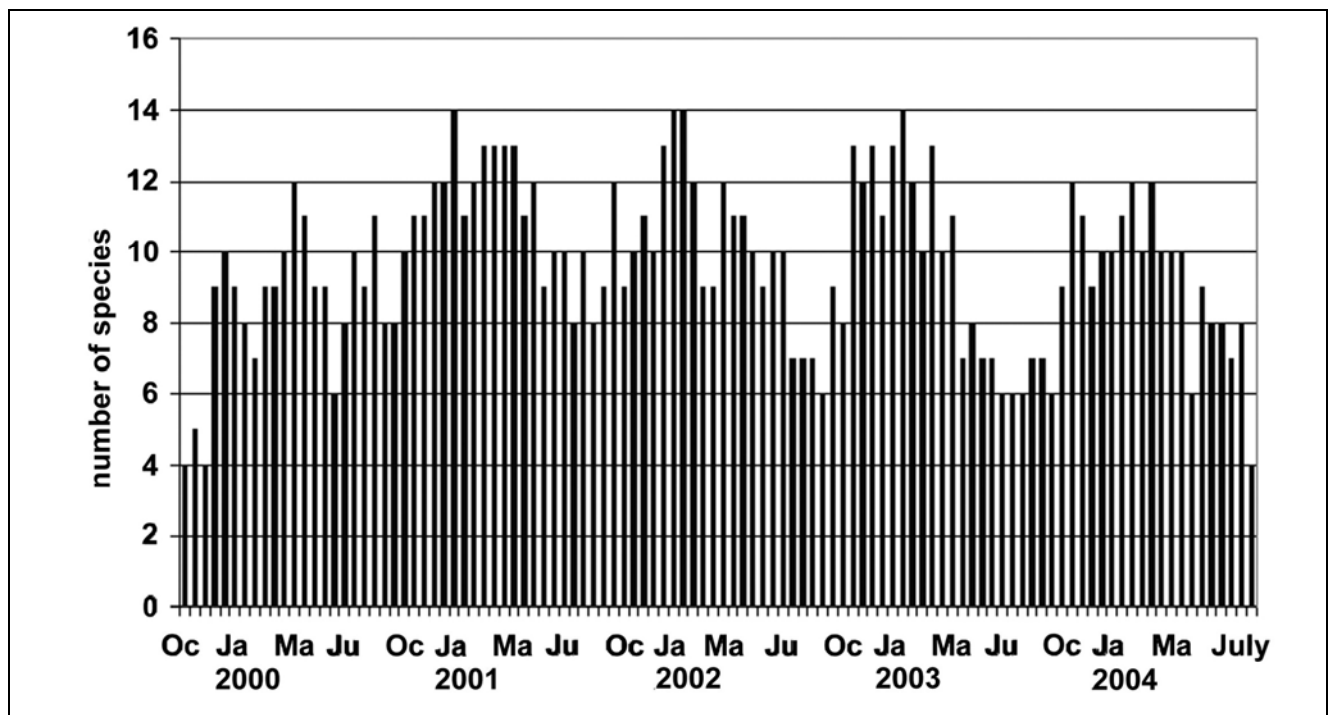

Figure 64. Seasonal changes in number of species of tardigrades found in *Rhytidiadelphus squarrosus* (Figure 19) clumps. (n = 108). Redrawn from Schuster & Greven 2007.
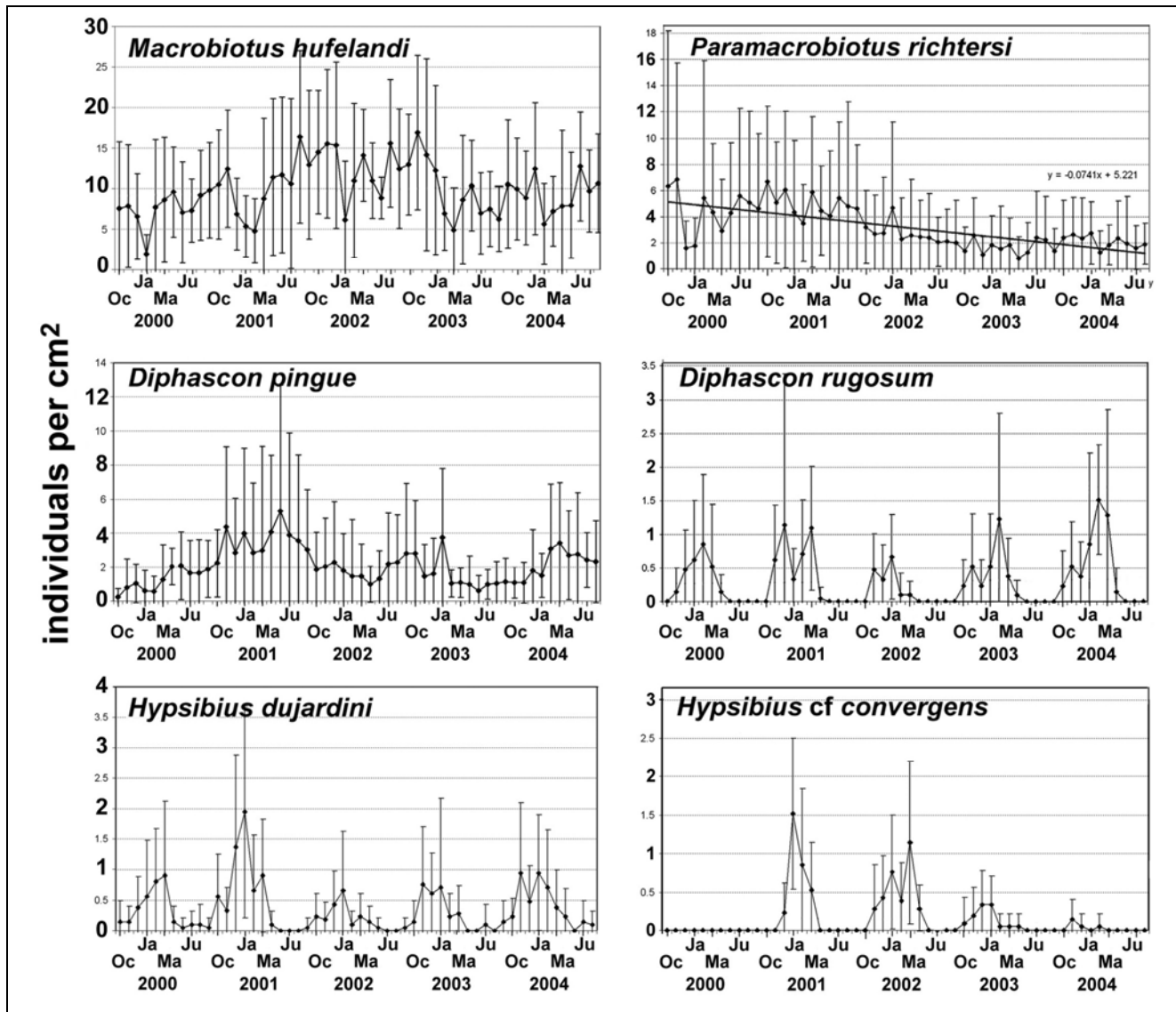
Figure 65.　Seasonal changes in number of individuals of the dominant tardigrades found in ***Rhytidiadelphus squarrosus*** (Figure 19)*. **Paramacrobiotus richtersi*** shows a trend of decline during the sampling years, as shown by the regression line. Modified from Schuster and Greven 2007.

Using a Baermann funnel (Merrifield & Ingham 1998), Merrifield (1992) reported 5 tardigrades per gram on ***Eurhynchium oreganum*** (Figure 67) in Oregon, USA, from April to August, with an increase to 15 in September and October, then a crash to 1 for winter months of November through March (Figure 66). Were the bears hibernating elsewhere, or were numbers crashing in the damp Oregon winter?
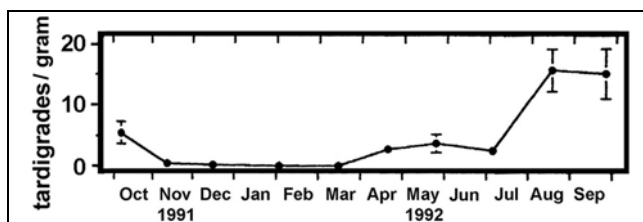

Figure 66.　Seasonal changes in numbers of tardigrades on mosses at Mary's Peak, Oregon, USA. Redrawn from Merrifield & Ingham 1998.

Figure 67.　***Eurhynchium oreganum***, a non-winter habitat for tardigrades. Photo from University of British Columbia bryophyte website, with permission.

Romano *et al*. (2001) attempted to determine the seasonal effects on tardigrades among mosses along Choccolocco Creek, Alabama, USA. They surveyed mosses on three trees each in six sites for 18 months and found no correlation between occurrence and season. However, they did find seasonal differences in the number of species and abundance when they pooled samples.

Patchiness

A number of studies suggest that the distribution of tardigrades within a given area or on a particular type of substrate is patchy. Degma *et al*. (2005) actually did both cluster analysis and CCA, demonstrating that most of the differences in species diversity were the result of randomly found species and that colonization of any given substrate is a random process. It would appear that the greatest determining factor in their specific location and species composition is their dispersal to that location, a process that is as random as it is for the mosses and liverworts they sit on. Further support for this randomness is their random distribution among populations of the moss *Hypnum cupressiforme* (Figure 68), supported by a Chi-square goodness of fit test.

Figure 68. *Hypnum cupressiforme*, a ubiquitous moss that seems suitable for many taxa of tardigrades. Photo by Michael Lüth, with permission.

Degma *et al*. (2009, 2011) found that the horizontal distribution of the tardigrades on a moss clump is aggregated, but that aggregation is not related to moisture in the moss cushion. They hypothesized that once a tardigrade arrives through random recruitment it is able to establish a micro-population. From that beginning slow radiation occurs. The result is that large substrates have more tardigrades but some parts of these larger patches will lack tardigrades while other parts will house aggregations. They continued their study (Degma *et al*. 2011) using *Hypnum cupressiforme* (Figure 68) with a 5x5 matrix of circular plots and determined that there was no significant moisture gradient along that moss slope. Nevertheless, the tardigrades existed in clumps or patches. With a large number of individuals (224) in seven species [*Milnesium tardigradum* (Figure 3), *Hypsibius convergens* (Figure 18), *H. microps*, *Diphascon pingue* (Figure 21), *Astatumen trinacriae* (Figure 36), *Macrobiotus hufelandi* (Figure 15), *Minibiotus* sp. (Figure 47)], they found that species number was random, but that species distribution

was aggregated. That aggregated distribution was NOT related to moisture in the moss mat. They concluded, therefore, that the best hypothesis to explain the patchy distribution of the tardigrades within the moss cushion was that recruitment of eggs and specimens on the moss was random and that these recruits subsequently reproduced, creating micro-populations where density gradually increased over time. This hypothesis makes the assumption that tardigrades migrate little from the location of their birth. Following this reasoning on a larger scale would account for the patchy distribution observed on larger moss clumps. Larger patches of mosses are more likely to be the recipients of dispersed tardigrades or their eggs and hence are more likely to have tardigrades than would small patches. This would also account for the high degree of variation encountered in random sampling from various moss cushions in the same location. While the individuals are aggregated, the aggregations are random.

Meyer (2006b) did a careful study on the spatial variability of tardigrade populations among moss patches on trees and rocks at three locations in the USA. He examined the fauna on patches ranging 0.1 to >5 cm^2. He found very high variation among the patches. One interesting discovery was that very small patches rarely had tardigrades. Could it be that they did not retain moisture long enough, or was it a matter of dispersal, with small patches having endured too short a time for colonization to be common?

Perhaps it is predictable that patchiness would characterize Antarctic moss dwellers. In the Antarctic, bryophytes, as well as lichens and algae, provide an important habitat for tardigrades, rotifers, and nematodes (Utsugi & Ohyama 1991; Sohlenius *et al*. 2004). Here tardigrades are also patchily distributed, nevertheless having the highest densities among these three groups of organisms. One might assume that bryophytes must arrive first, or that the tardigrades arrive with their bryophyte home. Hence, dispersal to the continent and its remote islands most likely plays a major role in their location.

Studies by the Swedish Museum of Natural History (2009) likewise found patchy distribution of tardigrades on the nunataks of the Antarctic. These windswept peaks emerge above the ice sheets and provide the substrate needed for bryophytes, lichens, and inhabiting tardigrades. Moss cushions and humus enriched by bird colonies provided the greatest numbers of tardigrades, with 400 samples yielding only nine tardigrade taxa. Nevertheless, 32% of the samples had tardigrades (Sohlenius & Boström 2006). The importance of the stochastic process of colonization is supported by the presence of different developmental stages in various samples, suggesting that dispersal may be a dynamic, albeit random, process occurring constantly on the windy peaks. Further population control may exist through competition with the co-occurring nematodes, whereas it appears that the poor rotifers serve as dinner for at least some of these tardigrades.

Bettis (2008) tested differences in tardigrade distribution on *Grimmia* (Figure 69) on exposed granitic outcrops vs protected seasonally riparian forms in California, USA. Again, the distribution was "very patchy" and did not support the hypothesis that more tardigrades would be on the more protected, more moist mosses.

Figure 69. *Grimmia laevigata* in Europe. Photo by Michael Lüth, with permission.

Both Meyer (2006b) (in the terrestrial system) and Romano *et al*. (2001) (in the aquatic system), emphasized the importance of accounting for this patchiness in designing a sampling strategy. Meyer suggested that the variability of a given location should be understood before determining the number of samples to take. Romano emphasized the need for a greater sampling effort.

In short, it appears that the major factor accounting for tardigrade distribution and patchiness is dispersal. If the tardigrade lands in an appropriate habitat, it is able to withstand considerable environmental variation there, and the habitat itself seems to offer little to discriminate against any tardigrade species. Rather, factors like reproductive potential may play the greater role in determining the abundance, and possibly even the diversity, once the tardigrades arrive.

Dispersal plays a large role in both geographic distribution and local patchiness. Within the cushions the tardigrades are often aggregated, but there appears to be no relationship with moisture. On the other hand, small patches seem to lack tardigrades, suggesting that moisture is important. But arrival is a major factor, and from that arrival of one tardigrade, a population develops. Since their movement is slow, they accumulate. But small patches of mosses indicate a short time in which arrival could have occurred.

Acknowledgments

Literature Cited

Bartels, P. J. and Nelson, D. R. 2007. An evaluation of species richness estimators for tardigrades of the Great Smoky Mountains National Park, Tennessee and North Carolina, USA. Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 104-110.

Bartos, E. 1963. Die Tardigraden der chinesischen und javanischen Mossproben. Vìst. Èsl. Spol. Zool., Acta Soc. Zool. Bohemoslov. 27: 108-114.

Beasley, C. W. and Miller, W. R. 2007. Tardigrada of Xinjiang Uygur Autonomous Region, China. Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(suppl. 1): 49-55.

Beasley, C. W., Kaczmarek, Ł. and Michalczyk, Ł. 2006. New records of tardigrades from China, with zoogeographical remarks. Biol. Lett. 2006, 43: 13-20.

Bertolani, R. 1983. Tardigardi muscicoli delle dune costiere Italiane, con descrizione di una nuova specie. Atti Soc. Tosc. Sci. Nat. Mem. Ser. B 90: 139-148.

Bettis, C. J. 2008. Distribution and abundance of the fauna living in two *Grimmia* moss morphotypes at the McKenzie Table Mountain Preserve, Fresno County, California. M.S. Thesis, California State University, Fresno, CA, 65 pp.

Binda, M. G. 1984. Notizie sui tardigradi dell'Africa Meridionale con descrizione di una nuova specie di *Apodibius* (Eutardigrada). [Remarks on some species of tardigrades from Southern Africa and description of *Apodibius nuntius* n. sp.]. Animalia 11(1-3): 5-15.

Summary

Tardigrades can range in numbers from none to 22,000 individuals per gram of dry moss. More than 2 million are known from a single square meter. On Vancouver Island in Canada, 39 species have been found among bryophytes. They do not seem to prefer any particular moss, and they often occur equally as frequently on lichens and liverworts as they do on mosses. In New Zealand, 30 species are known from liverworts.

Macrobiotus and *Echiniscus* (and their more recent segregates) are among the most abundant tardigrades in the bryophyte fauna. Although most invertebrates decrease in numbers toward the poles, tardigrades actually increase. However, their numbers are highly variable from one place to another. Here, even more so than elsewhere, distribution of the tardigrades is patchy. Even adjacent trees in some localities are known to differ greatly in their tardigrade fauna. Yet, on Wilkes Land in the Antarctic, 74% of the fellfield mosses had tardigrades.

They are known to increase up to 100-fold, but it appears that 3- to 4-fold is more typical. Their abundance can be seasonal, with some peaking in winter and others in summer or spring/fall. Some respond to the rainy season. Others don't seem to respond to season.

Briones, M. J. I., Ineson, P., and Piearce, T. G. 1997. Effects of climate change on soil fauna; responses of Enchytraeids, Diptera larvae and tardigrades in a transplant experiment. Appl. Soil Ecol. 6: 117-134.

Brusca, R. C. and Brusca, G. J. 1990. Invertebrates. Sinauer Associates, Sunderland, Massachusetts.

Claps, M., Rossi, G., and Rocha, A. 2009. Tardigrade fauna from two biogeographic regions of Patagonia (Río Negro province, Argentina). In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009.

Degma, P. 2003. First records of six Hypsibiidae species (Tardigrada, Eutardigrada) in Slovakia. Biologia, Bratislava 58: 1003-1005.

Degma, P. 2006. First records of two Heterotardigrada (Tardigrada) species in Slovakia. Biologia, Bratislava 61: 501-502.

Degma, P. and Pecalková, M. 2003. First records of Tardigrada in Slovakia from Stuzica National Nature Reserve (Bukovské vrchy Mts, NE Slovakia). Biologia, Bratislava 58: 274, 286.

Degma, P., Bertolani, R., and Guidetti, R. 2010. Actual checklist of Tardigrada species (Ver. 10: 15-12-2009). Accessed on 23 January 2010 at <http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf>.

Degma, P., Gulánová, S., and Simurka, M. 2004. First records of three Hypsibiidae species (Tardigrada: Eutardigrada) in Slovakia. Biologia, Bratislava 59(Suppl 15): 29-11.

Degma, P., Katina, S., and Sabatovicová, L. 2009. Horizontal distribution of moisture and Tardigrada in a single moss cushion. In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009.

Degma, P., Simurka, M., and Gulánová, S. 2005. Community structure and ecological macrodistribution of moss-dwelling water bears (Tardigrada) in central European oak-hornbeam forests (SW Slovakia). Ekológia (Bratislava) 24(Suppl 2): 59-75.

Degma, P., Stanislav, K., and Sabatovicova, L. 2011. Horizontal distribution of moisture and Tardigrada in a single moss cushion. J. Zool. Syst. Evol. Res. 49 (Suppl 1): 71–77.

Felföldy, L. and Iharos, A. 1947. Relation between moss-association and Tardigrada-fauna on the northern shores of the Tihany Peninsula. Borbasia 7(1-6): 31-38.

Franceschi, T., Loi, M. L., and Pierantoni, R. 1962-1963. Risultati di una prima indagine ecologica condotta su popolazioni di Tardigradi. Bollettino dei Musei e degli Istituti Biologici dell'Universita di Genova 32: 69-93.

Hallas, T. E. and Yeates, G. W. 1972. Tardigrada of the soil and litter of a Danish beech forest. Pedobiologia 12: 287-304.

Hofmann, I. and Eichelberg, D. 1987. Faunistisch-oekologische Untersuchungen zur Habitat-praeferenz moosbewohnender Tardigraden. [Ecological investigations of the habitat preference of moss-inhabiting tardigrades.]. Zool. Beitr. 31(1): 61-76.

Horikawa, D. D. 2008. The tardigrade *Ramazzottius varieornatus* as a model animal for astrobiological studies. Biological Sciences in Space 22: 93-98.

Horikawa, D. D. and Higashi, S. 2004. Desiccation tolerance of the tardigrade *Milnesium tardigradum* collected in Sapporo, Japan, and Bogor, Indonesia. Zool. Sci. 21: 813-816.

Horning, D. S., Schuster, R. O., and Grigarick, A. A. 1978. Tardigrada of New Zealand. N. Zeal. J. Zool. 5: 185-280.

Iharos, G. 1966. Neue tardigraden-arten aus Ungarn (Neuere beiträge zur kenntnis der tardigraden-fauna Ungarns, VI.). Acta Zool. Acad. Scien. Hung. 12: 111–122.

Ito, M. 1999. Ecological distribution, abundance and habitat preference of terrestrial tardigrades in various forests on the northern slope of Mt. Fuji, central Japan. Zool. Anz. 238: 225-234.

Jennings, P. G. 1979. The Signy Island terrestrial reference sites: X. Population dynamics of Tardigrada and Rotifera. Brit. Antarct Surv. Bull. 47: 89-105.

Jerez Jaimes, J. H., Narváez, E. X., and Restrepo, R. 2002. Tardígrados en musgos de la Reserva el Diviso (Santander, Colombia). Revista Colombiana de Entomología 28(2): 199-206.

Jönsson, K. I. 2003. Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography 26: 356-364.

Kaczmarek, Ł., Michalczyk, Ł., Gołdyn, B., and Welnicz, W. 2009. Ecological factors determining distribution of Tardigrada in Costa Rican rain forests. In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009, p. 49.

Kaczmarek, Ł., Gołdyn, B., Wełnicz, W., and Michalczyk, Ł. 2011. Ecological factors determining Tardigrada distribution in Costa Rica. J. Zool. Syst. Evol. Res. 49 (Suppl 1): 78-83.

Kathman, R. D. and Cross, S. F. 1991. Ecological distribution of moss-dwelling tardigrades on Vancouver Island, British Columbia, Canada. Can. J. Zool. 69: 122-129.

Kinchin, I. M. 1994. The Biology of Tardigrades. Blackwell Publishing Co., London. 186 pp.

Kristensen, R. M., Michalczyk, Ł., and Kaczmarek, Ł. 2009. Tardigrades from Easter Island, Rapa Nui (Pacific Ocean, Chile) with the description of a new species of *Bryodelphax* (Heterotardigrada: Echiniscidae). In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009.

Marcus, E. 1928a. Spinnentiere oder Arachnoidea IV: Bärtierchen (Tardigrada). Springer, Jena.

Marcus, E. 1928b. Zur Ökologie und Physiologie der Tardigraden. Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere 44: 323-370.

Marcus, E. 1929. Tardigrada. In: Bronn, H. G. (ed.). Klassen und Ordnungen des Tierreichs. Section 4, part 3, 5. Akademische Verlagsgesellschaft, pp. 1-608.

Mathews, G. B. 1936/1937. Tardigrada from Japan. Pek. Nat. Hist. Bull. 2: 411-412.

Mathews, G. B. 1937a. The Tardigrada or water bears. China J. 26: 97-105.

Mathews, G. B. 1937b. More tardigrades from the Far East. China J. 27: 32-35.

Mathews, G. B. 1938. Tardigrada of North America. Amer. Midl. Nat. 19: 619-627.

Maucci, W. 1980. Analisi preliminare di alcuni dati statistici sulla ecologia dei tardigradi muscicoli. [Some statistical data about ecology of moss-dwelling Tardigrada.]. Boll. Mus. Civ. Stor. Nat. Verona 7: 1-47.

McInnes, S. J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. J. Nat. Hist. 28: 257-352.

McInnes, S. J., Chown, S. L., Dartnall, H. J. G., and Pugh, P. J. A. 2001. *Milnesium* cfr. *tardigradum* (Milnesiidae, Apochela, Tardigrada): A monitor of high altitude meiofauna on sub-Antarctic Marion Island. Zool. Anz. 240: 461-465.

Merrifield, K. 1992. Population dynamics of forest floor moss-dwelling nematodes and tardigrades. J. Nematol. 24: 607.

Merrifield, K. and Ingham, R. E. 1998. Nematodes and other aquatic invertebrates in *Eurhynchium oreganum* (Sull.) Jaeg., from Mary's Peak, Oregon Coast Range. Bryologist 101: 505-511.

Meyer, H. A. 2006a. Interspecific association and substrate specificity in tardigrades from Florida, southeastern United States. Hydrobiologia 558: 129-132.

Meyer, H. A . 2006b. Small-scale spatial distribution variability in terrestrial tardigrade populations. Hydrobiologia 558: 133-139.

Meyer, H. A. and Hinton, J. G. 2009. The Tardigrada of Southern Africa, with the description of *Minibiotus harrylewisi*, a new species from KwaZulu-Natal, South Africa (Eutardigrada: Macrobiotidae). African Invertebrates 50: 255-268.

Meyer, H. A., Hinton, J. G., and Trahan, K. 2003. Spatial variability of tardigrade populations in leaf litter, moss, and lichens. Abstracts of the 88th meeting of the Ecological Society of America, Savannah, GA, 3-8 August 2003. Accessed on 3 July 2004, at <http://abstracts.co.allenpress.com/pweb/esa2003/document/?ID=26552>.

Meyer, H. A., Lyons, A. M., Nelson, D. R., and Hinton, J. G. 2011. Tardigrada of Michigan, Northern USA, with the description of *Minibiotus jonesorum* sp. n. (Eutardigrada: Macrobiotidae). J. Zool. Syst. Evol. Res. 49 (Suppl. 1): 40-47.

Mitra, B. 1999. Studies on moss inhabiting invertebrate fauna of Schirmacher Oasis. Fifteenth Indian Expedition to Antarctica, Scientific Report, 1999, Department of Ocean Development, Technical Publication No. 13: 93-108.

Morgan, C. I. 1974. Studies on the Biology of Tardigrades. Unpublished Ph. D. thesis, University College, Swansea. 220 pp. 2287x103 m-2 in Welsh mosses

Morgan, C. I. 1976. Studies on the British tardigrade fauna. Some zoogeographical and ecological notes. J. Nat. Hist. 10: 607-632.

Morgan, C. I. 1977. Population dynamics of two species of Tardigrada, *Macrobiotus hufelandii* (Schultze) and *Echiniscus* (*Echiniscus*) *testudo* (Doyère), in roof moss from Swansea. J. Anim. Ecol. 46: 263-279.

Nelson, D. R. 2002. Current status of the Tardigrada: Evolution and ecology. Integrat. Compar. Biol. 42: 652-659.

Nelson, J. and Hauser, D. 2012. A survey of invertebrate communities on epiphytic mosses and liverworts in Tryon Creek State Natural Area. Unpublished Undergraduate Report, Lewis & Clark College, Portland, OR.

Ono, F., Sigusa, M., Uozumi, T., Matsushima, Y., Ikeda, H., Sainic, N. L., and Yamashita, M. 2008. Effect of high hydrostatic pressure on to life of the tiny animal tardigrade. J. Phys. Chem. Solids 69: 2297-2300.

Ottesen, P. S. and Meier, T. 1990. Tardigrada from the Husvik area, South Georgia, sub-Antarctic. Polar Res. 8: 291-294.

Peters, T. and Dumjahn, P. 1999. Ecological aspects of tardigrade distribution on Disko Island, West Greenland. In: Brandt, A., Thomsen, H. A., Heide-Jørgensen, H., Kristensen, R. M., and Ruhberg, H. (eds.). The 1998 Danish-German Excursion to Disko Island, West Greenland. Ber. Polarforsch. 330: 64-75.

Petz, W. 1997. Ecology of the active soil microfauna (Protozoa, Metazoa) of Wilkes Land, East Antarctica. Polar Biol. 18: 33-44.

Pilato, G. 1974. Tre nuove specie di Tardigradi muscicoli di Cina. [Three new species of Tardigrada from mosses in China.]. Animalia 1(1-3): 59-68.

Pilato, G. and Pennisi, G. 1976. Prime notizie sui Tardigradi di Cirenaica. [First account on Tardigrada of Cyrenaica.]. Animalia 3(1-3): 243-258.

Pilato, G. and Sperlinga, G. 1975. Tardigradi muscicoli di Sardegna. [Moss-inhabiting Tardigrada from Sardinia.]. Animalia 2(1-3): 79-90.

Ramazzotti, G. and Maucci, W. 1983. II. Phylum Tardigrada. 3rd ed. Mem. 1st. Ital. Idrobiol. Dott. Marco di Marchi, Vol. 41.

Riggin, G. T. Jr. 1962. Tardigrada of Southwest Virginia: With the Addition of a Description of a New Marine Species from Florida. Technical Bull. 152, Virginia Agricultural Experiment Station, Blacksburg, VA. 145 pp.

Romano, F. A. III., Barreras-Borrero, B., and Nelson, D. R. 2001. Ecological distribution and community analysis of Tardigrada from Choccolocco Creek, Alabama. Zool. Anzeiger 240: 535-541.

Schuster, R. and Greven, H. 2007. A long-term study of population dynamics of tardigrades in the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst. J. Limnol, 66(Suppl. 1): 141-151.

Sohlenius, B. and Boström, S. 2006. Patch-dynamics and population structure of nematodes and tardigrades on Antarctic nunataks. Eur. J. Soil Biol. 42(suppl. 1): S321-S325.

Sohlenius, B., Boström, S., and Jönsson, K. I. 2004. Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. Pedobiologia 48: 395-408.

Steiner, W. 1994a. The influence of air pollution on moss-dwelling animals: 1. Methodology and composition of flora and fauna. Rev. Suisse Zool. 101: 533-556.

Steiner, W. A. 1994b. The influence of air pollution on moss-dwelling animals: 2. Aquatic fauna with emphasis on Nematoda and Tardigrada. Rev. Suisse Zool. 101: 699-724.

Steiner, W. A. 1994c. The influence of air pollution on moss-dwelling animals: 4. Seasonal and long-term fluctuations of rotifer, nematode and tardigrade populations. Rev. Suisse Zool. 101: 1017-1031.

Steiner, W. A. 1995. The influence of air pollution on moss - dwelling animals: 5. Fumigation experiments with SO_2 and exposure experiments. Rev Suisse Zool. 102: 13-40.

Swedish Museum of Natural History. 2009. Microfauna on Antarctic nunataks. Accessed on 14 February 2010 at <http://www.nrm.se/en/menu/researchandcollections/departments/invertebratezoology/research/researchprojects/nematodaandtardigrada/microfaunaonantarcticnunataks.5892_en.html>.

Utsugi, K. and Ohyama, Y. 1989. Antarctic Tardigrada. Proc. NIPR Symp. Polar Biol. 2: 190-197.

Utsugi, K. and Ohyama, Y. 1991. Tardigrades in King George Island (Antarctica). Zool. Sci. 8: 1198.

Yang, T. 2002. The tardigrades from some mosses of Lijiang County in Yunnan Province (Heterotardigrada: Echiniscidae; Eutardigrada: Parachela: Macrobiotidae, Hypsibiidae). A. Zootaxon. Sin. 27: 53-64.

CHAPTER 5-6
TARDIGRADE ECOLOGY

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CHAPTER 5-6
TARDIGRADE ECOLOGY

Figure 1. ***Echiniscus***, the genus with the most species among mosses.   Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Dispersal

As already discussed, one suggested reason for patchy distribution of tardigrades is the difficulty of dispersal for this small organism. Miller *et al*. (1994) concluded that tardigrade distribution in the Antarctic is influenced more strongly by dispersal limitations than it is by climate or habitat interactions. McInnes and Convey (2005) found a low species richness of tardigrades (6 taxa) in the South Sandwich Islands in the sub-Antarctic. They found indications that the tardigrades originated from both sub-Antarctic and maritime Antarctic populations.

Wind dispersal is considered the major means by which tardigrades move to new locations (Christenberry & Higgins 1979; Bertolani *et al*. 2009). The anhydrobiotic state is very light weight and can easily survive the various dangers of space (see below).

Faurby *et al*. (2008) suggested dispersal rate may be coupled with survival in the anhydrobiotic state. Based on these assumptions, Bromley (2009) has considered the possibilities that habitats such as rooftops with mosses serve as islands for tardigrades.

Sudzuki (1972) experimented with wind dispersal to moss mats and found that smaller micro-organisms (<10x30x50 µm) were easily blown from their location to reach the mosses 100-500 cm away. Tardigrades, on the other hand, almost never reached the mosses during two months of experimentation with the fan-generated wind operating alternate days.

Although wind is probably the most common means, other means of dispersal may be afforded by water, insects, and other invertebrates. Resting eggs offer another dispersible propagule. Eggs of tardigrades are about the same size as a pollen grain and may similarly be dispersed by wind (Ramazzotti 1972). Many species have eggs with decorations on them (Figure 2) reminiscent of allergenic pollen grains, *i.e.*, those adapted for wind pollination.
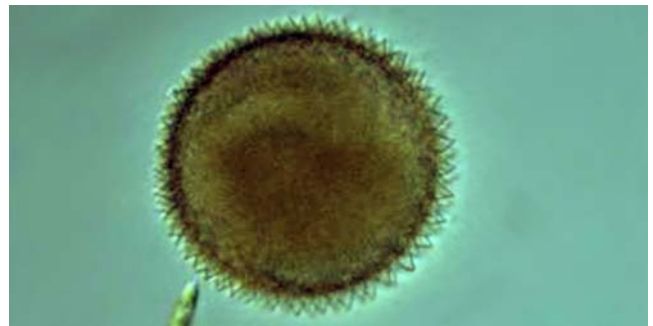

Figure 2. Egg of ***Dactylobiotus*** sp. showing decorated wall similar to that of a pollen grain. Photo by Yuuji Tsukii, with permission.

Whereas tardigrades don't seem to be dispersed very easily by themselves, their dispersal as hitch-hikers on bryophytes is a more feasible means (Janiec 1996). Not only live animals, but also tuns, cysts, and eggs can be dispersed when their bryological home is dispersed. Storms, animals, wind, and water are all means by which the substrate and fauna could travel together.

Peninsula Effect

The **peninsula effect** postulates that the number of species will decrease as one approaches the tip of a peninsula. This presumably is the case because of the lack of opportunity for invasion of new species from the more seaward positions. Simpson (1964) suggested that there is a peninsular effect in animal distribution similar to that seen in island biogeography. Jenkins and Rinne (2008) defined the peninsula effect as "the prediction that the number of species declines from a peninsula's base to its tip." They considered whether this concept might be a "red herring" and expressed concern that most studies had not controlled for other possible explanations or from unequal sampling effort. In the Florida, USA, peninsula, they found that 82.5% of variation in freshwater microcrustacean species richness was attributed to habitat and sampling effort, and there was no peninsular effect. In fact, they suggest that the evidence previously published only supported the concept for mammals.

Taylor and Regal (1978) tested the concept of extinction and recolonization, a tenet of the peninsular effect, for rodents in Baja California and concluded that for an effective peninsular effect the habitable sites "are few and widely spaced." Brown (1987) examined the effect on butterflies (Lepidoptera) in the Baja peninsula and determined that there was no peninsular effect for that group. Likewise, Busack and Hedges (1984) found no peninsular effect for lizards and snakes on the Baja peninsula.

Taylor and Regal (1978) suggest that changes in vegetation in Florida that are due to rising water levels and climate change may account for the decline in species richness southward on that peninsula. They argue that other large peninsulas where Simpson demonstrated a peninsular effect represent major topographic changes or harsh climate gradients that could account for changes in species number.

Nevertheless, Meyer (2008) found a peninsular effect in tardigrades when bryophyte and lichen epiphytes in all 67 Florida counties were sampled, with species richness diminishing from north to south. If we combine the information gained from the above studies, it appears that Taylor and Regal (1978) may have been correct in suggesting that the peninsular effect required few and widely spaced habitable sites. And widely spaced for one species may be a lazy day's walk for another. To put this into the perspective of bryophyte-dwelling tardigrades, isolation and difficulty of dispersal are typical, *i.e.*, widely spaced habitable sites (see dispersal discussion above), and would make tardigrades more likely to have the extinction-recolonization limits required for the peninsular shape to have a "peninsular effect." The apparent need for wind dispersal of tardigrades is further complicated by their residence within the bryophyte mat, and it seems they would most likely be dispersed only when they and their bryophyte substrate are dry and fragments to which they adhere become airborne. This dispersal limitation has been discussed earlier (Chapter 5-5 of this volume) in relation to the patchiness of populations within a given small area.

There is a possible caveat to this discussion. The definition of a peninsular effect seems to have drifted from that of Simpson (1964). He considered peninsulas to have fewer species (lower richness) than continental areas and did not treat them as having fewer species toward the tip. However, he did consider that spread of species could occur in one direction only (*i.e.*, no new mammal species were likely to arrive from the tip in the short term). He suggested that this would make them more liable to local extinctions (*i.e.*, reducing the density of species richness).

Distribution

Tardigrades, like the protozoa, have many taxa with worldwide distributions (McInnes 1994; McInnes & Pugh 1998). With so little attention paid to this group, relative to that for the protozoa, it is difficult to draw distributional conclusions. There are hints of the "**everything is everywhere**" principle (in Wit & Bouvier 2006) for these small, lightweight creatures. (See Chapter 2-6 on Protozoa Ecology.) Their ability to become anhydrobiotic for long periods of time increases their chances for successful dispersal over great distances.

Among the moss dwellers, we find that *Doryphoribius flavus* has been found in Europe, Asia, Africa, and Central America (McInnes 1994). It is now known from Tennessee, USA (Bartels *et al*. 2007). Its original discovery was in moss subjected to desiccation (Iharos 1966). On the other hand, *Doryphoribius polynettae* had been found only in Russia in a small lake and in mosses [*Sphagnum* sp. (Figure 3), *Polytrichum juniperinum* (Figure 4)] near the lake, but was likewise recently found in Tennessee (Bartels *et al*. 2007).

Figure 3. *Sphagnum warnstorfii*. Photo by Michael Lüth, with permission.

Meyer (2008) found *Minibiotus fallax*, previously known only from Australia (Pilato *et al*. 1989), in moss and lichen collections from Florida, USA. Other surprising disjunctions are likely to appear because the group is poorly known and sampling is spotty.

Jørgensen *et al*. (2007) explored the microspecies concept in *Echiniscus testudo* (Figure 5), using DNA sequences. Their study included 13 localities on three continents. They found high haplotype diversity and low sequence diversity, suggesting that this species had

haplotype evolution with distinct asexual lineages and a high dispersal, as suggested by lack of isolation by distance. Although there were geographic differences, there was low genetic diversity. *Echiniscus* (Figure 6) is the largest genus, and the authors suggest that the high number of species may result from the combination of large potential for dispersal coupled with the lack of need of a partner for reproduction. Bryologists might benefit from understanding the distribution of these organisms as bryophytes and tardigrades seem to have similar dispersal advantages and disadvantages and both are capable of asexual reproduction.

Although it does not appear that *E. testudo* (Figure 5) has a large number of microspecies, it is likely that some of the more common species may actually be species complexes, further complicating our understanding of species distributions and diversity. These include taxa such as *Macrobiotus hufelandi* (Figure 7), *Minibiotus intermedius* (Figure 8), *Diphascon scoticum* (Figure 9), and *Milnesium tardigradum* (Figure 10) (Bertolani & Rebecchi 1993; Claxton 1998, 1999; Pilato 1987).

Figure 4. *Polytrichum juniperinum*. Photo by Michael Lüth, with permission.

Figure 5. *Echiniscus testudo* tun. Photo by Power & Syred through Creative Commons.

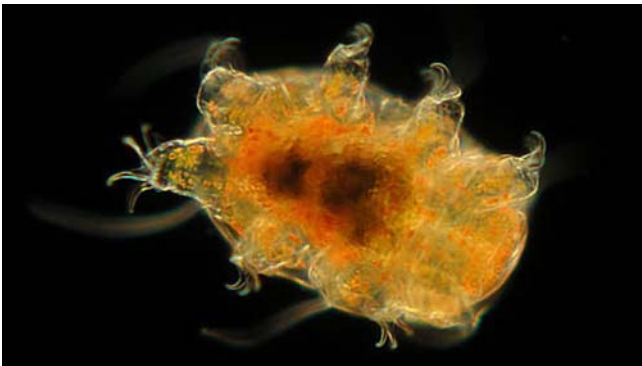Figure 6. *Echiniscus*, the largest tardigrade genus and a common bryophyte inhabitant. Photo by Martin Mach, with permission.

Figure 7. *Macrobiotus hufelandi*. Photo by Martin Mach, with permission.

Figure 8. SEM of *Minibiotus intermedius*. Photo by William Miller through Flickr Commons.

Figure 9. *Diphascon scoticum*. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Figure 10. *Milnesium tardigradum*. Photo by Björn Sohlenius at Swedish Museum of Natural History, with permission.

Blaxter *et al*. (2004) examined the Scottish tardigrade fauna for genetic differences. They found that some **Molecular Operational Taxonomic Units** (**MOTU**) were shared between the two rural collections in the study, but that none were found in both the one urban and two rural sites. This lack of commonality conflicts with the generally accepted concept of ubiquity of this faunal group. They described the high variabiliy among the specimens as representing "**taxon flocks**." It suggests to me that once arriving at a new site, the tardigrades are likely to reproduce asexually and eventually diverge from their ancestors, creating **cryptic** species. **Genetic drift**, **Founder Principle**, and **microselection** factors could all contribute to enhancing this noted variability. I suspect we will find many similar examples among bryophytes.

Such possibilities of genetic variation without obvious morphological indicators demonstrate the folly of attempting to present a reasonable distribution pattern for the moss-dwelling tardigrades at this stage. Look for them. You will probably find a new species, or at least a new cryptic species, and certainly expand our understanding of their distributions and their ecology.

Distribution is the product of many factors, including dispersal, climate, niches available, microclimate, and attending factors such as altitude, rainfall, temperature range, and severity and length of winter or summer. Guil *et al*. (2009) sampled mosses and other vegetation to determine the effects of micro- and macro-environmental factors on the distribution of tardigrades in those habitats in the central Iberian mountain areas of Spain. They found that bioclimatic classification was the best predictor for species richness. Altitude had a relationship, but its effects could not be separated from the macro-environmental gradients of soil, climate, vegetation type, and litter type. Within the micro-scale plots, the micro- and macro-environmental variables could explain ca 60% of the species richness, particularly litter type and vegetation type. On the other hand, abundance was not explained by the macro-environment, but rather was explained by soil composition and litter type. I have to wonder if soil composition affected the availability of bryophytes and their growth forms.

Common Species

Among the many collections of bryophytes, species of *Echiniscus* (Figure 11)*, Hypsibius* (Figure 12)*,

Macrobiotus (and segregate genera; Figure 13)*, Milnesium* (Figure 10), and *Ramazzottius* (Figure 14) seem particularly common.

Table 1 lists some of the taxa known from the literature, but many more exist and would be an endless task to include here. A complete list of all tardigrade names with updated nomenclature (used here) is in Degma *et al.* 2010.

Figure 11. *Echiniscus* sp., member of one of the most common genera among bryophytes. Photo by Martin Mach, with permission.

Figure 12. *Hypsibius dujardini*, member of one of the most common moss-dwelling genera of tardigrades. Photo by Bob Goldstein, with permission.
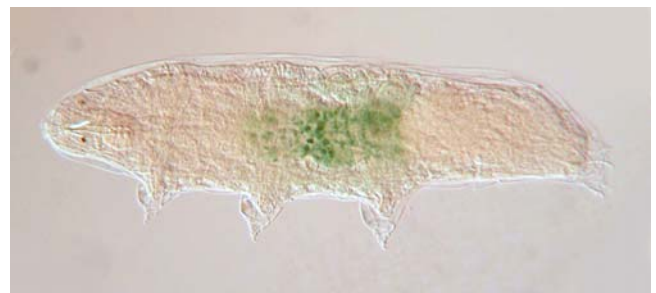

Figure 13. *Macrobiotus blocki*, an Antarctic member of one of the most common moss-dwelling genera. Photo by Martin Mach, with permission.

In Sweden, Jönsson (2003) found that **Macrobiotus hufelandi** (Figure 7) is far more common among mosses than are other tardigrade species. This species seems to be abundant in most bryophyte studies. It seems remarkable that as late as 2003, the common bryophyte dwellers **Murrayon dianae**, **Isohypsibius sattleri**, **Platicrista angustata** (Figure 18), **Diphascon belgicae**, and **Diphascon pingue** (Figure 15) were recorded from Sweden for the first time (Jönsson 2003).
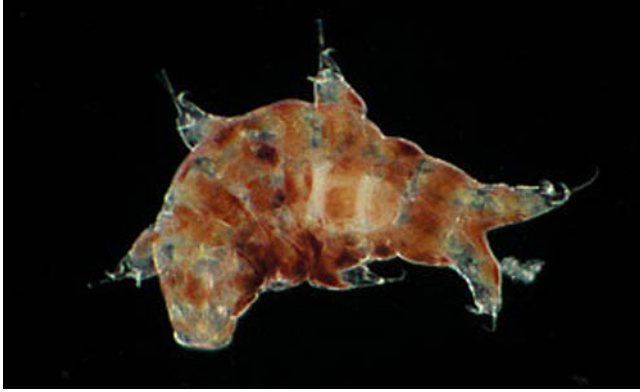

Figure 14. **Ramazzottius** sp., member of a common moss-dwelling genus. Photo by Martin Mach, with permission.

Figure 15. **Diphascon pingue**. Photo by Michael Collins, with permission.

A major problem in trying to define tardigrade distributions and habitats is the lack of sufficient study of this entire group of organisms. Guil and Cabrero-Sañudo (2000) stated that the "tardigrade species description process fails to show an asymptotic tendency." Meyer and Hinton (2007) reported that "30% of the Nearctic species have been reported from a single site!" In other words, it will be a long time before we even know most of the species in existence or even make reasonable estimates of how many species there are. And ecological descriptions necessarily lag behind the descriptions of the species.

Beasley *et al.* (2006) lamented the difficulty of assessing diversity of tardigrades in China because of the limited data available. In their report on 18 tardigrades of Sichuan, Yunnan, and Xizang Provinces, eight were new records for China. Among the 86 species known from China, 82 are terrestrial. Among these, **Echiniscus nepalensis**, **Pseudechiniscus jiroveci**, **Murrayon hibernicus**, **Hypsibius pallidus**, **Isohypsibius sattleri**, **Doryphoribius flavus**, **Diphascon pingue** (Figure 15), **D. scoticum** (Figure 9), **D. prorsirostre** (Figure 16), **Mesocrista spitsbergensis** (Figure 17), **Platicrista angustata** (Figure 18), **Milnesium tardigradum** (Figure 10) were from mosses. Only **Cornechiniscus lobatus** (see Figure 19) was found on liverworts.
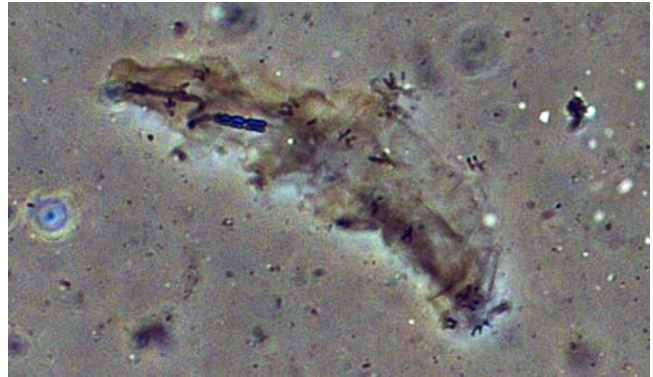

Figure 16. **Diphascon prorsirostre**. Photo by Michael Collins, with permission.

Figure 17. **Mesocrista spitsbergensis**. Photo by Björn Sohlenius, Swedish Museum of Natural History, with permission.

Figure 18. **Platicrista angustata**. Photo by Michael Collins, with permission.

Figure 19. **Cornechiniscus cornutus**. Photo by Martin Mach, with permission.

Communities

Séméria (1982) found that a typical bryophyte-dwelling (and lichen-dwelling) tardigrade had one predator and several detritivorous or herbivorous species in its community. Typical communities of bryophyte-dwellers have 2-6 tardigrade species, and sometimes even more than ten (Ramazzotti & Maucci 1983).

Specific bryophyte communities have rarely been identified, but Miller *et al*. (1996) identified significant positive associations between the three most common of the tardigrade species and certain bryophyte species in the Antarctic. On the other hand, these same three species had a strong negative association with algae and lichens. In Britain, Wright (1991) suggested that ***Milnesium tardigradum*** (Figure 10) might have a selective predatory association with ***Hypsibius dujardini*** (Figure 12) and ***Ramazzottius oberhaeuseri*** (Figure 24).

Among the Florida epiphytes, Meyer (2008) typically found one predatory species [***Milnesium tardigradum*** (Figure 10, Figure 46), ***Macrobiotus*** cf. ***harmsworthi*** (Figure 20), or ***Paramacrobiotus richtersi*** (Figure 21)], one other ***Macrobiotus*** species, one ***Minibiotus*** species, and one echiniscid species. One or more of these niches is frequently unoccupied, but when occupied the species followed this trophic hierarchy. In only one sample were there two predatory species.
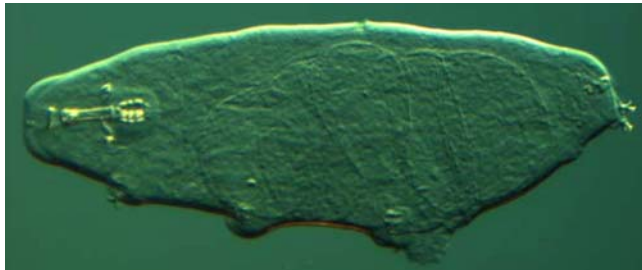

Figure 20. ***Macrobiotus harmsworthi***, a common tardigrade on bryophytes and elsewhere. Photo by Paul J. Bartels, with permission.

Figure 21. SEM of ***Paramacrobiotus richtersi***. Photo through Creative Commons.

Collins and Bateman (2001) found that ***Isohypsibius prosostomus*** (Figure 22) did not occur with ***Diphascon scoticum*** or ***Minibiotus intermedius*** (Figure 8) and only rarely occurred with ***Diphascon pingue*** (Figure 15) or ***Hypsibius convergens*** (Figure 23), attributing these negative associations to trophic overlap.
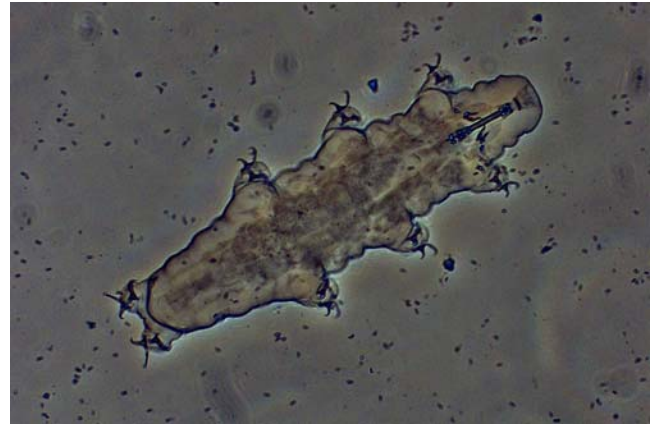

Figure 22. ***Isohypsibius prosostomus***. Photo by Michael Collins, with permission.

Figure 23. ***Hypsibius convergens***. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Competitive exclusion, most likely for food, also drives community associations. Wright (1991) found that ***Macrobiotus hufelandi*** (Figure 7), ***Paramacrobiotus richtersi*** (Figure 21), and ***Isohypsibius prosostomus*** (Figure 22) avoid each other's company.

But food webs are not the only controlling factors. Bryophytes can play a major role through the climate they create. For example, ***Macrobiotus hufelandi*** (Figure 7) and ***Hypsibius dujardini*** (Figure 12) are absent when the habitat desiccates rapidly, making some bryophytes ideal for them (Wright 1991). ***Milnesium tardigradum*** (Figure 10, Figure 46) and ***Ramazzottius*** (formerly ***Hypsibius***) ***oberhaeuseri*** (Figure 24) likewise avoid areas with a high rate of desiccation, as well as locations with high insolation, but also avoid poorly drained sites, excluding them from bryophytes in low-lying, wet areas. These limitations can result in predictable associations. Among these is a significant association among ***Milnesium tardigradum*** (Figure 10, Figure 46) – a predator – and two ***Hypsibius*** species among the British fauna. Further temporary community differences can be driven by the behavior of some species to migrate vertically in the moss cushion to a position of greater moisture, while other species such as ***Echiniscus testudo*** (Figure 5) remain behind. And of course this inability to migrate may limit the mosses it occupies in the first place.

But despite the importance of moisture in the continuing life cycle of the tardigrades, there is not always agreement on the moisture level needed. In Newfoundland, only ***Hypsibius dujardini*** (Figure 12) seems to follow a consistent relationship to a moisture category (Collins & Bateman 2001). It appears that rate of desiccation must be

considered along with moisture level in determining the community structure.

Even on the same moss species or growth form, macrohabitat makes a difference. Jönsson (2003) found that tardigrade abundance of the same species differed between a forest and a clearcut area, with greater numbers of species in the forest. Nevertheless, abundances were similar.

Unique Partnerships?

Despite the tiny size of this moss community, its relationships can be complex. The tardigrades *Ramazzottius oberhaeuseri* (Figure 24) and *Milnesium tardigradum* (Figure 10) both can carry the protozoan *Pyxidium tardigradum* (Figure 25) on their surface, a relationship known as that of a **symphoriont** (Morgan 1976). Morgan found both of these tardigrades with their passengers living among mosses. Van der Land (1964), who first discovered these protozoa on lichens, revived the dried tardigrades there, only to discover these protozoa that also were dormant became active after being placed in water. The accompanying rotifers and oribatid mites had no protozoan passengers, but Van der Land (1964) could not determine if these companions were alive at the time he rehydrated them. As noted earlier, this protozoan can at times be so abundant (up to 35 on a single water bear) that they slow down the tardigrade and might more appropriately be considered a parasite (Vicente *et al*. 2008). Although such symphoriont/parasitic(?) relationships have rarely been observed among the moss dwellers, we simply have not spent much time looking for them.

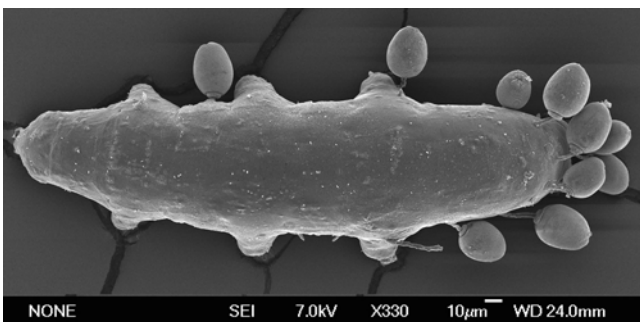Figure 24. *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.

Figure 25. Tardigrade infected with the protozoan *Pyxidium* sp. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Bryophyte Dangers – Fungal Parasites

This wonderful bryophyte home is not without its dangers to the moss-dwelling tardigrades. The phycomycetous fungus *Catenaria anguillulae* (Figure 26) is a widespread parasite on living, senescent, and dead microscopic animals (Barron 2009). The motile zoospores are attracted to exudations from openings on the bodies of nematodes and rotifers (Jansson & Thiman 1992), and it is likely that this opportunist also occurs on tardigrades (George Barron, pers. comm. 2010). The zoospores encyst on the cuticular covering near the opening or a wound. Hence, it is typical for them to colonize around the mouth or other opening. They subsequently germinate and penetrate through the orifice or wound to attack the animal on the inside.
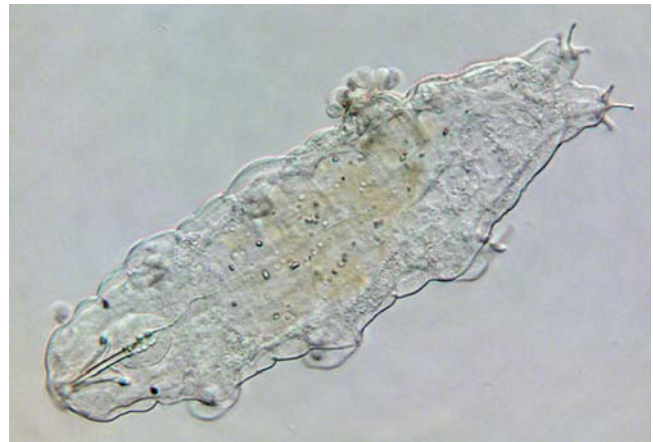

Figure 26. Tardigrade with fungus on its side. It is likely that this fungus is *Catenaria anguillulae* and that the attack location is a wound. Photo by Martin Mach, with permission.

Two fungi in the Ascomycota, *Lecophagus* [=*Cephaliophora*] *muscicola* (Figure 27-Figure 28) and *Lecophagus* [=*Cephaliophora*] *longispora* (Figure 29-Figure 30), capture rotifers and possibly tardigrades within the bryophytes and bryophyte-covered soil (Barron *et al*. 1990). This rather strange pair of fungi is endowed with elongate, hyaline, multiseptate, canoe-shaped conidia produced in small clusters at the apex of the conidiophores. When rotifers are present, the conidia germinate and produce one or several adhesive pegs that capture rotifers and tardigrades. Such fungal predators have been identified from moss-covered soil and forest debris in New Zealand and mosses in Canada, as well as from leaf mold in Japan, but their actual attack on tardigrades dwelling in mosses lacks documentation.

McInnes (2003) reports that the predatory fungus *Lecophagus antarcticus* attacks tardigrades that occur on cyanobacterial mats in lake sediments. Since *Lecophagus muscicola* (Figure 27-Figure 28) and *L. longispora* (Figure 29) are in the same genus and occur in wet or mossy soil (Barron *et al*. 1990; George Barron, pers. comm. 25 January 2010), it is reasonable to suggest that they might attack tardigrades in the same habitat, but can they? In fact, rotifers are attracted to the adhesive pegs of the fungus and then attach to the tip of the peg. That is their downfall, as the predator becomes the prey when the host (rotifer, nematode, or tardigrade) adheres to these adhesive pegs, possibly by lectin/carbohydrate bonding. But instead of

providing a meal for the invertebrate, the fungus penetrates and parasitizes it, making it a host. Barron considers this "a stretch" to consider that the tardigrades would attack the fungi just as readily as would the rotifers. But tardigrades are known to consume other filaments such as those of algae, so it is at least a possibility.

Figure 27. *Lecophagus muscicola* with two captured rotifers and two adhesive pegs. Photo by George Barron, with permission.

Figure 28. Conidia (X600) of *Lecophagus muscicola*. Photo by George Barron, with permission.
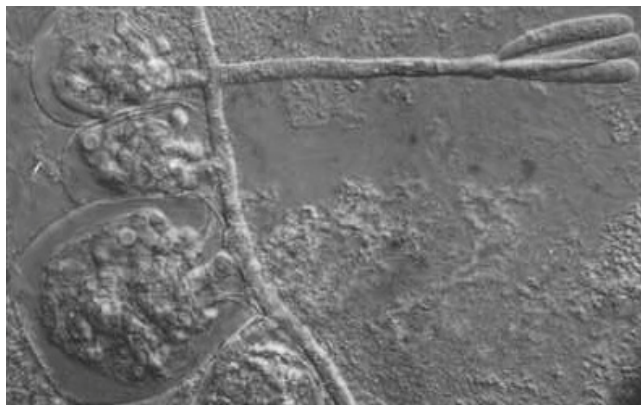

Figure 29. *Lecophagus longispora* infecting rotifers. Note also the elongate branch with terminal conidiogenous cell bearing a cluster of developing conidia. (X450). Photo by George Barron, with permission.

Several species of fungi in the genus *Ballocephala* (Zygomycota) (Figure 31-Figure 34) are known to attack tardigrades (Pohlad & Bernard 1978; Barron 2007). *Ballocephala pedicellata* was first described from individuals attacking the tardigrades *Hypsibius dujardini* (Figure 12) and the *Diphascon pingue* (Figure 15) complex living among mosses (Pohlad & Bernard 1978).

Adhesive conidia of *Ballocephala* species (Figure 31) attach to the cuticle surrounding the mouth of the tardigrade (Figure 31). These adhesive spores then encyst on the outside of their host. The spores extend long, narrow penetration pegs that are used to "snake" their way into the host, bypassing the defense mechanisms of the

animal (Barron 2007). As the hyphae grow they penetrate the host and fill it with hyphae (Figure 32) that secrete extracellular enzymes to digest the tardigrade and assimilate its tissue.
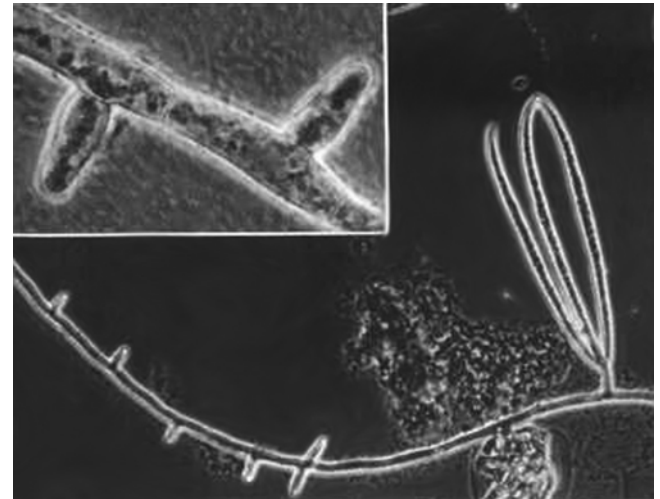
Figure 30. Hypha of *Lecophagus longispora* with a cluster of conidia and adhesive pegs, a fungus that traps rotifers and that may be a threat to some tardigrades living in mosses. Inset shows the adhesive pegs. Photos by George Barron, with permission.

Eventually the fungus breaks from the inside to the outside where it produces asexual spores (**conidiospores**) (Figure 33) that attack additional hosts. The fungus even has its own means to survive when the environment dries up and the tardigrade with it. Like many algae faced with declining conditions, this fungus undergoes sexual reproduction and produces zygotes that in turn form a thick wall and become **zygospores** (Figure 34). These resting spores permit the fungus to survive long periods of time (weeks to years) until favorable growing conditions return. Upon germination the zygospores presumably will divide to produce conidiophores and conidia that infect later generations of the tardigrade, but so far, this event has not been witnessed.

Figure 31. Spores of the fungus *Ballocephala sphaerospora* surrounding the mouth of a tardigrade. Photo by George Barron, with permission.

Figure 32. Tardigrade with hyphae of fungus *Ballocephala* sp. that have completely taken over its body. Photo by George Barron, with permission.

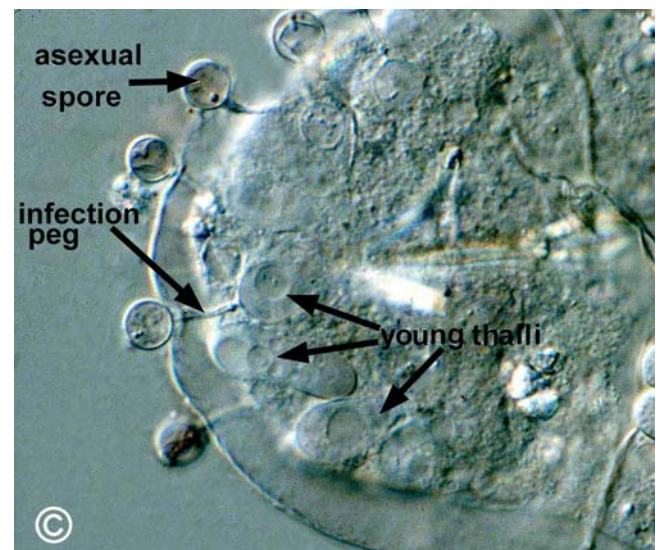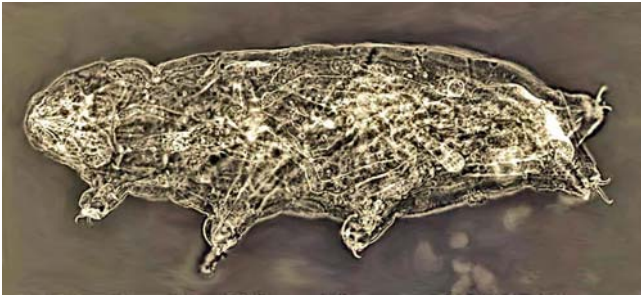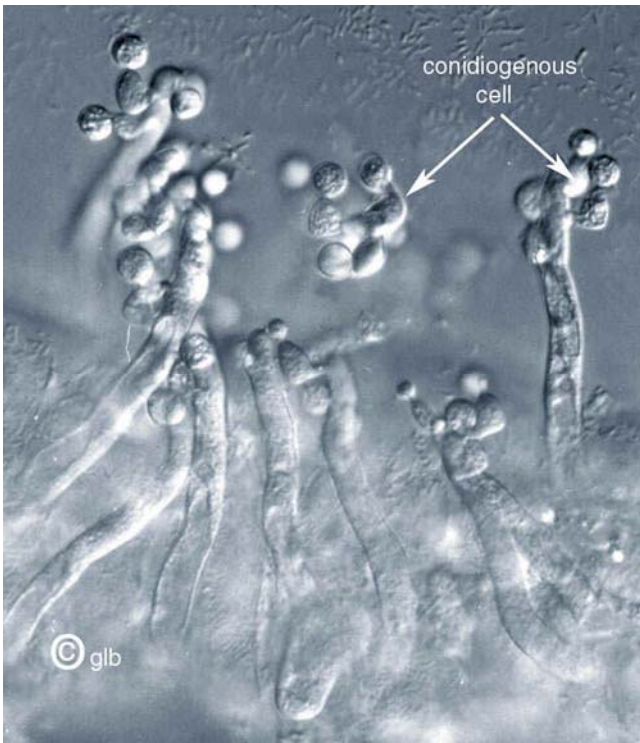Figure 33.   Conidiospores of the fungus *Ballocephala sphaerospora* on a tardigrade. Photo by George Barron, with permission.

Figure 34. Zygospores of the parasitic fungus *Ballocephala sphaerospora* filling a tardigrade. The tiny balls inside the cells are oil droplets used as a reserve food supply. Photo by George Barron, with permission.

The parasitic fungus *Haptoglossa mirabilis* (Figure 35) attacks tardigrades using the most complicated cellular mechanism known among the fungi (Robb & Barron 1982). It uses a gun-shaped attack cell that "shoots" into the host (Figure 36-Figure 37). At the anterior end of its cell is an elongated, barrel-shaped form. It is invaginated deeply into the cell, forming a bore. At the base of the bore is a walled chamber to house a missile-like attack apparatus. Robb and Barron hypothesize its mechanism:

This apparatus is able to fire the projectile at a high speed like a hypodermic needle, penetrating and injecting the host with the sporidium that infects the host. To accomplish this it has a basal vacuole with very high osmotic power. Water enters it rapidly and pumps the protoplasm and nucleus from the gun cell, through the hypodermic tube, and into the body of the host. If rotifers touch this fungus, they are hit within 0.1 second! But the proficiency of this apparatus doesn't end there. The head of this "harpoon" projectile is laminated, making it compressible. Thus, once it penetrates its host, it expands to seal the wound.

Once within the tardigrade, the fungus forms a cylindrical thallus (Figure 35) that consumes the poor tardigrade in a matter of days (Robb & Barron 1982). The hyphae eventually form zoospores. These exit through tubes and swim away to encyst. When the cysts germinate they form new clusters of gun cells. Some species can attack rotifers and nematodes as well.

George Barron (personal communication 1 March 2010) has commented to me that parasitic and predatory fungi are likely if both stylet-feeding and ingestion-feeding tardigrades coexist among mosses. But he finds it surprising that they have not yet been discovered.
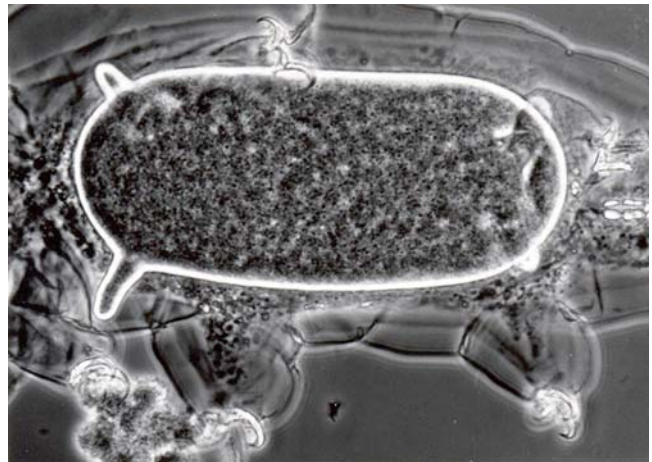
Figure 35. Fungus *Haptoglossa mirabilis* in a tardigrade, where it has formed a single thallus. Photo by George Barron, with permission.

The parasitic fungal genus *Harposporium* (Figure 38) has members that attack tardigrades (Saikawa *et al*. 1991), but the fungus must be ingested to function (George Barron, pers. comm. 25 January 2010). Barron (2008) suggests, as a hypothesis, that the spore is pumped down the oesophagus, where "it spirals and screws into the muscle fibres, thus lodging in the oesophagus."
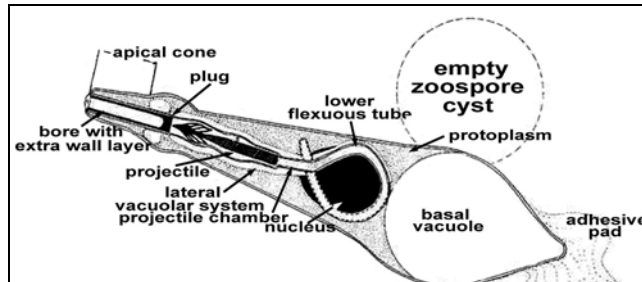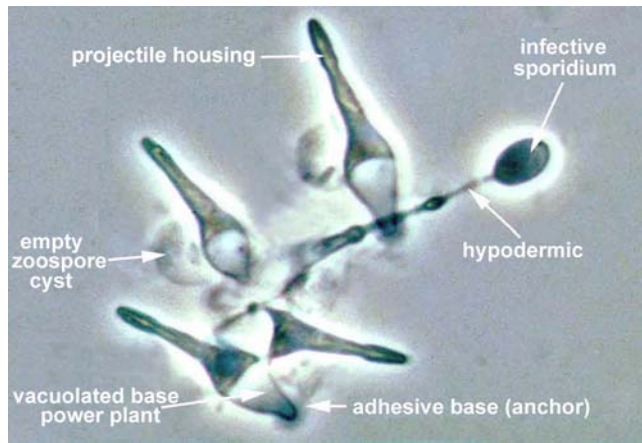
Figure 36. *Haptoglossa mirabilis* attack cells. Photo and diagram by George Barron, with permission.
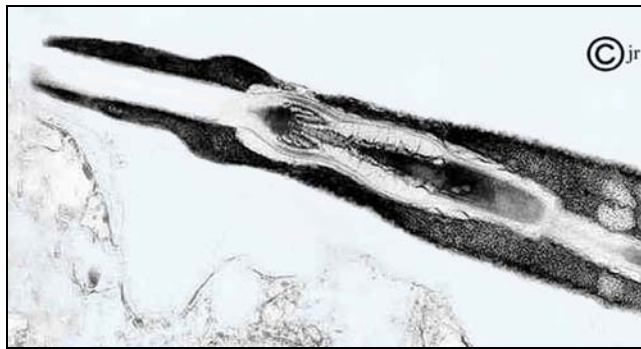
Figure 37. *Haptoglossa mirabilis* attack cell. Photo by Jane Robb and George Barron, with permission.
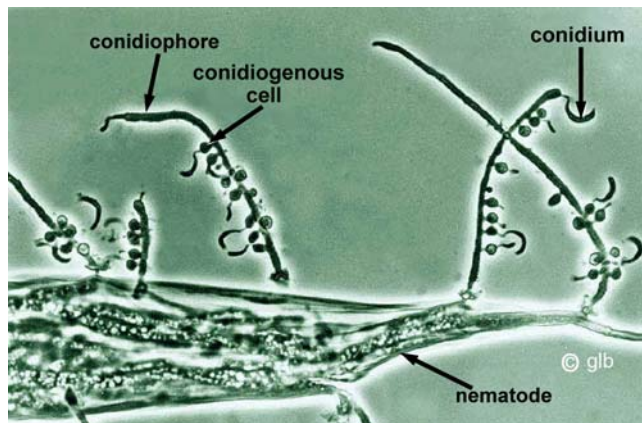
Figure 38. *Harposporium anguillulae*, a parasite on nematodes and tardigrades. Photo by George Barron, with permission.

Role of Bryophytes in Fungal Interactions

Milnesium tardigradum (Figure 10) can be parasitized by a chytridiomycetous fungus, a zoosporic fungus that is common in soil (Dewel & Dewel 1987). Letcher and Powell (2002) attempted to determine the role that mosses play in the establishment of **Chytridiomycota** in four sites in the Blue Ridge and Allegheny Mountains of Virginia, USA. They first determined that frequency of **Chytridiomycota** was greatest in soil under and surrounding the rhizoids of two moss species [*Polytrichastrum* (formerly in *Polytrichum*) *ohioense* (Figure 39), *Dicranum polysetum* (Figure 40)]. They also found that random point sampling suggested differences existed in zoosporic fungal frequency between the moss-covered soil and the exposed soil adjacent to these mosses, as well as between the two taxa. Similar differences were demonstrated using linear transect sampling. But, at last, the statistical analysis of the random point samples failed to demonstrate that the differences were significant for frequency of zoosporic fungi between the exposed soil and the moss-covered soil. However, they did find a significant difference between different moss/soil complexes for the frequency of the 15 common zoosporic fungal species they identified.

Figure 39. *Polytrichastrum ohioense* males with new growth. Photo by Janice Glime

Figure 40. *Dicranum polysetum*. Photo by Robert Klips, with permission.

One reason to suspect fungal differences under mosses compared to exposed soil is moisture. Using a scale of 0-10 (dry to saturated), Letcher and Powell found that the 48 moss-covered soil samples had a mean soil moisture

content of 1.89, whereas the 24 exposed soil samples had a mean of 1.33.

Pollution

In addition to fungal dangers, the bryophytes also become dangerous to tardigrades when they become polluted (Steiner 1994b). Vargha *et al.* (2002) found that the concentrations of metals in tardigrades bore a relationship to the concentrations in the mosses. Elevated concentrations of heavy metals correlated with a decrease in tardigrade fauna. Meininger *et al.* (1985) found that poor air quality (SO_2) could reduce the number of tardigrades in otherwise suitable moisture regimes among epiphytic mosses in the area of Cincinnati, Ohio, USA (Figure 41).
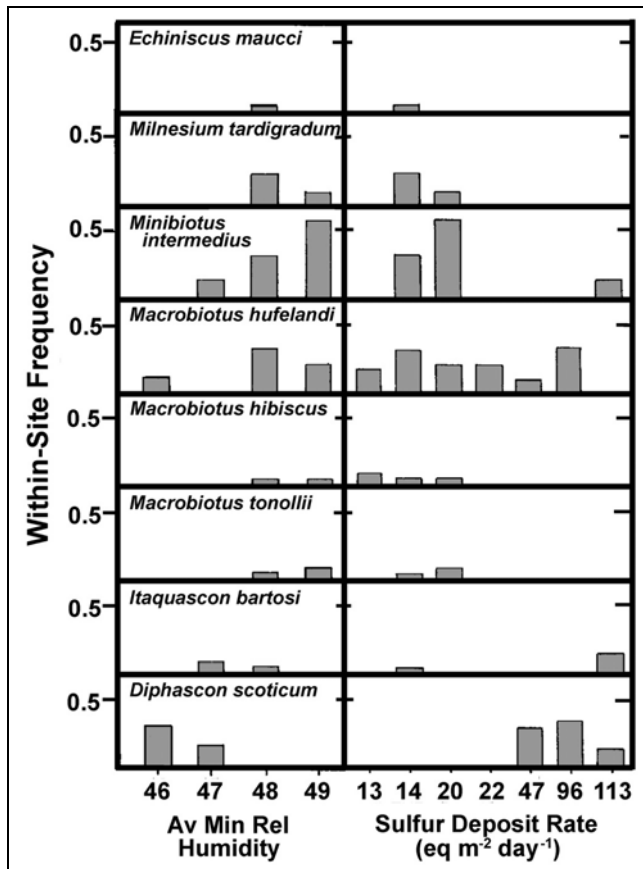

Figure 41. Effects of relative humidity and sulfur deposits from SO_2 on the frequency of **tardigrades** inhabiting lichens and mosses on red oak (*Quercus rubra*) in the Cincinnati area of Ohio, USA. n=20. Redrawn from Meininger *et al.* 1985.

Just as bryophytes and lichens have often been used as indicators of clean air or biomonitors of air pollution, so have their tardigrade inhabitants in both aquatic (Steiner 1994a) and terrestrial (Steiner 1995) conditions. And their responses are much the same. In a study in the Cincinnati, Ohio, USA area, Meininger *et al.* (1985) found that both epiphytic (tree-dwelling) bryophytes and their tardigrade fauna had the greatest species richness in areas with high humidity and clean air. Humidity is typically depressed in urban areas, and depression is likewise more rapid there, resulting from the more open environment (fewer trees) and reradiation of heat trapped by buildings and asphalt.

Two moss-dwelling tardigrades provided good indicators. *Diphascon scoticum* (Figure 9) was dominant in urban park sites with low air quality and reduced humidity. They feed on protococcal algae on the tree bark, and the low *p*H tolerance of this species may have played a role in its prevalence there. *Minibiotus intermedius* (Figure 8), on the other hand, occurs more often in the more humid rural sites and is apparently unable to tolerate the rapid evaporation rates that may characterize more urban environments.

Hohl *et al.* (2001) investigated tardigrades upwind and downwind of a coal-burning power plant in Missouri, USA. They found that the tardigrade density was greater upwind, but *Echiniscus* sp. was found only downwind. The more sensitive *Ramazzottius* sp. occurred only upwind. *Macrobiotus* sp., *Minibiotus* sp., and *Milnesium tardigradum* occurred in both locations, but in different numbers. Although these species were mostly named only to genus, all were in genera or species known from bryophytes.

Acid Rain, SO_2, and NO_2

Acid rain poses a threat to at least some tardigrades. *Hypsibius dujardini* (Figure 12) has reduced activity at *p*H 4.0 (Bartels 2005). Even at *p*H 5, activity is reduced compared to controls at *p*H 7 (Thompson 2008). At *p*H 2.8 they are killed (Bartels 2005). Acevedo (2008) examined one of the most common bryophyte dwellers, *Milnesium tardigradum* (Figure 10), for its tolerance to *p*H, as measured by activity. Although *M. tardigradum* could survive from *p*H 1.54 to 12.5 for 1 minute, this species, like *Hypsibius dujardini* (Figure 12), had increased survival time as the *p*H approached 7. This species proved to be more sensitive at the higher *p*H levels.

Nevertheless, Steiner (1994a) found that neither tardigrades nor rotifers seemed to vary in abundance in response to SO_2 or NO_2. But in 1995, Steiner reported that the community composition did change in response to SO_2.

In the aquatic environment, using both experiments and air quality at 12 sites, Steiner (1995) showed that changes to the tardigrade and other aquatic fauna composition correlated negatively with the SO_2 concentrations. They used untreated control mosses and those subjected to concentrations of 0.025, 0.075, and 0.225 ppm SO_2 for 18 months and found that the highest level caused significant decreases in the numbers of several tardigrade species. SO_2 caused a significant decrease in *p*H in the moss cushions. The populations of *Macrobiotus persimilis* actually increased as pollution increased. Steiner (1994a) suggested that the tardigrade (and other invertebrate) fauna of epilithic moss cushions could be appropriate biomonitors for air quality.

Urban Environment

Even if the urban habitat is not always polluted in the usual sense, it is a drastic contrast in environment to that of more natural rural areas. Cities themselves both create and hold heat. That makes them susceptible to rapid drying, a condition lethal to many tardigrades.

Johansson *et al.* (2011) compared tardigrades from 73 urban and 24 rural locations in Fresno County, California. Only 22% of the urban samples had tardigrades, whereas 74% of the rural samples had tardigrade inhabitants. The urban samples likewise had fewer species, but the densities

of these species differed little from that of the rural sites. Of the 26 species found, 7 were found only in the urban samples, 16 only in rural samples, and 3 in both. The rural communities differed more among themselves than did the urban samples. Interestingly, tardigrades seemed to prefer lower *p*H levels in both community types. Hence, the researchers excluded acid rain as the cause of differences. It is likely that the microclimate of the city is less favorable due to greater exposure and heat, leading to more rapid drying.

Moly de Peluffo *et al*. (2006) conducted a more controlled study of urban conditions by using samples from the same tree species from the city of General Pico in Central Argentina. They included paved areas with different traffic intensities, non-paved areas, an industrial area, and a bus station. They examined 11 mm diameter samples of lichens and mosses from the trees. Only 5 tardigrade species were collected in total [***Echiniscus rufoviridis***, ***Milnesium cf. tardigradum*** (Figure 10), ***Ramazzottius oberhaeuseri*** (Figure 24), ***Paramacrobiotus areolatus*** (Figure 42), ***Macrobiotus*** sp.]. ***Milnesium cf. tardigradum*** and ***Ramazzottius oberhaeuseri*** were the most abundant. Mean density of tardigrades was 10, but the maximum reached 50 per cm^2. ***Milnesium cf. tardigradum*** dominated along paved streets where there was intense traffic, suggesting that it was the most tolerant species. In periurban areas, ***Ramazzottius oberhaeuseri*** dominated despite the high suspension dust and high sun exposure. In the Neotropical region of Santa Rosa, Argentina, Peluffo *et al*. (2006) found the same five species. Again, only ***Milnesium cf. tardigradum*** occurred in areas with high levels of vehicle traffic.

Figure 42. ***Paramacrobiotus areolatus*** head region. Photo by Martin Mach, with permission.

Dust can be a factor in tardigrade distribution. Meininger and Spatt (1988) found that both tardigrades and their bryophyte environment are affected by road dust such as that created by the trans-Alaska Pipeline haul road (Dalton Highway). In this case, the dust is calcium-rich. ***Sphagnum*** (Figure 3) is absent within 10 m of the road. Instead, calcium-tolerant moss species such as ***Aulacomnium turgidum*** (Figure 43) and ***Dicranum angustum*** (Figure 44) occupy these alkaline areas. The tardigrades that survive here are xeric species such as ***Diphascon scoticum*** (Figure 9) and ***Hypsibius dujardini*** (Figure 12). Farther from the road, these fungal and algal

feeding species are replaced by omnivores and carnivores [***Macrobiotus hufelandi*** (Figure 7) and ***M. harmsworthi*** (Figure 20), respectively].

Tardigrades in Space

Imagine living through conditions of 151ºC, -273ºC, the vacuum of space, radiation 500 times that humans can tolerate, 2000X normal atmospheric pressure, and ten years with no water! (Ramløv & Westh 2001; Jönsson *et al*. 2008; ZAMP Wiki 2009). Tardigrades are sometimes considered to be **extremophiles**, and their ability to survive these conditions would seemingly put them among the champions. But extremophiles are organisms that *live* at extremes; tardigrades must become dormant to survive these, and the longer they are in this condition, the lower their chances of survival (Mullen 2002). These abilities to become dormant - enter **cryptobiosis** - have made them popular animals for space travel, surviving and carrying back the evidence of the effects of space on subcellular components. And they were the first space travellers without space suits to survive.

Figure 43. ***Aulacomnium turgidum***, a calcium-tolerant species, in Norway. Photo by Michael Lüth, with permission.

Figure 44. ***Dicranum angustum***, a calcium-tolerant species. Photo by Jan-Peter Frahm, with permission.

The ability to shut down makes tardigrades ideally suited for space travel (Jönsson *et al*. 2008; Rebecchi *et al*. 2009a). They can survive a vacuum (Gavaret 1859) and its

resulting extreme dehydration (Jönsson *et al*. 2008). They also survive both solar and cosmic radiation. They survive extreme ionizing radiation (570,000 roentgens kills only 50%; humans can be killed by 500) (May *et al*. 1964) and can return from space travel under these extremes and become active again with no reduction in survival (Jönsson *et al*. 2008; Rebecchi *et al*. 2009a). They have even survived temperature extremes from -200ºC to 151ºC (Doyère 1842; Rahm 1923, 1937; Becquerel 1950; Keilin 1959; Seki & Toyoshima 1998; Lindahl & Balser 1999).

   Richtersius coronifer (Figure 45), *Milnesium tardigradum* (Figure 46) (Jönsson 2008), and *Paramacrobiotus richtersi* (Figure 21; Rebecchi *et al*. 2009a, b) began their space adventure on 17 September 2007. They arrived back from their adventure in space on 26 September, and not only were they alive, but they could also reproduce! *Milnesium tardigradum* did better than *R. coronifer*. About 12% of those exposed to UV-A and UV-B managed to recover, although they exhibited reduced egg production compared to the control animals that stayed on Earth. Those exposed to the full range of UV radiation only partially recovered, then died. Jönsson *et al*. (2005) suggested that during anhydrobiosis there are no biochemical protectants to protect against radiation. Rather, it appears that survival of tardigrades may be due to efficient DNA repair.

Figure 45. *Richtersius coronifer* and its close relatives have a beautiful deep yellow color. This species has been a successful space traveller. Photo by Martin Mach, with permission.

   Paramacrobiotus richtersi (Figure 21) and *Ramazzottius oberhaeuseri* (Figure 24) were able to withstand high doses of ultraviolet radiation in both active and anhydrobiotic states (Altiero *et al*. 2011). Nevertheless, the survival rate of hydrated or desiccated specimens was inversely related to the UV doses, with *P. richtersi* tolerating the increase of UV dose better than *R. oberhaeuseri*.

   The survival of *Richtersius coronifer* (Figure 45) in the adventure beginning 17 September 2007 (Jönsson 2008) was at least somewhat predictable. In the lab, this species survived instant freezing to -195.8ºC and vacuum conditions with 96-100% survival (Persson *et al*. 2009). In low Earth orbit it experienced little effect from cosmic radiation or microgravity (68, 89, 82% survival). On the other hand, when Persson *et al*. (2009) subjected *R. coronifer*, *Ramazzottius oberhaeuseri* (Figure 24), and *Echiniscus testudo* (Figure 47) to desiccation on moss, the survival rate was very low (0-22.5%). They emphasized

that the protocol for desiccation is important and may not have been appropriate for survival.
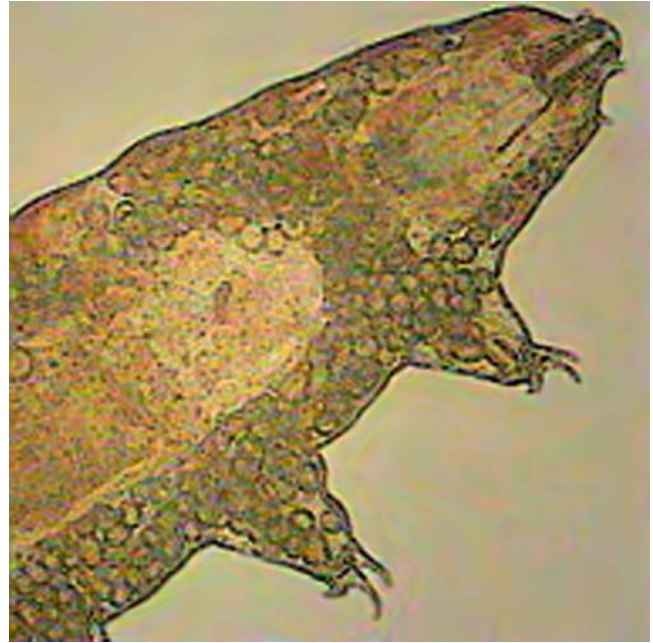
Figure 46. *Milnesium tardigradum*, a space traveller. Photo by Martin Mach, with permission.
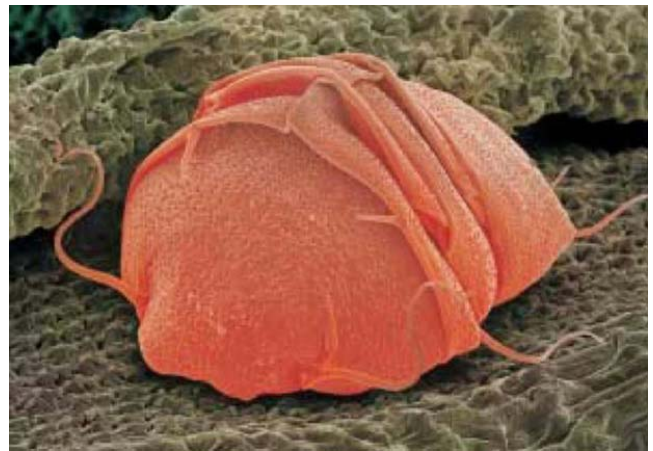
Figure 47. *Echiniscus testudo* tun, a way to survive space travel. Picture by Power & Syred through Creative Commons.

   Another moss-dweller (among other substrata), *Hypsibius dujardini* (Figure 12), was scheduled to be travelling in space on a Russian spacecraft as I write (Milstein 2009). Following its liftoff in October 2009, it was scheduled to reside there for three years! Its habitat is a bit of polymer microcosm with conditions mimicking those of a bit of meteor rock. But, alas, technical and safety issues have caused a two-year delay before lift off (Madrigal 2009). The objective is to determine if life can survive interplanetary space travel, a test of the **Transpermian Theory**. The Transpermian Theory suggests that life might have travelled on a bit of meteoric rock from Mars or other planet and landed on Earth billions of years ago. The tough part, both for the real meteoric rock traveller and the spacecraft test subjects may be the landing event on Earth.

Most tardigrades have gone on their space adventure in an anhydrobiotic state. Rebecchi *et al*. (2011) used *Paramacrobiotus richtersi* (Figure 21) to determine the effects of the anhydrobiotic state on survivorship in space. They flew these animals for twelve days in a low-earth orbit (250-290 km altitude). The desiccated animals had high survival rate (79-95%), similar to that of non-desiccated ground controls. There was no visible damage to their double-stranded DNA, but their heat shock proteins (HSP expression at 70 and 90 kDa) increased compared to ground controls.

Persson *et al*. (2011) assessed the effects of cosmic radiation on tardigrades, as well as rotifers. The tardigrades spent time in a low Earth orbit where they were exposed to cosmic radiation and microgravity. *Richtersius coronifer* (Figure 45) was dried for two years on Whatman-3 filters during its space travel. However, none of the members of this species could be revived. In a different microcosmos experiment, Persson *et al*. desiccated *R. coronifer*, *Ramazzottius oberhaeuseri* (Figure 24), and *Echiniscus testudo* (Figure 47) as well as rotifers and nematodes on a moss substrate. Species in this experiment had some, but very low, survival. Embryos of *Milnesium tardigradum* (Figure 10), on the other hand, all survived cosmic radiation from space flight. *Richtersius coronifer* also survived extreme cold and vacuum while in anhydrobiosis.

Evolutionary Similarities to Bryophytes

What could tardigrades and bryophytes possibly have in common? Their habitat requirements, for one thing. Hence, both do well with asexual reproduction (Pilato 1979). And both do well under circumstances requiring dormancy. Pilato points out that there is marked uniformity in both size and morphology in the four invertebrate groups of rotifers, gastrotrichs, nematodes, and tardigrades. That character does not seem to fit so well with bryophytes, but if one compares them to tracheophytes, perhaps it does. Both groups have "a less common pattern of geographic distribution than usually believed" for organisms having only passive transport. They furthermore have maintained their antiquity and have given rise to entire orders. But there are also some strong differences. Compared to tardigrades, bryophytes do not have a constant cell number, have no marine members, and are capable of regeneration. Pilato suggests that parthenogenesis would have significantly delayed evolution, a concept that has come into question for bryophytes. He furthermore suggests that by entering cryptobiosis, these invertebrate organisms escape the variability of the environment and thus escape many of the selection pressures that drive evolution.

Sampling and Extraction

Sampling from mosses is not a very standardized procedure. The moss must first be collected from its substrate. What works for a flat mat does not work for a thick mat, weft, or tall turf. Sayre and Brunson (1971) suggested using a metal test-tube cap 2.5 cm in diameter to cut a core from the moss (see also Guil *et al*. 2009) – a technique that should work well for thin mats with stems that are not tough, but many growth forms would not

sample well by this method. Since tardigrades are slow-moving, one need not worry about their escaping during the cutting procedure. However, I prefer the hand removal, followed by a dry weight of bryophyte for quantification. Fortunately, both bryophytes and tardigrades can survive such drying. A large clump could later be cut down the middle and still permit sampling of all the sub-habitats.

Fortunately, mosses need little care once collected and can easily be kept in small paper bags – the kind that was once used in the candy store (~10 x 20 cm when folded). If it is not too hot or severely dry, these will permit the mosses to dry slowly, provided they are not in the path of a fan or other drying agent. Using plastic bags can foster growth of fungi if the bag is kept for more than a day or two, and it will not permit the drying needed for both kinds of organisms. Morgan and King (1976 in Clifford 2005) suggested using an open plastic bag that would permit air drying.

Obtaining tardigrades from mosses may be a bit tedious. One method is to immerse the bryophytes in water (Claxton 1998; Guidetti *et al*. 2008) in something like a finger bowl or Petri dish. Tap water may be okay, depending on chlorine levels; if pond water is used, it should be boiled and filtered to avoid contamination. Distilled water should NOT be used because it will cause the tardigrade to take on water by osmosis and it is likely to become too extended to move. Placing the bryophyte upside down with the soil portion above the water will give the best results; muddy water makes it difficult to spot the bears (Sayre & Brunson 1971). If you are interested in vertical positioning, place aluminum foil or other cover over the container to keep it dark.

Sayre and Brunson (1971) recommend keeping the moss submersed for 24 hours at room temperature. The moss should then be removed and the absorbed water squeezed into the dish. The water in the dish should then be stirred and poured into a counting dish. After the tardigrades have settled they can be counted with a dissecting microscope. They recommend 60X, but most dissecting microscopes don't go that high, so one might need a pair of 20X oculars. If there is too much water, they suggest decanting off the excess, but for quantitative purposes, I suspect that will lead to inaccuracies for these light-weight animals.

Nelson (1991) considered this to be a common method. She recommended letting the collected bryophytes dry in paper bags for at least several days, then placing them in water for several hours to awaken the tardigrades. The wet bryophytes are then squeezed to remove water with water bears into a Petri dish or other container. As in the Sayre and Brunson method, excess water can be decanted off and the sediment examined.

Guidetti *et al*. (2008) recommend that after immersing the bryophytes, the water should be sifted repeatedly to collect the tardigrades. From there, the animals can be extracted while observing them with a dissecting microscope.

Schuster *et al*. (1977) used a similar method. Collection material (bryophytes, soil, detritus) was swirled in water and once the dense particles settled, the water was decanted and poured through a US Standard #325 filter, pore size ~44 μm. These were then washed from the filter into a preservation jar or onto a glass slide. Specimens can

be preserved by adding 5% formalin or glutaraldehyde, or alcohol, as discussed later.

Other researchers choose to use the Baermann funnel to extract the animals (Hohberg 2006) as already described for invertebrates (Chapter 4-1 of this volume).

A slight modification of these methods is to clean a bryophyte clump of its loose soil and place it into a Petri plate upside down (Deep Data 2009). Fill the plate with water. When the bryophyte absorbs all the water, add more. Maintain a few mm of water in the plate after the bryophyte is fully hydrated. The bryophyte should be kept hydrated for several hours or overnight before examination. Remove the bryophyte from the water and examine it with a dissecting microscope at 20X or higher. Alternatively, this website also recommends squeezing out the water and examining the liquid. Look for yellow, red, or whitish animals that move "like puppies."

Nelson and Bartels (2007) used a different method when working with samples from soil, lichens, mosses, and leaf litter from the Smoky Mountains. They extracted the tardigrades using centrifugation with Ludox AM™ and mounted them on microscope slides in Hoyer's medium.

Tardigrades can be put to sleep with $MgCl_2$ or shocked with freshwater, then decanted into a fine mesh (20-40 µm) net (Nelson 2002). An intriguing method is to use an aquarium air stone to bubble the tardigrades that can then be collected with a piece of paper towel laid on the surface of the water. (If that works, I doubt that decanting is good for quantitative studies.) The towel should then be rinsed through a 30-40 µm net. For aquatic samples, water can be used from the habitat.

Clifford (2005) draws heavily on the experience of Morgan and King (1976) in his Tardigrada website. He suggests narcotizing the water bears with 20% alcohol in a volume equal to that of the bryophyte plus water. The bears will no longer be able to cling to the bryophytes and can be more easily dislodged into the alcohol/water solution. The bryophyte and associated organisms can be removed after about 10 minutes and wrung into a Petri dish to remove the adhering water and bears. This immersion

and wringing should be repeated several times, with immersion up to 15 minutes. The extracted water can be examined at about 50X magnification. (As you can see, recommendations for magnification vary from 20X to 60X. You will have to see what works for you.)

Clifford (2005) suggests that a somewhat simpler method is to flood the bryophyte with enough water to cover it for 48-72 hours. Then remove the moss to a new dish and start with the alcohol at 70%. Force the alcohol through the bryophyte clump with a pipette that has a strong bulb. A baster might work for larger mosses, but I haven't tried it. This alcohol method seems a bit cruel to the bears.

Nelson and Hauser (2012) complain that extraction from the limnoterrestrial habitats, those habitats of water adhering in spaces such as the ones among bryophytes, is often done with the Berlese funnel. They contend that this method has a strong bias toward arthropod diversity and does not work well for relatively slow-moving or immobile animals such as tardigrades. Rather, they found much more diversity among the microscopic invertebrates such as tardigrades when they used water flooding for extraction. In fact, they found almost no taxa overlaps when comparing these two methods!

Tardigrades can be preserved in 70% alcohol, but they can be difficult to locate again, and one must check periodically to be sure the alcohol isn't about ready to dry up. A few drops of glycerine in the vial of alcohol helps when the alcohol does dry out. The preferable method seems to be to mount them in one of the standard mounting media used for bryophytes and other things. Pennak (1953) gives instructions for various media. Unfortunately (or fortunately) a number of these media have been declared carcinogenic or toxic and are no longer available.

Checklist of Bryophyte Dwellers

I won't even pretend that I can provide a complete list of these taxa. I have not covered all the literature, and certainly many have yet to be discovered, but at least Table 1 is a start.

Table 1. Partial list of tardigrades known to inhabit bryophytes, based on literature.

Heterotardigrada (armored tardigrades)

Bryodelphax aaseae	Kristensen *et al*. 2010	*Echiniscus jenningsi*	Margulis & Chapman 1998
Bryodelphax asiaticus	Kaczmarek & Michalczyk 2006	*Echiniscus kofordi*	Christenberry 1979
Bryodelphax brevidentatus	Kaczmarek *et al*. 2005	*Echiniscus laterosetosus*	Ito 1993
Bryodelphax mateusi	Guil 2002	*Echiniscus lichenorum*	Guil 2002
Bryodelphax parvulus	Guil 2002	*Echiniscus madonnae*	Michalczyk & Kaczmarek 2006a
Bryodelphax sinensis	Pilato 1974	*Echiniscus marinellae*	Guil 2002
Bryodelphax tatrensis	Guil 2002	*Echiniscus mauccii*	Kathman & Cross 1991
Cornechiniscus cornutus	Mach, The Water Bear	*Echiniscus mediantus*	Guil 2002
Cornechiniscus lobatus	Guil 2002	*Echiniscus merokensis*	Kaczmarek & Michalczyk 2006
Cornechiniscus holmeni	Beasley & Miller 2007	*Echiniscus multispinosus*	Guil 2002
Cornechiniscus subcornutus	Guil 2002	*Echiniscus nelsonae*	Li *et al*. 2007
Echiniscus arctomys	Mehlen 1969	*Echiniscus* cf. *oihonnae*	Hooie & Davison 2001
Echiniscus barbarae	Kaczmarek & Michalczyk 2002	*Echiniscus palmai*	Dastych 1997
Echiniscus bigranulatus	Horning *et al*. 1978	*Echiniscus perarmatus*	Binda *et al*. 2001
Echiniscus bisculptus	Guil 2002	*Echiniscus perviridis*	Nelson & Adkins 2001
Echiniscus blumi	Kaczmarek & Michalczyk 2006	*Echiniscus polygonalis*	Ito 1993
Echiniscus brachyspinosus	Horning *et al*. 1978	*Echiniscus quadrispinosus*	Kathman & Cross 1991
Echiniscus canadensis	Kaczmarek & Michalczyk 2006	*Echiniscus reticulatus*	Kaczmarek & Michalczyk 2006
Echiniscus capillatus	Jennings 1979	*Echiniscus scabrospinosus*	Guil 2002
Echiniscus cavagnaroi	Christenberry 1979	*Echiniscus semifoveolatus*	Ito 1993
Echiniscus ganczareki	Michalczyk & Kaczmarek 2007	*Echiniscus shaanxiensis*	Li *et al*. 2007
Echiniscus granulatus	Kaczmarek & Michalczyk 2006	*Echiniscus sinuloides* =???	Guil 2002
Echiniscus horningi	Kathman & Cross 1991	*Echiniscus spiniger*	Horning *et al*. 1978

Echiniscus spinulosus	Guil 2002
Echiniscus taibaiensis	Wang & Li 2005
Echiniscus testudo	Beasley & Miller 2007
Echiniscus trisetosus	Kaczmarek & Michalczyk 2006
Echiniscus velaminis	Horning *et al.* 1978
Echiniscus vinculus	Horning *et al.* 1978
Echiniscus virginicus	Hooie & Davison 2001
Echiniscus viridianus	Pilato *et al.* 2007
Echiniscus viridissimus	Nelson & Adkins 2001
Echiniscus wendti	Kathman & Cross 1991
Echiniscus zetotrymus	Horning *et al.* 1978
Hypechiniscus exarmatus	Horning *et al.* 1978
Hypechiniscus gladiator	Kathman & Cross 1991
Oreella minor	Horning *et al.* 1978
Oreella mollis	Horning *et al.* 1978
Parechiniscus chitonides	Guil 2002
Parechiniscus unispinosus	Guil 2002
Pseudechiniscus beasleyi	Li *et al.* 2007
Pseudechiniscus brevimontanus	Nelson & Bartels 2007
Pseudechiniscus clavatus	Guil 2002
Pseudechiniscus facettalis	Ito 1993
Pseudechiniscus goedeni	Kathman & Cross 1991
Pseudechiniscus gullii	Pilato & Lisi 2006
Pseudechiniscus insolitus	Guil 2002
Pseudechiniscus jiroveci	Kaczmarek & Michalczyk 2006
Pseudechiniscus juanitae	Pilato & Lisi 2006
Pseudechiniscus lateromamillatus	Horning *et al.* 1978
Pseudechiniscus novaezeelandiae	Horning *et al.* 1978
Pseudechiniscus papillosus	Li *et al.*.2005
Pseudechiniscus pseudoconifer	Franceschi *et al.* 1962-63
Pseudechiniscus spinerectus	Pilato *et al.* 2001
Testechiniscus laterculus	Kathman & Cross 1991
Testechiniscus meridionalis	Jennings 1979
Testechiniscus spitsbergensis	Kaczmarek & Michalczyk 2006

Eutardigrada (unarmored/naked tardigrades)

Acutuncus antarcticus	Miller *et al.* 1996
Amphibolus weglarskae	Bartels & Nelson 2007
Astatumen trinacriae	Nelson & Bartels 2007
Bertolanius nebulosus	Westh & Kristensen 1992
Bertolanius volubilis	Guidetti *et al.* 2008
Bertolanius weglarskae	Kathman & Cross 1991
Bindius triquetrus	Pilato 2009
Biserovus bindae	Christenberry & Higgins 1979
Calohypsibius maliki	Michalczyk & Kaczmarek 2005
Calohypsibius ornatus	Kaczmarek & Michalczyk 2006
Calohypsibius placophorus	Guil 2002
Calohypsibius schusteri	Nelson & Bartels 2007
Calohypsibius verrucosus	Guil 2002
Dactylobiotus ambiguus	Guil 2002
Dactylobiotus dispar	Suren 1992
Dactylobiotus macronyx	Guil 2002
Diphascon alpinum	Horning *et al.* 1978
Diphascon belgicae	Kathman & Cross 1991
Diphascon bullatum	Horning *et al.* 1978
Diphascon carolae	Bartels & Nelson 2009
Diphascon chilenense	Horning *et al.* 1978
Diphascon dolomiticum	Pilato & Bertolani 2005.
Diphascon granifer	Bartels & Nelson 2009
Diphascon higginsi	Nelson & Bartels 2007
Diphascon iltisi	Kathman & Cross 1991
Diphascon modestum	Kathman & Cross 1991
Diphascon nobilei	Schuster & Greven 2007
Diphascon nodulosum	Kathman & Cross 1991
Diphascon oculatum	Schuster & Greven 2007
Diphascon patanei	Nelson & Bartels 2007
Diphascon pingue	Kaczmarek & Michalczyk 2006
Diphascon prorsirostre	Kaczmarek & Michalczyk 2006
Diphascon ramazzottii	Bartels & Nelson 2007
Diphascon recamieri	Kathman & Cross 1991
Diphascon rugosum	Schuster & Greven 2007
Diphascon scoticum	Kaczmarek & Michalczyk 2006
Diphascon sexbullatum	Kaczmarek & Michalczyk 2006
Doryphoribius flavus	McInnes 1994
Doryphoribius gibber	Pilato & Lisi 2006
Doryphoribius huangguoshuensis	Wang *et al.* 2007
Doryphoribius mexicanus	Beasley *et al.* 2008
Doryphoribius picoensis	Fontoura *et al.* 2008
Doryphoribius polynettae	Bartels *et al.* 2007
Doryphoribius quadrituberculatus	Kaczmarek & Michalczyk 2004a
Doryphoribius zappalai	Wang *et al.* 2007
Doryphoribius zyxiglobus	Horning *et al.* 1978
Hebesuncus conjungens	Kaczmarek & Michalczyk 2006
Hypsibius arcticus	Utsugi & Ohyama 1989
Hypsibius camelopardalis	Guil 2002
Hypsibius convergens	Kaczmarek & Michalczyk 2006
Hypsibius dujardini	Kaczmarek & Michalczyk 2006
Hypsibius maculatus	Kaczmarek & Michalczyk 2004b
Hypsibius microps	Guil 2002
Hypsibius pallidus	Kaczmarek & Michalczyk 2006
Hypsibius roanensis	Nelson & Bartels 2007
Hypsibius scabropygus	Schuster & Greven 2007
Insuetifurca arrowsmithi	Kathman & Cross 1991
Insuetifurca xiae	Li 2009
Isohypsibius arbiter	Binda *et al.* 2001
Isohypsibius archangajensis	Kaczmarek & Michalczyk 2006
Isohypsibius asper	Jennings 1979
Isohypsibius baslovi	Bartels & Nelson 2009
Isohypsibius bertolanii	Manicardi 1989
Isohypsibius brevispinosus	Bartels & Nelson 2009
Isohypsibius brulloi	Pilato & Pennisi 1976
Isohypsibius cameruni	Horning *et al.* 1978
Isohypsibius josephi	Guil 2002
Isohypsibius lunulatus	Kathman & Cross 1991
Isohypsibius mammillosus	Guil 2002
Isohypsibius mihelcici	Kaczmarek & Michalczyk 2006
Isohypsibius monstruosus	Guil 2002
Isohypsibius montanus	Guil 2002
Isohypsibius pappi	Pilato & Sperlinga 1975
Isohypsibius prosostomus	Schuster & Greven 2007
Isohypsibius qinlingensis	Li *et al.* 2005
Isohypsibius sattleri	Kaczmarek & Michalczyk 2006
Isohypsibius taibaiensis	Li & Wang 2005
Isohypsibius tuberculatus	Nelson & Bartels 2007
Isohypsibius wilsoni	Horning *et al.* 1978
Isohypsibius woodsae	Kathman & Cross 1991
Itaquascon bartosi	Meininger *et al.* 1985
Itaquascon mongolicus	Kaczmarek & Michalczyk 2006
Itaquascon pawlowskii	Kathman & Cross 1991
Itaquascon unguiculum	Pilato *et al.* 2002
Limmenius porcellus	Horning *et al.* 1978
Macrobiotus almadai	Fontoura *et al.* 2008
Macrobiotus anderssoni	Horning *et al.* 1978
Macrobiotus arguei	Pilato & Sperlinga 1975
Macrobiotus baltatus	Guil 2002
Macrobiotus barabanovi	Tumanov 2005
Macrobiotus barbarae	Kaczmarek *et al.* 2007
Macrobiotus blocki	Bertolani pers. comm. 2010
Macrobiotus bondavallii	Manicardi 1989
Macrobiotus contii	Pilato & Lisi 2006
Macrobiotus coronatus	Horning *et al.* 1978
Macrobiotus crenulatus	Kathman & Cross 1991
Macrobiotus echinogenitus	Kathman & Cross 1991
Macrobiotus furciger	Horning *et al.* 1978
Macrobiotus grandis	Guil 2002
Macrobiotus harmsworthi	Kaczmarek & Michalczyk 2006
Macrobiotus hibiscus	Meininger *et al.* 1985
Macrobiotus hufelandi	Kaczmarek & Michalczyk 2006
Macrobiotus islandicus	Kathman & Cross 1991
Macrobiotus kazmierskii	Kaczmarek & Michalczyk 2009
Macrobiotus kirghizicus	Tumanov 2005
Macrobiotus kovalevi	Tumanov 2004
Macrobiotus lazzaroi	Nelson & Bartels 2007
Macrobiotus liviae	Nelson & Bartels 2007
Macrobiotus lusitanicus	Guil 2002
Macrobiotus magdalenae	Michalczyk & Kaczmarek 2006b
Macrobiotus mandalaae	Pilato 1974
Macrobiotus marlenae	Kaczmarek & Michalczyk 2004c
Macrobiotus martini	Bartels & Nelson 2009
Macrobiotus mauccii	Pilato 1974
Macrobiotus montanus	Kaczmarek & Michalczyk 2006
Macrobiotus nuragicus	Pilato & Sperlinga 1975
Macrobiotus occidentalis	Guil 2002
Macrobiotus orcadensis	Horning *et al.* 1978
Macrobiotus pallarii	Guil 2002
Macrobiotus patiens	Schuster & Greven 2007
Macrobiotus persimilis	Fontoura *et al.* 2008
Macrobiotus polaris	Venkataraman 1998

Macrobiotus polyopus	Kaczmarek 2003	*Minibiotus weglarskae*	Kaczmarek & Michalczyk 2006
Macrobiotus priviterae	Binda *et al.* 2001	*Minibiotus xavieri*	Fontoura *et al.* 2009
Macrobiotus pseudofurcatus	Guil 2002	*Murrayon dianae*	Jönsson 2003
Macrobiotus radiatus	Guil 2002	*Murrayon hibernicus*	Kaczmarek & Michalczyk 2006
Macrobiotus ragonesei	Binda *et al.* 2001	*Murrayon pullari*	Guil 2002
Macrobiotus rawsoni	Horning *et al.* 1978	*Murrayon stellatus*	Bartels & Nelson 2009
Macrobiotus recens	Horning *et al.* 1978	*Parhexapodibius pilatoi*	Hinton & Meyer 2009
Macrobiotus reinhardti	Michalczyk & Kaczmarek 2003	*Paramacrobiotus alekseevi*	Beasley & Miller 2007
Macrobiotus sapiens	Bertolani pers. comm. 2010	*Paramacrobiotus areolatus*	Kaczmarek & Michalczyk 2006
Macrobiotus sklodowskae	Michalczyk *et al.* 2006	*Paramacrobiotus danielae*	Pilato *et al.* 2001
Macrobiotus snaresensis	Horning *et al.* 1978	*Paramacrobiotus derkai*	Degma *et al.* 2008
Macrobiotus spectabilis	Kaczmarek & Michalczyk 2006	*Paramacrobiotus halei*	Bartels & Nelson 2009
Macrobiotus subjulietae	Horning *et al.* 1978	*Paramacrobiotus huziori*	Michalczyk & Kaczmarek 2006b
Macrobiotus szeptyckii	Kaczmarek & Michalczyk 2009	*Paramacrobiotus richtersi*	Kaczmarek & Michalczyk 2006
Mesocrista spitzbergensis	Kathman & Cross 1991	*Paramacrobiotus tonollii*	Hooie & Davison 2001
Milnesium asiaticum	Beasley & Miller 2007	*Parhexapodibius pilatoi*	Hinton & Meyer 2009
Milnesium cf. *eurystomum*	Kaczmarek & Michalczyk 2006	*Platicrista angustata*	Jönsson 2003
Milnesium longiungue	Beasley & Miller 2007	*Platicrista cheleusis*	Kathman & Cross 1991
Milnesium tardigradum	Kaczmarek & Michalczyk 2006	*Platicrista horribilis*	Nelson & Bartels 2007
Minibiotus aculeatus	Horning *et al.* 1978	*Platicrista ramsayi*	Marley 2006
Minibiotus africanus	Binda *et al.* 2001	*Ramajendas renaudi*	Jennings 1979
Minibiotus continuus	Pilato & Lisi 2006	*Ramajendas frigidus*	Miller *et al.* 1996
Minibiotus fallax	Claxton 1998	*Ramazzottius baumanni*	Kathman & Cross 1991
Minibiotus gumersindoi	Fontoura *et al.* 2008	*Ramazzottius novemcinctus*	Guil 2002
Minibiotus hufelandioides	Guil 2002	*Ramazzottius oberhaeuseri*	Kaczmarek & Michalczyk 2006
Minibiotus intermedius	Kaczmarek & Michalczyk 2006	*Richtersius coronifer*	Kaczmarek & Michalczyk 2006
Minibiotus cf. *poricinctus*	Schuster & Greven 2007	*Thulinius augusti*	Guil 2002
Minibiotus pustulatus	Hooie & Davison 2001		
Minibiotus cf. *scopulus*	Schuster & Greven 2007		

Summary

The patchy distribution of tardigrades may be due to dispersal. The tardigrades (unlike their mossy habitats) are not easily blown 100 cm by typical winds. Nevertheless, they easily survive space travel on during those uncommon events of dispersal. Highly resistant eggs may be a common means of dispersal.

The most common genera are ***Echiniscus***, ***Hypsibius***, ***Macrobiotus*** and segregate genera, ***Milnesium***, and ***Ramazzottius***. Many taxa are widespread, and many more are likely to be discovered. Cryptic species are likely.

Despite their cosmopolitan distribution and widespread occurrence in moss, lichen, and certain soil habitats, it is relatively easy to find new species anywhere in the world. Whole states in the USA (16!) lack any tardigrade records.

Literature Cited

Acevedo, C. A. 2008. Analyzing the active time and dissolved oxygen levels of *Melnesium* (sic) *tardigradum* in its anoxybiotic state in both acidic and basic conditions. Biology 402 Senior Seminar, Research project and thesis. Available at AccessScience@McGraw-Hill <http://www.accessscience.com>.

Altiero, T., Guidetti, R., Caselli, V., Cesari, M., and Rebecchi, L. 2011. Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades. J. Zool. Syst. Evol. Res. 49 (Suppl 1): 98-103.

Barron, George. 2007. War of the Microworlds. #1. Accessed on 25 January 2010 at <http://www.uoguelph.ca/~gbarron/MISC2007/aug2007.htm>.

Barron, George. 2008. War of the Microworlds. # 6. Accessed on 6 February 2010 at <http://www.uoguelph.ca/~gbarron/2008/april.htm>.

Barron, George. 2009. War of the Microworlds. How do I kill Thee? Let me count the Ways! # 16. Accessed on 28 February 2009 at <http://www.uoguelph.ca/~gbarron/2008/catenar0.htm>.

Barron, G. L., Morikawa, C., and Saikawa, M. 1990. New *Cephaliophora* species capturing rotifers and tardigrades. Can. J. Bot. 68: 685-690.

Bartels, P. 2005. "Little known" water bears? ATBI Quarterly 6(2): 4.

Bartels, P. J. and Nelson, D. R. 2007. An evaluation of species richness estimators for tardigrades in the Great Smoky Mountains National Park. J. Limnol. 66(Suppl. 1): 104-110.

Bartels, P. J. and Nelson, D. R. 2009. Status report on the multihabitat inventory of tardigrades in the Great Smoky Mountains National Park (NC & TN, USA). 1 p.

Bartels, P. J., Nelson, D. R., Kaczmarek, Ł. and Michalczyk, Ł. 2007. Doryphoribius smokiensis, a new species of Eutardigrada (Hypsibiidae) from the Great Smoky Mountains National Park, TN, USA (North America). Zootaxa 1646: 59-65.

Beasley, C. W. and Miller, W. R. 2007. Tardigrada of Xinjiang Uygur Autonomous Region, China. Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 49-55.

Beasley, C. W., Kaczmarek, Ł. and Michalczyk, Ł. 2006. New records of tardigrades from China, with zoogeographical remarks. Biol. Lett. 2006, 43: 13-20.

Beasley, C. W., Kaczmarek, Ł, and Michalczyk, Ł. 2008. Doryphoribius mexicanus, a new species of Tardigrada (Eutardigrada: Hypsibiidae) from Mexico (North America). Proc. Biol . Soc. Wash. 121: 34-40.

Becquerel, P. 1950. La suspension de la vie au dessous de 1/20 K absolu par demagnetization adiabatique de l'alun de fer dans le vide les plus eléve. C. R. Hebd. Séance. Acad. Sci. Paris 231: 26.

Bertolani, R., and Rebecchi, L. 1993. A revision of the Macrobiotus hufelandi group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades. Zool. Scripta 22: 127-152.

Bertolani, R., Altiero, T., and Nelson, D. R. 2009. Tardigrada (water bears). Chapter 188. In: Likens, G. E. (ed.). Volume 2. Encyclopedia of Inland Waters. Elsevier, Oxford, pp. 443-465.

Binda, M. G., Pilato, G., Moncada, E., and Napolitano, A. 2001. Some tardigrades from Central Africa with the description of two new species: Macrobiotus ragonesei and M. priviterae (Eutardigrada Macrobiotidae). Trop. Zool. 14: 233-242.

Blaxter, M., Elsworth, B., and Daub, J. 2004. DNA taxonomy of a neglected animal phylum: An unexpected diversity of tardigrades. Proc. Royal Soc. Lond. B (Suppl.) 271: S189-S192.

Bromley, C. 2009. Do buildings in Cambridge, United Kingdom act as "islands" for Tardigrada and associated meiofauna? In: Greven, H., Hohberg, K., and Schill, R. O. 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009.

Brown, J. W. 1987. The peninsular effect in Baja California: An entomological assessment. J. Biogeogr. 14: 359-365.

Busack, S. D. and Hedges, S. B. 1984. Is the peninsular effect a red herring? Amer. Nat. 123: 266-275.

Christenberry, D. 1979. On the distribution of Echiniscus kofordi and E. cavagnaroi (Tardigrada). Trans. Amer. Microsc. Soc. 98: 469-471.

Christenberry, D. and Higgins, R. P. 1979. A new species of Pseudodiphascon (Tardigrada) from Alabama. Trans. Amer. Microsc. Soc. 98: 508-514.

Claxton, S. 1998. A revision of the genus Minibiotus (Tardigrada: Macrobiotidae) with descriptions of eleven new species from Australia. Records Austral. Mus. 50: 125-160.

Claxton, S. 1999. Milnesioides exsertum gen. n. sp., a new tardigrade from Australia (Tardigrada: Milnesiidae). In: Greven, H. (ed.). Proceedings of the Seventh International Symposium on the Tardigrada, August 1997, Düsseldorf, Germany. Zool. Anz. 238: 183-190.

Clifford, H. F. 2005. Tardigrada (Water Bears). Aquatic Invertebrates of Alberta. Accessed on 14 February 2010 at <http://sunsite.ualberta.ca/Projects/Aquatic_Invertebrates/?Page=21>.

Collins, M. and Bateman, L. 2001. The ecological distribution of tardigrades in Newfoundland. Zool. Anz. 240: 291-297.

Dastych, H. 1997. A new species of the genus Echiniscus (Tardigrada) from New Zealand. Entomol. Mitt. zool. Mus. Hamburg 12: 209-215.

Deep Data. 2009. Microorganisms. Accessed 5 August 2009 at <http://hostprods.net/ongoing/deep-data-prototype/>.

Degma, P., Bertolani, R., and Guidetti, R. 2010. Actual checklist of Tardigrada species (Ver. 10: 15-12-2009). Accessed on 23 January 2010 at <http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf>.

Degma, P., Michalczyk, Ł., and Kaczmarek, Ł. 2008. Macrobiotus derkai, a new species of Tardigrada (Eutardigrada, Macrobiotidae, huziori group) from the Colombian Andes (South America). Zootaxa 1731: 1-23.

Dewel, W. C. and Dewel, R. A. 1987. Study of a moss community containing Milnesium tardigradum parasitized by a chytridiomycetous fungus. In: Bertolani, R. (ed.). Selected Symposia and Monographs U.Z.I., 1, Mucchi, Modena, pp. 45-56.

Doyère, P. L. N. 1842. Memories sur les tardigrades. Sur le facilité que possedent les tardigrades, les rotifers, les anguillules des toits et quelques autres of animalcules, de revenir à la vie après été complétement déssechées. Ann. Sci. Nat. (Ser. 2) 18: 5.

Faurby, S., Jönsson, K. I., Rebecchi, L., and Funch, P. 2008. Variation in anhydrobiotic survival of two eutardigrade morphospecies: a story of cryptic species and their dispersal. J. Zool. 275: 139-145.

Fontoura, P., Pilato, G., and Lisi, O. 2008. New records of eutardigrades (Tardigrada) from Faial and Pico Islands, the Azores, with the description of two new species. Zootaxa 1778: 37-47.

Fontoura, P., Pilato, G., Morais, P., and Lisi, O. 2009. Minibiotus xavieri, a new species of tardigrade from the Parque Biológico de Gaia, Portugal (Eutardigrada: Macrobiotidae). Zootaxa 2267: 55-64.

Franceschi, T., Loi, M. L., and Pierantoni, R. 1962-1963. Risultati di una prima indagine ecologica condotta su popolazioni di Tardigradi. Bollettino dei Musei e degli Istituti Biologici dell'Universita di Genova 32: 69-93.

Gavaret, J. 1859. Quelques expériences sur les rotifères, les tardigrades et les anguillules des mousses des toits. Ann. Sci. Nat. (Ser. 4) 11: 315.

Guidetti, R., Boschini, D., Altiero, T., Bertolani, R., and Rebecchi, L. 2008. Diapause in tardigrades: A study of factors involved in encystment. J. Exper. Biol. 211: 2296-2302.

Guil, N. 2002. Diversity and distribution of tardigrades (Bilateria, Tardigrada) from the Iberian Peninsula, Balearic Islands and Chafarinas Islands. Graellsia 58(2): 75-94.

Guil, N. and Cabrero-Sañudo, F. 2000. Analysis of the species description process for a little known invertebrate group: The limnoterrestrial tardigrades (Bilateria, Tardigrada). Biodiv. Conserv. 16: 1063-1086.

Guil, N., Hortal, J., Sánchez-Moreno, S., and Machordom, A. 2009. Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment. Landscape Ecol. 24: 375-390.

Hinton, J. G. and Meyer, H. A. 2009. Tardigrades from Fayette County, Georgia. Georgia J. Sci. 67(2): 30-32.

Hohberg, K. 2006. Tardigrade species composition in young soils and some aspects on life history of *Macrobiotus richtersi* J. Murray, 1911. Pedobiologia 50: 267-274.

Hohl, A. M., Miller, W. R., and Nelson, D. R. 2001. The distribution of tardigrades upwind and downwind of a Missouri coal-burning power plant. Zool. Anz. 240: 395-401.

Hooie, A. K. and Davison, P. G. 2001. Tardigrade diversity in the moss *Ulota crispa* from tree canopies in the Great Smoky Mountains National Park – A preliminary report. J. Alabama Acad. Sci. 72: 91. Abstract.

Horning, D. S., Schuster, R. O., and Grigarick, A. A. 1978. Tardigrada of New Zealand. N. Zeal. J. Zool. 5: 185-280.

Iharos, G. 1966. Neue tardigraden-arten aus Ungarn (Neuere beiträge zur kenntnis der tardigraden-fauna Ungarns, VI.). Acta Zool. Acad. Scien. Hung. 12: 111-122.

Ito, M. 1993. Taxonomic study on the class Heterotardigrada (Tardigrada) from the northern slope of Mt. Fuji, Central Japan. Edaphologia 59: 1-13.

Janiec, K. 1996. Short distance wind transport of microfauna in maritime Antarctic (King George Island, South Shetland Islands). Polish Pol.Res. 17: 203-211.

Jansson, H.-B. and Thiman, L. 1992. A preliminary study of chemotaxis of zoospores of the nematode-parasitic fungus *Catenaria anguillulae*. Mycologia 84: 109-112.

Jenkins, D. G. and Rinne, D. 2008. Red herring or low illumination? The peninsula effect revisited. J. Biogeogr. 35: 2128-2137.

Jennings, P. G. 1979. The Signy Island terrestrial reference sites: X. Population dynamics of Tardigrada and Rotifera. Brit. Antarct. Surv. Bull. 47: 89-105.

Johansson, C., Calloway, S., Miller, W. R., and Linder, E. T. 2011. Are urban and rural tardigrade (Tardigrada) communities distinct and determined by pH: A case study from Fresno County, California. Pan-Pacific Entomol. 87(2): 86-97.

Jönsson, K. I. 2003. Population density and species composition of moss-living tardigrades in a boreo-nemoral forest. Ecography 26: 356-364.

Jönsson, K. Ingemar. 2008. Tardigrades In Space (TARDIS). Space tardigrades stood the test! Accessed on 7 August 2009 at <http://tardigradesinspace.blogspot.com/>.

Jönsson, K. I., Harms-Ringdahl, M., and Torudd, J. 2005. Radiation tolerance in the eutardigrade *Richtersius coronifer*. Internat. J. Radiation Biol. 81: 649-656.

Jönsson, K. I., Rabbow, E., Schill, R. O., Harms-Ringdahl, M., and Rettberg, P. 2008. Tardigrades survive exposure to space in low Earth orbit. Current Biol. 18: R729-R731 (9 September 2008).

Jorgensen, A., Mobjerg, N., and Kristensen, R. M. 2007. A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. Proceedings of the Tenth International Symposium on Tardigrada. J. Limnol. 66(Suppl. 1): 77-83.

Kaczmarek, Ł. 2003. New records of and key to Tardigrada from Costa Rica. Zootaxa 177: 1-4.

Kaczmarek, Ł. and Michalczyk, Ł. 2002. *Echiniscus barbarae*, a new species of tardigrade from Cuba Island (Tardigrada: Heterotardigrada, Echiniscidae, 'arctomys' group'). Zootaxa 53: 1-4.

Kaczmarek, Ł. and Michalczyk, Ł. 2004a. First record of the genus *Doryphoribius* Pilato, 1969 from Costa Rica (Central America) and description of a new species *Doryphoribius*

quadrituberculatus (Tardigrada: Hypsibiidae). Genus 15: 447-453.

Kaczmarek, Ł. and Michalczyk, Ł. 2004b. Notes on some tardigrades from South Africa, with the description of *Diphascon* (*Diphascon*) *zaniewi* sp. nov. (Eutardigrada: Hypsibiidae). Zootaxa 576: 1-6.

Kaczmarek, Ł. and Michalczyk, Ł. 2004c. New records of Tardigrada from Cyprus with a description of the new species *Macrobiotus marlenae* (*hufelandi* group) (Eutardigrada: Macrobiotidae). Genus 15: 141-152.

Kaczmarek, Ł. and Michalczyk, Ł. 2006. The Tardigrada fauna of Mongolia (Central Asia) with a description of *Isohypsibius altai* sp. nov. (Eutardigrada: Hypsibiidae). Zool. Stud. 45(1): 11-23.

Kaczmarek, Ł. and Michalczyk, Ł. 2009. Two new species of Macrobiotidae, *Macrobiotus szeptyckii* (*harmsworthi* group) and *Macrobiotus kazmierskii* (*hufelandi* group) from Argentina. Acta Zool. Cracoviensia, Seria B - Invertebrata 52: 87-99.

Kaczmarek, Ł., Michalczyk, Ł., and Degma, P. 2005. A new species of Tardigrada *Bryodelphax brevidentatus* sp. nov. (Heterotardigrada: Echiniscidae) from China (Asia). Zootaxa 1080: 33-38.

Kaczmarek, Ł., Michalczyk, Ł., and Degma, P. 2007. Description of a new Tardigrade, *Macrobiotus barbarae* (Eutardigrada: Macrobiotidae) from the Dominican Republic. Ann. Zool. 57: 363-369.

Kathman, R. D. and Cross, S. F. 1991. Ecological distribution of moss-dwelling tardigrades on Vancouver Island, British Columbia, Canada. Can. J. Zool. 69: 122-129.

Keilin, D. 1959. The problem of anabiosis or latent life: History and current concept. Proc. Royal Soc. London B 150: 149-191.

Kristensen, R. M., Michalczyk, Ł., and Kaczmarek, Ł. 2010. The first record of the genus *Bryodelphax* (Tardigrada: Heterotardigrada: Echiniscidae) from Easter Island, Rapa Nui (Pacific Ocean, Chile) with the description of a new species, *Bryodelphax aaseae*. Zootaxa 2343: 45-56.

Land, J. Van der. 1964. A new peritrichous ciliate as a symphoriont on a tardigrade. Zool. Mededelingen 1964: 85-88.

Letcher, P. M. and Powell, M. J. 2002. Frequency and distribution patterns of zoosporic fungi from moss-covered and exposed forest soils. Mycologia 94: 761-771.

Li, X.-C. 2009. Revision of *Biserovus xiae* Li, Su & Yu, 2004 (Tardigrada: Macrobiotidae). Zootaxa 2076: 63-68.

Li, X. and Wang, L. 2005. *Isohypsibius taibaiensis* sp. nov. (Tardigrada, Hypsibiidae) from China. Zootaxa 1036: 55-60.

Li, X.-C., Wang, L.-Z., and Yu, D. 2005. *Isohypsibius qinlingensis* sp. nov. (Tardigrada, Hypsibiidae) from China. Zootaxa 980: 1-4.

Li, X.-C., Wang, L.-Z., and Yu, D. 2007. The Tardigrada fauna of China with descriptions of three new species of Echiniscidae. Zool. Stud. 46(2): 135-147.

Lindahl, Karen and Balser, Susie. 1999. Tardigrade Facts. Illinois Wesleyan University. Accessed on 29 April 2009 at <http://www.iwu.edu/~tardisdp/tardigrade_facts.html>.

Madrigal, Alexis. 2009. Can Life Survive Deep Space? Let's Send It There! Wired Science 9 October 2009. Accessed on 28 February 2010 at <http://www.wired.com/wiredscience/2009/10/can-life-survive-deep-space-lets-send-it-there/>.

Manicardi, G. C. 1989. Two new species of soil moss eutardigrades (Tardigrada) from Canada. Can. J. Zool. 67: 2282-2285.

Margulis, L. and Chapman, M. J. 1998. Kingdoms & Domains. An Illustrated Guide to the Phyla of Life on Earth. W. H. Freeman & Co., New York.

Marley, N. J. 2006. A new species of Tardigrada, *Platicrista ramsayi* sp. nov. from the páramo of Volcán Chiles, Ecuador. Zootaxa 1166: 35-48.

May, R. M., Maria, M., and Guimard, J. 1964. Action différentielle des rayons x et ultraviolets sur le tardigrade *Macrobiotus areolatus*, a l'état actif et desséché. Bull. Biol. France Belgique 98: 349-367.

McInnes, S. J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. J. Nat. Hist. 28: 257-352.

McInnes, S. J. 2003. A predatory fungus (Hyphomycetes: *Lecophagus*) attacking Rotifera and Tardigrada in maritime Antarctic lakes. Polar Biol. 26: 79-82.

McInnes, S. and Pugh, P. 1998. Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. J. Biogeogr. 25: 31-36.

McInnes, S. J. and Convey, P. 2005. Tardigrade fauna of the South Sandwich Islands, maritime Antarctic. Zootaxa 1058: 43-49.

Mehlen, R. H. 1969. Tardigrada: Taxonomy and distribution in Costa Rica. Trans. Amer. Microsc. Soc. 88: 498-505.

Meininger, C. A. and Spatt, P. D. 1988. Variations of tardigrade assemblages in dust-impacted Arctic mosses. Arct. Alp. Res. 20: 24-30.

Meininger, C. A., Uetz, G. W., and Snider, J. A. 1985. Variation in epiphytic microcommunities (tardigrade-lichen-bryophyte assemblages) of the Cincinnati, Ohio area. Urban Ecol. 9(1): 45-62.

Meyer, H. A. 2008. Distribution of tardigrades in Florida. Southeast. Nat. 7: 91-100.

Meyer, H. A. and Hinton, J. G. 2007. Limno-terrestrial Tardigrada of the Nearctic realm. J. Limnol. 66(Suppl. 1): 97-103.

Michalczyk, Ł. and Kaczmarek, Ł. 2003. A description of the new tardigrade *Macrobiotus reinhardti* (Eutardigrada: Macrobiotidae, *harmsworthi* group) with some remarks on the oral cavity armature within the genus *Macrobiotus* Schultze. Zootaxa 331: 1-24.

Michalczyk, Ł. and Kaczmarek, Ł. 2005. The first record of the genus *Calohypsibius* Thulin, 1928 (Eutardigrada: Calohypsibiidae) from Chile (South America) with a description of the new species *Calohypsibius maliki*. N. Z. J. Zool. 32: 287-292.

Michalczyk, Ł. and Kaczmarek, Ł. 2006a. Revision of the *Echiniscus bigranulatus* group with a description of a new species *Echiniscus madonnae* (Tardigrada: Heterotardigrada: Echiniscidae) from South America. Zootaxa 1154: 1-26.

Michalczyk, Ł. and Kaczmarek, Ł. 2006b. *Macrobiotus huziori*, a new species of Tardigrada (Eutardigrada: Macrobiotidae) from Costa Rica (Central America). Zootaxa 1169: 47-59.

Michalczyk, Ł., and Kaczmarek, Ł. 2007. *Echiniscus ganczareki*, a new species of Tardigrada (Heterotardigrada: Echiniscidae, *bigranulatus* group) from Costa Rica. Zootaxa 1471: 15-25.

Michalczyk, Ł., Kaczmarek, Ł., and Weglarska, B. 2006. *Macrobiotus sklodowskae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae, *richtersi* group) from Cyprus. Zootaxa 1371: 45-56.

Miller, W. R., Miller, J. D., and Heatwole, H. 1996. Tardigrades of the Australian Antarctic Territories: The Windmill Islands, East Antarctica. Zool. J. Linn. Soc. 116: 175-184.

Miller, W. R., Heatwole, H., Pidgeon, R. W. J., and Gardiner, G. R. 1994. Tardigrades of the Australian Antarctic Territories: The Larsemann Hills, East Antarctica. Trans. Amer. Microsc. Soc. 113: 142-160.

Milstein, M. 2009. World's Hardiest Organisms to Face 3 Years in Space. Popular Mechanics January 2009. Accessed on 28 February 2010 at <http://www.popularmechanics.com/science/air_space/4295 922.html>.

Moly de Peluffo, M. C., Peluffo, J. R., Rocha, A. M., and Doma, I. L. 2006. Tardigrade distribution in a medium-sized city of Central Argentina. Hydrobiologia 558: 141-150.

Morgan, C. I. 1976. Studies on the British tardigrade fauna. Some zoogeographical and ecological notes. J. Nat. Hist. 10: 607-632.

Morgan, C. I. and King, P. E. 1976. British Tardigrada. Tardigrada keys and notes for the identification of the species. Synopses of the British Fauna, No. 9. Academic Press, London, 132 pp.

Mullen, Leslie. 2002. Extreme Animals. Astrobiology Magazine. Accessed on 2 February 2010 at <http://www.astrobio.net/exclusive/261/extreme-animals>.

Nelson, D. R. 1991. Tardigrada. Chapt. 15. In: Thorp, J. H. and Covich, A. P. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, New York, pp. 501-521.

Nelson, D. R. 2002. Current status of the Tardigrada: Evolution and ecology. Integrat. Compar. Biol. 42: 652-659.

Nelson, D. R. and Adkins, R. G. 2001. Distribution of tardigrades within a moss cushion: Do tardigrades migrate in response to changing moisture conditions? Zool. Anzeiger 240: 493-500.

Nelson, D. R. and Bartels, P. J. 2007. "Smoky Bears" – Tardigrades of Great Smoky Mountains National Park. Southeast. Nat. Spec. Iss. 1: 229-238.

Nelson, J. and Hauser, D. 2012. A survey of invertebrate communities on epiphytic mosses and liverworts in Tryon Creek State Natural Area. Unpublished Undergraduate Report, Lewis & Clark College, Portland, OR.

Peluffo, J. R., Rocha, A. M., and Moly de Peluffo, M. C. 2007. Species diversity and morphometrics of tardigrades from a medium-size city in the Neotropical Region: Santa Rosa (La Pampa, Argentina). Anim. Biodiv. Conserv. 30.1: 43-51.

Pennak, R. W. 1953. Fresh-water Invertebrates of the United States. Ronald Press Co., N. Y., 769 pp.

Persson, D., Halberg, K. A., Jorgensen, A., Ricci, C., Mobjerg, N., and Kristensen, R. M. 2011. Extreme stress tolerance in tardigrades: Surviving space conditions in low Earth orbit. J. Zool. Syst. Evol. Res. 49 (Suppl 1): 90-97.

Persson, D., Ricci, C., Halberg, K. A., and Kristensen, R. M. 2009. Extreme stress tolerance in tardigrades – Surviving space conditions in low earth orbit. In: Greven, H., Hohberg, K., and Schill, R. O. (eds.). 11th International Symposium on Tardigrada. Conference Guide. Tübingen, Germany, 3-6 August 2009.

Pilato, G. 1974. Tre nuove specie di Tardigradi muscicoli di Cina. [Three new species of Tardigrada from mosses in China.]. Animalia 1(1-3): 59-68.

Pilato, G. 1979. Correlations between cryptobiosis and other biological characteristics in some soil animals. Bol. Zool. 46: 319-342.

Pilato, G. 1987. Revision of the genus *Diphascon* Plate, 1889, with remarks on the subfamily Itaquasconinae (Eutardigrada, Hypsibiidae). In: Bertolani, R. (ed.). Biology of Tardigrades. Proceedings of the Fourth International

Symposium on the Tardigrada, September 1985, Modena, Italy. Selected Symposia and Monographs 1: 337-357.

Pilato, G. 2009. *Bindius triquetrus* gen. nov. sp. nov. (Eutardigrada, Hypsibiidae) from Sicily (Italy). Zootaxa 2058: 62-68.

Pilato, G. and Bertolani, R. 2005. *Diphascon* (*Diphascon*) *dolomiticum*, a new species of Hypsibiidae (Eutardigrada) from Italy. Zootaxa 914: 1-5.

Pilato, G. and Lisi, O. 2006. Notes on some tardigrades from southern Mexico with description of three new species. Zootaxa 1236: 53-68.

Pilato, G. and Pennisi, G. 1976. Prime notizie sui Tardigradi di Cirenaica. [First account on Tardigrada of Cyrenaica.]. Animalia 3(1-3): 243-258.

Pilato, G. and Sperlinga, G. 1975. Tardigradi muscicoli di Sardegna. [Moss-inhabiting Tardigrada from Sardinia.]. Animalia 2(1-3): 79-90.

Pilato, G., Binda, M. G., and Claxton, S. 2002. *Itaquascon unguiculum* and *Itaquascon cambewarrense*: Two new species of eutardigrades from Australia. N. Zeal. J. Zool. 29(2): 87-93.

Pilato, G., Binda, M. G., Napolitano, A., and Moncada, E.. 2001. Notes on South American tardigrades with the description of two new species: *Pseudechiniscus spinerectus* and *Macrobiotus danielae*. Tropical Zool. 14: 223-231.

Pilato, G., Claxton, S., and Binda, M. G. 1989. Tardigrades from Australia. II. The evaluation of *Calohypsibius ornatus* (Richters, 1900) *caelatus* (Marcus, 1928) as a valid species and description of *Minibiotus fallax* n. sp. (Eutardigrada). Animalia 16: 21-27.

Pilato, G., Fontoura, P., and Lisi, O. 2007. Remarks on the *Echiniscus viridis* group, with the description of a new species (Tardigrada, Echiniscidae). J. Limnol. 66(Suppl. 1): 33-39.

Pohlad, B. R. and Bernard, E. C. 1978. A new species of Entomophthorales parasitizing tardigrades. Mycologia 70: 130-139.

Rahm, P. G. 1923. Biologische und physiologische Beiträge zur Kenntnis der Moosfauna. Z. Allg. Physiol. 20: 1-34.

Rahm, G. 1937. A new order of tardigrades from the hot springs of Japan (Furuyu section, Unzen). Annot. Zool. Japan. 16: 345-352.

Ramazzotti, G. 1972. Phylum Tardigrada (Seconda edizione aggionata). Vol. II. Mem. Ist. Ital. Idrobiol. 28: 1-732.

Ramazzotti, G. and Maucci, W. 1983. Il Philum Tardigrada. Mem. Istit Italiano Idrobiol. 41: 1-1011.

Ramlov, H. and Westh, P. 2001. Cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*: Tolerance to alcohols, temperature and de novo protein synthesis. Zool. Anz. 240: 517-523.

Rebecchi, L., Altiero, T., Guidetti, R., Cesari, M., Bertolani, R., Negroni, M., and Rizzo, A. M. 2009a. Tardigrade resistance to space effects: First results of experiments on the LIFE-TARSE Mission on FOTON-M3 (September 2007). Astrobiology 9: 581-591.

Rebecchi, L., Cesari, M., Altiero, T., Frigieri, A., and Guidetti, R. 2009b. Survival and DNA degradation in anhydrobiotic tardigrades. J. Exper. Biol. 212: 4033-4039.

Rebecchi, L., Altiero, T., Cesari, M., Bertolani, R., Rizzo, A. M., Corsetto, P. A., and Guidetti, R. 2011. Resistance of the anhydrobiotic eutardigrade *Paramacrobiotus richtersi* to space flight (LIFE-TARSE mission on FOTON-M3). J. Zool. Syst. Evol. Res. 49 (Suppl 1): 98-103.

Robb, J. and Barron, G. L. 1982. Nature's ballistic missile. Science 218: 1221-1222.

Saikawa, M., Oyama, Y., and Yamaguchi, K. 1991. *Harposporium anguillulae* and *Haptoglossa intermedia* parasitizing tardigrades. Trans. Mycol. Soc. Japan 32: 501-508.

Sayre, R. M. and Brunson, L. K. 1971. Microfauna of moss habitats. Amer. Biol. Teacher Feb. 1971: 100-102, 105.

Schuster, R. and Greven, H. 2007. A long-term study of population dynamics of tardigrades in the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst. J. Limnol, 66(Suppl. 1): 141-151.

Schuster, R. O., Toftner, E. C., and Grigarick, A. A. 1977. Tardigrada of Pope Beach, Lake Tahoe, California. Wasmann J. Biol. 35: 115-136.

Seki, K. and Toyoshima, M. 1998. Preserving tardigrades under pressure. Nature 395: 853-854.

Séméria, Y. 1982. Recherches sur la faune urbaine et semi-urbaine des tardigrades muscicoles et lichénicoles. II. L'éspace sub-urbain: Les hauteurs orientales de Nice-Ville. Bull. Soc. Linné. Lyon 51: 315:328.

Simpson, G. G. 1964. Species density of North American recent mammals. System. Zool. 13: 57-73.

Steiner, W. A. 1994a. The influence of air pollution on moss-dwelling animals: 2. Aquatic fauna with emphasis on Nematoda and Tardigrada. Rev. Suisse Zool. 101: 699-724.

Steiner, W. 1994b. The influence of air pollution on moss-dwelling animals: 4. Seasonal and long-term fluctuations of rotifer, nematode and tardigrade populations. Rev. Suisse Zool. 101: 1017-1031.

Steiner, W. A. 1995. The influence of air pollution on moss-dwelling animals: 5. Fumigation experiments with SO_2 and exposure experiments. Rev. Suisse Zool. 102: 13-40.

Sudzuki, M. 1972. [An analysis of colonization in freshwater micro-organisms. II. Two simple experiments on the dispersal by wind]. Jap. J. Ecol. 22(5): 222-225.

Suren, A. M. 1992. Meiofaunal communities associated with bryophytes and gravels in shaded and unshaded alpine streams in New Zealand. N. Z. J. Marine Freshwat. Res. 26: 115-125.

Tardigrada. 2005. Water Bears. Accessed 14 June 2010 at < http://www.answers.com/topic/tardigrada-water-bears-biological-family>.

Taylor, R. J. and Regal, P. J. 1978. The peninsular effect on species diversity and the biogeography of Baja California. Amer. Nat. 112: 583-593.

Thompson, S. 2008. Tardigrades and long-term exposure to specific pH values (*Thulinius stephaniae*). Natural Science Seminar Abstracts. University of North Alabama, Florence, Alabama, Fall 2008: 3 November.

Tumanov, D. V. 2004. *Macrobiotus kovalevi*, a new species of Tardigrada from New Zealand (Eutardigrada, Macrobiotidae). Zootaxa 406: 1-8.

Tumanov, D. V. 2005. Two new species of *Macrobiotus* (Eutardigrada, Macrobiotidae) from Tien Shan (Kirghizia), with notes on *Macrobiotus tenuis* group. Zootaxa 1043: 33-46.

Utsugi, K. and Ohyama, Y. 1989. Antarctic Tardigrada. Proc. NIPR Symp. Polar Biol. 2: 190-197.

Vargha, B., Oetvoes, E., and Tuba, Z. 2002. Investigations on ecological effects of heavy metal pollution in Hungary by moss-dwelling water bears (Tardigrada), as bioindicators. Ann. Agric. Environ. Med. 9(2): 141-146.

Venkataraman, K. 1998. Studies on Phylum Tardigrada and Other Associated Fauna, South Polar Skua and Bird and Mammal Logging During 1994 - 1995 Expedition. Fourteenth Indian Expedition to Antarctica, Scientific

Report, 1998. Department of Ocean Development, Technical Publication No. 12: 221-243.

Vicente, F., Michalczyk, Ł., Kaczmarek, Ł., and Boavida, M.-J. 2008. Observations on *Pyxidium tardigradum* (Ciliophora) a protozoan living on Eutardigrada: Infestation, morphology and feeding behaviour. Parasitol. Res. 103: 1323-1331.

Wang, L. H. and Li, X. C. 2005. *Echiniscus taibaiensis* sp. nov. and a new record of *Echiniscus bisetosus* Heinis (Tardigrada, Echiniscidae) from China. Zootaxa 1092: 39-45.

Wang, H.-P., Wang, Li.-Z., and Li, X.-C. 2007. Tardigrades from Guizhou Province (China) with description of a new species of genus *Doryphoribius* (Tardigrada: Hypsibiidae). Zootaxa 1626: 59-67.

Westh, P. and Kristensen, R.-M. 1992. Ice formation in the freeze-tolerant eutardigrades *Adorybiotus coronifer* and *Amphibolus nebulosus* studied by differential scanning calorimetry. Polar Biol. 12: 693-699.

Wit, R. de and Bouvier, T. 2006. 'Everything is everywhere, but, the environment selects;' what did Baas Becking and Beijerinck really say? Environ. Microbiol. 8: 755-758.

Wright, J. C. 1991. The significance of four xeric parametres in the ecology of terrestrial Tardigrada. J. Zool. 224: 59-77.

ZAMP Wiki. 2009. Tardigrades. Accessed on 7 August 2009 at <http://www.zampwiki.com/?t=Tardigrada>.