

CHAPTER 5-1

TARDIGRADE SURVIVAL

TABLE OF CONTENTS

Tardigrades – Water Bears.....	5-1-2
Suitability of Bryophytes as Habitat	5-1-3
Adaptations of Tardigrades	5-1-5
Survival of Hazardous Conditions	5-1-6
Physical Adaptations.....	5-1-7
Pigments	5-1-8
Physiological Adaptations.....	5-1-8
Light response.....	5-1-8
Cryptobiosis.....	5-1-9
Tun Formation	5-1-10
Dangers in a Tun.....	5-1-13
Effects of Size.....	5-1-13
Longevity.....	5-1-14
Dangers and Protective Mechanisms	5-1-15
Anhydrobiosis.....	5-1-16
Osmobiosis	5-1-17
Anoxybiosis	5-1-17
Cryobiosis	5-1-18
Temperature	5-1-19
Diapause (Encystment)	5-1-19
Eggs	5-1-21
Migration?.....	5-1-21
Summary	5-1-22
Acknowledgments.....	5-1-22
Literature Cited	5-1-22

CHAPTER 5-1

TARDIGRADE SURVIVAL



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.

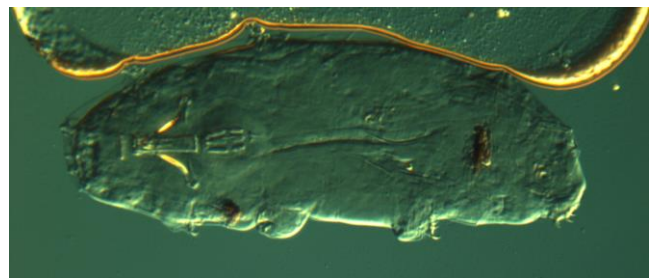


Figure 2. *Macrobiotus hufelandi*, a common tardigrade that is among those inhabiting mosses. Photo by Paul Bartels, with permission.

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).

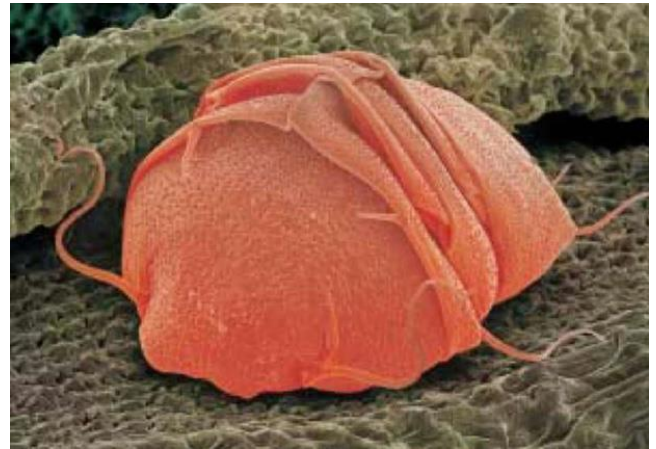


Figure 5. *Echiniscus testudo* dormant stage (tun), demonstrating the rigid nature of its armor that prevents it from extensive changes in size. Photo by Power & Syred through Creative Commons.

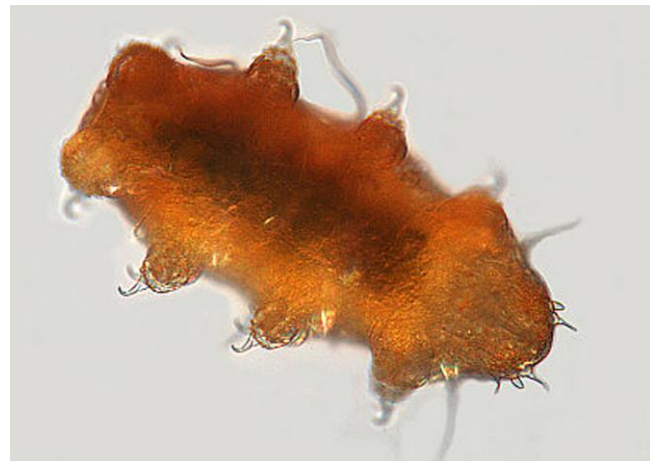


Figure 6. *Echiniscus* species (*E. testudo* occurs almost exclusively on bryophytes) seem to be unresponsive to moisture changes. Photo by Martin Mach, with permission.

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; Brueggemann 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.)

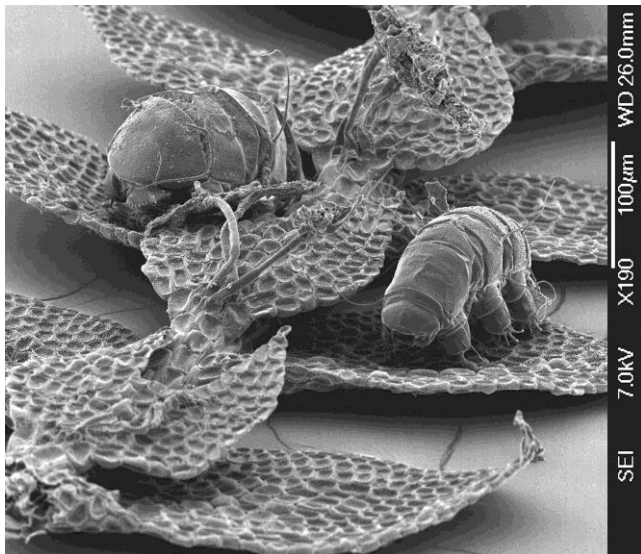


Figure 7. SEM view of tardigrades on the lower sides of leaves of a leafy liverwort. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

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?

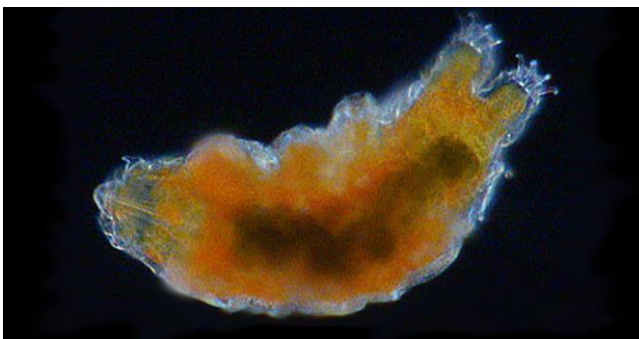


Figure 14. *Cornechiniscus cornutus*. Photo by Martin Mach, with permission.

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.

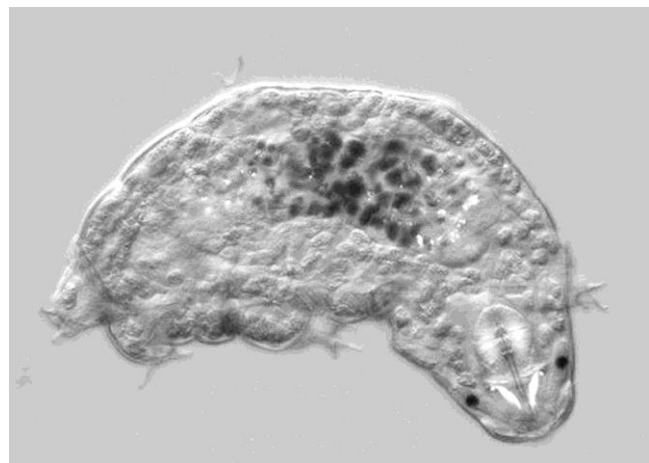


Figure 16. *Hypsibius dujardini* with the alga *Chlorococcum* in its gut. Photo by Willow Gabriel through EOL Creative Commons.



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. Hmm...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 could 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!

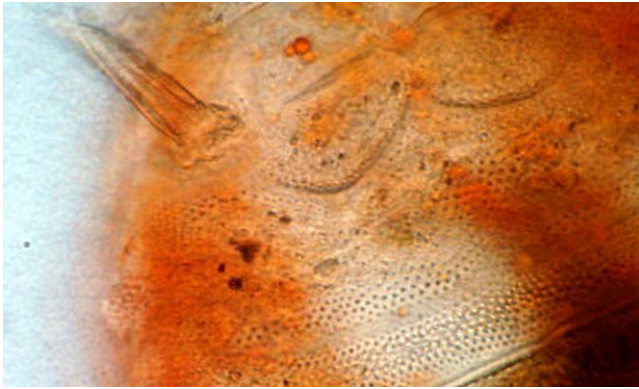


Figure 21. *Cornechiniscus cornutus* showing armor. Photo by Martin Mach, with permission.



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.

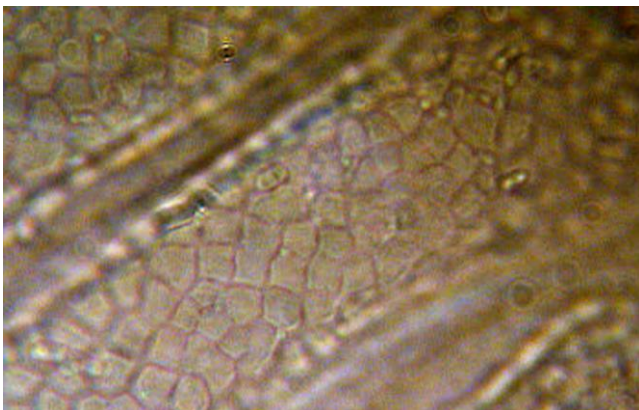


Figure 24. Armor on *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.



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 *Macrobotus 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.

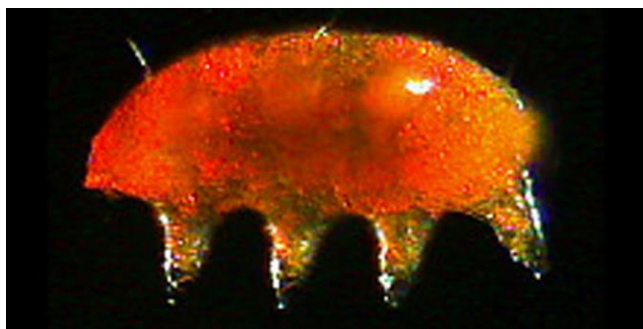


Figure 26. Adult *Echiniscus* sp., demonstrating one of the bright colors found in tardigrades. Photo by Martin Mach, with permission.



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 Leeuwenhoek 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.

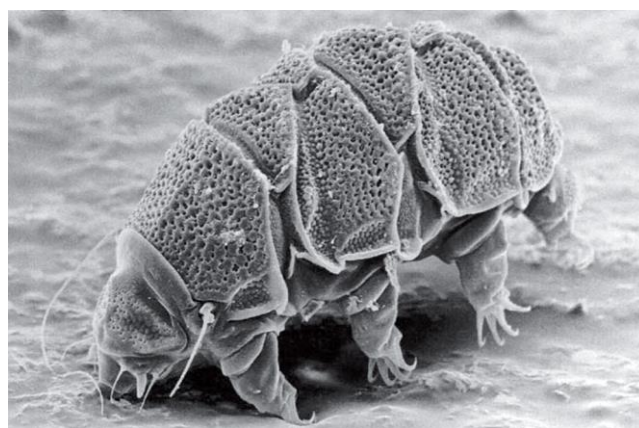


Figure 29. *Echiniscus mauccii* showing the plates that make shrinkage during desiccation all but impossible. Photo by Diane Nelson and Paul Bartels, with permission.

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)
- **anoxibiosis** (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 (-272.95°C) for 20 hours or -200°C for 20 months (Miller 1997). They have even survived 151°C 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.

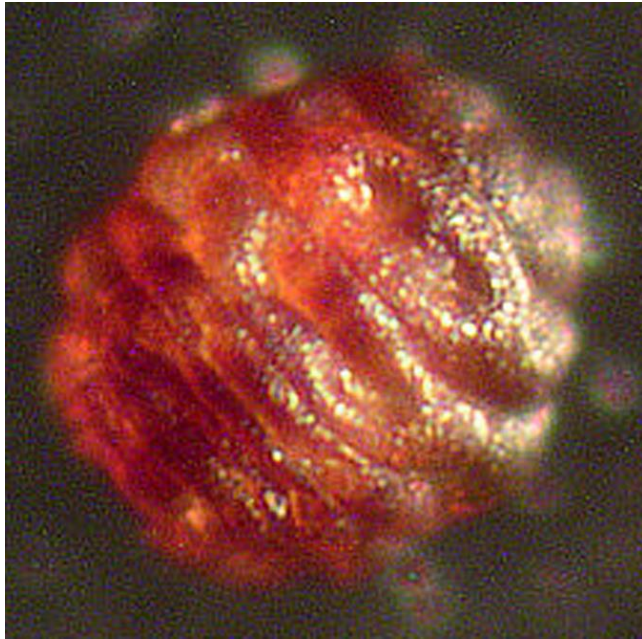


Figure 33. Tun of *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.

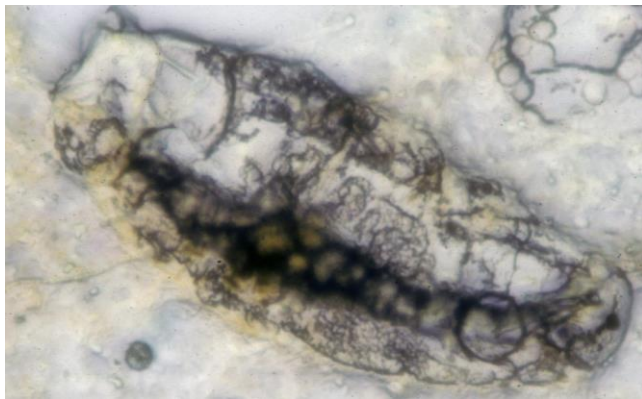


Figure 34. **Tardigrade** tun – water bear in a state of anhydrobiosis. Photo by Janice Glime.

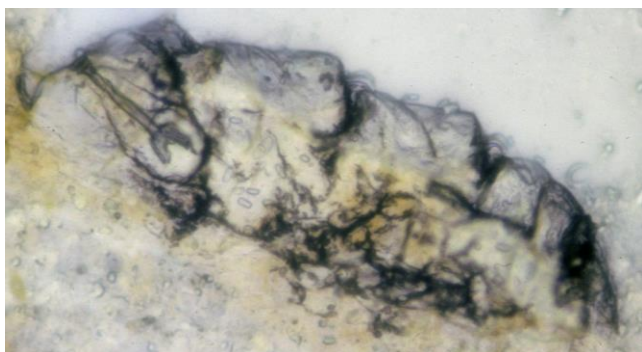


Figure 35. **Tardigrade** tun – water bear in a state of anhydrobiosis. Note the buccal apparatus (resembles a tuning fork on left end). Photo by Janice Glime.

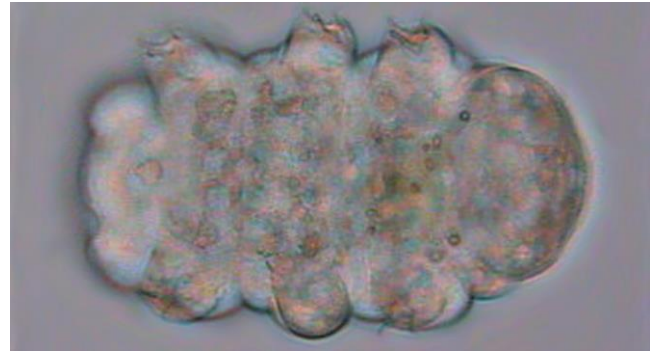


Figure 36. Tun of *Hypsibius* sp. Photo by Martin Mach, with permission.



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

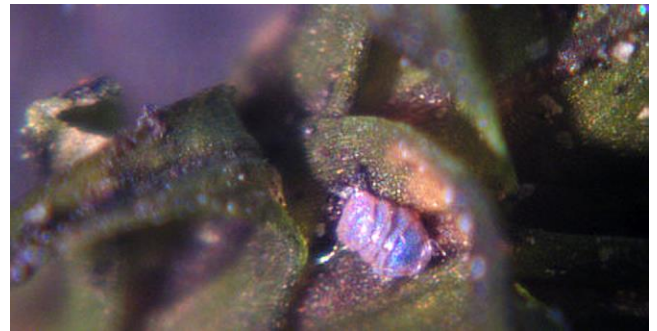


Figure 38. Tun of *Echiniscus* sp. on moss leaf. Photo by Martin Mach, with permission.

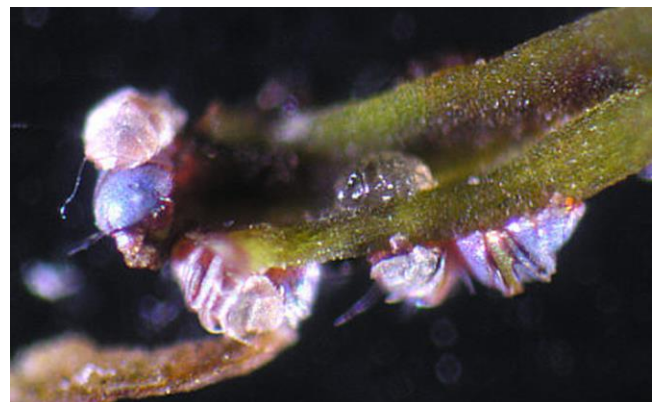


Figure 39. Multiple tuns of *Echiniscus* sp. on a single moss leaf. Photo by Martin Mach, with permission.

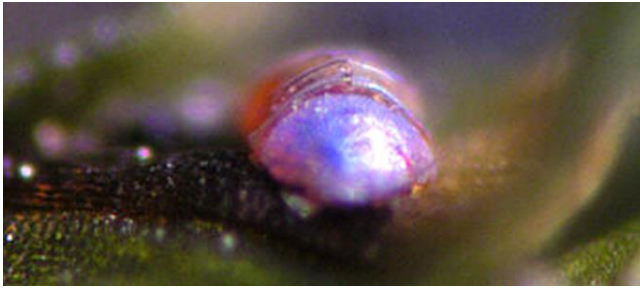


Figure 40. Tun of *Echiniscus* sp. on moss leaf. Photo by Martin Mach, with permission.

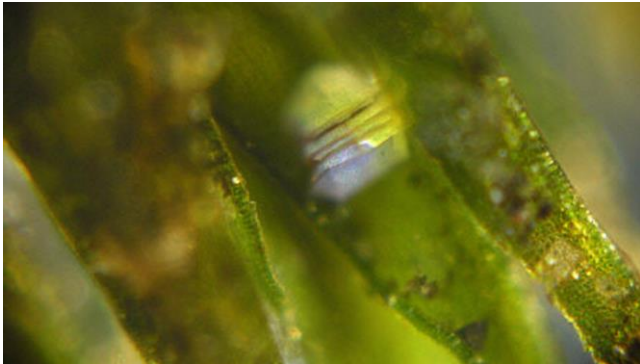


Figure 41. Tun of *Echiniscus* sp. on a moss leaf. Photo by Martin Mach, with permission.

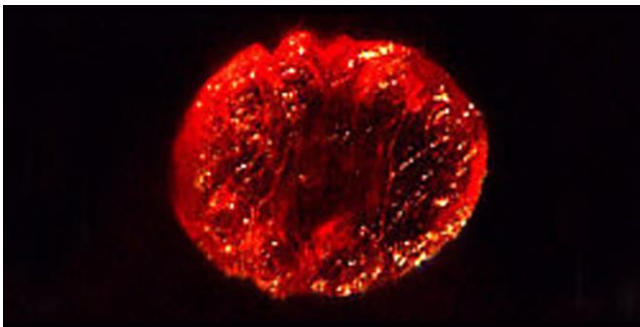


Figure 42. Tun of *Echiniscus* sp. Photo by Martin Mach, with permission.

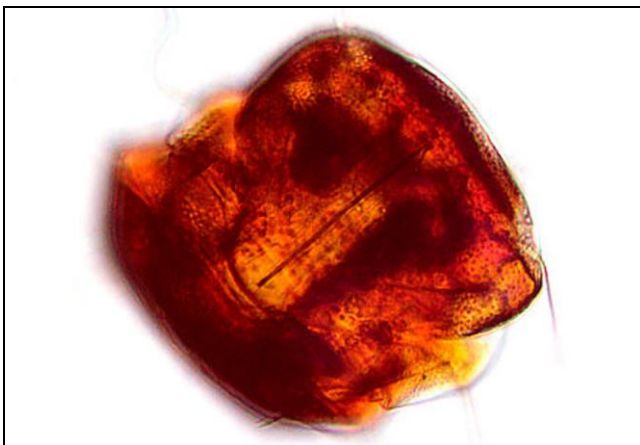


Figure 43. Tun of *Echiniscus* sp. Photo by Martin Mach, with permission.

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.

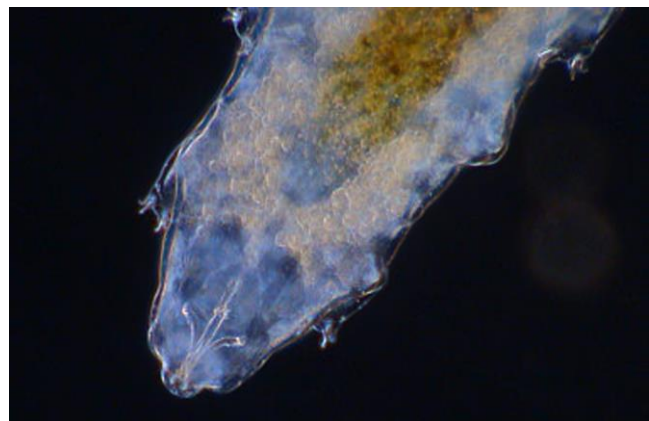


Figure 44. *Paramacrobiotus areolatus*. Photo by Martin Mach, with permission.

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.

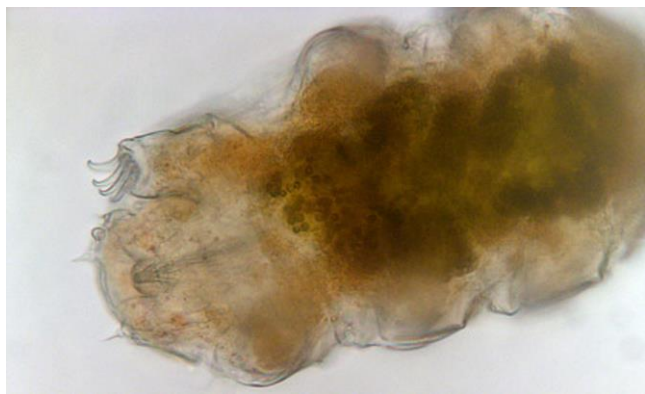


Figure 45. *Echiniscus* sp. rehydrated after four years of desiccation. Photo by Martin Mach, with permission.

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 μ m 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.

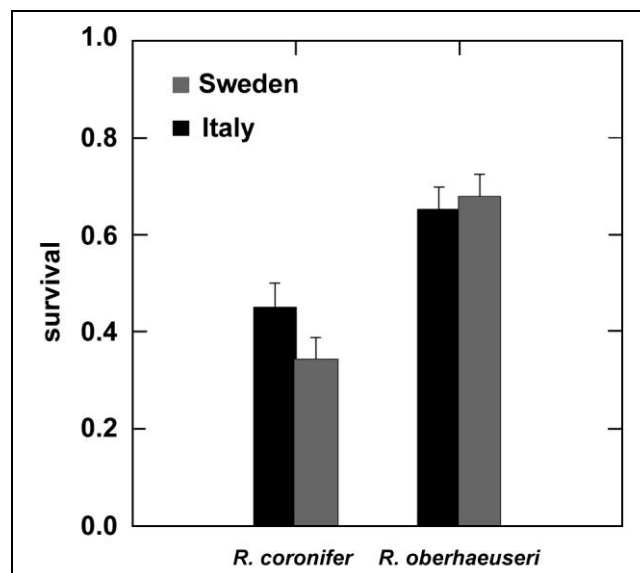


Figure 47. Comparison of survival during encystment for *Richtersius coronifer* and *Ramazzottius oberhaeuseri* from Italy and Sweden. Vertical line represents standard error. Redrawn from Bertolani *et al.* 2004, based on Jönsson *et al.* 2001.

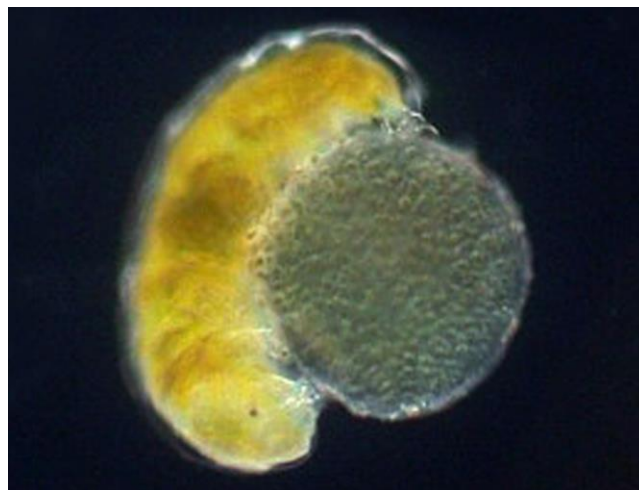


Figure 48. *Richtersius coronifer*, clinging to an algal cell. Photo by Martin Mach, with permission.

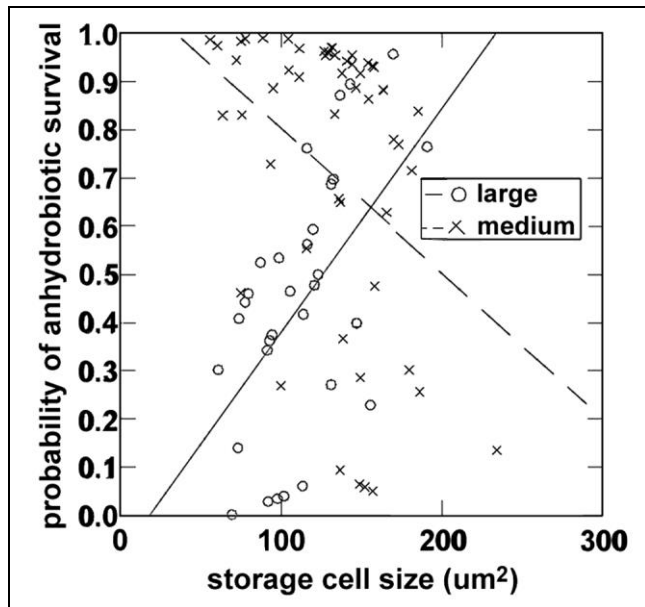


Figure 49. Probability of survival from anhydrobiosis for large and medium-sized *Richtersius coronifer* as a function of storage cell size. Probability is based on the predicted values from a logistic regression model, using buccal tube length, category, storage cell size, and interaction between the 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.

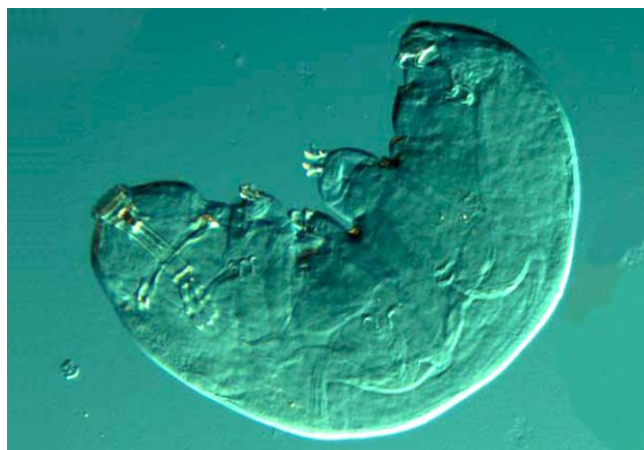


Figure 50. *Paramacrobiotus tonollii*. Photo by P. J. Bartels, with permission.



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.

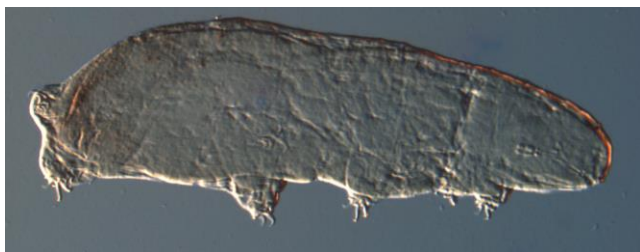


Figure 54. *Hypsibius convergens*, a common bryophyte inhabitant. Photo by Paul Bartels, with permission.



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.

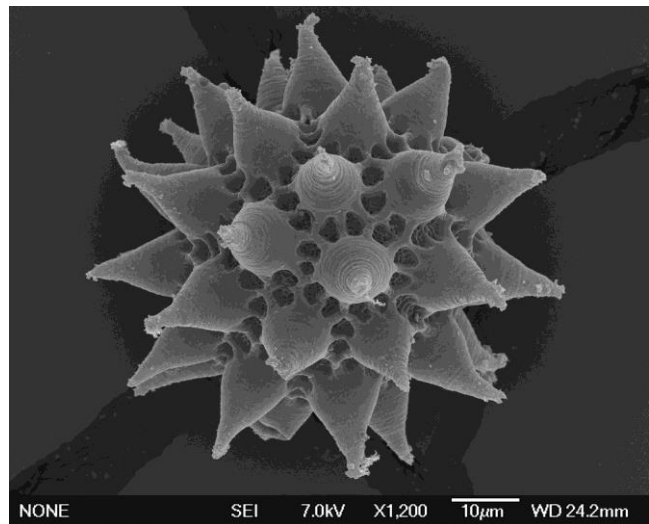


Figure 57. *Macrobiotus magdalenae* egg showing the highly decorated nature that is typical of eggs laid free from the **exuvia** (shed body shells). In this state the organism can survive as well as in a tun. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

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, Schokriai *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.

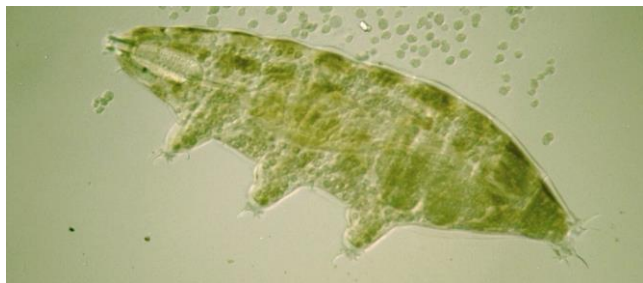


Figure 58. *Milnesium tardigradum*, a bryophyte dweller whose younger stages are the most susceptible to desiccation. Photo by Yuuji Tsukii, with permission.

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.

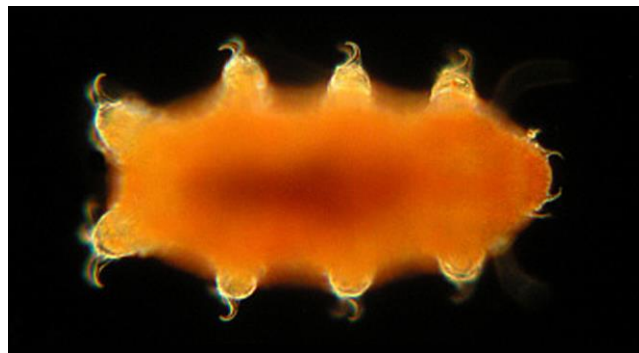


Figure 60. Tardigrade showing **anoxybiosis**, where water has entered through the cuticle by osmosis and caused swelling and turgidity. Note the extended legs and perfectly symmetrical body. The animal cannot move in this state. Photo by Martin Mach, with permission.



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 is 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* 7x10³ 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.

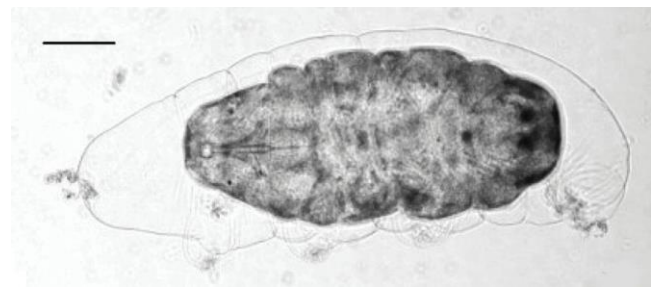


Figure 63. *Bertolanius volubilis* type A cyst. Photo by Roberto Bertolani from Bertolani *et al.* 2004, with permission.

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.

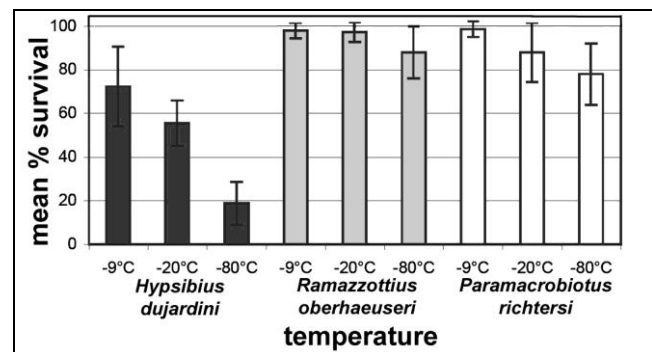


Figure 64. Comparison of survival of three bryophyte-dwelling tardigrades subjected to sub-zero temperatures. Redrawn from Bertolani *et al.* 2004.

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 $33.0 \pm 0.5^\circ\text{C}$. 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 . Ramløv 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 **anoxymbiosis**. 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 Matryoshka Russian doll.

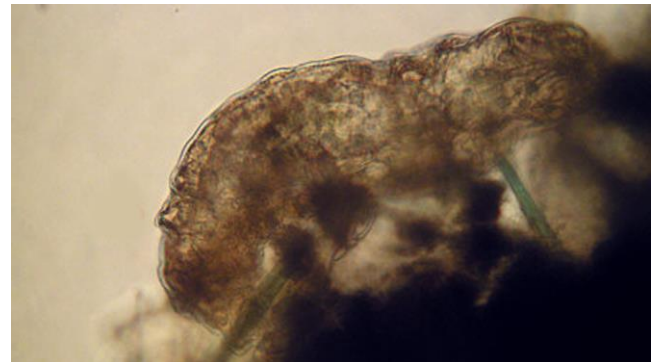


Figure 66. *Dactylobiotus dispar*. Photo by Martin Mach, with permission.



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).

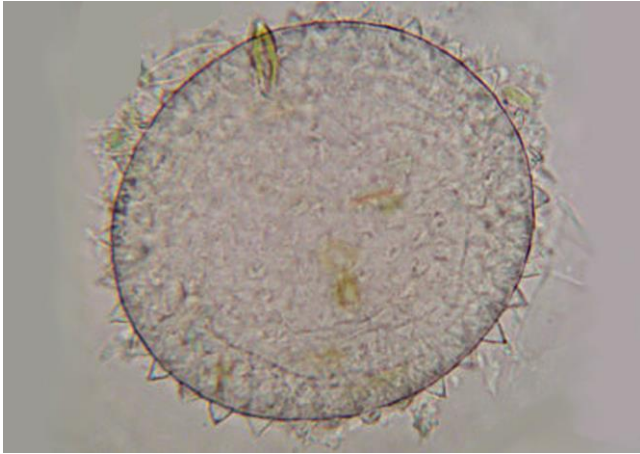


Figure 68. Egg of *Dactylobiotus dispar*. Photo by Martin Mach, with permission.

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.

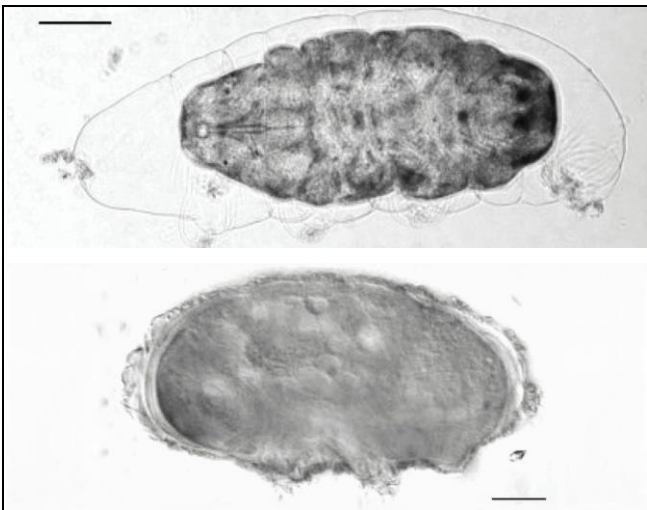


Figure 69. **Upper:** Type 1 cyst. **Lower:** Type 2 cyst (surrounded by several layers of cuticle), both of *Bertolanius volubilis*. Photos by Roberto Bertolani in Bertolani *et al.* 2004, reproduced with permission.



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

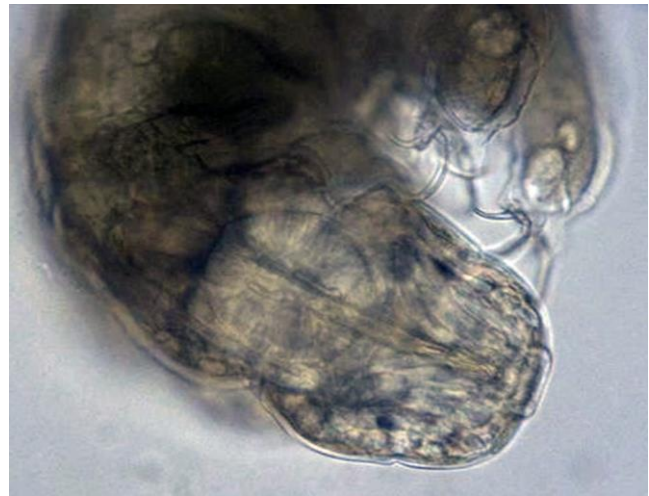


Figure 71. *Dactylobiotus* sp., a tardigrade with only one type of diapause cyst. Photo by Martin Mach, with permission.

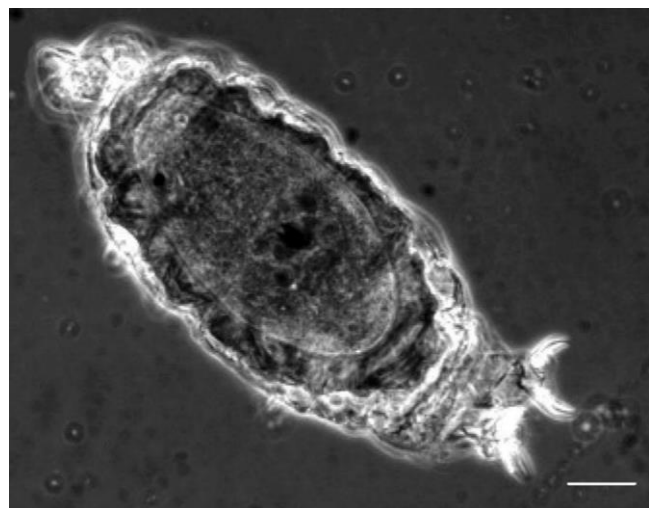


Figure 72. *Dactylobiotus* sp. cyst. Photo by Roberto Bertolani in Bertolani *et al.* 2004, reproduced with permission.

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**.

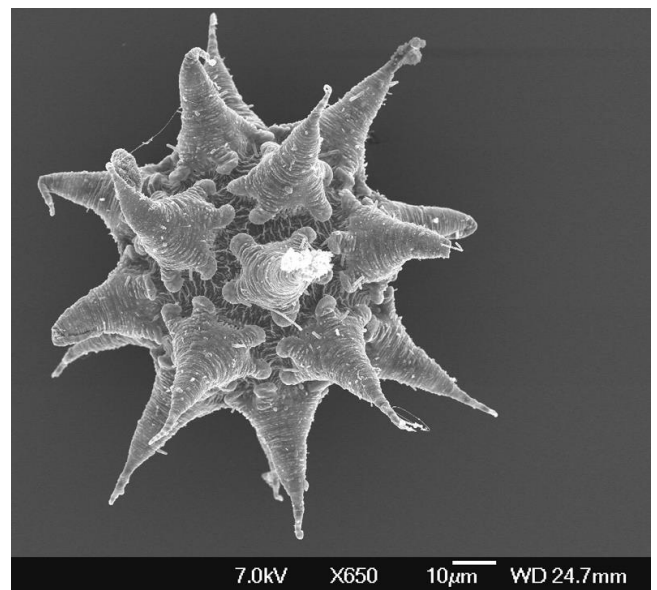


Figure 76. *Macrobiotus szeptyckii* egg showing the highly decorated surface of eggs laid free from the exuvia. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

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 0°C; **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.

Acknowledgments

Like all of my chapters, this one is really the product of the efforts of many biologists. Roberto Bertolani provided an invaluable update to the tardigrade taxonomic names, offered several suggestions on the text to provide clarification or correct errors, and obtained permission to use his published photographs from the Journal of Limnology. Paul Davison and Des Callahan have been helpful in providing suggestions and offering images. Filipe Osorio has sent me images several times, thinking of this project even when I was not soliciting help. Martin Mach and Yuuji Tsukii have given permission to use their many images that illustrate the species and life cycle stages. Martin Mach's website has been invaluable. Łukasz Kaczmarek has provided me with references, images, contact information, and many valuable comments on early stages of the manuscript. Martin Mach was kind enough to send me corrections for typos in the previous online version. Marty Janners and Eileen Dumire provided me with the views of two novices in the readability of the text. Thank you to Michael Lüth for permission to use his many images and to all those who have contributed their images to Wikimedia Commons and other public domain sites for all to use. I fear I have forgotten some who have helped – I have worked on this chapter for too many years!

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. Tardigradi 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., Guidetti, 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.cognicaop.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., Bagnúá, 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 *Ulotia crista* 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 *Ulotia crista* 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. Tardigrados 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/~tardisd/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 Lebensstätten für 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 *Diphyscon* 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., Brummer, 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.