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Review

Anhydrobiosis: the extreme limit of desiccation tolerance

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Abstract

Extreme habitats give rise to strong stressors that lead organisms to die or to possess specific adaptations to those stressors. One of the most widespread adaptations is quiescence, a common term for several strategies, including anhydrobiosis, a highly stable state of suspended animation due to complete desiccation pending recovery by rehydration. Anhydrobiosis is widespread in nature in a wide taxonomic variety among bacteria, protists, metazoans and plants. Using as model organisms, mainly tardigrades, micrometazoans able to enter anhydrobiosis in any phase of their life cycle from egg to adult, this review presents the response to desiccation from molecules to cells and organisms. Particular emphasis has been done with studies devoted to elucidate phenomena such as the long-term resistance in a desiccated state, the extraordinary resistance to chemical and physical extremes, the morphological, physiological, biochemical, and molecular constraints allowing organisms to enter and to survive anhydrobiosis, and the evolutionary meaning of life without water.

Key words: anhydrobiosis; desiccation tolerance; anhydrobiotic constraints; molecular response; evolution; tardigrades

Introduction

Extreme habitats give rise to strong stressors that very often cause organisms to die or, alternately, to allocate specific amounts of energy toward survival. These habitats are highly selective and require organisms to be tolerant and to possess specific adaptations to stressors. One of the most widespread adaptations is dormancy. Dormancy includes any form of resting stage, regardless of cues required for induction or termination (Hand, 1991). It involves a temporary suspension of active life, a reduced or suspended metabolism, and a developmental standstill. Dormancy can be subdivided into diapause and quiescence. Diapause is under endogenous control and is not directly induced by environmental stressors. It is temporary and may persist even though environmental conditions return (Womersley, 1981). The term quiescence is applied to a decrease of metabolic activity under exogenous control. It is directly induced and maintained by a variety of environmental stressors: increase or decrease of the temperature, desiccation, high salt concentration, lack of oxygen, and so on. It is immediately reversed by the removal of external stimuli. Some quiescent organisms are capable of reducing their metabolic activities to an undetectable level. This extreme form of quiescence is called cryptobiosis (latent life), a term introduced by Keilin in 1959. According to this author cryptobiosis indicates “the state of an organism when it shows no visible signs of life and when its metabolic activity becomes hardly measurable, or comes to a reversible standstill”. Cryptobiosis is actually accepted as a common term for several strategies including cryobiosis, anoxybiosis, osmobiosis, and anhydrobiosis, respectively induced by cooling, lack of oxygen, extremely high levels of solutes in the surrounding environment, and desiccation. According to Clegg (2001), cryptobiosis originated independently several times in the history of life, being present in diverse evolutionary lines within groups of unicellular (bacteria and protists) and multicellular organisms such as mosses, lichens, liverworts, plants, and metazoans (nematodes, rotifers, tardigrades, insects and crustaceans).

The most widespread and best known form of cryptobiosis is anhydrobiosis (Giard, 1894). The term is derived from Greek and indicates “life without water”. Anhydrobiosis is a highly stable state

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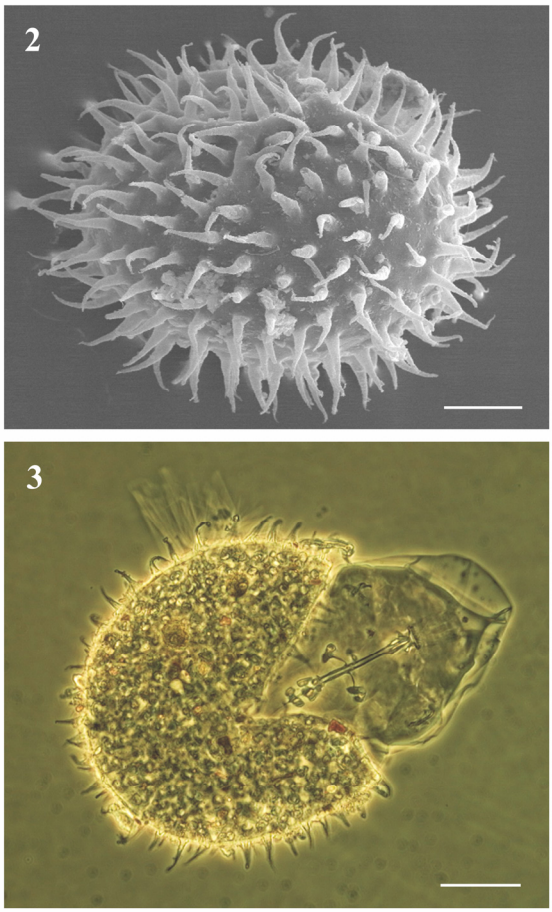
Fig. 1 Female of the eutardigrade *R. coronifer* (*in vivo* and Nomarski contrast). The bucco-pharyngeal apparatus (arrow), the ovary containing two big oocytes (asterisk) ready to be laid, and black eyes spot are clearly visible. The yellow colour is due to pigment present inside the storage cells (arrow head). Bar = 100 µm.

of suspended animation of an organism due to the complete desiccation pending recovery by rehydration. This state seems always characterized by cessation of measurable metabolism. A quantitative definition of complete desiccation is probably drying to < 0.1 g H_2O g^{-1} dry mass; this is equivalent to air dryness at 50 % of relatively humidity at 20 °C (Alpert, 2005). According to Alpert (2005), anhydrobiosis could be considered a synonymous of "desiccation tolerance", a term most frequently used for plants. Desiccation tolerance could be defined as the ability to dry, to equilibrium, with air that is moderately to extremely dry and then to recover normal function after rehydration.

This review presents the factors in anhydrobiosis analyzing the response to desiccation from molecules to cells and organisms. Emphasis will be placed on the anhydrobiotic ability in metazoans, particularly on the desiccation ability of tardigrades. Tardigrades will be considered here as the animal model, exhibiting anhydrobiosis in any stage of their life cycle, from egg to adult (Bertolani *et al.*, 2004).

Tardigrades (Figs 1-3) are microscopical metazoans, often with mature adults averaging 250-500 µm in length. Due to their small size, tardigrades have begun to be known only after the introduction of microscopy. The first tardigrade, found in inland waters, was described as a "kleiner Wasser Bär" (small water bear) in 1773 by JAE Goeze. Their current name has been attributed to these animals by L Spallanzani (1776), an Italian clergyman and professor of Natural History at the University of Pavia (Italy), who first analyzed the ability of tardigrades to enter anhydrobiosis. He hydrated sediment from a rain gutter, in which he observed, other than rotifers, what he considered a true aquatic specimen, naming it "il Tardigrado" (slow-stepper), due to its slow movement. Actually, tardigrades are considered a separate phylum. Recent molecular studies with 18S rRNA, in agreement with previous morphological studies, have lead biologists to consider Tardigrada a sister group of the Arthropoda, combining with Onychophora, the evolutionary line of Panarthropoda (Nielsen, 1997; Ruppert *et al.*, 2004). The systematic position of Panarthrpoda is still an open debate. According to some authors who use a molecular approach, Panarthropoda belongs to Ecdysozoa (moulting animals) (Garey *et al.,* 1996; Giribet *et al.,* 1996; Aguinaldo *et al.*, 1997), together with nematodes (as tardigrades characterized by anhydrobiosis) and other blastocoelomate phyla. According to others, together with Annelida, Panarthropoda belong to Articulata (Brusca and Brusca 2003; Ruppert *et al.*, 2004). Tardigrada comprise two main classes: Heterotardigrada and Eutardigrada (Ramazzotti and Maucci, 1983; Garey *et al.*, 1999; Jørgensen and Kristensen, 2004; Nichols *et al.*, 2006). The existence and position of a third class, Mesotardigrada, are uncertain. Tardigrades probably originated within the sea (Renaud-Mornant, 1982) and then colonized both inland waters and terrestrial environments, thus



Figs 2-3 Eggs of the eutardigrade *R. coronifer*. 2) Scanning electron micrograph of the egg showing the shell ornamented by long and spine-shaped processes. Bar = 20 µm. 3) Hatching of a new born (phase contrast). Bar = 50 µm.

acquiring or developing the anhydrobiotic ability. At present tardigrades are known from marine, freshwater, and terrestrial environments in which they are represented by the highest number of species, limited to specific terrestrial niches (e.g. moss, lichen, leaf litter, and turf) (Ramazzotti and Maucci, 1983). The ability to enter cryptobiosis (in particular, anhydrobiosis and cryobiosis) also allows tardigrades to colonize extreme environments exposed to rapid desiccation and/or freezing such as deserts, high mountains, and polar regions (Bertolani *et al.*, 2004).

Anhydrobiosis

History of anhydrobiosis discovering

The study of anhydrobiosis began in the eighteenth century when Dutch microscopist Van Leeuwenhoek (1702), using the microscope he developed, discovered that when dry, apparently lifeless, dust from the roof gutter of a house was re-hydrated, many small “animalcules” became active within an h, swimming, adhering, or crawling in a cup. With this simple experiment Van Leeuwenhoek observed that “animalcules” (bdelloid rotifers) can apparently survive desiccation. Van Leeuwenhoek never suggested that his dried “animalcules” were completely desiccated. Although he demonstrated that these animals can be kept dry for several months without showing any visible sign of life, he never discussed the phenomenon he discovered in terms of concepts of life, latent life, resuscitation, and death (Keilin, 1959). In addition, Van Leeuwenhoek observed the longevity of the dried animals and speculated that the phenomenon was wide spread in nature. Other researchers following Van Leeuwenhoek were not directly interested if rotifers were dry or not, although clearly the idea that they might be in a resting stage was pervasive at the time (Tunnacliffe and Lapinski, 2003). In 1743, Needham reported that blighted wheat grains contained “white fibres” (nematodes) which took life when hydrated. These observations were repudiated by Spallanzani (1776) who disputed the animal nature of the “white fibres” found by Needham. Spallanzani’s view, backed by his great scientific reputation, prompted Needham to abandon his original idea. In the meantime Roffredi (1775a, b) and Fontana (1776) each independently unravelled the main stage of the life history of eel-worms (nematodes) found in blighted wheat (Keilin, 1959). The work of Fontana and Roffredi demonstrated the animal nature of the “white fibres” and that these animals could revive within a few min when they are brought in contact with water. The results obtained by these two researchers must have caused Spallanzani (1776) to revisit his idea about anhydrobiosis and furthermore to demonstrate that rotifers and nematodes and another moss-dwelling group of metazoans, tardigrades, shared the remarkable ability to reversible desiccation, often called as “resuscitation” or “resurrection”. He was the first researcher to propose that dried rotifers did not retain water in their bodies and that they are ametabolic. Moreover, Spallanzani observed that these animals can be stored dried for long time and that dried rotifers could survive exposure to very low or high temperature, while hydrated animals treated in the same ways were killed. Similar data were obtained by Doyère (1842) who demonstrated that the moss-dwelling eutardigrade *Macrobiotus hufelandi,* in the desiccated state, was able to revive after few min of exposure to temperature up to 120 °C to 125 °C and that slow drying was crucial for survival.

During the last quarter of the eighteenth century, debates arose about the status of dry organisms. Are they really living? Have they a metabolism? Are they dead? Following two decades of decline of interest about desiccation resistance and meaning, a new interest appeared when the French biologists Doyère and Pouchet, working on tardigrades and rotifers respectively, reached different results. Doyère (1842) asserted that desiccated organisms can be revived after complete desiccation and cessation of their life processes, while Pouchet (1859a, b) asserted that organisms cannot survive after desiccation once all life processes have been arrested. The main problem posed by anhydrobiosis was the apparent cessation of metabolism. These different concepts attracted the attention of several scientists probably because

they were strictly related to the discussion of spontaneous generation (Keilin, 1959).

Originally, “anabiosis” or “return to life” was used by Preyer (1872, 1891) to indicate extreme resistance to desiccation and the ability of resuscitation, or resurrection of completely lifeless but viable organisms (Keilin, 1959). This term was then extended to the state of viable lifelessness itself generating a lot of confusion because from this statement emerge two possible states of an organism: lifeless and viable (= anabiotic) and lifeless and not viable (= death) respectively (Keilin, 1959). To avoid this confusion Schmidt (1948) proposed the term “abiosis” to indicate the state of viable lifelessness. Nevertheless, this word was too closely linked to abiotic and abiogenesis and generated further confusion. In 1959 Keilin proposed the term cryptobiosis (divided in anhydrobiosis, cryobiosis, anoxybiosis and osmobiosis) that is currently used. Cryptobiosis is the term indicating a state in which organism does not feed, does not grow, does not reproduce, does not replicate its DNA, and so on.

Anhydrobiosis indicates a fundamental concept about the nature of living systems. An anhydrobiotic organism lacks all dynamic features of living organism due to the absence of an ongoing metabolism. In that sense it is not alive, but it is not dead since rehydration produces a living organism (Clegg, 2001). The extreme desiccated anhydrobiont is indeed reversibly ametabolic. Clegg (2001) affirms that there are three states of biological organisation: alive, dead and cryptobiotic. In cryptobiotic, and specifically in anhydrobiotic organisms, the suspension of metabolism is reversible, and a kind of resuscitation routinely occurs (Tunnacliffe and Lapinski, 2003).

The review of Keilin (1959) has rediscovered the interest in the anhydrobiosis, as demonstrated by several meetings having desiccation tolerance as a topic. As example we can cite the symposium entitled “*Drying Without Dying: Mechanisms and Evolution of Desiccation Tolerance in Animals, Microbes and Plant*” hosted at the annual meeting of the Society for Integrative Biology (California, 2005). The rediscovery of interest for anhydrobiosis was also indicated by the amount of literature published in the last decades over the morphological, physiological, biological, and molecular aspects of anhydrobiosis with the aim to discover the secrets of the life without water.

Anhydrobiotic ability among organisms

Anhydrobiosis is widespread in nature. A wide taxonomic variety of bacteria, protists, animals, and plants are able to undergo anhydrobiosis suggesting that this ability, developed by ancient cell types, may have been essential for efficient colonization of land mass (Tunnacliffe and Lapinski, 2003). Certain bacteria such as the radiotolerant species, *Deinococcus radiodurans,* and the cyanobacteria, *Nostoc commune* and *Chroococcidiopsis* spp. are capable of anhydrobiosis (Mattimore and Battista, 1996; Potts *et al*., 2005). *N. commune* shows no significant DNA damage after 13 years of being dry and can resume growth after 55 years in herbarium (Shirkey *et al*., 2003).

Among plants, desiccation tolerance is common in lichens and bryophytes. The form of anhydrobiosis present in these primitive plants seems to comprise constitutively expressed cell protection mechanisms associated with inducible repair systems which are activated after rehydration (Oliver, 1997). Also among algae, it is possible to find species able to tolerate desiccation, either in terrestrial or in marine intertidal algae (Trainor and Gladych, 1995; Abe *et al*., 2001). Higher plants do not have desiccation ability; nevertheless, the vegetative tissues of the so called “resurrection plants” (e.g. *Craterostigma plantagineum* and *Selaginella lepidophylla*) are able to enter anhydrobiosis even though they require a slow rehydration phase (Oliver *et al*., 2000; Alpert, 2005). In any case, spores, seeds, pollens, and other propagules of many angiosperms are known to be able to tolerate high levels and long periods of desiccation (Clegg, 2001). The most famous example of long term survival in a desiccate state is represented by the seeds of the ancient sacred lotus, *Nelumba nucifera*. Seeds dated about 1100 years old successfully germinated (Shen *et al*., 1995). Mosses and liverworts have recovered after 20-25 years at air-dryness, and adult angiosperms and pteridophytes after 5 years (Alpert, 2005). Among fungi, some yeasts tolerate desiccation. For example we can cite the dried granules of the baker’s yeast, *Saccaromyces cerevisiae,* whose desiccation tolerance has an important role either from a historic and commercial point of view or from a genetic and molecular point of view.

Among metazoans, anhydrobiosis is known only in invertebrates. It is very frequent in three phyla of small animals: rotifers, nematodes, and tardigrades, in which anhydrobiosis occurs at any stage of their life cycle (from the egg to the adult) (Wright *et al*., 1992; Ricci, 2001; Bertolani *et al*., 2004; Ricci and Caprioli, 2005). The life cycle of these animals consists of active periods for growth and reproduction, interrupted by periods of metabolic inactivity induced by desiccation of their environment. According to Jönsson (2005), these animals may be called holo-anhydrobiotic in order to distinguish them from animals where anhydrobiosis is restricted to a specific phase of their life cycle. Belonging to this latter group are larvae of African chironomid, *Polypedilum vanderplanki* (Diptera; Hinton, 1951, 1960a) and the cysts (embryonic eggs) of the brine shrimp *Artemia* spp. (Crustacea; Clegg, 2001, 2005). The larvae of *P. vanderplanki* (from 6 to 7 mm in length) represent the largest multicellular organism with cryptobiotic ability (Watanabe *et al*., 2004). The well documented survival record for dried animals is in the order of decades. Egg cysts of brine shrimp can survive 15 years after desiccation (Clegg, 1967), while survival record for the larvae of *P. vanderplanki* is 17 years (Adams, 1985). The longest verified period of survival for rotifers (*Mniobia* spp.) after desiccation appears to be 9 years (Guidetti and Jönsson, 2002). Most reports on long-term desiccated survival of nematodes have been on species of commercial

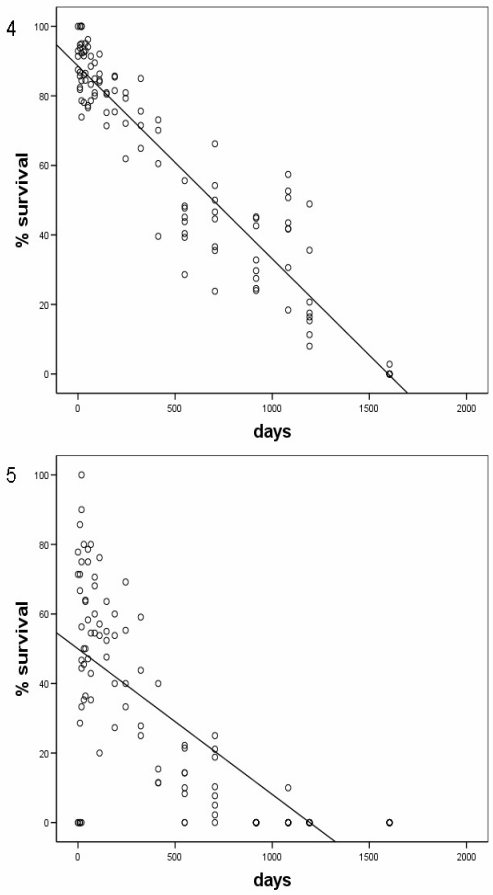
importance such as parasitic plant nematodes. They can survive dry 20-40 years (see Guidetti and Jönsson, 2002).

Anhydrobiosis in tardigrades

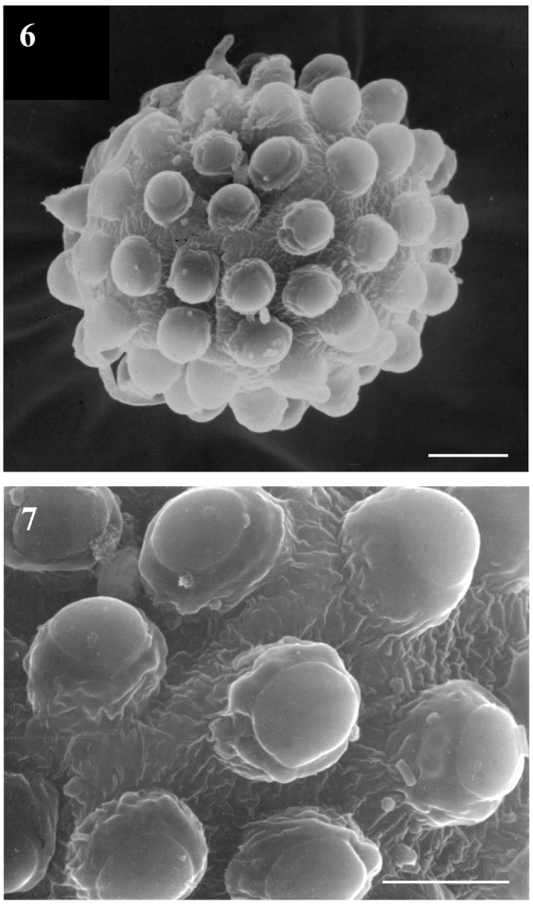
After Spallanzani (1776), in the nineteenth and twentieth centuries, other than at the beginning of this century, several other scientists have continued to dedicate their research to anhydrobiotic ability of tardigrades with the aim to discover the secret of anhydrobiosis. A huge amount of interesting data has been published covering a wide range of different topics. Among them, we can remember studies devoted to elucidate phenomena such as a) long-term resistance in a desiccated state; b) extraordinary resistance to chemical and physical extremes; c) morphological, physiological, biochemical, and molecular constraints allowing survival during anhydrobiosis; and d) ecological and evolutionary meanings of this survival strategy that allow tardigrades to live even long periods without water.

Long-term anhydrobiotic ability

Regarding long-term survival of desiccated tardigrades, the most famous tale is that they can survive more than a century in a desiccated state. This idea originated from an observation made by Franceschi (1948), who noted a single extending movement followed by retraction of the front leg in only one tardigrade soon after rehydration of a 120 years old moss stored in a museum herbarium. This gave the impression of a well documented phenomenon and the story that tardigrades can survive a century in an anhydrobiotic state has been very popular not only in popular science articles (Crowe and Cooper, 1971; Copley, 1999), but also in well-reputed books of invertebrate zoology (Brusca and Brusca, 2003). The story about century long anhydrobiotic survival in tardigrades provides either an example of how some biological phenomena easily give rise to sensational journalism in popular science or of how incorrect data may persist for several years inside the scientific community (Jönsson and Bertolani, 2001). Current evidence suggests that the limit of anhydrobiotic survival in tardigrades is shorter than a century and that it is within a decade. In fact, Baumann (1927) obtained living specimens of eutardigrades belonging to the genus *Macrobiotus* after 7 years of desiccation. Sømme and Meyer (1995) reported a high survival rate for three species (*Macrobiotus furgicer*, *Diphascon chilenens*e and *Echiniscus jenningsi*) kept in an anhydrobiotic state for 8 years (but these species were stored at -22 °C). In order to obtain data about the ability to recover after a long-term desiccated period, Guidetti and Jönsson (2002) evaluate the presence of survivors of tardigrades (as well as nematodes and rotifers) in 63 samples of lichens or mosses from public and private herbariums kept dry for 9-138 years. No live juvenile or adult tardigrades and nematodes were found; nevertheless, 4 eggs of the eutardigrade, *Ramazzottius oberhaeuseri* extracted from 9-year-old lichen hatched after rehydration. With the same aim, Crowe (1975) periodically re-hydrated specimens of the eutardigrade *Macrobiotus areolatus.* He found that since the experiment was started in 1968, the proportion that survived has gradually declined from initial values of 95 % to the value of about 50 % in 1974 (i.e. after 4 years and 8 months in a desiccated state) when he stopped his experiment. A further and complete experiment to understand the long-term ability of tardigrades to recover from long-term desiccation under atmospheric oxygen and temperature conditions was recently performed by Rebecchi *et al*. (2006) on lichen-dwelling tardigrades (*R. oberhaeuseri*, *Echiniscus testudo,* and *Echiniscus trisetosus*). Significant inter-specific differences were found, other than the survival decrease with time spent in anhydrobiosis. The first significant decrease in recovery of *R. oberhaeuseri* was observed after 86 days from the beginning of the experiment, while recovery of adult and juvenile specimens was zero after 1604 days (i.e. about 4 years, Fig. 4). At this time 4 eggs of *R. oberhaeuseri* (Figs 6-7) and 8 eggs of *Milnesium tardigradum* hatched after lichen hydration. The anhydrobiotic survival of *Echiniscus* spp. was lower than the previous species; the first significant decrease in recovery was after 41 days from the beginning of the experiment and its survival was zero after 917 days (i.e. 2 years and 6 months, Fig. 5).



Figs 4-5 Long-term anhydrobiotic survival of the lichen-dwelling tardigrades 4) *R. oberhaeuseri* and 5) *Echiniscus* spp. (Modified from Rebecchi *et al*., 2006).



Figs 6-7 Scanning electron micrographs of the egg of *oberhaeuseri*. 6) *In toto* egg. Bar = 20 µm. 7) Detail of the egg shell showing the species-specific ovoidal processes. Bar = 10 µm. (6: From Bertolani and Rebecchi, 1999). (Reprinted with permission).

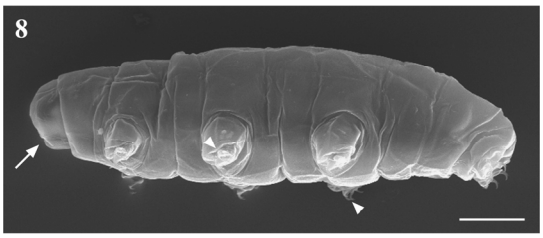
In general, we can affirm that a recovery after about 10 years of anhydrobiosis has to be considered a very good long-term survival although the ecological and evolutionary importance of such a phenomenon remains unclear.

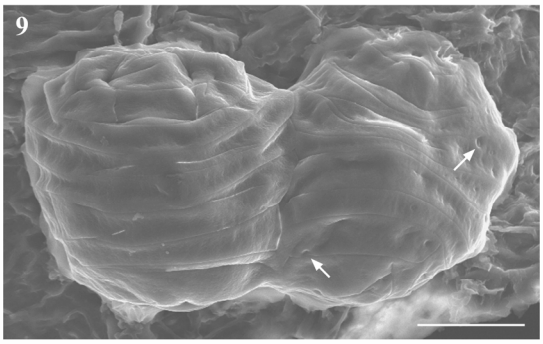
Morphological and physiological traits of anhydrobiotic ability

Despite the presence of anhydrobiosis in species belonging to several distant evolutionary lines and its adaptive potentiality, in metazoans anhydrobiosis can be found only in a restricted number of taxa whose animal sizes do not exceed 5-7 mm, and often it is much smaller. These apparent morphological and ecological limits could be linked to physiological limits in order to tolerate physical and physiological constraints (Alpert, 2005). Several authors evidenced that anhydrobiotes cannot either have a very rapid desiccation rate or have any pain at their death (Crowe, 1972; Crowe and Madin, 1975; Wright, 1989a, b; Ricci *et al.*, 2003; Ivarsson and Jönsson, 2004). All tardigradologists know that if they put an animal on a glass slide within a water drop and wait until it desiccates, certainly the tardigrade dies because it desiccates too quickly. In order to prepare desiccated viable tardigrades a group of active animals suspended in about 10 µl of water was placed on a piece of filter paper (0.25 cm^2 in size; thickness of 0.4 mm) in a plastic Petri dish and desiccated for one day in a climatic chamber with 80 % of relative humidity (RH) of the air and 18 °C. Then the Petri dish was stored at 50 % RH and at 20 °C (Rebecchi, personal communication). To avoid risk of rapid dehydration, anhydrobiotes carry out morphological modifications, other than production of bioprotectans. When the natural desert pools dry up, larvae of the anhydrobiotic chironomid *P. vanderplanki* gradually dry out and fold in the middle (Watanabe *et al*., 2004). In nematodes there is a coiling of the body (see Crowe, 1971). In addition, certain nematodes are reported to congregate into masses of “nematode wool”, with better survival of specimens in the center of the mass (Hellemby, 1968). Aggregation effect has also been experimentally evidenced in tardigrades (Ivarsson and Jönsson, 2004), but not yet verified in nature. Entering anhydrobiosis, tardigrades and rotifers show anterior-posterior body contraction (also withdraw their limbs) to form a so-called “tun” (Baumann, 1922). In particular, bdelloid rotifers contract their body into a compact shape by withdrawing its cephalic and caudal extremities into the trunk (Ricci and Melone, 1984; Ricci, 2001). In addition, the proper contraction of the rotifer body into a tun shape and probably the correct packaging of internal structures are necessary for survival desiccation (Ricci *et al*., 2003).

Tun formation represents a structural adaptation to desiccation, which acts on the permeability of their body surface. Tun formation reduces the evaporation surface and in particular removes high permeability areas of the cuticle from direct contact with the air. Tun formation has been well studied in eutardigrades that seem to be able to reduce their surface area more than heterotardigrades (Wright, 1989b), which have thick cuticular plates (Figs 10-11). According to Crowe (1972, 1975), tun formation is an active phenomenon. In fact, his experiments carried out on *M. areolatus* evidenced that tuns are formed only by active animals when desiccated at opportune conditions of relative humidity, whereas at the same conditions anesthetized (anoxic) animals simply flatten or crumple. Forming tuns, tardigrades contract their intersegmental areas forming transverse cuticular folds and reducing the body surface (Figs 8-9). The thin cuticle areas are drawn into the body wall and removed from contact with the air. Crowe *et al.* (1971) reported that cuticular folds of *M. areolatus* are about 1/20 the thickness of the rest of the cuticle, suggesting that they may be more highly permeable than the rest of the cuticle. A clearly different permeability has been confirmed by the use of dyes and, at ultrastructural level, using a solution of $PbNO_3$ (Crowe, 1972).

Wright (1988) stated that when tardigrades enter anhydrobiosis, a rapid dehydration is followed soon after tun formation by a “permeability slump”, which is a non-metabolic process induced by dehydration of the cuticle although delayed by increasing rates of dehydration (Wright, 1988, 1989a).





Figs 8-9 Scanning electron micrographs of eutardigrades. 8) Specimen of *richtersi* in a fully extended position, as in active state. Anterior region of the animal (head) and legs with claws (arrow heads) are identifiable. Bar = 50 µm. 9) Specimens of *M. richtersi* in tun state. Limbs and intersegmental cuticle plates are inflexed. The cuticular depressions are area of muscle insertion (arrows). Bar = 50 µm.

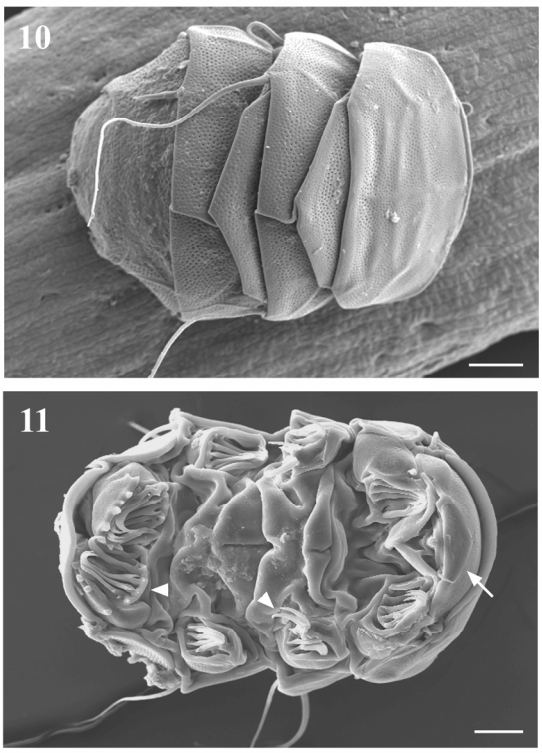
Permeability slump permits animals to retain considerable amounts of internal water when desiccation has begun and to produce bioprotectants (Wright, 1988). Comparing four different species, three eutardigrades and one heterotardigrade, Wright (1989a) noted that dynamics of permeability decline are similar, but the point at which it is initiated differs significantly between species, in particular the species most tolerant to desiccation. Among eutardigrades, *R. oberhaeuseri* shows the earliest permeability slump and the greatest infolding, the less tolerant, *Hypsibius dujardini*, the latest permeability slump and the least infolding, while the intermediate tolerant species *M. hufelandi* exhibits an intermediate situation. The heterotardigrade *E. testudo* has a different situation because its very thick cuticle is difficult to fold. Wright (1988, 1989a) underlines that lipids, particularly rich in the intracuticle and comparable with those of the insects, seem to compose the main transpiration barrier. He also notes that in the three eutardigrades considered, thickness of the intracuticle is directly related with the ability to tolerate desiccation. The intracuticle of *E. testudo* is thicker ventrally than dorsally but in any case it is even thicker than the intracuticle of *R. oberhaeuseri*, the most tolerant species to desiccation. May (1946) reported intracellular modifications in the anhydrobiotic eutardigrade *M. hufelandi*. Walz (1979), directly fixing desiccated specimens and conventionally fixing hydrated specimens of the same species and comparing them at ultrastructural level, concluded that the basic morphology of the cells and their organelles is not changed in the anhydrobiotic specimens with respect to hydrated ones.

The necessity to lose water slowly has also been verified in tardigrades by desiccation experiments with different values of relative humidity (RH) of air. In his experiences on *M. areolatus*, Crowe (1972) verified that the highest number of survivals was obtained when the animals were dried at RH greater than 70 %. He explained this result with the fact that at high RH values tardigrades retain significant proportions of their body water for extended periods of time (up to 100 h). At lower RH they can still form tuns, but they lose water at a much greater rate. Animals dried at low RH or when anesthetized become irregularly crumpled or simply flattened and do not form tuns. According to Wright (1989b), a lower lethal value of humidity of the air for initial desiccation ranges from 78 % to 53 %. Species most tolerant of rapid initial drying also show the most rapid acquisition of tolerance to low values of humidity (25-31 %) following drying in high humidity. If specimens of *M. areolatus* are transferred from 95 % RH to dry air at various phases of the dehydration, their water content rapidly falls to 2 % or less of the water present in a hydrated specimen (Crowe, 1972). The number of animals that survives following the exposure to dry air varies directly with the length of the time they have been kept at 95 % RH (Crowe, 1975). After 12 h in moist air, none survive to exposure to dry air, after 72 h in moist air, nearly all survive.

Anhydrobiotic ability and extreme resistance

Anhydrobiotic organisms show extraordinary resistance to physical and chemical extremes that far exceed tolerance ranges of active organisms. This ability allows anhydrobiotic organisms to persist in environments excluded to most other organisms and to resist against unnatural abiotic extremes. According to Clegg (2005), even tough the word extremophile is usually applied to certain bacteria showing extraordinary resistance to extreme conditions, there is no reason why this term cannot also be used for plant or animal taxa characterized by the same extraordinary resistance. Among metazoans, examples of extremophiles can be represented by the cysts of the brine shrimp *Artemia* spp., by the larvae of the chironomid *P. vanderplanki*, by the eggs of monogonont rotifers and by eggs, juveniles, and adults of bdelloid rotifers and most tardigrades. Extremophile invertebrates, when in a desiccated state, are able to resist stressors such as temperatures near absolute zero or above 100 °C, immersion in various organic solvents, high and low extremes of hydrostatic pressure as well as very high levels of ionizing radiation. Resistance to those extremes can be attributed to the inert state of a dehydrated organism and to the absence of a sensitive aqueous phase, only through which sensitive biochemical reactions can proceed (Wright, 2001).

With regard to low temperature stress, Rahm (1923, 1924, 1926) found that desiccated tardigrades of several species, other than nematodes and rotifers, survived immersion in liquid air (-190 °C and -200 °C) for 21 months, in liquid nitrogen (-253 °C)



Figs 10-11 Scanning electron micrographs of heterotardigrades. 10) Dorsal view of a specimen of *trisetosus* in tun state. Note the armadillo-shaped tun due to hollow of thin intersegmental cuticular plates under the thick cuticular plates. Bar = 20 µm. 11) Ventral view of a specimen of *E. trisetosus* in tun state. Claws (arrow heads) and the retracted head (arrow) are clearly identifiable. Bar = 20 µm.

for 26 h and in liquid helium (-272 °C) for 8 h. The association between anhydrobiosis and temperatures near absolute zero was also evidenced by Becquerel (1950), who was able to revive animals after adiabatic cooling at -272.8 °C. Recently, Sømme and Meyer (1995) observed a high degree of survival in three desiccated species of Antarctic tardigrades after 8 years at -22 °C, while Newsham *et al.* (2006) evidenced that some soil Antarctic tardigrade species (*Diphascon* sp. and *Hypsibius* cf. *dujardini*) survive at -80 °C for 6 years and two months. In any case, the ability to persist for long time at -80 °C is not an exclusive characteristic of Antarctic tardigrades. In our lab we usually conserve desiccated terrestrial tardigrades (lichen-, moss- and leaf-litter-dwelling) collected in our temperate region for a long time at –80 °C. For example, the survival rate at -80 °C of the moss-dwelling *Richtersius coronifer* and of the lichen-dwelling *R. oberhaeuseri* was very high (about 95 %) after 6 and 3 years. In contrast, the ability of hydrated tardigrades to survive at low temperatures (-9 °C, -22 °C, -80 °C) is limited to short periods and decreases with the duration time of exposure to low temperature (Sømme and Meier, 1995; Guidetti, personal communication). Ramløv and Westh (1992) observed that hydrated specimens of *R. coronifer* survive cooling to -196 °C for 15 min, but their viability decreases with faster cooling rates. Recall that the freeze-resistance of anhydrobiotic tardigrades should be distinguished from cryobiosis, which is related to the ability of the hydrated animal to freeze and survive after thawing (Wright, 2001).

With regard to hot temperature stressors, as said above, the eutardigrade *M. hufelandi* was able to revive after few min of exposure to temperature up to 120-125 °C (Doyère, 1842). Rahm (1923, 1924, 1926) found that desiccated tardigrades survived exposure to 151 °C for 15 min. Recent experiments indicate that the eutardigrade *R. coronifer,* heat stressed in a dry state and inside moss cushions (its habitat), survived temperatures up to about 70 °C for 1 h without any decrease in survival. Nevertheless, its survival decreased quickly when animals were exposed to a temperature above 70 °C and no animals survived exposure of 100 °C (Ramløv and Westh, 2001). Among other extremophile metazoans able to resist temperature stress, we can cite the cryptobiotic larvae of *P. vanderplanki*. They show an extremely high thermal tolerance from -270 °C to 103 °C and can revive after immersion in pure ethanol or glycerol (Hinton, 1960b; Watanabe *et al*., 2002).

According to Baumann (1922), desiccated tardigrades are able to resist exposure to CO_2 and H_2S. Recently, Ramløv and Westh (2001) demonstrated that experimentally desiccated specimens of *R. coronifer* survived exposure to ethanol for less than 10 min. In addition, their survival decreased slowly to about 42 % in 7 days when exposed to 1-butanol, while the exposure to 1-hexanol did not show any decline in survival over a period of 7 days. In addition, desiccated tardigrade specimens of *R. coronifer* are able to escape the treatments with the biocide methyl bromide gas (50 g/m^3 for 70 h; Jönsson and Guidetti, 2001). Similar resistance to exposure of CO_2 or N_2 has been found in desiccated bdelloid rotifer *Macrotrachela quadricornifera* (Ricci *et al*., 2005).

As mentioned above, desiccated tardigrades are able to resist high hydrostatic pressure. In a dry state, specimens of eutardigrade *Macrobiotus occidentalis* and of heterotardigrade *Echiniscus japonicus* are able to survive hydrostatic pressure up to 600 MPa (rate survival of 95 % and 80 % respectively), while specimens of the same species in active state in water have a high survival (about 90 %) only up to 100 MPa (Seki and Toyoshima, 1998).

Regarding resistance to irradiation, in general eukaryotic organisms (both uni- and multicellular organisms) have lower tolerance to irradiation than prokaryotes. In any case, the ability of the bacteria *D. radiodurans* and *Chroococcidiopsis* spp. to survive high doses of ionizing radiation is positively related to the tolerance of desiccation (Mattimore and Battista, 1996; Battista *et al.*, 1999; Billi *et al*., 2000). It was speculated that their extraordinary radio-tolerance is a consequence of their capability to repair DNA damages that are caused by desiccation (Battista, 1997; Billi *et al.*, 2000). In fact, in *D. radiodurans* the inactivation of a gene encoding a DNA repair protein reduces its survival to desiccation and ionizing radiation (Kumaraswamy *et al*., 1994; Mattinore and Battista, 1996). On the

contrary, according to Shirkey *et al.* (2003) whether the capacity for desiccation tolerance is a consequence of the evolution of a capacity for DNA repair following desiccation is questionable. Among multicellular organisms too, ability to tolerate radiation exposures seems related to anhydrobiotic ability, as indicated by studies of the brine shrimp *Artemia* sp. (Iwasaki, 1964; Clegg, 2001, 2005), the larvae of chironomid *P. vanderplanki* (Watanabe *et al.*, 2006), some nematodes (Chinnasri *et al.*, 1997), bdelloid rotifers (Ricci *et al.*, 2005) and, of course, tardigrades (May *et al*., 1964; Jönsson *et al*., 2005; Horikawa *et al*., 2006).

The reputation that desiccated tardigrades are able to better resist irradiation than hydrated ones came from the pioneering experiments of May *et al*. (1964) who exposed hydrated or desiccated specimens of the eutardigrade *M. areolatus* to X-rays. With a median lethal dose (LD_{50}) of about 5000 Gy X-rays, tolerance was slightly higher in desiccated specimens than in hydrated ones (May *et al*., 1964). New data come from very recent experiments made up on two different eutardigrade species, *M. tardigradum* and *R. coronifer* (Jönsson *et al*., 2005; Horikawa *et al*., 2006). Desiccated specimens of *R. coronifer* were exposed to γ-rays at doses between 1000-9000 Gy, while hydrated to doses between 500-5000 Gy (Jönsson *et al*., 2005). Both desiccated and hydrated specimens of *R. coronifer* exposed to 500 or 1000 Gy did not deviate in survival from their controls and hydrated tardigrades were found to be about as resistant to γ-rays as hydrated tardigrades (Jönsson *et al*., 2005). Specimens of *M. tardigradum* survive high doses of γ-rays (doses from 1000 to 7000 Gy) in both hydrated and desiccated states, but irradiation with doses higher than 1000 Gy makes them sterile. In addition, specimens of *M. tardigradum* are more tolerant to heavy ions (doses from 1000 to 8000 Gy) than that to γ-rays in both sates (Horikawa *et al*., 2006). Up to date, only one study analyzed the effect of UV radiation on tardigrade survival (May *et al*., 1964). The survival of desiccated specimens of *M. areolatus* irradiated with UV rays for a time ranging between 2 and 6 h was high. Hydrated specimens showed an opposite trend. In fact, the rate of survival was of 58.6 % after 1 h of exposure and only of 8.6 % after 2 h (May *et al*., 1964). A similar trend was obtained when desiccated or hydrated specimens of the bdelloid rotifer *M. quadricornifera* were exposed to UV rays (180 nm) (Ricci *et al*., 2005). Even though data about resistance of tardigrades to UV radiation are very scarce, and further studies are certainly necessary, we can hypothesize that hydrated tardigrades are more vulnerable to UV rays than to γ-rays.

The data about tardigrade radio-tolerance seem to indicate that anhydrobiosis is not always effective for protection against radiation in terms of tardigrade lethality and lead to consider desiccation tolerance in a new perspective. It is difficult to explain why hydrated tardigrades are radiation tolerant because, up to now, tolerance to extreme environmental stress conditions, including radiation, has been related with the desiccated state. According to Horikawa *et al*. (2006), this answer is related to the fact that desiccated tardigrades, having extremely low water contents with respect to hydrated ones, are expected to be damaged less by the indirect action of radiation compared to hydrated animals. In addition, dry animals have high levels of biochemical molecules such as the disaccharide trehalose that prevent cellular damages. On the contrary, according to Jönsson *et al*. (2005), radiation tolerance might be the result of a high ability to repair biological damage, in particular DNA damages, rather than biochemical prevention of damage itself. Repair mechanisms are well documented in radio- and desiccation-tolerant bacteria (Battista *et al.*, 1999; Billi *et al.*, 2000), but little considered and studied in multicellular organisms. In any case, further studies are needed to verify if radiation induces biological damages, especially on DNA, in hydrated or desiccated tardigrades, other than to understand the eventual repair mechanisms. The study of mechanisms underlying anhydrobiosis and radio-tolerance is relevant not only in the frame of a better understanding of an intriguing natural phenomenon, but also for the development of novel technologies in the field of radioprotection and for the preservation in the dry state of cells otherwise susceptible to desiccation.

The extraordinary ability of desiccated tardigrades to resist extreme stressors, especially their radiation tolerance, has lead to defined the tardigrade as the “toughest animal on the Earth” (Copley, 1999). In addition, these extreme abilities have led to affirm that tardigrades “semble les désigner comme matériel de choix dans l’étude des conditions extrêmes que peut supporter le protoplasme dans le espaces interplanétaires” (May *et al*., 1964). More recently, tardigrades have been proposed as suitable model organisms in space research (Bertolani *et al*., 2001). Natural exposure to space environment provides a more realistic evaluation than simulated tests on the Earth of mechanisms that allow anhydrobiotic organisms to survive space stressors (Bertolani *et al.*, 2001). As a consequence, we are involved in a space project (*TARSE: Tardigrades Resistance to Space Effects*) using tardigrades as a model organism scheduled for launch in September 2007.

Molecules involved in anhydrobiosis

The removal of intracellular water causes drastic changes in inter- and intra-molecular interactions. Upon drying, intracellular proteins and membranes compensate the loss of hydrogen bonding with water by hydrogen bonding with other molecules. This produces intermolecular interactions between molecules that normally would not interact whit each other in presence of bulk water (Wolkers *et al.*, 2002). Drying of cells generally leads to massive damages to the cellular membranes and proteins, which eventually result in cell death and, consequently, in the death of the organism. In desiccation-tolerant cells, molecular interactions during drying are controlled in such a way that loss of water is compensated by reversible interactions with other molecules. In this way, bio-molecules and cellular structures are protected and

retain their native configuration again after rehydration (Wolkers *et al.*, 2002).

One of the survival strategies against desiccation is the accumulation of high concentrations of small non-reducing disaccharides such as trehalose (e.g. in bacteria, fungi, lower plants and animals) and sucrose (e.g. in higher plants) (Clegg, 2001). The role of these sugars in desiccation tolerant organisms is two-fold. First, sugars play a role in the protection of cells and bio-molecules by replacing water that is normally bonded to hydrogen. Secondly, sugars are involved in the formation of a glassy matrix in the cytoplasm (Wolkers *et al.*, 2002). In addition, it was reported that *in vitro* trehalose stabilized dried DNA (Shirkey *et al*., 2003). Trehalose was first associated with anhydrobiosis in the cyst of the brine shrimp *Artemia* spp. In its dry cysts this sugar is present at concentration of about 15 % of the total dry weight (Clegg, 2001). The biosynthesis of trehalose also correlates with the acquisition of desiccation tolerance in nematodes (Madin and Crowe, 1975; Womersley, 1981; Higa and Womersley, 1993) in which the content of trehalose is species-specific (e.g. in *Aphelenchus avenae* is 12-13 % of dry weight; Madin and Crowe, 1975) and in the larvae of the chironomid *P. vanderplanki (*Watanabe *et al*., 2004). During desiccation processes larvae of *P. vanderplanki* accumulate trehalose to around 20 % of dry weight (Watanabe *et al*., 2004). The knowledge of the role of trehalose in relationship to anhydrobiotic ability of tardigrades is based on studies performed on two eutardigrade species, namely *M. areolatus* and *R. coronifer* (Crowe, 1975; Westh and Ramløv, 1991). The pioneering experiment on *M. areolatus* suggested a synthesis of trehalose and glycerol during desiccation (Crowe, 1975). Nevertheless, the first quantitative study is that of Westh and Ramløv (1991) who analyzed accumulation of trehalose in *R. coronifer* during exposure to decreasing water potential. This species accumulated trehalose from 0.1 % to 2.3 % of the dry weight within 5-7 h reaching a 23-fold increase with respect to hydrated specimens. The accumulated trehalose was subsequently metabolized during the restoration of active life. Although substantial, the accumulation of trehalose in tardigrades is modest compared to levels detected in other anhydrobiotic invertebrates. In addition, in *R. coronifer* trehalose may have some protective effect at low cooling rates during cooling at -196 °C (Ramløv and Westh, 1992).

Even though considerable emphasis has been placed on the role of non-reducing disaccharides in both unicellular and multicellular organisms, molecular mechanisms governing anhydrobiosis is not fully understood. Recently it has become increasingly apparent that trehalose is insufficient to induce anhydrobiosis by itself. In fact, some anhydrobiotic organisms seem do not use trehalose and that other adaptations are required (Clegg 2001; Kshamata *et al.*, 2003; Tunnacliffe and Lapinski, 2003). The bdelloid rotifers exhibit excellent desiccation tolerance, but trehalose or other disaccharides are absent from sugars extracted from dry specimens (Lapinski and Tunnacliffe, 2003; Caprioli *et al.*, 2004). Furthermore, trehalose synthase genes (*tps*) have not been found in rotifer genomes (Lapinski and Tunnacliffe, 2003). In contrast, trehalose was detected in the anhydrobiotic resting egg of monogonont rotifer (Caprioli *et al*., 2004)

Attention has been turned to the definition of other molecular adaptations required for anhydrobiosis. Several stress protein families seem to be further keys to understand the anhydrobiotic mechanisms. In fact, upon drying, the stress response entails rapid synthesis of a suite of stress proteins. Proteins commonly indicated as “Heat shock proteins” (Hsps), being up-regulated by heat stress, also seem to be implicated in desiccation tolerance (Goyal *et al.*, 2005). Genes encoding Hsps are highly conserved and occur in every species in which they have been sought (Feder and Hofmann, 1999). As in any biochemical system, these proteins function as molecular chaperones, even in unstressed cells, and play primary roles in protein biosynthesis, folding, assembly, intracellular localization, secretion, and degradation of other proteins (Feder and Hofmann, 1999). Intracellular proteins can compensate the loss of hydrogen to water by hydrogen’s bonding to other molecules. It is likely that proteins and sugars interact through hydrogen bonding in the dry state in the cytoplasm of anhydrobiotes. Thus, both stress proteins and sugars could play an important role in the molecular organization of the dry cell when they are present in the same cellular compartment (Wolkers *et al.*, 2002).

Small heat shock proteins are expressed in the vegetative tissue of the resurrection plant during water stress (Alamillo *et al*., 1995) and in the cysts of the brine shrimp *Artemia franciscana* under anoxia (α-crystallin protein p26) or desiccation stresses (Clegg *et al*., 1999; Clegg, 2005). Genes coding Hsps are up-regulated in response to desiccation in the cyanobacterium *Anabaena* sp. 7120 (Katoh *et al*., 2004). In response to both desiccation and ionizing radiation, the bacterium *D. radiodurans* over-expresses a gene encoding a Hsp20 (Tanaka *et al*., 2004). In tardigrades, the evaluation of stress protein expression under desiccation stress is very recent and leads to contradictory data. *De novo* synthesis of a protein with a molecular weight of about 71 kDa, maybe belonging to the Hsp70 family, has been found during the entrance into anhydrobiosis in *R. coronifer* (Ramløv and Westh, 2001). Instead, Jönsson and Schill (2007), using the same model eutardigrade species, evidenced that desiccated specimens of *R. coronifer* have lower levels of Hsp70 than hydrated ones, while re-hydrated tardigrades after a period of desiccation show a higher level of Hsp70 than untreated controls. In addition, high levels of Hsp70 were detected in *R. coronifer* exposed to ionizing radiation and high temperature (Jönsson and Schill, 2007). Using the eutardigrade *Amphibolus volubilis,* Boschini *et al.* (2006) found a level of Hsp70 higher in dried specimens with respect to hydrated ones. In another eutardigrade, *M. tardigradum*, three heat-shock protein (Hsp70 family) genes and their different expression are evidenced during the transition from an active to a desiccated state and back to an active

state again (Schill *et al.*, 2004). In particular, one isoform shows an up-regulation during the induction of the desiccated state, while the other two isoforms seem not directly involved in anhydrobiosis since desiccated animals had significantly lower levels of mRNA expression than tardigrades in the hydrated state (Schill *et al.*, 2004). Certainly Hsp70 are involved in tardigrade desiccation, but further studies are necessary to understand how these proteins work to protect anhydrobiotic organisms.

An important role in anhydrobiotes is also played by "Late Embryogenesis Abundant" proteins (LEA), that are a complex of proteins accumulated during the seed maturation and which were first reported in the "resurrection plants" (Hoekstra *et al.*, 2001). Recently, LEA proteins have been found in non-plant organisms. Gene encoding LEA-like proteins have been identified in the genome of the bacterium *D. radiodurans*, and the inactivation of a gene coding for a group-3 LEA protein reduces its desiccation resistance (Battista *et al.,* 2001). LEA-like proteins and a novel protein called anhydrin are induced by dehydration in the nematode A*. avenae* (Browne *et al.,* 2002, 2004; Goyal *et al.*, 2005). Anhydrin has not yet been described in other species. In addition, LEA-like protein's expression is induced by dehydration in the entomopathogenic nematode (Gal *et al.*, 2003). On desiccation, the bdelloid rotifer *Philodina roseola* up-regulated a hydrophilic protein related to the LEA proteins associated with desiccation tolerance in plants (Tunnacliffe *et al.*, 2005). A similar protein was recently also evidenced in the chironomid *P. vanderplanki* (Kikawada *et al.*, 2006). These data suggest LEA-like proteins could be widespread in invertebrates, and also that bacteria, plants, and animals use these proteins in similar ways to combat desiccation stress.

Evolutionary and ecological traits of anhydrobiosis

Anhydrobiosis is considered an ancestral condition in plants, while in metazoans its origin is still unclear. However, the apparent homology of some genes (e.g. LEA genes) associated with anhydrobiosis in some animal groups (e.g. nematodes and rotifers) and even across, plants, and bacteria, could lead biologists to presume a common origin of this phenomenon (Alpert, 2005). For plants, anhydrobiosis is lost when organisms are no longer subject to desiccation, and associated genes can still be still present but not expressed or used (Alpert, 2006). Retention of unexpressed genes for desiccation tolerance leads to suppose that tolerance may have been selected against, rather than just no longer selected for (Alpert, 2006). On the contrary, the fact that anhydrobiosis has been found in different metazoan taxa that are not strictly phylogenetically correlated could be explained by convergent adaptive evolution (Hinton, 1968).

Water availability is one of the most important ecological factors acting on high selective pressure of terrestrial life. Selective advantages of anhydrobiosis are related to: a) the opportunity of continuous persistence in habitats under desiccation; b) the opportunity of dispersion in time from unfavorable conditions; c) the increased ability of dispersal in space thanks to increased resistance of the desiccated state; d) low interspecific competition because anhydrobiotic species can permanently live in habitats not suitable for other non-anhydrobiotic species.

Evolution of anhydrobiosis (and of cryobiosis) is the result of trade-offs between selective advantages, anhydrobiosis energetic costs, and physical and physiological constraints related to the process. In the anhydrobiotic process, needed energy is related to up-regulation of gene expression leading to synthesis and accumulation of bioprotectans (e.g. proteins, sugars, antioxidant) during the initial phase, while energetic costs are probably null during anhydrobiotic state because metabolism either is absent or is almost absent.

The recovery time of active life after a period of cryptobiosis (anhydrobiosis or cryobiosis) is directly related both to the harshness condition during the cryptobiotic initial phase (e.g. low humidity rate during desiccation in anhydrobiosis or high cooling rate in cryobiosis; higher stressors lead to longer recovery time; Wright, 1989a, b; Westh and Ramløv, 1991; Guidetti personal communication) and to time spent in cryptobiosis (Baumann, 1922; Crowe and Higgins, 1967; Guidetti, personal communication). The recovery time is probably a function of metabolic activities linked to repair of damages caused by desiccation (e.g. DNA damages recorded in bacteria, see Mattimore and Battista, 1996) and/or to restoration of metabolic pathways. The need of repair damages accumulated while desiccation occurs and the need to metabolize bioprotectans lead to a post-anhydrobiotic metabolic cost. There is very little information on anhydrobiosis costs in terms of energy. Jönsson and Rebecchi (2002) quantified energetic costs of a single anhydrobiotic period in the eutardigrade *R. coronifer* by measuring the size of storage cells (free moving cells in the body cavity that accumulate lipids and sugars; Fig. 1). Storage cell area decreases after a desiccation period, suggesting a substantial energetic cost of anhydrobiosis. On the other hand, energy consumption in connection with desiccation has also been shown in some nematodes (Madin and Crowe, 1975; Demeure *et al.*, 1978; Womersley, 1981).

Anhydrobiotic process need energy that is withdrawn from other possible uses such as growth and reproduction. This should have strong effects on the evolution of life histories. Even though there is not direct evidence for a trade-off between anhydrobiosis and fitness, the few ecological studies on this topic are consistent with the hypothesis that in a suitable habitat for non-anhydrobiotic species, the anhydrobiotic species have lower fitness (Alpert, 2006). Moreover, theoretical considerations (Jönsson, 2005) and empirical comparisons (Ricci and Caprioli, 2005) seem to suggest that low fecundity is associated with desiccation tolerance. Different life histories probably linked to anhydrobiotic ability are evidenced in a long-term study of a tardigrade community living in beech leaf litter. *Macrobiotus richtersi* (with good anhydrobiotic ability) shows different population dynamics and different reproductive strategies from *Hypsibius convergens*

(with lower anhydrobiotic performance) (Guidetti *et al.*, 2007). Generally, tardigrade species living in beech leaf litter and sharing low anhydrobiotic performances show similar population dynamics (Guidetti, personal communication).

Effects of desiccation on age-specific survival have been studied in rotifers and tardigrades. A tendency towards higher survival of older animals has been reported in rotifers (Ricci *et al.*, 1987; Ricci, 2001). In tardigrades, both positive and negative relationships between body size (age) and desiccation survival have been obtained at the intra-specific and inter-specific level. In the eutardigrade *R. coronifer*, desiccation survival generally tends to increase with body size, although survival declined dramatically in very large tardigrades, perhaps due to senescence (Jönsson and Rebecchi, 2002), whereas in *R. oberhaeuseri*, survival tends to decline with body size (Jönsson *et al.*, 2001).

Evolutionary studies on anhydrobiosis remain scarce, so there is little information related to the nature of selection on this phenomenon. Current opinion suggests that tardigrades originated within the sea (Renaud-Mornant, 1982), therefore the acquisition of anhydrobiosis was a crucial step in their colonization of terrestrial habitats. The first step towards anhydrobiotic evolution could have occurred in littoral zones, where temporally desiccating conditions are created by fluctuations in water level (May, 1953; Jönsson, 2001). Some marine heterotardigrades living in littoral zone (e.i. *Echiniscoides hoepneri*, *E. sigismundi groenlandicus* and *Archechiniscus marci*) support this hypothesis showing some degrees of anhydrobiotic abilities (Marcus, 1929; Kristensen and Hallas, 1980). Many heterotardigrades are bound to marine habitats, others colonize terrestrial (rarely freshwater) habitats, whereas, eutardigrades are limnic and/or terrestrial, rarely marine (no primarily marine eutardigrades are known). If both classes have originated within the sea starting from non anhydrobiotic tardigrades, anhydrobiosis has originated at least twice, and it may be the result of adaptive convergence. Alternatively, anhydrobiosis has been lost in several species, supporting the idea of "selection against, rather than just no longer selected for" desiccation tolerance. The action of natural selection in the evolution of anhydrobiotic ability can be evidenced by comparative studies on species living in different habitats. Species not strictly phylogenetically related and dwelling different environments show different degrees of anhydrobiosis (and cryobiosis) performances, with lower anhydrobiotic and cryobiotic abilities in species living in moister habitats (Wright 1991; Bertolani *et al.*, 2004). This finding leads to suppose that also in tardigrades lost or reduction of anhydrobiosis ability is due to a lack of positive selection to maintain it or to a negative selection against it. Similar results can be found in other types of organisms, for example in plants, in which although several plants can tolerate desiccation, the rate of tolerance is negatively associated with occurrence in moist habitats (see Alpert, 2005).

If natural selection acts on the anhydrobiotic performances, quantitative differences among individuals should have a genetic component. Studies on comparative differences in anhydrobiotic tardigrade performances show differences in specimens belonging to the same population and subject to the same experimental conditions. According to Jönsson and Rebecchi (2002), these differences could be bound to genetic differences and/or contingent factors, such as nutritional state, level of molecular protectans and some traits of the life history, including age, reproductive stage, and phenotypic plasticity. Differences in anhydrobiotic survival among geographically isolated populations of the eutardigrade *M. tardigradum* have been found by Horikawa and Higashi (2004), while Jönsson *et al*. (2001) did not find differences in isolated populations of *R. coronifer* and *R. oberhaeuseri*. Contrasting results also come from similar studies on nematodes (Menti *et al.*, 1997; Solomon *et al.*, 1999).

A last consideration regards the tardigrade rate of evolution. Thank to anhydrobiosis, selection by environment is enormously reduced (Pilato, 1979). In fact these animals are active solely under adequate level of free water. Therefore, anhydrobiotic periods also have an obvious impact on the generation time, which in turn influences the potential rate of evolution. This could be the cause of the surprising diversity of marine tardigrades with respect to terrestrial anhydrobiotic forms that appear more uniform at species, genera, and family level. The fossil *Milnesium swolenskyi* (terrestrial eutardigrade) found in Turonian amber 92 million years old (Bertolani and Grimaldi, 2000) is differentiated from some current co-generic species only by morphometric characters, indicating high levels of conservativeness.

Concluding remarks

From bacteria to different multicellular organisms, even though they are widespread in nature and belong to very diverse taxa, the complete removal of water from cells, storage in the dried state, and the rewetting impose physiological constraints that few organisms can tolerate. Anhydrobiotes can be able to avoid and/or to repair damages and alterations to proteins, lipids, nucleic acids, and alterations of the permeability features of the phospholipid bilayers which lead to death in the majority of organisms. Hence, the ability to withstand desiccation is a complex phenomenon that takes place at every level of the cellular organization, through mechanisms not yet completely understood. In addition, numerous observations suggest that some of the mechanisms underlying anhydrobiosis might contribute to the resistance of anhydrobiotes to other stresses. Further studies are necessary to better understand the mechanisms by which anhydrobiotes are able to tolerate the total suspension of metabolism due to the complete desiccation. These most recent studies are addressed to understand repair mechanisms of damages to biological structures induced by desiccation, especially of DNA damages, and to characterize genes and their proteins which allow dehydration without death in anhydrobiotic organisms.

Finally, using what we have learned from nature, can we engineer desiccation tolerance in species or cells that are not naturally anhydrobiotic? The stabilization of cells or tissues in a dry state of non anhydrobiotic organisms could be of considerable practical importance. The engineer of properties of trehalose represents a good response and a stimulus to continue the basic research to discover the secret of life without water. In fact, from the first report that biomolecules, membranes, and organisms can be stabilized in a dry state -thanks to the presence of high levels of trehalose - an astonishing array of implications for trehalose have been reported. These implications range from the stabilization of vaccines, lysosomes, and platelets to the hypothermic storage of humans organs (for review see Crowe *et al*., 2005). It is important to stress that all these discoveries have their roots in basic research on anhydrobiotic organisms, work that initially did not appear to have any practical applications in biotechnology (Crowe and Crowe, 2000).

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References

Abe S, Kurashima Y, Tanaka J. The cellular ability of desiccation tolerance in Japanese interdital seaweeds. Bot. Mar. 44: 125-131, 2001.

Adams S. Cryptobiosis in Chironomidae (Diptera) – two decades on. Antenna 8: 58-61, 1985.

Aguinaldo AMA, Turbeville JM, Linford L, Rivera MC, Garey JR, Raff RA, *et al*. Evidence for a clade nematodes, arthropods and other moulting animals. Nature 387: 489-493, 1997.

Alamillo J, Almoguera C, Bartels D, Jordano J. Constitutive expression of small heat shock proteins in vegetative tissues of the resurrection plant *Craterostigma plantagineum.* Plant Mol. Biol. 29: 1093-1099, 1995.

Alpert P. The limits and frontiers of desiccation-tolerant life. Integr. Comp. Biol. 45: 685-695, 2005.

Alpert P. Constraints of tolerance: why are desiccation-tolerant organisms so small or so rare? J. Exp. Biol. 209: 1575-1584, 2006.

Battista JR. Against all odds: the survival strategies of *Deinococcus radiodurans*. Ann. Rev. Microbiol. 51: 203-224, 1997.

Battista JR, Earl AM, Park M-J. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? Trends Microbiol. 7: 362-365, 1999.

Battista JR, Park MJ, McLemore AE. 2001. Inactivation of two homologues of proteins presumed to be involved in the desiccation tolerance of plants sensitizes *Deinococcus radiodurans* R1 to desiccation. Cryobiology 43: 133-139, 2001.

Baumann H. Die Anabiose der Tardigraden. Zool. Jahrb. 45: 501-556, 1922.

Baumann H. Bemerkungen zur Anabiose von Tardigraden. Zool. Anz. 72: 175-127, 1927.

Becquerel P. La suspension de la vie au desessous de 1/20 K absolu par demagnetisation adiabatique de l'alun de fer dans le vide les plus elève. C. R. Acad. Séan. Paris 231: 261-263, 1950.

Bertolani R, Grimaldi D. A new eutardigrade (Tardigrada: Milnesiidae) in amber from the upper cretaceous (Turonian) of New Jersey. In: Grimaldi D (ed), Studies on fossils in amber, with particular reference to the cretaceous of New Jersey, Backhuys Publishers, Leiden Netherlands, pp 103-110, 2000.

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

Bertolani R, Rebecchi L. Tardigrada. In: Knobil E and Neill JD (eds), Encyclopedia of Reproduction, Academic Press, San Diego, vol 4, pp 703-717, 1999.

Bertolani R, Rebecchi L, Jönsson KI, Borsari S, Guidetti R, Altiero T. Tardigrades as a model for experiences of animal survival in the space. In: Monti R and Bonifazi C (eds), La Scienza e la Tecnologia Spaziale sulla Stazione Internazionale (ISS). Special Issue on ASI National Workshop, Turin, 2001, MSSU - Microgravity and Space Station Utilization, 2: 211-212, 2001.

Billi D, Friedmann EI, Hofer KG, Grilli Caiola M, Ocampo-Friedmann R. Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl. Environm. Microbiol. 66: 1489-1492, 2000.

Boschini D, Guidetti R, Rebecchi L. Desiccation tolerance and Heat-shock proteins expression in active and dormant *Amphibolus volubilis* (Eutardigrada). Abstracts of 10th International Symposium on Tardigrada, University of Catania and University of Modena and Reggio Emilia, Catania, Italy, p 68, 2006.

Browne JA, Dolan KM, Tyson T, Goyal K, Tunnacliffe A, Burnell AM. Dehydration-specific induction of hydrophilic protein genes in the anhydrobiotic nematode *Aphelenchus avenae*. Eukaryotic Cell 3: 966-975, 2004.

Browne JA, Tunnacliffe A, Burnell A. Anhydrobiosis – plant desiccation gene found in a nematode. Nature 416: 38, 2002.

Brusca C, Brusca GJ. Invertebrates. Second edition. Sinauer Associates, Inc., Publishers, Sunderland, Massachussettes, 2003.

Caprioli M, Kathiolm AK, Melno G, Ramløv H, Ricci C, Santo N. Trehalose in desiccated rotifers: a comparison between a bdelloid and a monogonont species. Comp. Biochem. Physiol. 139A: 527-532, 2004.

Clegg JS. Metabolic studies of cryptobiosis in encysted of *Artemia salina*. Comp. Biochem.

Physiol. 20: 801-809, 1967.
Clegg JS. Cryptobiosis - a peculiar state of biological organization. Comp. Biochem. Physiol. 128B: 613-624, 2001.
Clegg JS. Desiccation tolerance in encysted embryos of the animal extremophiles, *Artemia*. Integr. Comp. Biol. 45: 715-724, 2005.
Clegg SJ, Willsie JK, Jackson SA. Adaptive significance of a small heat shock/α-crystallin protein (p26) in encysted embryos of the brine shrimp, *Artemia franciscana*. Am. Zool. 39: 836-847, 1999.
Chinnasri B, Moy JH, Spires BS, Schmidt DP. Effect of gamma-irradiation and heat on root-knot nematode *Neloidogyne javanica.* J. Nematol. 29: 30-34, 1997.
Copley J. Indestructible. New Scientist 164: 45-46, 1999.
Crowe JH. Anhydrobiosis: an unsolved problem. Am. Nat. 105: 563-573, 1971.
Crowe JH. Evaporative water loss by tardigrades under controlled relative humidity. Biol. Bull. 142: 407-416, 1972.
Crowe JH. The physiology of cryptobiosis in tardigrades. Mem. Its. Ital. Idrobiol. 32 Suppl.: 37-59, 1975.
Crowe JH, Cooper F jr. Cryptobiosis. Sci. Am. 225: 30-36, 1971.
Crowe JH, Crowe LM. Preservation of mammalian cells – learning nature's tricks. Nature Biotechnol. 18: 145-146, 2000.
Crowe JH, Crowe LM, Wolkers WF, Oliver AE, Ma X, Auh J-H, *et al*. Stabilization of dry mammalian cells: lessons from nature. Integr. Comp. Biol. 45: 810-820, 2005.
Crowe JH, Higgins B. The revival of *Macrobiotus areolatus*: (Tardigrada) from the cryptobiotic state. Trans. Am. Microsc. Soc. 86: 286-294, 1967.
Crowe JH, Madin KAC. Anhydrobiosis in nematode: evaporative water loss and survival. J. Exp. Biol. 193: 323-334, 1975.
Crowe JH, Newell IM, Thomson WW. Fine structure and chemical composition of the cuticle of the tardigrade, *Macrobiotus areolatus* Murray. J. Microsc. 11: 107-120, 1971.
Demeure Y, Reversat G, Van Gundy SD, Freckman DW. The relationship between nematode reserves and their survival to desiccation. Nematropica 8: 7-8, 1978.
Doyère PLN. Memoires sur les Tardigrades. Sur le facilité possedent les tardigardes, les rotifers, les anguilleles des toit et quelques autres animacules, de renvenir à la vie après été complement désesschées. Ann. Sci. Nat. 18: 5, 1842.
Feder ME, Hofmann GE. Heat-shock proteins, molecular chaperomes, and the stress response: evolutionary and ecological physiology. Ann. Rev. Physiol. 61: 243-282, 1999.
Fontana F. Lettre a un de ses amis sur l'ergot et la Tremella. F Physique Par l'Abbé Rozier 7: 42, 1776.
Franceschi T. Anabiosi nei tardigradi. Boll. Mus. Ist. Biol. Univ. Genova 22: 47-49, 1948.
Gal TZ, Glatzer I, Koltai H. Differential gene expression during desiccation stress in the insect-killing nematode *Steinernema feltiae* IS-6. J. Parasitol. 89: 761-766, 2003.
Garey JR, Krotec M, Nelson DR, Brooks J. Molecular analysis supports a tardigrade-arthropod association. Invertebr. Biol. 115: 79-88, 1996.
Garey JR, Nelson DR, Mackey LY, Li J. Tardigrade phylogeny: congruency of morphological and molecular evidence. Zool. Anz. 238: 205-210, 1999.
Giard A. L'anhydrobiose ou ralentisswement des phénomènes vitaux. C. R. Soc. Biol. Paris 46 : 497-500, 1894.
Giribet G, Carranza S, Baguña J, Riutort M, Ribera C. First molecular evidence for the Existence of a Tardigrada + Arthropoda clade. Mol. Biol. Evol. 13: 76-84, 1996.
Goeze JAE. Über den kleinen Wasserbär. In: Bonnet HK (ed), Abh. Insektologie, vol 2, Halle, pp 367-375, 1773.
Goyal K, Walton LJ, Browne JA, Burnell AM, Tunnacliffe A. Molecular anhydrobiosis: identifying molecules implicated in invertebrate anhydrobiosis. Integr. Comp. Biol. 45: 702-709, 2005.
Guidetti R, Colavita C, Altiero T, Bertolani R, Rebecchi L. Energy allocation in two species of Eutardigrada. In: Pilato G and Rebecchi L (eds), Proceedings of the Tenth International Symposium on Tardigrada, J. Limnol. (66 Suppl.) 1: 111-18, 2007.
Guidetti R, Jönsson KI. Long-term anhydrobiotic survival in semi-terrestrial micrometazoans. J. Zool. London 257: 181-187, 2002.
Hand SC. Metabolic dormancy in aquatic invertebrates. Adv. Comp. Environm. Physiol. 8: 1-50, 1991.
Hellenby C. Desiccation survival in the plant parasitic nematodes. *Heterodera rostochiensis* Wollenweber and *Dirylenchus dispaci* (Kuhn) Filipjev. Proc. R. Soc. 169B: 203-213, 1968.
Higa LM, Womersley CZ. New insights into the anhydrobiotic phenomenon: the effects of trehalose content and differential rates of evaporative water loss on the survival of *Aphelenchus avenae.* J. Exp. Zool. 267: 120-129, 1993.
Hinton HE. A new chironomid from Africa, the larva of which can be dehydrated without injury. Proc. Zool. Soc. London 121: 371-380, 1951.
Hinton HE. Cryptobiosis in the larva of *Polypedilum vanderplanki* Hint (Chironomidae). J. Insect Physiol. 5: 286-300, 1960a.
Hinton HE. A fly larva that tolerates dehydration and temperatures of -270° to + 102°C. Nature 188: 336-337, 1960b.
Hinton HE. Reversible suspension of metabolism and the origin of life. Proc. R. Soc. London Biol. Sci. 171B: 43-57, 1968.
Hoekstra FA, Golovina EA, Buitink J. Mechanisms of plant desiccation tolerance. Trends Plant Sci. 6: 431-438, 2001.
Horikawa DD, Higashi S. Desiccation tolerance of the tardigrade *Milnesium tardigradum* collected in Sapporo, Japan and Bogor, Indonesia. Zool. Sci. 21: 813-816, 2004.
Horikawa DD, Sakashita T, Katagiri C, Watanabe M, Kikawada T, Nakahara Y, *et a*l. Radiation

tolerance in the tardigrade *Milnesium tardigradum.* Int. J. Radiation Biol. 82: 843-848, 2006.
Ivarsson H, Jönsson KI. Aggregation effects on anhydrobiotic survival in the tardigrade *Richtersius coronifer.* J. Exp. Zool. 301A: 195-199, 2004.
Iwasaki T. Sensitivity of *Artemia* eggs to the γ-irradiation. III. The sensitivity and the duration of hydration. J. Radiation Res. 5: 91-96, 1964.
Jönsson KI. The nature of selection on anhydrobiotic capacity in tardigrades. Zool. Anz. 240: 409-417, 2001.
Jönsson KI. The evolution of life histories in holo-anhydrobiotic animals: a first approach. Integr. Comp. Biol. 45: 764-770, 2005.
Jönsson KI, Bertolani R. Facts and fiction about long-term survival in tardigrades. J. Zool. London 255: 121-123, 2001.
Jönsson KI, Borsari S, Rebecchi L. Anhydrobiotic survival in populations of the tardigrades *Richtersius coronifer* and *Ramazzottius oberhaeuseri* from Italy and Sweden. Zool. Anz. 240: 419-423, 2001.
Jönsson KI, Guidetti R. Effects of Methyl-bromide fumigation on anhydrobiotic micrometazoans. Ecotox. Environm. Safety 50: 72-75, 2001.
Jönsson KI, Harms-Ringdhal M, Torudd J. Radiation tolerance in the eutardigrade *Richtersius coronifer*. Int. J. Radiation Biol. 81: 649-656, 2005.
Jönsson KI, Rebecchi L. Experimentally induced anhydrobiosis in the tardigrade *Richtersius coronifer*: phenotypic factors affecting survival. J. Exp. Zool. 293: 578-584, 2002.
Jönsson KI, Schill RO. Induction of Hsp70 by desiccation, ionizing radiation and heat-shock in the eutardigrade *Richtersius coronifer*. Comp. Biochem. Physiol. 146B: 456-460, 2007.
Jørgensen A, Kristensen RM. Molecular phylogeny of Tardigrada - investigation of the monophyly of Heterotardigrada. Mol. Phyl. Evol. 32: 666-670, 2004.
Katoh H, Asthana RK, Ohmori M. Gene expression in the cyanobacterium *Anabaena* sp. PCC7120 under desiccation. Microbiol Ecol. 47: 164-174, 2004.
Keilin D. The problem of anabiosis or latent life: history and current concepts. Proc. Roy. Soc. 150B: 149-191, 1959.
Kikawada T, Nakahara Y, Kanamori Y, Iwata KI, Watanabe M, McGee B, *et al*. Deydration induced expression of LEA proteins in an anhydrobiotic chironomid. Biochem. Biophis. Res. Com. 348: 56-61, 2006.
Kristensen RM, Hallas TE. The tidal Genus *Echiniscoides* and its variability, with erection of Echiniscoididae fam. n. (Tardigrada). Zool. Scripta 9: 113-127, 1980.
Kshamata G, Tisi L, Basran A, Browne J, Burnell A, Zurdo J, *et al*. Transition from natively unfolded to folded state induced by desiccation in an anhydrobiotic nematode protein. J. Biol. Chem. 278: 12977-12984, 2003.
Kumaraswamy S, Udupa S, O'Cain V, Mattimore V, Battista jr. Novel ionizing radiation sensitive mutants of *Deinococcus radiodurans*. J. Bacteriol. 176: 7439-7446, 1994.
Lapinski J, Tunnacliffe A. Anhydrobiosis without trehalose in bdelloid rotifers. FEBS Letters 553: 387-390, 2003.
Madin KAC, Crowe JH. Anhydrobiosis in nematodes: carbohydrates and lipids metabolism during dehydration. J. Exp. Zool. 193: 335-342, 1975.
Marcus E. Tardigrada. In: Bronns HG (ed), Klassen und Ordnungen des Tierreichs., Leipzig vol 5, pp 1-608, 1929.
Mattimore V, Battista JR. Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J. Bacteriol. 178: 633-637, 1996.
May RM. Cytologie des globules cavitaires actifs et dormants chez le tardigrade *Macrobiotus hufelandi* Schultze. Arch. Anat. Microsc. Morphol. Exp. 36 : 136-150, 1946.
May RM. L'evolution des tardigrades de la vie aquatic a la vie terrestre. Bull. Fran. Pisciculture 25: 93-100, 1953.
May RM, M Maria, J Guimard. Actions différentielles des rayons x et ultraviolets sur le tardigrade *Macrobiotus areolatus*, à l´état actif et desséché. Bull. Biol. France Belgique 98: 349-367, 1964.
Menti H, Wright DJ, Perry RN. Desiccation survival of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. J. Helminthol. 71: 41-46, 1997.
Needham JT. Concerning chalky concretions called malm; with some microscopical observations on the farina of Red Lily, and worms discovered in smutty corn. Phil. Trans. 42: 634, 1743.
Newsham KK, Maslen NR, McInnes SJ. Survival of Antarctic soil metazoans at -80°C for six years. CrioLetters 27: 269-280, 2006.
Nichols PB, Nelson DR, Garey JR. A family level analysis of tardigrade phylogeny. Hydrobiologia 558: 53-60, 2006.
Nielsen C. Animal Evolution: Interrelationships of the Living Phyla. Oxford University Press, 1997.
Oliver MJ. How some plants recover from vegetative desiccation: a repair based strategy. Acta Physiol. Plant. 19: 419-425, 1997.
Oliver MJ, Tuba Z, Mishler BD. The evolution of vegetative desiccation tolerance in land plants. Plant Ecol. 151: 85-100, 2000.
Pilato G. Correlation between cryptobiosis and other biological characteristics in some soil animals. Boll. Zool. 46: 319-332, 1979.
Potts M, Slaughter SM, Hunneke F-R, Garst JF, Helm RF. Desiccation tolerance of prokaryotes: application of pronciples to human cells. Integr. Comp. Biol. 45: 80-809, 2005.
Pouchet F. Expériences sur la résistance vitale des animalcules pseudo-ressuscitans. Comp. Rend. Acad. Paris, 49: 492-494, 1859a.
Pouchet F. Nouvelles expériences sur les animaux pseudo-ressuscitans. Comp. Rend. Acad. Paris 49: 886-888, 1859b.
Preyer W. Mittheilung aus dem Vortrag des Herrn Prof. Dr Preyer. Tagebl. Versamml. dtsch. Naturf. Aertze, Leipzig 18: 46, 1872.
Preyer W. Über die Anabiose. Biol. Zbl. 1: 1, 1891.

Rahm PG. Biologische und Physiologische Beiträge zur Kenntnis der Moosfauna. Z. Allg. Physiol. 20: 1-34, 1923.
Rahm PG. Weitere physiologische Versuche mit niederen Temperaturen. Ein Beitrag zur Lösung des Kalteproblems. Verhand. Deut. Zool. Ges. 29: 106-111, 1924.
Rahm PG. Die Trochenstarre (Anabiose) de moostierwelt (ihr Verlauf. ihre Bedeutung und ihr Unterschied on der Cystebildung). Biol. Zbl. 46: 452-477, 1926.
Ramazzotti G, Maucci W. Il Phylum Tardigrada. III edizione riveduta e aggiornata. Mem. Ist. Ital. Idrobiol. 41: 1012, 1983.
Ramløv H, Westh P. Survival of the cryptobiotic eutardigrade *Adorybiotus coronifer* during cooling to –196°C: effect to cooling rate, trehalose level, and short-term acclimation. Cryobiology 29: 125-130, 1992.
Ramløv H, Westh P. Cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*: tolerance to alcohols, temperature and *de novo* protein synthesis. Zool. Anz. 240: 517-523, 2001.
Rebecchi L, Guidetti R, Borsari S, Altiero T, Bertolani R. Dynamics of long-term anhydrobiotic survival of lichen-dwelling tardigrades. Hydrobiologia 558: 23-30, 2006.
Renaud-Mornant J. Species diversity in marine Tardigrada. In: Nelson DR (ed), Proceedings of the Third International Symposium Tardigrada. East Tennessee State University Press, Johnson City, Tennessee, pp 149-178, 1982.
Ricci C. Dormancy patterns in rotifers. Hydrobiologia 446/447: 1-11, 2001.
Ricci C, Caprioli M. Anhydrobiosis in bdelloid species, populations and individuals. Integr. Comp. Biol. 45: 759-763, 2005.
Ricci C, Caprioli M, Boschetti C, Santo N. *Macrotrachela quadricornifera* featured in a space experiment. Hyrobiologia 534: 239-244, 2005.
Ricci C, Melone G. *Macrotrachela quadricornifera* (Rotifera Bdelloidea): a SEM study on active and cryptobiotic animals. Zool. Scripta 13: 195-200, 1984.
Ricci C, Melone G, Santo N, Caprioli N. Morphological response of a bdelloid rotifer to desiccation. J. Morphol. 257: 246-253, 2003.
Ricci C, Vaghi L, Manzini ML. Desiccation of rotifers (*Macrotrachela quadricornifera*): survival and reproduction. Ecology 68: 1488-1494, 1987.
Roffredi A. Sur l'origine des petits verms ou Anguilles du bled rachitiches. F. Physique Par l'Abbé Rozier 5: 1-19, 1775a.
Roffredi A. Seconde lettre on suite d'observations sur le rachitism du Bled, sur les Anguilles de la colle de farine et sur le grain charbonne. F. Physique Par l'Abbé Rozier 5 : 197-225, 1775b.
Ruppert EE, Barnes RD, Fox RS. Zoologia degli Invertebrati. Un approccio funzionale-evoluzionistico. IV edizione italiana condotta sulla VII edizione americana. Piccin, 2004.
Schill RO, Steinbrück GHB, Köhler H-R. Stress gene (hsp70) sequences and quantitative expression in *Milnesium tardigradum* (Tardigrada) during active and cryptobiotic stages. J. Exp. Biol. 207: 1607-1613, 2004.
Schmidt P. Anabiosis. SSSR. Academy of Science, Moscow and Leningrad, 376 pp, 1948.
Seki K, Toyoshima M. Preserving tardigrades under pressure. Nature 395: 853-854, 1998.
Shen MJ, Mudgett MB, Schorf JW, Clarke S, Berger R. Exceptional seed longevity and robust growth: ancient sacred lotus from China. Am. J. Botany 82: 1367-1380, 1995.
Shirkey B, McMaster NJ, Smith SC, Wright DJ, Rodriguez H, Jaruga P, *et al*. Genomic DNA of *Nostoc commune* (Cyanobacteria) becomes covalently modified during long-term (decades) desiccation but is protected from oxidative damage and degradation. Nucleic Acids Res. 31: 2995-3005, 2003.
Solomon A, Paperna I, Glatzer I. Desiccation survival of the entomopathogenic nematode *Steinernema feltiae*: induction of anhydrobiosis. Nematology 1: 61-68, 1999.
Sømme L, Meier T. Cold tolerance in Tardigrada from Dronning Maud Land, Antarctica. Polar Biol. 15: 221-224, 1995.
Spallanzani L. Opuscoli di Fisica animale e Vegetabile. Società Tipografica, Modena, vol 2, 1776.
Tanaka MM, Earl AM, Howell HA, Park M-J, Eisen JA, Peterson SN, *et al*. Analysis of *Deinococcus radiodurans*'s transcriptional response to ionizing radiation reveals novel proteins that contribute to extreme radioresistance. Genetics 168: 21-33, 2004.
Trainor FR, Gladych R. Survival of algae in a desiccated soil: a 35 year study. Phycologia 34: 191-192, 1995.
Tunnacliffe A, Lapinski J. Resurrecting Van Leeuwenhoek's rotifers: a reappraisal of the role of disaccharides in anhydrobiosis. Phil. Trans. R. Soc. London 358B: 1755-1771, 2003.
Tunnacliffe A, Lapinski J, McGree B. A putative LEA protein, but no trehalose, is present in anhydrobiotic bdelloid rotifers. Hydrobiologia 546: 315-321, 2005.
Van Leeuwenhoek A. On certain animalcules found in the sediments in gutter of the roofs of houses. Letter 144 to Hendrik van Bleyswijk, dated 9 February 1702 in The Select Works of Anton van Leeuwenhoek, London, vol 2, pp 207-213, 1702.
Walz B. The morphology of cells and cell organelles in the anhydrobiotic tardigrade, *Macrobiotus hufelandi*. Protoplasma 99: 19-30, 1979.
Watanabe M, Kikawada T, Fujita A, Forczek E, Adati T, Okuda T. Physiological traits of invertebrates entering cryptobiosis in a post-embryonic stage. Eur. J. Entomol. 101: 439-444, 2004.
Watanabe M, Kikawada T, Minagawa N, Yukuhiro F, Okuda T. Mechanism allowing an insect to survive complete dehydration and extreme temperatures. J. Exp. Biol. 205: 2799-2802, 2002.
Watanabe M, Sakashita T, Fujita A, Kikawada T, Horikawa DD, Nakahara Y, *et al*. Biological effects of anhydrobiosis in an African chironomid, *Polypedilum vanderplanki* on radiation tolerance. Int. J. Radiation Biol. 82: 587-592, 2006.

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

Wolkers WF, Tablin F, JH Crowe. From anhydrobiosis to freeze-drying of eukaryotic cells. Comp. Biochem. Physiol. 131A: 535-543, 2002.

Womersley C. Biochemical and physiological aspects of anhydrobiosis. Comp. Biochem. Physiol. 70B: 669-678, 1981.

Wright JC. Structural correlates of permeability and tun formation in tardigrade cuticle: an image analysis study. J. Exp. Ultrastruct. Mol. Res. 101: 23-29, 1988.

Wright JC. The tardigrade cuticle II. Evidence for a dehydration-dependent permeability barrier in the cuticle. Tissue Cell 21: 263-279, 1989a.

Wright JC. Desiccation tolerance and water-retentive mechanisms in tardigrades. J. Exp. Biol. 142: 267-292, 1989b.

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

Wright JC. Cryptobiosis 300 Years on from van Leeuwenhoek: what have we learned about tardigrades? Zool. Anz. 240: 563-582, 2001.

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